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Molecular and morphological diversity of surviving *on-farm* hazelnut (*Corylus avellana* L.) landraces from southern Europe and their role in the origin and diffusion of the cultivated germplasm

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Abstract

Hazelnut (*Corylus avellana* L.) is a traditional nut crop in southern Europe. As a result of germplasm exploration conducted *on-farm*, 77 landraces were surveyed in five countries (Portugal, Spain, Italy, Slovenia, and Greece). The present work describes phenotypic variation in nut and husk traits and investigates genetic relationships among 42 landraces, 57 well-known references cultivars, and 19 wild accessions using 10 microsatellite (SSR) markers. Among the 77 landraces, 42 had unique fingerprints while 35 turned out to be potential synonyms, showing an identical SSR profile with some cultivars. Based on the 42 unique landraces, morphological observations revealed high phenotypic diversity and some had characteristics appreciated by the market. The analysis of the genetic relationships and population structure contributed to investigate the origin and diffusion of the cultivated germplasm in southern Europe. Our results indicating the existence of three primary centre of domestication in the Mediterranean basin: northwestern Spain (Tarragona) and southern Italy (Campania) in the West and Black Sea (Turkey) in the East. Moreover, data suggested the existence of secondary hazelnut domestication centre in the Iberian (Asturias) and Italian (Liguria and Latium) Peninsula, where local varieties have been domesticated in subsequent times from wild forms and/or from the introduction of ancient domesticate varieties.

Key words: filbert; *in situ* conservation; biodiversity; simple sequence repeat (SSR) markers; microsatellite; domestication

Introduction

Corylus avellana L., the European hazelnut, is diploid (2n=2x=22), monoecious, dichogamous, wind-pollinated, and has a sporophytic incompatibility that enforces cross-pollination. Its geographical distribution extends from Europe and North Africa to the Caucasus region and Asia Minor. It is the source of important cultivars in Europe and Turkey, that show a high level of genetic diversity for plant size, growth habit, husk length, nut size, nut shape, and shell thickness. Many of them were selected over many centuries from local wild populations (Thompson et al. 1996), but some were recognized as superior varieties and spread outside the area of origin by trade and migrations. In spite of the long cultivation history, still little is known about their origin and domestication. European hazelnut is one of the most important tree nut crops in terms of worldwide production. The Black Sea countries account for the majority of world production: Turkey (610,264 tons, average of 2009-2011), Azerbaijan (28,564 tons), and Georgia (20,567 tons). Other important producers are Italy (114,991 tons), the USA (35,079 tons), and Spain (16,988 tons) followed by Iran, China, France and Greece (FAOstat 2011). About 90% of the world crop is shelled and sold as kernels, while the remaining 10% is sold in-shell for fresh consumption. The primary user of kernels, the food industry, has precise requirements for morphological, chemical, and physical characteristics of the kernels as well as absence of defects.

In recent years efforts to improve the efficiency and effectiveness of agro-biodiversity conservation have been done for most crop species as required by the Convention on Biological Diversity (CBD 1992), the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO 2001), and the Global Plant Conservation Strategy (CBD 2002). Agro-biodiversity includes plant genetic resources (PGR) for food and agriculture: i) modern cultivars, breeding lines, and genetic stocks that are widely and actively conserved by plant breeders and gene banks; ii) obsolete cultivars, landraces (e.g., farmer populations of crop plants), ecotypes (e.g., natural plant populations), wild and weedy relatives that still need to be actively conserved (Polegri and Negri 2010). PGR are the raw material required for the genetic improvement, in order to adapt a crop to unpredictable environmental changes, and to guarantee the food security for the future generations (FAO 2001). In the last decades, PGR have usually been conserved by *ex situ* methods. More recently *in situ* conservation, sometimes

referred to as "on-farm conservation", has been proposed as a better conservation strategy. It allows the maintenance of genetic resources in natural settings where their processes of evolution and adaptation to environment are maintained (Jarvis et al. 2000).

In hazelnut about 400 cultivars have been described and are maintained in different international germplasm repositories (Thompson et al. 1996). A total of 510 accessions are conserved in 13 European hazelnut collection fields: 4 are located in Italy (Università degli Studi di Torino - UNITO, Agenzia Regionale per lo Sviluppo e l'Innovazione dell'Agricoltura del Lazio - ARSIAL, and Centro Ricerche per l'Agricoltura - CRA); 3 in Portugal (Universidade de Trás-os-Montes e Alto Douro – UTAD, Direcção Regional de Agricultura e Pescas do Norte, Sergude – DRAPN, and Direcção Regional de Agricultura e Pescas do Centro, Viseu – DRAPC); 2 in Spain (Institut de Recerca i Tecnologia Agroalimentàries - IRTA and Servicio Regional de Investigación y Desarollo Agroalimentario - SERIDA) and in Slovenia (University of Ljubljana, Biotechnical Faculty); 1 in France (Conservatoire Végétal Régional d'Aquitanie) and in Greece (National Agricultural Research Foundation - NAGREF). (Rovira et al. 2011). The United States Department of Agriculture-Agricultural Research Service-National Clonal Germplasm Repository (USDA-ARS-NCGR) and Oregon State University (OSU) in Corvallis (Oregon, USA) preserves more than 800 *Corylus* accessions (Bassil et al. 2009). A collection at the Hazelnut Research Institute (HRI) in Giresun (Turkey) contain 20 registered cultivars and more than 400 accessions collected from the Black Sea coasts of Turkey (Gürcan et al. 2010a). In contrast, *ex situ* conservation strategies are not been applied in hazelnut PGR, although a first *on-farm* exploration was conducted in northern Spain (Asturias) by Ferreira et al. (2010). Thus, during over three consecutive years (2008-2010) the EU AGRI GEN RES project SAFENUT ('Safeguard of almond and hazelnut genetic resources: from traditional uses to modern agro-industrial opportunities') has been conducted with the aim of increasing the knowledge on the European hazelnut genetic diversity. Objectives included the description of major cultivars as well as the *on-farm* exploration, description, and *in situ* conservation of local endangered PGR. This characterization was carried out under different points of view: morphological, biochemical, molecular, as well as in ecological and cultural aspects (Bacchetta et al. 2011)

Identification of accessions and analysis of genetic diversity in *in situ* and *ex situ* collections are important points in the management and utilization of PGR. Traditional methods to characterize and identify hazelnut accessions or cultivars are based on morphological and phenological descriptors (Biodiversity International 2008). In recent years, DNA markers have proven to be convenient for accurately identifying cultivars due to their high discriminating power at a relatively low cost. In *C. avellana* microsatellite or simple sequence repeat (SSR) markers have been recently developed (Bassil et al. 2005a, 2005b; Boccacci et al. 2005; Gürcan and Mehlenbacher 2010a, 2010b; Gürcan et al. 2010b) and placed in linkage genetic maps (Mehlenbacher et al. 2006; Sathuvalli et al. 2011). Loci have been used to fingerprint accessions in collections, identify synonyms, determine parentage, and assess genetic relationships among cultivars (Boccacci et al. 2006, 2008; Gökirmak et al. 2009; Gürcan et al. 2010a). SSR markers have been also used to investigate the genetic diversity and structure of different populations (Boccacci and Botta 2010; Gökirmak et al. 2009; Gürcan et al. 2010a) or between local cultivars and wild hazelnuts (Campa et al. 2011).

The present work reports the results of a hazelnut germplasm exploration conducted *on-farm* within the SAFENUT project in five southern European countries (Portugal, Spain, Italy, Slovenia, and Greece). The main aims were: i) to characterize hazelnut landraces using morphological descriptors and SSR markers; ii) to investigate their genetic relationships with wild forms and well-known reference cultivars; iii) to understand their role in the domestication events. The information will be useful to identify landraces for *in situ* preservation, further evaluations in *ex situ* collections, and use in breeding programs.

Materials and methods

Plant material

A total of 153 hazelnut accessions were analyzed in this study: i) 77 landraces mostly surveyed *on-farm* during the SAFENUT project (2008-2010) (Table 1); ii) 57 true-to-type reference cultivars collected in different European and Turkish collection fields (Supplementary Table 1) ; iii) 19 wild hazelnuts sampled in the sites of Vejano (Latium, Central Italy) and Benevento (Campania, South Italy), where wild populations are still present.

The landraces were surveyed in the traditional areas of hazelnut cultivation in five southern European countries (Table 1). Among them, 15 accessions were collected in the Iberian Peninsula, 5 in northern Portugal and 10 in northern Spain (Asturias); 52 accessions were surveyed in six Italian regions: 6 in Piedmont (North-West Italy), 10 in Liguria (North-West Italy), 1 in Marche (Central Italy), 12 in Latium (Central Italy), 3 in Calabria (South Italy), and 20 in Sicily (South Italy); 10 accessions were from the Balkans, 5 from Slovenia, and 5 from northern Greece. Farmers were contacted explaining the reasons of the project; they were interviewed about the presence of old endangered cultivars in their farm. Information on agronomic and qualitative traits, as well as use, local names, tradition, and social context were also collected.

Morphological observations

A total of 20-50 nuts were collected *in situ* from each surveyed landrace. Husks or involucres, nuts and kernels were characterized using 14 qualitative standard descriptors specific for hazelnut (Table 2), following Thompson et al. (1978), the UPOV (1979), and Biodiversity International (2008) guidelines.

DNA extraction and SSR analysis

Total genomic DNA was extracted from 0.25 g of young leaves or immature catkins using the modified procedure described by Thomas et al. (1993).

A total of 10 SSR loci, selected by Boccacci and Botta (2010) for the SAFENUT project, were analyzed: CaT-B107, CaT-B501, CaT-B502, CaT-B503, CaT-B504, CaT-B505, CaT-B507, CaT-B508 (Boccacci et al. 2005), CaC-B020, and CaC-B028 (Bassil et al. 2005a). PCR amplifications were performed in a volume of 15 µl containing 40 ng DNA, 0.5 U Taq-DNA polymerase (AmpliTaq Gold, Applied Biosystems, Foster City, CA, USA), 1.5 µl 10x PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 2 mM $MgCl_2$, 200 µM dNTPs, and 0.5 µM of each primer. The PCR conditions were: a first denaturation step at 95°C for 9 min, followed by 26 cycles of denaturation (30 s at 95°C), annealing (45 s at 55°C and 50°C for CaT-B502), and extension (90 s at 72°C). The final elongation step was carried out at 72 °C for 30 min. Amplification products were analyzed using an ABI-PRISM

3130 Genetic Analyzer capillary electrophoresis instrument (Applied Biosystems, Foster City, CA, USA). Results of the run were processed with GeneMapper software (Applied Biosystems) and alleles were designated by their size in base pairs (bp) using a GeneScan-500 LIZ standard (Applied Biosystems).

Data analysis

Microsatellite data obtained at 10 SSR loci for 153 hazelnut accessions were processed using the software Identity 4.0 (Wagner and Sefc 1999) to identify accessions with identical SSR profile and only one genotype was included in the following analysis.

The genetic relationships among the different genotypes were investigated using two types of analysis. An unweighted pair-group method using arithmetic average (UPGMA) was used to construct and draw a dendrogram from the genetic similarity matrix using MEGA v. 5.05 (Tamura et al. 2011). Genetic distances (1,000 bootstraps) were computed as: D=[1-(proportion of shared alleles)], using Microsat software (Minch 1997). A principal coordinate analysis (PCoA) was computed by GenAlEx 6.2 (Peakall and Smouse 2006).

The program STRUCTURE v. 2.3.3 (Pritchard et al. 2000), a model-based Bayesian clustering method, was used to infer population structure and assign individuals to sub-populations. STRUCTURE was run five independent times for each *K* value ranging from 1 to 10. The admixture model was applied and allele frequencies were assumed to be correlated. A burn-in period of 100,000 generations and 200,000 Markov Chain Monte Carlo replications were used. All individuals were also treated as having known origin and were divided in 8 geographical groups. Landraces and cultivars from Iberian Peninsula (18 accessions), North-West Italy (13), Central Italy (12), South Italy (7), Sicily (22), and Balkans-Black Sea (27) were assigned to 6 different groups, while the wild individuals from Latium (9) and Campania (10). were separated in further 2 groups. The statistic ΔK (Evanno et al. 2005) was calculated by STRUCTURE HARVESTER software (Earl et al. 2011) and used to selected the optimal *K* value.

Genetic diversity and differentiation among 8 geographical populations was investigated. The Popgene software (Yeh et al. 1997) was used to calculate: observed (N_a) and effective (N_e) number of

alleles; observed (H_o) and expected heterozygosity (H_e); Nei's (1978) coefficient of genetic identity (G_i) and genetic distance (Gd); and gene flow (N_m) (Slatkin and Barton 1989). The fixation index (F_{st}) was estimated according to Weir and Cockerman (1984) using the program F-STAT (Goudet et al. 1995); significance level of F_{st} values were determined after 560 permutations.

The Shannon-Weaver index was calculated to estimate the phenotypic diversity of each trait observed in 42 landraces. The diversity index was calculated as $H = - \Sigma p_i \ln p_i$, where p_i is the frequency of the phenotypic class *i* in each evaluated trait, as reported in Table 2 (Shannon and Weaver 1949). A principal component analysis (PCA) using the 14 morphological descriptors for 42 landraces and 11 well-known cultivars ('Casina', 'Barcelona', and 'Negret' from Spain; 'Nocchione', 'Tonda Gentile Romana', 'Tonda di Giffoni', and 'Tonda Gentile delle Langhe' from Italy; 'Tombul' from Turkey; 'Cosford' from England; 'Istrska dolgoplodna' and 'Istrska okrogloplodna' from Slovenia) was performed using PAST v. 2.12 software (Hammer et al. 2001).

Results

Set of duplicates

Microsatelite analysis identified 42 unique genotypes among the 77 investigated landraces (Supplementary Table 1). An identical SSR profile was observed between 35 landraces and some reference cultivars and a total of 10 sets of duplicates were identified.

Among the landraces surveyed in the Iberian Peninsula, 3 sets of duplicates were detected. The first set consisted of 3 accessions from Portugal: 'Cartuxeria/Tubulosa', 'Dawton', and 'Purpurea' that showed the same SSR profile with 'Fructo rubro' (syn. 'Pellicule rouge'), which had small, long, thin-shelled nuts and long tubular husks. The second set was the pair 'Raul' and the Turkish cultivar 'Karidaty' (syn 'Imperial de Trebizonde'). The third set grouped 6 accessions from northern Spain and 'Casina', the most common cultivar spread in this area.

Three sets of accessions with the same genotype were found among the accessions collected in the Italian regions. The first set included 6 accessions of 'Tonda di Biglini' (Piedmont) and 'Tonda Gentile della Langhe' ('TGL'). The latter cultivar dominates in over 90% of the local orchards for its unquestioned technological and quality characteristics. However, 'Tonda di Biglini' showed some

important phenological and carpological differences in comparison to 'TGL'. Harvest time was more precocious for 'Tonda di Biglini' (10-15 days before 'TGL'), nuts had thicker shell with a consequent lower percentage of kernel by weight, and a higher presence of double kernels in comparison to 'TGL' nuts (data not showed). The second set was 'Meloni' and 'Nocciola della Madonnella' from Latium and 'Tonda Gentile Romana' ('TGR'), that represents about the 85% of the local nut production. Also 'Meloni' showed a more precocious harvest time in comparison to 'TGR' (about 15 days). The third set consisted of 'Nocchia rosa' (Latium), 3 accessions of 'Tonda Calabrese' (Calabria), 6 accessions of 'Caraffara' (Sicily), and the cultivar 'Nocchione'. All of which had round-oblate nuts of medium size in short husks. 'Nocchione' is the main pollinizer of 'TGR' in the Latium region. It is also the widespread cultivar in Sicily, where is known under different names: 'Nostrale', 'Comune' or 'Mansa' (Catania and Messina provinces), 'Racinante' (Enna province), and 'Santa Maria del Gesù' (Palemo province). According to Alberghina (1982), the morphological differences observed in the above-mentioned cultivars to be due to environmental factors and thus he renamed them 'Siciliana'. Recently, Boccacci et al. (2006) and Gökirmak et al. (2009) confirmed these synonymies analyzing 24 and 21 SSR loci, respectively.

The landraces from the Balkans showed 4 set of duplicates. The first consisted of the accessions named 'CV/1' and 'CV/2' from Slovenia and 'Barcelona' (syn. 'Castanyera'). 'Barcelona' is commercially important only in the USA and in France where it is known as 'Fertile de Coutard'. In Spain, it is of minor importance and known as 'Castanyera' in northwestern Spain (Tarragona province) and 'Grande' in northern Spain. In Portugal is named 'Grada de Viseu' (Mehlenbacher and Miller 1989). The second set was the pair 'Patem small' from Greece and 'Fructo rubro'. The third set was 'Argiroupoli' and 'Patem large' from Greece and the cultivar 'Yassi Badem' from Turkey. Its kernels resembled almonds in size and shape and are consumed fresh, but are not suitable for processing. Finally, the fourth set was the pair 'Polykarpos' and 'Tombul Ghiaghli' from Greece, one of the common varieties cultivated in Greece.

Morphological characterization

Morphological observations revealed high phenotypic diversity in 42 unique landraces genotypes (Table 2). The H index calculated for each of the 14 morphological descriptors averaged 1.1, ranging from 0.26 (‘presence of double kernels’) to 1.57 (‘kernel shape’); the highest values were found for ‘nut shape’ (1.50) and ‘kernel shape’(1.57). Among the husk traits, the predominant nut number per cluster was 2-3 (46.2%) and 1-2 (30.8%) and the majority of landraces had an involucre longer than the nut (47.4%). Of the nut characters, the small (40.5%) and medium (37.5%) sizes were predominant among the individuals. Nut shape was highly variable, but the globular (33.3%) and long cylindrical (26.2%) shapes were the most represented. The majority of nuts had light brown shell color (64.3%), few (33.3%) or medium stripes (47.6%), and small (44.1%) or medium (44.1%) size of pistil scar. Among kernel descriptors, almost all accessions (92.9%) had no presence of double kernels. The majority of them showed medium (45.2%) or small (40.5%) kernel size and the most representative shapes were ovoid, long cylindrical and globular (28.6, 28.6 and 23.8%, respectively). The appearance of skin was slightly corky (57.1%) and the size of internal cavity was prevalently small (53.1%). Concerning the ‘percentage of kernel by weight’, 31.7% of landraces showed medium values (45.1-50.0%), while 58.6% had values less than 45.0%. Finally, 43.9% of them had a high ‘percentage of kernel caliber higher than 12 mm’ (75.1-100%). These descriptors can be used to define the suitability of the nuts for industry processing or for fresh consumption. The main morphological and technological traits evaluated in each of the 42 landraces are reported in Table 3.

In the PCA obtained from 14 morphological descriptors for 42 landraces and 11 reference cultivars, the first two components (PC1 and PC2) explained 38.7% of the total variation. PC1 accounted for 25.1% and was positively correlated with the nut and kernel size. PC2 accounted for an additional 13.6% and was mostly associate to nut and kernel shape. The PCA scatter-plot split the samples into three main groups (Fig. 1). Among Italian landraces, the northwestern accessions (Liguria) were separated by those from central (Latium) and southern (Sicily) Italy. Ligurian landraces, to exception of ‘Noscello’, were grouped on the right side of the scatter-plot with ‘Casina’, ‘Istrska dolgoplodna’, ‘Negret’, and ‘Tombul’. The accessions collected in Sicily and Latium, except ‘Selvaggiola lunga’, clustered together but in two separated groups. The group on the upper left part of

the plot contained: i) ‘Barrettona’, ‘Cappello del Prete’, ‘Itavex’, and ‘Madonnella’ from Latium; ii) ‘Selvaggiola riccia’, ‘Selvaggiola SIC6’, SIC13, and SIC16, ‘Selvaggiola agostara’, ‘Selvaggiola tardiva SIC8’, and ‘Trichette’ from Sicily; iii) ‘Barcelona’, ‘Nocchione’, ‘TGR’, ‘Tonda di Giffoni’, and ‘TGL’ as reference cultivas. In the group on the lower part of the graph were included: i) ‘Allungata’, ‘Nocciola Ada’, ‘Nocciola Benedetta’, ’Nocciola centenaria’, and ‘Nocciola lunga’ from Latium and ‘San Vicino Vittori’ from Marche; ii) ‘Minnulara’, ‘Selvaggiola tardiva SIC12’, and ‘Selvaggiola’ SIC4, SIC7, and SIC17 from Sicily; iii) ‘Cosford’. The PCA did not separate in distinct groups the landraces from the Iberian Peninsula, Slovenia, and Greece.

Genetic relationships and population structure analysis

A dendrogram depicting the genetic relationships among 42 unique landrace genotypes, 57 true-to-type cultivars, and 19 wild individuals was constructed. Accessions were grouped into 8 clusters (Fig. 2). Group A included cultivars from the Balkans and Black Sea (Turkey). In this group were placed 3 accessions surveyed in Italy (‘San Vicino Vittori’ from Marche, ‘Lunghera’ and ‘Seigretta’ from Liguria) and 1 surveyed in Greece (‘Philio’). In group B were clustered cultivars and landraces from different geographical areas. The accession ‘T/10’ surveyed in Slovenia was placed with the cultivars ‘TGL’ (Italy), ‘Trenet’, and ‘Morell’ (Spain). The landraces ‘Ciasetta’ from Liguria and ‘Nocciola Benedetta’, ‘Nocciola lunga’, and ‘Allungata’ from Latium were placed with the Turkish cultivars ‘Yassi Badem’ and ‘Badem’. Group C contained cultivars from the Iberian Peninsula together with the landraces surveyed in Asturias (‘Allande 3’, ‘Robriguedo 2’, ‘Las Cuevas 1’, and ‘Priero 1’) and in Liguria (‘Noscello’, ‘Menoia’, and ‘Bardina’). The landraces from northern Spain constituted a sub-group with ‘Casina’ and ‘Noscello’. Most Italian cultivars and landraces were placed in the main cluster D. They represents germplasm from Central Italy (Latium), South Italy (Campania), and Sicily. In this group were also placed the accessions ‘T/0’ and ‘T/16’ from Slovenia and ‘Quinta Vila Nova Do Rego’ from Portugal. Group E was formed by 4 landraces surveyed in North-West Italy (Liguria). Finally, the last three clusters (F, G, and H) were constituted by wild genotypes and some cultivated accessions. Clusters F and G included both wild and landraces from Latium, while almost all wild individuals from Latium and Campania were included in the main group H with the cultivars ‘Tonda

rossa' and 'Tonda bianca'. They are two varieties grown in the Avellino province only (Campania, South Italy), resulted distinct from the cultivars of the same geographical area and show similar morphological nut traits.

In the PCoA, the first two PCs explained 48.7% of the total variation. The first coordinate explained 26.1 % of the variation and the second coordinate an additional 22.6 %. The projection of 118 hazelnut accessions on a two-dimensional plane defined by the first two PCs (Fig. 3) showed a tendency to separate the cultivated accessions from the wild genotypes. Considering the geographical origin of the cultivars and landraces analyzed, the scatter-plot showed a tendency of the central-southern Italian accessions to cluster together in the half below of the graph. Accessions from Balkans-Black Sea were preferentially placed in the upper left part and those from Iberian Peninsula in the upper right part of the scatter-plot. Among the northern Italian accessions: three ('Lunghera', 'Seigretta', and 'Trietta') clustered with those from Balkans-Black Sea; seven ('Bardina', 'Del Rosso', 'Dell'Orto', 'Gianchetta', 'Menoia', 'Noscello', and 'Tapparona') were found with the Iberian accessions; three ('Catainetto', 'Ciasetta', and 'TGL') were placed in an intermediate position, along the axis X of the graph.

The 118 hazelnut genotypes were further evaluated for population stratification using the STRUCTURE software. SSR data were analyzed increasing the number of subpopulations (K) from 1 to 10. The estimation of ΔK revealed the highest value for $K = 3$ ($\Delta K = 48.1$), indicating the existence of three groups mainly constituted by Turkish, wild, and central-southern Italian accessions, respectively (Fig. 4). Several genotypes were not clearly placed in separated groups, such as those from Spain or Liguria that clustered both with the Turkish and wild accessions. Moving to $K = 4$ ($\Delta K = 21.2$) and $K = 5$ ($\Delta K = 12.0$) these three populations remained almost invariable, whereas several Spanish accessions showed the tendency to constitute a separate group; Ligurian accessions were placed both with the Turkish and Spanish accessions; some cultivated forms collected in Latium showed introgression with the local wild germplasm (Fig. 4). Comparing these results with the UPGMA dendrogram and the PCA scatter-plot, there was an agreement about the population subdivision and the genetic relationships among genotypes.

Differentiation among geographical gene pools

On the basis of their geographical area of origin, the 118 unique genotypes were divided in 8 gene pools (Table 4 and 5).

The observed (N_a) and effective (N_e) number of alleles and the observed (H_o) and expected heterozygosity (H_e) were calculated to evaluated the level of genetic diversity within each gene pool (Table 4). N_a and N_e ranged from 4.2 to 7.8 (average 6.3) and from 3.1 to 4.7 (average 3.8), respectively. H_o (average 0.81) was generally higher than H_e (average 0.71) in each group, with the exception of the wild individuals from Latium. The level of genetic diversity observed, was high and similar to that found by other authors (Boccacci and Botta 2010; Boccacci et al. 2006, 2008; Gökirmak et al. 2009; Gürcan et al. 2010a; Campa et al. 2011). Although this high heterozygosity is a consequence of the self-incompatibility mating system of *C. avellana* and wind pollination, it could reflect high levels of outcrossing in several gene pools (Boccacci and Botta 2010).

Genetic identity (G_i), genetic distances (Gd), fixation index (F_{st}), and gene flow (N_m) were calculated to investigate the genetic differentiation among gene pools (Table 5). G_i was highest among gene pools constituted by cultivated accessions, ranging from 0.667 to 0.918. On the contrary, G_i values were lower between wild and cultivated groups, and ranged from 0.400 to 0.694. Correspondingly, a higher Gd was found between cultivated and wild groups, and ranged from 0.086 (Iberian Peninsula vs. North-West Italy) to 0.916 (Wild Campania vs. Balkans-Black Sea). All pairwise comparisons yielded significant differentiation values, ranging from 0.015 (Iberian Peninsula vs. North-West Italy) to 0.194 (Wild Campania vs. Sicily) with $P_{(Fst\ not > 0)} < 0.05$ for each F_{st} value and an equally distribution of N_m values between gene pools was observed.

Discussion

Mislabeling and the existence of synonyms and homonyms are important challenges in the *in situ* conservation strategies. In the past decade, SSR markers have become very valuable tools in the management of hazelnut *ex situ* collections. In their study, Boccacci et al. (2006) reported 6 sets of synonyms among 78 accessions from European collections; Gökirmak et al. (2009) found 72 duplicates among 270 accessions from USDA-ARS-NCGR and OSU germplasm repositories; and 6

Turkish accessions conserved in the US collection fields were found to be synonyms of cultivars from the HRI collection by Gürcan et al. (2010a).

Among the 77 landraces surveyed in five southern European countries (Portugal, Spain, Italy, Slovenia, and Greece), the combination of SSR profiles across all loci resulted in 42 unique genotypes and 35 accessions turned out to be potential synonyms. A total of 10 sets of duplicates were found between landraces and some reference cultivars. Among them, landraces from Portugal, Slovenia, and Greece would result to be synonymous of foreign cultivars: 'Barcelona' (syn. 'Castanyera') from Spain; 'Fructo rubro' from Balkans; 'Karidaty' (syn. 'Imperial de Trebizonde'), 'Yassi Badem', 'Palaz', and 'Tombul Ghiaghli' from Turkey. Hazelnut is cultivated in very small amount in Portugal and Slovenia, where foreign cultivars were introduced. Since the 1980's, several commercial cultivars have been evaluated for growth and nut production in both Countries, in order to obtain more information about local and imported cultivars (Solar and Štampar 2011). In Greece hazelnut cultivation was originated by Greek immigrants coming from the Pontus region (northern Turkey). They brought Turkish cultivars which are cultivated until today, such as 'Extra Ghiaghli', 'Tombul Ghiaghli', and 'Sivri Ghiaghli'. Accessions surveyed in Spain and Italy showed a same SSR profile and similar morphological traits with some of the most important local cultivars: 'Casina' in northern Spain, 'TGL' in Piedmont, 'TGR' in Latium, and 'Nocchione' (syn. 'Siciliana') in central and southern Italy. Hazelnut growing has a strong tradition in Spain and Italy, where few cultivars dominates in the orchards. In Spain, the northeastern province of Tarragona (Catalonia) accounts for 88% of the total area planted to hazelnut and 'Negret' is the most widespread cultivar. Minor hazelnut-growing areas include the Asturias and adjacent regions in northern Spain, where cultivated forms are found in small orchards and gardens. In the past, hazelnut was an important crop in this region and 'Casina' was one of the most cultivated variety (Ferreira et al. 2010; Campa et al. 2011). In Italy, almost all of the producing surface is located in four regions that represent 98% of the hazelnut production: Campania, Latium, Piedmont, and Sicily. Other producers are Liguria, Sardinia, Emilia, Veneto, and Calabria. In the Italian Peninsula a wide and varied germplasm exists but in some regions it is often unknown, such as in Sicily and in some minor growing areas. In Campania, seven main varieties are cultivated for the food industry ('Mortarella', 'San Giovanni', and 'Tonda di Giffoni') or

fresh consumption ('Tonda bianca', Tonda rossa', 'Camponica', and 'Riccia di Talanico'). In Piedmont ('TGL') and Latium ('TGR') a monovarietal cultivation exists, while 'Nocchione' (syn. 'Siciliana') is the main pollinizer of 'TGR' in Latium.

In their studies, Boccacci et al. (2006) and Gökirmak et al. (2009) reported that 'Nocchione' and a group of Sicilian cultivars, renamed 'Siciliana' by Alberghina (1982), were synonyms at 24 and 21 SSR loci, respectively. It was an unexpected results, since these cultivars are grown in two distant Italian regions: Latium and Sicily (Boccacci et al. 2006). Our results contributes to confirm this hypothesis of synonymy and to clarify the origin of 'Nocchione'. STRUCURE analyses revealed that 'Nocchione' grouped (98%) with the accessions from South Italy and Sicily rather than with those from Latium (Central Italy). The Bayesian clustering and admixture analysis can be considered as a standard method to establish the cultivar origins in ancestral populations, quantifying the genetic relationships by probabilities and proportions (Breton et al. 2008). Thus, data indicated that 'Nocchione' was originated in southern Italy, most likely in Sicily, and was introduced in recent times in Latium as TGR's pollinizer. It is probable that 'Nocchione' was also introduced in Calabria, during the second half of XIX century (Piccirillo et al. 2007), as pointed out by the genetic identity between 'Nocchione' and 'Tonda Calabrese'. Moreover, the molecular analysis of most Sicilian accessions surveyed *in situ* contributed to confirm the existence of a dominant cultivars in the local orchards (Alberghina 1982). In fact, 6 'Caraffara' accessions, also named 'Nostrale' in the Enna province, showed the same SSR profile with 'Siciliana', indicating that Sicily was very likely the centre of origin of 'Nocchione' from which it spread in central and south Italy.

The morphological characterization of 42 unique genotypes revealed a wide diversity among the surveyed landraces. The H index was high (average of 1.1) and most of the phenotypic classes were represented in each evaluated descriptors (Table 2). These accessions should be considered original and valuable PGR; thus they should be regarded as additional local source of genetic diversity which need to be conserved *in situ*. In addition, some landraces showed morphological and technological traits appreciated by the market (Table 3). Accessions 'Robriguedo-2' (Asturias), 'Noscello' (Liguria), 'Barrettona', 'Itavex', 'Cappello del Prete', 'Madonnella' (Latium), and 'Selvaggiola Tardiva SIC12' (Sicily) were interesting for the food industry. Nuts with globular or ovoid shape, kernels with

medium size and a caliber ≥ 12 mm are the ideal traits for the industry processing (Garrone and Vacchetti 1994). On the contrary, 'Selvaggiola SIC3', 'Trichette' (Sicily), 'San Vicino Vittori' (Latium), and 'T/16' (Slovenia) showed traits suitable for fresh consumption, such as nuts and kernels with large size.

The study of the genetic relationship and population structure among wild forms, landraces, and cultivars in a geographical area can supply information about the putative domestication events, the evolutionary relationships or the gene flow between them.

The UPGMA tree (Fig. 2), the PCoA scatter-plot (Fig. 3), and the STRUCTURE analyses (Fig. 4) revealed a high level of differentiation between wild and cultivated forms. The wild genotypes from Latium and Campania resulted closely related and were found separated from cultivars and landraces. Nevertheless, an introgression and admixture of genotypes between wild accessions and some landraces from Campania ('Tonda bianca' and 'Tonda rossa') or from Latium ('Nocciola centenaria', 'Cappello del prete', and 'Barrettona') was observed. Similar results were also obtained by Campa et al. (2011) between 40 wild hazelnuts collected in northern Spain and 62 local cultivated accessions, investigated at 13 SSR markers. Then, SSR data are in agreement with the general idea that most currently cultivated hazelnut varieties were selected over centuries from local wild populations and some were spread outside their area of origin by trade and migrations (Thompson et al. 1996).

The cultivated forms showed the tendency to constitute two main groups located to Mediterranean basin in the West (Spain-Italy) and Black Sea basin in the East (Turkey). They are two of the four major geographical gene pools described in the European hazelnut (English, Central European, Spanish-Italian, and Black Sea) by Gökirmak et al. (2009). A high level of genetic similarity between cultivars grown in the Iberian and Italian Peninsula was also reported by other authors (Boccacci and Botta 2010; Boccacci et al. 2006; Gürcan et al. 2010a). In our study, almost all accessions from the Iberian Peninsula were separate by those from Italy. The cultivars from northeastern Spain (Tarragona) resulted closer to the accessions surveyed in the northern Spain (Asturias), rather than to the varieties cultivated in central and southern Italy. The landraces surveyed in Asturias showed the tendency to cluster into a separate 'Casina' group. This result was in agreement with those reported by Campa et al. (2011), suggesting that locally hazelnuts belong to the

northeastern Spanish gene pool but constitute a separate domestication group. Only three Iberian accessions ('Barcelona', 'Gironell', and 'Quinta Vila Nova Do Rego') grouped with the Italian cultivars, probably as consequence of the high number of Italian landraces analyzed. A significant genetic differentiation between the Spanish and Italian gene pools was also observed by Boccacci and Botta (2010). It is probable that Spain and Italy are two independently hazelnut domestication areas and the gene flow between western and central Mediterranean basin was a consequence of human migrations and trade during and after the Roman civilization (Boccacci and Botta 2009, 2010). Among the accessions from Italian Peninsula, most of central-southern ones constituted a largest gene pool, while some landraces surveyed in Liguria ('Gianchetta', 'Dell'Orto', 'Tapparona', and 'Del Rosso') were arranged in a separate group. A congruent topology was reported in the PCA scatter-plot obtained from morphological data (Fig. 1). Then, genetic and morphological data indicated less gene flow between northern and southern Italy, whereas exchange of plant material very likely occurred in South Italy between Campania and Sicily. The existence of a main gene pool in southern Italy supported the hypothesis that it was an important centre of origin and diffusion of hazelnut cultivars, as suggested by Boccacci and Botta (2009) analyzing 75 hazelnut cultivars from Spain, Italy, Turkey, and Iran at 13 chloroplast SSR (cpSSR) loci. Several Italian landraces were not grouped according to their geographical origin. Some accessions from Latium ('San Vicino Vittori', 'Nocciola Benedetta', 'Nocciola lunga', and 'Allungata') and Liguria ('Trietta', 'Lunghera', and 'Seigretta') were genetically closer to Turkish cultivars; others from Liguria ('Noscello', 'Menoia', 'Catainetto', and 'Bardina') showed a genetic similarity with Spanish accessions. Therefore, a gene flow occurred from western Mediterranean basin to northern Italy and from Black Sea to North and Central Italy, most likely as consequence of commercial exchanges. In fact, during the XI century, hazelnuts produced in Turkey were traded in Liguria on the Genoa market (Rosengarten 1984). These results confirm the hypothesis that hazelnut cultivation and cultivars were not introduced from the eastern Mediterranean/Black Sea basin into southern Italy by Greeks or by Arabs (Boccacci and Botta 2009).

The genetic diversity calculated between each geographical gene pool pair (Table 5) also supported the above mentioned considerations: i) high genetic differentiation between northern and southern Italian groups; ii) low genetic diversity among central-southern Italian gene pools; iii) higher

genetic similarity between Iberian and North-West Italy groups and between Balkans-Black Sea and northwestern and central Italian groups; iv) low gene flow between southern Italy and Black Sea. Finally, these results also indicated that northeastern Spain, southern Italy, and Black Sea were the three most important hazelnut domestication areas.

The archeological findings, historical documents, pollen data, and cpSSR analysis supported the hypothesis that Campania (Southern Italy) was an important centre of origin and diffusion of hazelnut cultivars (Boccacci and Botta 2009). It seems likely that this germplasm originated from the post-glacial refuge in southern Italy (Palmé and Vendramin 2002), and spread beginning from the Roman civilization around the Mediterranean Sea. Our data contributed to support this hypothesis, indicating that a varietal circulation occurred among Latium, Campania, and Sicily regions. Moreover, genetic relationships also showed that the Sicilian cultivars 'Napoletana' and 'Napoletanedda' were very close to those from Campania, confirming their introduction in Sicily from Campania. Thus, it can be hypothesized that a gene flow occurred in a first time from Campania southward to Sicily and northward to Latium, while in a second time the cultivar circulation continued from Sicily to Latium and Calabria. Results would confirm that hazelnut cultivation was not introduced in Sicily by Arabs but from Campania by the Romans. The Arabs dominated the Isle only from the second half of the IX century, whereas hazelnut was already cultivated at Roman time (Boccacci and Botta 2009).

In summary, the molecular and morphological characterization of surviving *on-farm* landraces were useful for eliminating duplications or mistakes in order to rationalize their *in situ* preservation and to identify the most interesting accessions. These materials have been grafted and propagated into their own roots. In the next future the materials will be planted in two hazelnut collections: IRTA in Reus (Spain), and in the country were the material comes, to be evaluated *ex situ* for further uses in breeding programs. Findings about genetic relationships and population structure also raise an interesting question about the origin and diffusion of the hazelnut germplasm cultivated in southern Europe. According to several authors (Boccacci and Botta, 2009, 2010; Gökirmak et al. 2009; Gürcan et al. 2010a), *C. avellana* seems to have been domesticated independently in six main different areas: British Islands, Central Europe, Spain, Italy, Black Sea, and Iran. Our results are in agreement with these conclusions, indicating the existence of three primary centre of domestication in the

Mediterranean basin: northwestern Spain and southern Italy in the West and Black Sea in the East. Moreover, data indicated the existence of secondary hazelnut domestication centre in the Iberian (Asturias) and Italian (Liguria and Latium) Peninsula, where local varieties have been domesticated in subsequent times from wild forms and/or from the introduction of ancient domesticate varieties, followed by a relatively local evolution that could include crosses among them and with local hazelnuts. The introduction of plant material from other areas influenced the local gene pool, but it is more likely that this was due to introgression of genes from foreign germplasm into local accessions followed by selection rather than to the direct adoption of introduced cultivars.

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References

Alberghina O (1982) Indagine sulla corilicoltura siciliana. Frutticoltura 2: 27-30

Bacchetta L, Di Giovanni B, Avanzato D, Botta R, Bellon B, Boccacci P, Drogoudi P, Metzidakis I, Rovira M, Sarraquigne JP, Silva AP, Solar A, Spera (2011) The AGRI GEN RES 'SAFENUT' action: a European strategy for the preservation and utilization of hazelnut and almond genetic resources. Nucis-Newsletter 15: 30-33

Bassil NV, Botta R, Mehlenbacher SA (2005a) Microsatellite markers in the hazelnut: isolation, characterization and cross-species amplification in *Corylus*. J Am Soc Hort Sci 130: 543-549

Bassil NV, Botta R, Mehlenbacher SA (2005b) Additional microsatellites of the European hazelnut. Acta Hort 686: 105-110

Bassil NV, Postman J, Hummer K, Botu M, Sezer A (2009) SSR fingerprinting panel verifies identities of clones in backup hazelnut collection at USDA genebank. Acta Hort 845:95-102

Bioversity, FAO, CIHEAM (2008) Descriptors for hazelnut (*Corylus avellana* L.). Biodiversity International, Rome, Italy; Food and Agriculture Organization of the United Nations, Rome, Italy; International Centre for Advanced Mediterranean Agronomic Studies, Zaragoza, Spain.

Boccacci P, Botta R (2009) Investigating the origin of hazelnut (*Corylus avellana* L.) cultivars using chloroplast microsatellites. Genet Resour Crop Evol 56: 851-859

Boccacci P, Botta R (2010) Microsatellite variability and genetic structure in hazelnut (*Corylus avellana* L.) cultivars from different growing regions. Sci Hortic 124: 128-133

Boccacci P, Akkak A, Bassil NV, Mehlenbacher SA, Botta R (2005) Characterization and evaluation of microsatellite loci in European hazelnut (*Corylus avellana* L.) and their transferability to other *Corylus* species. Mol Ecol Notes 5: 934-937

Boccacci P, Akkak A, Botta R (2006) DNA-typing and genetic relationships among European hazelnut (*Corylus avellana* L.) cultivars using microsatellite markers. Genome 49: 598-611

Boccacci P, Rovira M, Botta R (2008) Genetic diversity of hazelnut (*Corylus avellana* L.) germplasm in northeastern Spain. HortScience 43: 667-672

Breton C, Pinatel C, Médail F, Bonhomme F, Bervillé A (2008) Comparison between classical and Bayesian methods to investigate the history of olive cultivars using SSR-polymorphisms. Plant Science 175: 524-532

Campa A., Trabanco E, Pérez-Vega E, Rovira M, Ferreira JJ (2011) Genetic relationship between cultivated and wild hazelnuts (*Corylus avellana* L.) collected in northern Spain. Plant Breed 130: 360—366

CBD (1992) Convention on biological diversity: text and annexes. Secretariat of the Convention on Biological Diversity, Montreal

CBD (2002) Global strategy for plant conservation. Secretariat of the convention on biological diversity, Montreal. Available via DIALOG. http://www.biodiv.org/decisions/?lg=0&dec=VI/9. Accessed 23 Oct 2009

Earl DA, vonHoldt BM (2011) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genet Resour DOI: 10.1007/s12686-011-9548-7

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14: 2611-2620

FAO (2001) International treaty on plant genetic resources for food and agriculture. Available via DIALOG. http://www.planttreaty.org/texts_en.htm. Accessed 6 Nov 2009

FAOstat (2011) Agriculture data. Available from: http://faostat.fao.org/site/408/default.aspx. Accessed 31 May 2011

Ferreira JJ, C. Garcia-González, Tous J, Rovira M (2010) Genetic diversity revealed by morphological traits and ISSR markers in hazelnut germplasm from northern Spain. Plant Breed 129: 435-441

Garrone W, Vacchetti M (1994) Hazelnut quality in relation with the requirements of confectionary industry. Acta Hort 351: 641-648

Gökirmak T, Mehlenbacher SA, Bassil NV (2009) Characterization of European hazelnut (*Corylus avellana* L.) cultivars using SSR markers. Genet Resour Crop Evol 56:147–172

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. J. Hered. 86: 485–486.

Gürcan K, Mehlenbacher SA (2010a) Transferability of microsatellite markers in the Betulaceae. J Amer Soc Hort Sci 135: 159-173

Gürcan K, Mehlenbacher SA (2010b) Development of microsatellite marker loci for European hazelnut (*Corylus avellana* L.) from ISSR fragments. Mol Breed 26: 551–559

Gürcan K, Mehlenbacher SA, Erdoğan V (2010a) Genetic diversity in hazelnut (*Corylus avellana* L.) cultivars from Black Sea countries assessed using SSR markers. Plant Breed 129: 422-434

Gürcan K, Mehlenbacher SA, Botta R, Boccacci P (2010b) Development, characterization, segregation, and mapping of microsatellite markers for European hazelnut (*Corylus avellana* L.) from enriched genomic libraries and usefulness in genetic diversity studies. Tree Genetics & Genomes 6: 513-531

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica 4(1): 9pp.

Jarvis DI, Myer L, Klemick H, Guarino L, Smale M, Brown AHD, Sadiki M, Sthapit B, Hodgkin T (2000). A training guide for *in situ* conservation *on-farm*. Version 1. IPGRI, Rome, Italy

Mehlenbacher SA, Miller AN (1989) 'Barcelona' hazelnut. Fruit Varieties Journal 43: 90-95

Mehlenbacher SA, Brown RN, Nouhra EN, Gökirmak T, Bassil NV, Kubisiak TL (2006) A genetic linkage map for hazelnut (*Corylus avellana* L.) based on RAPD and SSR markers. Genome 49:122–133

Minch E (1997) MICROSAT. Available from: http://hpgl.stanford.edu/projects/microsat/ Accessed 07 July 2009

Nei M (1978) Estimation of average heterozygocity and genetic distance from a small number of individuals. Genetics 89:583–590

Palmé AE, Vendramin GG (2002) Chloroplast DNA variation, postglacial recolonization and hybridization in hazel, *Corylus avellana*. Mol Ecol 11: 1769–1779

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6: 288–295

Piccirillo P, Petriccione M, Clasadonte A, Rosato T, De Luca A (2007) Problematiche e prospettive di rilancio per la corilicoltura calabrese. Frutticoltura 11: 62-66

Polegri L, Negri V (2010) Molecular markers for promoting agro-biodiversity conservation: a case study from Italy. How cowpea landraces were saved from extinction. Genet Resour Crop Evol 57:867–880

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945-959

Rosengarten F (1984) The book of edible nuts. Walker and Company, New York

Rovira M, Avanzato D, Bachetta L, Botta R, Drogoudi P, Ferreira JJ, Sarraquigne JP, Silva AP, Solar A (2011) European *Corylus avellana* germplasm collections. Acta Hort 918:871-876

Sathuvalli VR, Chen H, Mehlenbacher SA, Smith DC (2011) DNA markers linked to eastern filbert blight resistance in "Ratoli" hazelnut (*Corylus avellana* L.). Tree Genetics & Genomes 7: 337-345

Shannon CE, Weaver W (1949) The mathematical theory of communication. University of Illinois Press, Urbana

Slatkin M, Barton NH (1989) A comparison of three indirect methods for estimating average levels of gene flow. Evolution 43: 1349-1368

Solar A, Štampar F (2011) Characterization of selected hazelnut cultivars: phenology, growing and yielding capacity, market quality and nutraceutical value. Jour Sci Food Agric 91(7): 1205-1212

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular Evolutionary Genetics Analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731-2739

Thomas MR, Matsumoto S, Cain P, Scott NS (1993) Repetitive DNA of grapevine: classes present and sequences suitable for cultivar identification. Theor Appl Genet 86: 173-180

Thompson MM, Romisondo P, Germain E, Vidal-Barraquer R, Tasias Valls J (1978) An evaluaton system for filberts (Corylus avellana L.). HortScience 13(5): 514-517

Thompson MM, Lagerstedt HB, Mehlenbacher SA (1996) Hazelnuts. In: Janick J, Moore JN (eds) Fruit breeding: nuts, vol 3. Wiley, New York, pp 125-184

UPOV (1979) Guidelines for the conduct of test for distinctness, homogeneity and stability (hazelnut). UPOV, Genéve, Switzerland. TG/71/3

Wagner HW, Sefc, KM (1999) IDENTITY 1.0. Available from: http://www.boku.ac.at/zag/forsch/identity.htm. Accessed 07 July 2009

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38: 1358-1370

Yeh FY, Boyle R, Ye T, Mao Z (1997) POPGENE, the user-friendly shareware for population genetic analysis, version 1.31. Molecular Biology and Biotechnology Centre, University of Alberta, Alberta

Table 1 – List and location of 77 landraces characterized in five southern European Countries.

Accession/Country	Region	Zone/Council	Town/Locality
Portugal			
Cartuxeira/Tubulosa	Norte	Viseu	Moimenta da Beira
Dawton	Norte	Viseu	Moimenta da Beira
Purpurea	Norte	Viseu	Moimenta da Beira
Quinta Vila Nova Do Rego	Norte	Viseu	Povolide
Raul	Norte	Penafiel	Entre-os-Rios
Spain			
Allande-3	Asturias	Allande	Pola de Allande
Barreiras-1	Asturias	Sta. Eulalia de Oscos	Barreiras
Las Cuevas-1	Asturias	Caso	Las Cuevas
Llanazares-2	Asturias	Aller	Casomera
Priero-1	Asturias	Caso	Prieres
Pumares-4	Asturias	Sta. Eulalia de Oscos	Pumares
Robriguedo-2	Asturias	Penamellera Baja	Robriguero
Rubiano-1	Asturias	Grado	Rubiano
San Pedro-1	Asturias	Grado	San Pedro de los Burros
Tuñon-3	Asturias	Santo Adriano	Tuñon
Italy			
Allungata	Latium	Viterbo	Caprarola
Bardina	Liguria	Genova	Mezzanego
Barrettona	Latium	Viterbo	Vico Matrino
Cappello del prete	Latium	Viterbo	Caprarola
Caraffara SIC 1	Sicily	Catania	Etna Natural Park
Caraffara SIC 10	Sicily	Catania	Etna Natural Park
Caraffara SIC 11	Sicily	Catania	Etna Natural Park
Caraffara SIC 14	Sicily	Catania	Etna Natural Park
Caraffara SIC 2	Sicily	Catania	Etna Natural Park
Caraffara SIC 3	Sicily	Catania	Etna Natural Park
Caraffara SIC 9	Sicily	Catania	Etna Natural Park
Ciasetta	Liguria	Genova	Mezzanego
Del Rosso	Liguria	Genova	Pian dei Cunei
Dell'Orto	Liguria	Genova	Mezzanego
Gianchetta	Liguria	Genova	Mezzanego
Itavex	Latium	Viterbo	Caprarola
Lunghera	Liguria	Genova	Mezzanego
Madonnella	Latium	Viterbo	Caprarola
Menoia	Liguria	Genova	Mezzanego
Minnulara	Sicily	Catania	Etna Natural Park
Nocchia rosa	Latium	Viterbo	Caprarola
Nocciola Ada	Latium	Viterbo	Caprarola
Nocciola Benedetta	Latium	Viterbo	Carbognano
Nocciola Centenaria	Latium	Viterbo	Ronciglione
Nocciola della Madonnella	Latium	Viterbo	Caprarola
Nocciola lunga	Latium	Viterbo	Ronciglione
Nocciola Meloni	Latium	Viterbo	Caprarola
Noscello	Liguria	Genova	Pian dei Cunei

San Vicino Vittori	Marche	Ascoli-Piceno	Castigliano
Seigretta	Liguria	Genova	Mezzanego
Selvaggiola agostara	Sicily	Catania	Etna Natural Park
Selvaggiola lunga	Sicily	Catania	Etna Natural Park
Selvaggiola riccia	Sicily	Catania	Etna Natural Park
Selvaggiola SIC 16	Sicily	Catania	Etna Natural Park
Selvaggiola SIC 17	Sicily	Catania	Etna Natural Park
Selvaggiola SIC 4	Sicily	Catania	Etna Natural Park
Selvaggiola SIC 6	Sicily	Catania	Etna Natural Park
Selvaggiola SIC 7	Sicily	Catania	Etna Natural Park
Selvaggiola SIC13	Sicily	Catania	Etna Natural Park
Selvaggiola tardiva SIC 8	Sicily	Catania	Etna Natural Park
Selvaggiola tardiva SIC12	Sicily	Catania	Etna Natural Park
Tapparona	Liguria	Genova	Mezzanego
Tonda di Biglini Bi/01	Piedmont	Cuneo	Biglini
Tonda di Biglini Bi/02	Piedmont	Cuneo	Biglini
Tonda di Biglini LeqBer/01	Piedmont	Cuneo	Lequio Berria
Tonda di Biglini LeqBer/02	Piedmont	Cuneo	Lequio Berria
Tonda di Biglini Ver/05	Piedmont	Cuneo	Verduno
Tonda di Biglini Ver/06	Piedmont	Cuneo	Verduno
Tonda di Calabria Ca/01	Calabria	Catanzaro	Torre di Ruggiero
Tonda di Calabria Ca/02	Calabria	Catanzaro	Gagliato
Tonda di Calabria Ca/03	Calabria	Vibo Valentia	Jonadi
Trichette	Sicily	Catania	Etna Natural Park
Slovenia			
CV/1	Lower Styria	Šentjur	Rifnik
CV/2	Lower Styria	Šentjur	Rifnik
T/0	Lower Styria	Slovenske Konjice	Novo Tepanje
T/10	Lower Styria	Slovenske Konjice	Novo Tepanje
T/16	Lower Styria	Slovenske Konjice	Novo Tepanje
Greece			
Argiroupoli	East Macedonia and Thrace	Drama	Drama
Philio	East Macedonia and Thrace	Drama	Drama
Patem large	East Macedonia and Thrace	Drama	Drama
Patem small	East Macedonia and Thrace	Drama	Drama
Polykarpos	East Macedonia and Thrace	Drama	Drama

Table 2 – Proportion of phenotypic classes of morphological descriptors of hazelnut fruits collected from landraces resulted in unique SSR genotype. N: number of landraces characterized; H: Shannon-Weaver diversity index

Descriptors	N	Phenotypic classes (number of samples/proportion %)						H
Predominant nut number per cluster	39	1 (0/0)	1-2 (12/30.8)	2-3 (18/46.2)	3-4 (3/7.7)	>4 (6/15.4)		1.20
Involucre length compared to nut length	38	Shorter (8/21.1)	Equal (12/31.6)	Longer (18/47.4)				1.05
Nut size (1)	42	Very large (1/2.4)	Large (9/21.4)	Medium (15/35.7)	Small (17/40.5)			1.15
Nut shape	42	Oblate (3/7.1)	Globular (14/33.3)	Conical (0/0)	Ovoid (7/16.7)	Short cylindrical (7/16.7)	Long cylindrical (11/26.2)	1.50
Nut shell colour	42	Greenish yellow (2/4.8)	Ligth brown (27/64.3)	Brown (12/28.6)	Dark brown (1/2.4)			0.88
Nut shell striping	42	Absent (0/0)	Few (14/33.3)	Medium (20/47.6)	Many (8/19,0)			1.04
Size of pistil scar	34	Small (15/44.1)	Medium (15/44.1)	Large (4/11.8)				0.97
Presence of double kernels	42	Absent (39/92.9)	Present (3/7.1)					0.26
Kernel size (2)	42	Very large (0/0)	Large (6/14.3)	Medium (19/45.2)	Small (17/40.5)			1.00
Kernel shape	42	Oblate (2/4.8)	Globular (10/23.8)	Conical (2/4.8)	Ovoid (12/28.6)	Short cylindrical (4/9.5)	Long cylindrical (12/28.6)	1.57
Appearence of skin	42	Smooth (4/9.5)	Sligthly corky (24/57.1)	Medium corky (11/26.2)	Strongly corky (3/7.1)			1.08
Size of internal cavity of kernel	32	Absent (5/15.6)	Small (17/53.1)	Medium (6/18.8)	Large (4/12.5)			1.20
Percentage of kernel by weight (3)	41	Very low (12/29.3)	Low (12/29.3)	Medium (13/31.7)	High (4/9.8)	Very high (0/0)		1.31
Percentage of kernel calibre >12mm (4)	41	Very low (13/31.7)	Low (3/7.3)	Medium (7/17.1)	High (18/43.9)			1.22

(1) Nut size: 1= Very large (>4 g) 2= Large (3,1-4 g) 3= Medium (2,1-3 g) 4= Small (< 2,0 g); (2) Kernel size1= Very large (>1,65 g) 2= Large (1,26-1,65 g) 3= Medium (0,86-1,25 g) 4= Small (<0,85 g); (3) Percentage of kernel by weight1=Very low (< 40%) 2=Low (40,1-45%) 3=Medium (45,1-50%) 4=High (50,1-55%) 5=Very high (>55%); (4) Percentage of kernel calibre >12mm 1= Very low (0-25%) 2= Low (25,1-50%) 3= Medium (50,1-75%) 4= High (75,1-100%)

Table 3 – Morphological and technological parameters of 42 landraces resulted in unique SSR genotype

Identification	Code in the PCA	Nut size	Nut shape	Presence of double kernels	Kernel size	Kernel shape	Appearence of skin	Percentage of kernel by weight (%)	Percentage of kernel calibre >12mm (%)
Allande-3	All_3	Large	Short cylindrical	Absent	Small	Ovoid	Medium corky	38	70
Allungata	Allung	Medium-Small	Long cylindrical	Absent	Medium	Long cylindrical	Slightly corky	47	100
Bardina	Bard	Small	Ovoid	Absent	Small	Ovoid	Medium corky	54	14
Barrettona	Barrett	Medium	Globular	Absent	Medium	Globular	Slightly corky	50	100
Cappello del Prete	Capp_Prete	Medium	Globular	Absent	Medium	Globular	Slightly corky	44	100
Ciasetta	Cias	Small	Long cylindrical	Absent	Small	Long cylindrical	Medium corky	49	0
Dall'Orto	D_Orto	Small	Long cylindrical	Present	Small	Long cylindrical	Slightly corky	45	0
Del Rosso	D_Rosso	Small	Ovoid	Absent	Small	Ovoid	Not corky	45	4
Philio	Philio	Large	Oblate	Absent	Medium	Ovoid	Medium corky	40	100
Gianchetta	Gianch	Small	Long cylindrical	Absent	Small	Long cylindrical	Slightly corky	53	0
Itavex	Itavex	Small	Globular	Absent	Medium	Globular	Slightly corky	44	100
Las Cuevas-1	L_Cuev1	Medium	Globular	Absent	Small	Globular	Slightly corky	45	20
Lunghera	Lungh	Small	Long cylindrical	Absent	Small	Long cylindrical	Slightly corky	45	0
Madonnella	Madon	Small	Globular	Absent	Small	Globular	Slightly corky	45	90
Menoia	Menoia	Small	Ovoid	Absent	Small	Ovoid	Slightly corky	47	5
Minnulara	Minnu	Large	Short cylindrical	Absent	Medium	Short cylindrical	Slightly corky	34	40
Nocciola Ada	Nocc_Ada	Medium	Long cylindrical	Absent	Medium	Long cylindrical	Slightly corky	50	70
Nocciola Benedetta	Nocc_Ben	Medium	Ovoid	Absent	Medium	Ovoid	Slightly corky	40	100
Nocciola Centenaria	Nocc_Cent	Medium	Long cylindrical	Absent	Medium	Long cylindrical	Slightly corky	44	90
Nocciola lunga	Nocc_lung	Medium	Long cylindrical	Absent	Medium	Long cylindrical	Slightly corky	50	100
Noscello	Nosc	Medium	Ovoid	Absent	Medium	Ovoid	Medium corky	49	93
Prieres-1	Pri_1	Small	Globular	Absent	Small	Ovoid	Not corky	-	-
Quinta Vila Nova do Rego	QVNdR	Small	Globular	Absent	Small	Globular	Slightly corky	47	48
Robriguedo-2	Robr_2	Medium	Globular	Absent	Medium	Globular	Medium corky	44	90
San Vicino Vittori	San_Vic_Vitt	Very large	Short cylindrical	Absent	Large	Short cylindrical	Medium corky	49	100

Seigretta	Seigr	Small	Ovoid	Present	Small	Ovoid	Medium corky	51	0
Selvaggiola agostara	Selv_ago	Large	Globular	Absent	Medium	Conical	Strongly corky	30	70
Selvaggiola lunga	Selv_lunga	Small	Long cylindrical	Present	Small	Long cylindrical	Medium corky	45	0
Selvaggiola riccia	Selv_ric	Medium	Globular	Absent	Medium	Globular	Slightly corky	42	55
Selvaggiola SIC13	Selv_SIC13	Large	Globular	Absent	Large	Ovoid	Slightly corky	40	100
Selvaggiola SIC16	Selv_SIC16	Small	Globular	Absent	Small	Globular	Slightly corky	39	45
Selvaggiola SIC17	Selv_SIC17	Medium	Short cylindrical	Absent	Medium	Ovoid	Strongly corky	34	100
Selvaggiola SIC4	Selv_SIC4	Medium	Short cylindrical	Absent	Large	Long cylindrical	Slightly corky	40	60
Selvaggiola SIC6	Selv_SIC6	Large	Globular	Absent	Medium	Conical	Strongly corky	36	70
Selvaggiola SIC7	Selv_SIC7	Medium	Long cylindrical	Absent	Medium	Long cylindrical	Medium corky	39	55
Selvaggiola tardiva SIC12	Selv_SIC12	Small	Short cylindrical	Absent	Small	Short cylindrical	Slightly corky	44	80
Selvaggiola tardiva SIC8	Selv_SIC8	Large	Oblate	Absent	Medium	Oblate	Medium corky	39	100
T/0	T/0	Small	Oblate	Absent	Small	Oblate	Slightly corky	47	17
T/10	T/10	Small	Ovoid	Absent	Small	Ovoid	Not corky	46	8
T/16	T/16	Large	Short cylindrical	Absent	Large	Short cylindrical	Not corky	43	92
Tapparona	Tapp	Medium	Long cylindrical	Absent	Medium	Long cylindrical	Slightly corky	51	3
Tricchete	Trich	Large	Globular	Absent	Large	Globular	Slightly corky	48	85
TGL	TGL	Medium	Globular	Present	Medium	Globular	Medium corky	48	90
Negret	Negret	Medium	Ovoid	Present	Medium	Ovoid	Not corky	49	65

1

Table 4 –Genetic diversity for hazelnut accessions classified in 8 geographical groups. Ni: number of individuals; Na: observed number of alleles; Ne: effective number of alleles; H_0: observed heterozygosity; H_e: expected heterozygosity.

Gene pools	**Ni**	**Na**	**Ne**	**H_o**	**H_e**
Iberian Peninsula	18	6.5	3.9	0.87	0.73
North-West Italy	13	7.1	4.1	0.78	0.72
Central Italy	12	6.6	4.2	0.89	0.75
South Italy	7	4.2	3.1	0.86	0.66
Sicily	22	7.2	3.5	0.75	0.67
Balkans-Black Sea	27	7.8	3.7	0.83	0.71
Wild Latium	9	6.4	4.7	0.73	0.76
Wild Campania	10	4.8	3.2	0.74	0.67

Table 5 – Genetic identity (Gi), genetic distances (Gd), gene flow (Nm) and genetic differentiation (Fst) among and between hazelnut gene pools analyzed with SSR markers

Gene pools	Iberian Peninsula	NW Italy	Central Italy	South Italy	Sicily	Balkans-Black Sea	Wild Latium	Wild Campania	Iberian Peninsula	NW Italy	Central Italy	South Italy	Sicily	Balkans-Black Sea	Wild Latium	Wild Campania
	Gi								**Nm**							
Iberian Peninsula	-	0.918	0.857	0.735	0.747	0.812	0.674	0.475	-	16.07	7.19	2.80	2.61	4.03	2.99	1.33
North-West Italy	0.086	-	0.853	0.687	0.684	0.880	0.601	0.452	0.015	-	7.90	2.43	2.09	7.39	2.54	1.30
Central Italy	0.154	0.159	-	0.827	0.866	0.891	0.614	0.445	0.034	0.031	-	5.23	5.63	8.59	2.91	1.36
South Italy	0.308	0.376	0.190	-	0.824	0.667	0.428	0.413	0.082	0.093	0.046	-	3.87	2.01	1.46	1.05
Sicily	0.292	0.380	0.144	0.194	-	0.732	0.490	0.436	0.087	0.107	0.043	0.061	-	2.32	1.42	1.04
Balkans-Black Sea	0.209	0.127	0.115	0.405	0.313	-	0.557	0.400	0.058	0.033	0.028	0.111	0.098	-	1.85	1.07
Wild Latium	0.395	0.509	0.488	0.848	0.713	0.586	-	0.694	0.077	0.090	0.079	0.146	0.150	0.119	-	3.12
Wild Campania	0.745	0.795	0.811	0.885	0.830	0.916	0.366	-	0.159	0.162	0.155	0.192	0.194	0.189	0.074	-
	Gd								**Fst**							

Fig. 1 - PCA two-dimensional scatter plot based on the first two principal components (PC1 and PC2) generated for 42 landraces and 11 reference cultivars on 14 morphological traits. Legenda:

× Liguria; ○ Sicily; □ Latium; △ Spain; + Potugal; ✳ Slovenia; ▽ Greece; ● Reference cultivars

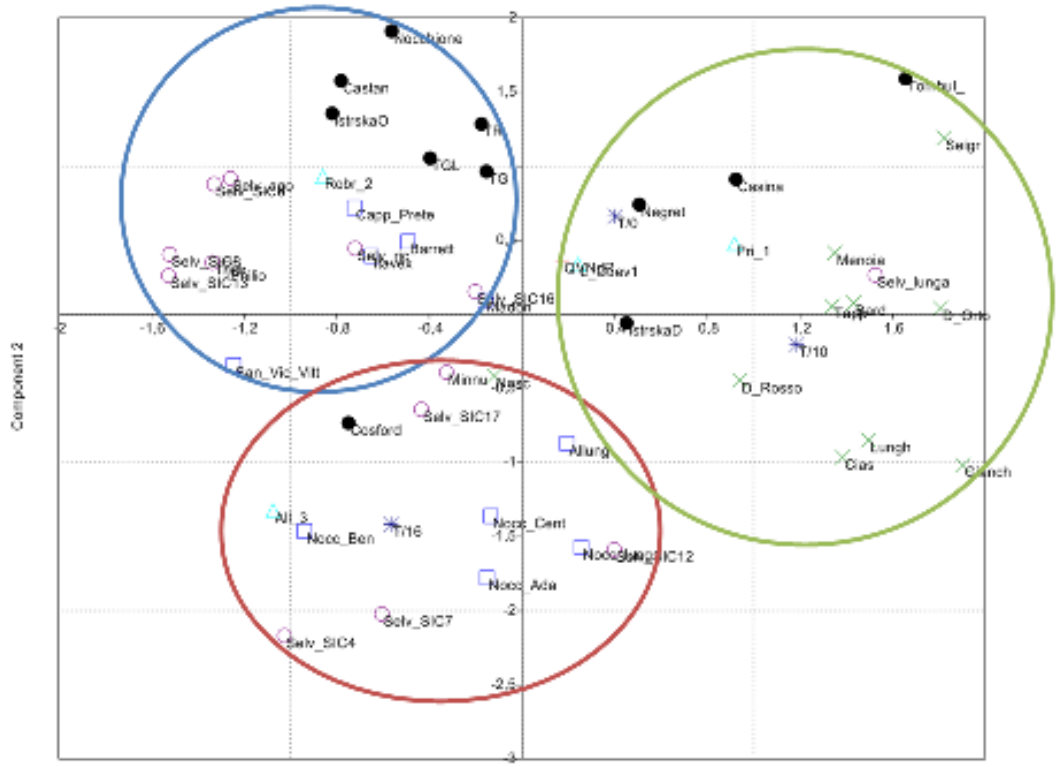


Fig. 2 – UPGMA dendrogram obtained from the SSR analysis in 42 unique landrace genotypes (LR), 57 true-to-type cultivars (CV), and 19 wild individuals (W).

Fig. 3 – Two-dimensional plot obtained from PCoA for 153 hazelnut genotypes classified in 8 geographical groups and analyzed at 10 SSR loci.

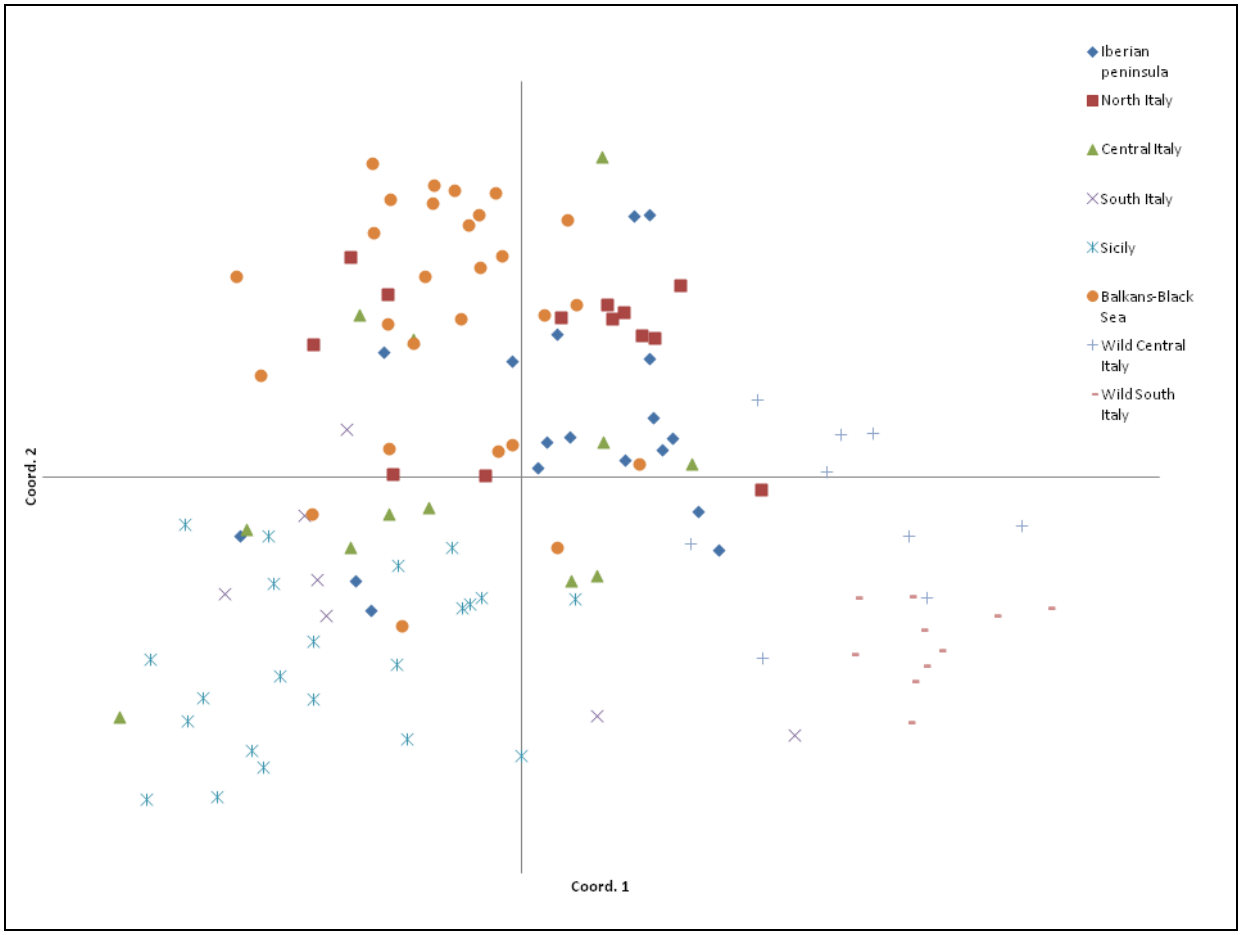
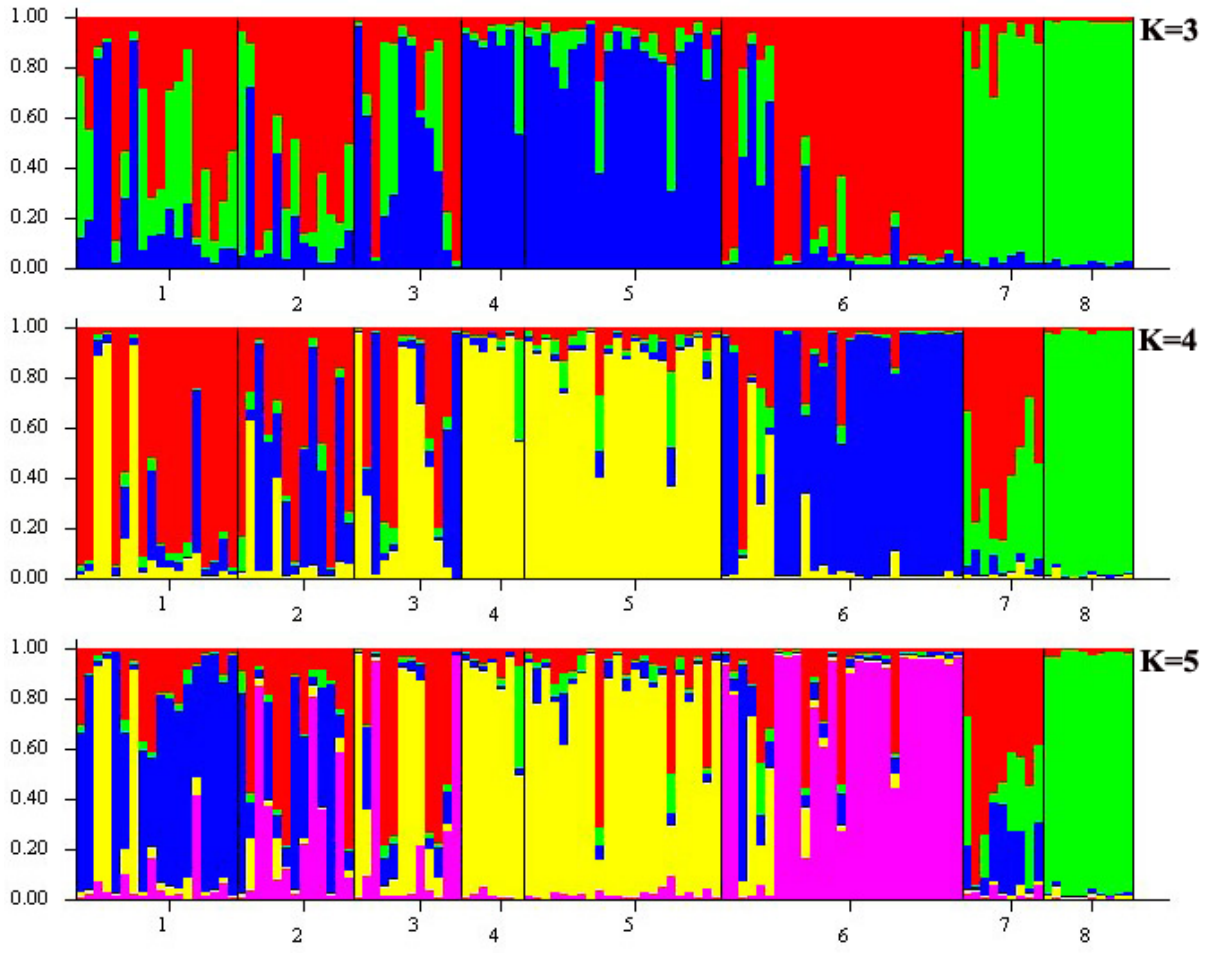


Fig. 4 – Hierarchical organization of genetic relatedness of 153 unique genotypes based on 10 SSR markers and analyzed by the STRUCTURE program, considering 3, 4, and 5 populations (K = 3, K = 4, and K = 5). Legenda geographical groups: 1 = Iberian Peninsula; 2 = North-West Italy; 3 = Central Italy; 4 = South Italy; 5 = Sicily; 6 = Balkans-Black Sea; 7 = wild Latium; 8 = wild Campania



Supplemetary Table 1 – Alleles sizes (in bps) at 10 SSR loci in 42 landraces (LR) surveyed *on-farm* and 57 reference cultivars (CV) collected from different European germplasm repositories

Accession name	Type	Origin	Collection[a)]	CAT-B501		CAT-B504		CAT-B502		CAT-B107		CAC-B028		CAT-B507		CAT-B508		CAT-B503		CAT-B505		CAT-B020	
Iberian peninsula																							
Allande 3	LR	Spain-Asturias	on-farm	116	130	177	179	189	191	132	136	257	278	190	198	158	162	129	133	120	128	285	291
Barcelona	CV	Spain-Tarragona	UNITO	116	130	161	185	189	191	114	136	255	263	182	192	158	158	123	125	108	122	279	285
Casina	CV	Spain-Asturias	UNITO	130	130	179	179	189	189	132	136	269	278	190	192	158	162	125	129	120	128	285	291
Comum	CV	Portugal	DRAPC	124	130	177	185	191	197	124	136	269	269	192	196	148	158	113	125	116	124	285	287
Culplà	CV	Spain-Tarragona	UNITO	116	130	173	185	185	191	126	136	257	269	192	198	146	158	125	125	116	128	285	285
Da Viega	CV	Portugal	DRAPN	116	130	175	185	189	197	124	136	267	269	192	196	158	166	115	125	116	128	285	287
Gironell	CV	Spain-Tarragona	UNITO	116	130	161	185	191	191	114	122	255	263	182	186	158	164	123	125	116	122	285	293
Grifoll	CV	Spain-Tarragona	IRTA	122	130	173	179	191	197	122	124	257	269	182	186	158	162	125	133	124	128	275	285
Las Cuevas 1	LR	Spain-Asturias	on-farm	122	130	179	179	189	189	132	136	269	278	190	192	158	162	125	129	120	128	285	291
Morell	CV	Spain-Tarragona	UNITO	130	130	165	185	191	191	124	124	257	263	186	190	160	164	115	125	108	120	285	285
Negret	CV	Spain-Tarragona	IRTA	130	134	179	185	191	191	122	136	257	269	192	196	158	160	123	125	116	128	275	285
Pauetet	CV	Spain-Tarragona	UNITO	130	134	179	179	189	191	122	136	257	257	182	196	158	160	123	125	116	128	285	287
Priero 1	LR	Spain-Asturias	on-farm	116	130	171	185	187	189	124	136	267	269	190	192	158	162	115	125	108	120	277	285
Quinta Vila Nova Do Rego	LR	Portugal	on-farm	122	130	161	179	189	191	114	122	255	263	182	186	158	158	123	123	116	122	279	285
Ribet	CV	Spain-Tarragona	IRTA	122	130	177	179	187	189	124	136	257	263	192	196	158	166	115	123	108	116	285	295
Robriguedo 2	LR	Spain-Asturias	on-farm	116	130	177	179	189	189	132	132	278	278	184	190	158	162	123	129	116	128	285	291
Segorbe	CV	Spain-Tarragona	IRTA	116	130	177	179	185	189	114	136	267	278	180	192	158	166	115	129	108	116	287	291
Trenet	CV	Spain-Tarragona	IRTA	130	130	179	185	187	191	132	136	257	263	190	192	158	164	123	125	108	128	279	285
Italy																							
Allungata	LR	Latium	on-farm	122	130	179	185	189	191	118	124	263	263	186	192	154	164	123	129	120	128	281	285
Bardina	LR	Liguria	on-farm	130	130	173	185	185	189	122	136	269	271	192	198	158	164	125	125	116	128	281	285
Barrettona	LR	Latium	on-farm	124	130	179	179	185	191	128	145	257	263	192	194	148	158	119	123	108	124	285	287
Camponica	CV	Campania	ARSIAL	116	130	161	179	185	191	114	136	263	278	182	192	158	168	123	129	120	128	285	285
Cappello del prete	LR	Latium	on-farm	124	130	179	179	185	191	124	145	257	263	192	194	148	158	119	123	108	124	285	287
Carrello	CV	Sicily	UNIPA	116	116	161	171	185	191	124	145	257	263	180	192	158	158	123	123	116	128	279	287
Catainetto	CV	Liguria	IFP	128	130	177	179	189	189	122	145	257	269	196	196	158	158	123	125	116	122	285	287

Ciasetta	LR	Liguria	on-farm	130	130	179	181	191	197	122	124	263	263	186	188	148	164	123	123	122	128	285	285
Del Rosso	LR	Liguria	on-farm	124	130	171	179	185	189	126	136	265	271	198	198	158	158	113	129	108	142	275	279
Dell'Orto	LR	Liguria	on-farm	130	130	179	181	187	189	136	145	269	278	186	196	158	158	125	129	108	122	281	287
Ghirara	CV	Sicily	UNITO	116	130	179	185	187	187	114	114	257	263	192	196	148	158	123	129	120	128	279	285
Gianchetta	LR	Liguria	on-farm	130	130	179	181	187	189	122	136	267	267	186	190	154	168	123	129	108	120	279	285
Iannusa Racinante	CV	Sicily	UNITO	116	130	179	185	185	191	114	147	255	257	182	192	148	158	123	123	120	122	285	287
Itavex	LR	Latium	on-farm	124	130	163	185	185	189	114	136	255	263	182	186	158	158	115	123	120	128	285	287
Lunghera	LR	Liguria	on-farm	122	130	171	185	189	201	136	136	257	263	192	198	152	158	117	123	128	128	285	289
Madonnella	LR	Latium	on-farm	116	130	161	163	189	191	124	145	263	263	190	192	158	158	115	123	120	122	279	285
Menoia	LR	Liguria	on-farm	128	130	171	177	189	189	122	136	257	269	196	198	158	158	123	127	116	128	281	285
Minnulara	CV	Sicily	UNIPA	116	122	161	179	185	191	122	124	255	263	180	192	150	158	115	123	120	128	279	287
Minnulara	LR	Sicily	on-farm	116	130	161	179	185	185	124	132	255	257	182	182	158	164	123	125	122	122	279	287
Minnulara Don Ciccio	CV	Sicily	UNIPA	116	130	161	177	187	187	114	122	257	263	192	196	158	164	123	127	120	128	285	287
Mortarella	CV	Campania	ARSIAL	116	130	161	185	185	189	114	136	263	288	182	192	158	164	123	123	108	116	285	287
Napoletana	CV	Sicily	CRA-FRC	126	130	171	185	185	201	114	134	255	280	182	182	146	158	123	125	116	128	285	287
Napoletanedda	CV	Sicily	CRA-FRC	116	130	161	173	185	191	114	114	261	263	182	192	158	158	123	127	116	128	285	285
Nocchione	CV	Latium	ARSIAL	116	130	161	185	185	191	114	124	255	263	182	192	158	158	123	123	122	128	279	285
Nocciola Ada	LR	Latium	on-farm	116	116	179	185	185	191	114	122	263	267	192	198	158	164	123	125	120	128	279	285
Nocciola Benedetta	LR	Latium	on-farm	116	122	177	185	191	199	124	134	263	269	186	192	148	148	113	123	120	128	285	297
Nocciola centenaria	LR	Latium	on-farm	124	124	161	163	187	189	130	136	255	269	186	196	158	160	125	127	120	122	273	287
Nocciola lunga	LR	Latium	on-farm	116	122	171	179	189	197	124	124	257	263	180	186	160	164	123	129	120	142	281	285
Nociara	CV	Sicily	UNITO	116	126	161	161	185	191	124	134	255	263	192	192	158	158	123	123	120	122	279	287
Noscello	LR	Liguria	on-farm	107	130	179	179	189	197	124	136	269	269	190	192	158	164	125	129	108	118	275	285
Panottara	CV	Sicily	UNIPA	116	122	175	181	187	203	122	122	263	267	180	182	158	158	115	123	122	128	275	279
Riccia di Talanico	CV	Campania	CRA-FRC	124	130	171	185	189	199	120	136	255	263	182	186	158	168	123	123	120	128	285	285
San Giovanni	CV	Campania	ARSIAL	126	130	161	185	191	211	120	136	255	263	182	186	158	168	117	123	120	128	285	287
San Vicino Vittori	LR	Latium	on-farm	122	130	171	185	189	201	132	136	263	269	186	198	154	164	127	129	108	116	279	281
Seigretta	LR	Liguria	on-farm	130	132	171	185	189	197	132	136	263	263	192	192	158	164	113	123	120	128	285	289
Selvaggiola agostara	LR	Sicily	on-farm	116	130	161	185	191	201	124	134	255	263	182	192	158	158	123	123	116	128	279	285
Selvaggiola lunga	LR	Sicily	on-farm	116	116	161	161	189	191	114	130	255	276	192	196	158	160	123	123	128	128	279	287
Selvaggiola riccia	LR	Sicily	on-farm	103	116	161	185	185	191	114	134	255	269	182	192	158	158	123	123	128	128	275	279
Selvaggiola SIC13	LR	Sicily	on-farm	116	130	161	185	185	189	114	136	263	278	182	192	148	164	123	129	108	116	285	285

Selvaggiola SIC16	LR	Sicily	on-farm	116	130	173	185	185	211	114	122	263	263	190	192	158	158	123	123	116	122	285	289
Selvaggiola SIC17	LR	Sicily	on-farm	116	116	161	181	189	191	124	124	255	259	182	192	158	158	123	123	120	128	279	281
Selvaggiola SIC4	LR	Sicily	on-farm	118	122	173	185	185	191	122	122	255	257	190	196	158	158	123	127	120	122	279	287
Selvaggiola SIC6	LR	Sicily	on-farm	116	132	161	173	189	191	122	124	255	257	192	192	158	158	123	123	120	128	-9	-9
Selvaggiola SIC7	LR	Sicily	on-farm	126	130	173	185	187	191	124	145	263	263	192	198	158	158	123	123	122	128	283	285
Selvaggiola tardiva SIC12	LR	Sicily	on-farm	116	132	161	161	191	205	124	124	257	263	192	192	158	158	123	123	116	122	279	287
Selvaggiola tardiva SIC8	LR	Sicily	on-farm	122	126	173	185	187	191	124	145	255	263	184	198	158	158	123	123	128	128	283	289
Tapparona	LR	Liguria	on-farm	130	130	171	175	189	211	128	136	259	265	182	198	158	158	125	129	108	124	279	287
Tonda bianca	CV	Campania	UNITO	116	124	161	171	185	199	114	120	255	278	180	182	156	168	123	129	116	120	285	287
Tonda di Giffoni	CV	Campania	CRA-FRC	116	130	161	185	185	189	120	136	263	278	182	192	158	158	123	123	120	128	285	287
Tonda Gentile Langhe	CV	Piedmont	UNITO	130	130	173	185	187	191	136	154	257	263	186	192	148	164	115	123	116	128	283	285
Tonda Gentile Romana	CV	Latium	ARSIAL	124	130	163	185	189	193	136	145	263	269	186	190	158	158	123	125	120	128	285	287
Tonda rossa	CV	Campania	CRA-FRC	116	124	161	177	185	185	114	120	269	280	180	182	148	148	115	129	116	116	285	285
Trichette	LR	Sicily	on-farm	116	130	161	179	185	191	114	136	263	278	182	192	158	158	123	129	120	128	285	285
Trietta	CV	Liguria	IFP	122	130	171	173	189	191	124	136	263	263	186	186	158	158	125	129	128	142	279	285
Balkans-Black Sea																							
Acı	CV	Turkey	HRI	122	130	183	185	185	189	118	136	263	269	192	196	152	158	123	125	108	128	259	279
Allahverdi	CV	Turkey	HRI	116	122	171	181	189	191	122	130	263	273	190	198	158	164	125	127	120	120	279	287
Badem	CV	Turkey	HRI	122	130	175	179	189	191	118	124	263	263	186	192	154	164	123	129	120	128	281	285
Çakıldak	CV	Turkey	HRI	122	132	181	185	189	197	122	132	257	263	192	194	158	166	123	127	116	128	279	285
Extra Ghiaghli	CV	Greece	NAGREF	122	122	171	185	189	189	118	122	263	263	190	198	158	164	123	125	108	120	279	285
Foşa	CV	Turkey	HRI	116	122	171	185	189	191	118	126	263	269	198	198	158	164	119	125	116	120	279	285
Fructo rubro	CV	Unkonwn	UNITO	130	130	171	179	189	191	124	136	269	269	186	198	158	158	127	129	108	128	279	281
Incekara	CV	Turkey	HRI	130	134	171	175	189	191	122	136	263	267	186	198	152	158	123	127	116	128	279	281
Istrska dolgoplodna	CV	Slovenia	BF	130	132	179	185	189	191	124	136	261	269	198	198	154	158	125	127	108	120	279	285
Istrska okrogloplodna	CV	Slovenia	BF	116	130	177	179	185	189	124	136	269	278	192	196	158	168	125	127	116	128	285	287
Kalınkara	CV	Turkey	HRI	122	134	171	175	189	189	122	130	263	267	186	198	152	158	123	127	116	128	281	281
Kan	CV	Turkey	HRI	122	130	171	185	189	191	122	136	263	269	194	198	158	158	125	127	108	128	279	285
Karafındık	CV	Turkey	HRI	118	122	171	185	189	201	132	136	263	269	190	190	158	164	-9	-9	120	128	285	285
Kargalak	CV	Turkey	HRI	122	130	171	187	185	189	122	132	263	269	188	192	158	160	123	123	116	128	279	279
Kuş	CV	Turkey	HRI	122	130	171	175	191	191	122	132	263	269	198	198	158	158	119	127	116	120	279	281
Palaz	CV	Turkey	HRI	122	130	171	185	189	191	130	136	263	263	192	198	158	158	123	127	120	128	259	279
Philio	LR	Greece	on-farm	122	124	179	181	185	189	124	136	259	263	182	186	158	158	123	127	108	116	279	285

Sivri	CV	Turkey	HRI	122	122	171	185	191	191	122	132	263	269	180	198	158	158	119	125	120	128	279	285
Sivri Ghiaghli	CV	Greece	NAGREF	122	130	171	185	189	201	118	122	263	263	180	198	158	164	119	127	120	128	279	285
T/0	LR	Slovenia	on-farm	124	130	161	163	191	193	124	145	263	269	182	186	158	158	123	125	128	128	285	287
T/10	LR	Slovenia	on-farm	116	130	173	185	191	203	122	136	263	267	180	192	148	164	115	123	116	124	285	285
T/16	LR	Slovenia	on-farm	116	116	161	175	189	197	136	136	255	265	186	192	158	158	115	123	118	122	279	287
Tombul	CV	Turkey	HRI	122	130	171	185	189	201	132	136	263	269	190	190	158	164	123	127	120	128	285	285
Tombul Ghiaghli	CV	Greece	NAGREF	122	130	171	185	189	189	118	136	263	263	190	198	158	164	123	127	120	128	285	285
Uzunmusa	CV	Turkey	HRI	122	130	171	185	189	191	122	136	263	269	192	198	158	158	125	127	108	128	279	285
Yassı Badem	CV	Turkey	HRI	122	130	179	185	191	191	118	124	263	263	186	192	-9	-9	123	129	120	128	281	285
Yuvarlak Badem	CV	Turkey	HRI	122	122	171	179	189	189	118	136	263	263	186	192	158	158	123	127	108	120	279	281

[a)] ARSIAL: Agenzia Regionale per lo Sviluppo e l'Innovazione dell'Agricoltura del Lazio (Italy); BF: Biotechnical Faculty, University of Ljubljana (Slovenia); CRA: Centro Ricerche e sperimentazione in Agricoltura – Unità di Ricerca per la Frutticoltura of Caserta (Italy); UNITO: Università degli Studi di Torino (Italy); UNIPA: Università degli Studi di Palermo (Italy); HRI: Hazelnut Research Institute of Giresun (Turkey); IFP: Istituto di Frutticoltura of Piacenza (Italy); IRTA: Istitut de Recerca i Tecnologia Agroalimentàries of Reus (Tarragona, Spain); NAGREF: National Agricultural Research Foundation of Naoussa (Greece); UTAD: Universidade de Trás-os-Montes e Alto Douro (Portugal).