A multidisciplinary approach to enhance the conservation and use of hazelnut Corylus avellana L. genetic resources.

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A multidisciplinary approach to enhance the conservation and use of hazelnut genetic resources

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Abstract:	During recent years, there has been increasing awareness of the importance of adopting a holistic view of biodiversity, including agricultural biodiversity, conservation for sustainable utilization and development. These principles have been underlined in the Convention on Biological Diversity and the European efficiency resources towards 2050. Thus one critical issue is now to understand the distribution and extent of genetic diversity available to breeders and stakeholders, the kind and range of characterization, how to face the problem of continuous expanding of germplasm to be conserved. Focusing on the case study of hazelnut which is a crop of great importance for European Countries, the paper describes a resourceful strategy for re-organizing and sharing hazelnut genetic resources through an upgrading of knowledge on their value and uses. The paper summarizes the progresses so far and provides a 'launching pad' for future researches. The brief review discusses also the recent progresses in recovery, characterization conservation and uses of European hazelnut germplasm achieved by 068 AGRI GEN RES SAFENUT which was one of the 17 Action financed by the European Commission - Directorate General for Agriculture and Rural Development. The current status on the morphological and molecular characterization of the in situ and ex situ of the most important European collections,

	the rescue and safeguards of new accessions recovered on farm were discussed underling critical aspects. A better understanding of hazelnut genetic diversity and its distribution is essential for its conservation and use as well as the harmonization of the morphological and biochemical descriptors. The importance of traditional knowledge is also considered as integrated part of the multidisciplinary approach useful to rationalize genetic resources maintained in the collections. Thus improving the characterization on cultivated and wild forms through the development of a core collection, is the further step to achieve a more effective management and use of European nuts germplasm.

A multidisciplinary approach to enhance the conservation and use of hazelnut genetic resources

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Abstract

During recent years, there has been increasing awareness of the importance of adopting a holistic view of biodiversity, including agricultural biodiversity, conservation for sustainable utilization and development. These principles have been underlined in the Convention on Biological Diversity and the European efficiency resources towards 2050. Thus one critical issue is now to understand the distribution and extent of genetic diversity available to breeders and stakeholders, the kind and range of characterization, how to face the problem of continuous expanding of germplasm to be conserved. Focusing on the case study of hazelnut which is a crop of great importance for European Countries, the paper describes a resourceful strategy for re-organizing and sharing hazelnut genetic resources through an upgrading of knowledge on their value and uses. The paper summarizes the progresses so far and provides a 'launching pad' for future researches. The brief review discusses also the recent progresses in recovery, characterization conservation and uses of European hazelnut germplasm achieved by 068 AGRI GEN RES SAFENUT which was one of the 17 Action financed by the European Commission - Directorate General for Agriculture and Rural Development. The current status on the morphological and molecular characterization of the in situ and ex situ of the most important European collections, the rescue and safeguards of new accessions recovered on farm were discussed underling critical aspects. A better understanding of hazelnut genetic diversity and its distribution is essential for its conservation and use as well as the harmonization of the morphological and biochemical descriptors. The importance of traditional knowledge is also considered as integrated part of the multidisciplinary approach useful to rationalize genetic resources maintained in the collections. Thus improving the characterization on cultivated and wild forms through the development of a core collection, is the further step to achieve a more effective management and use of European nuts germplasm.

Keywords: genetic resources, *Corylus avellana*, collections, conservation, traditional knowledge.

Introduction

The European hazelnut (*Corylus avellana* L.) is one of the world's major nut crops. Total worldwide production (FAOstat 2014) is sixth after that of cashew (*Anacardium occidentale* L.), walnut (*Juglans regia* L.), almond [*Prunus dulcis* (Miller) D.A. Webb], chestnut (*Castanea* spp.), and pistachio (*Pistacia vera* L.). The Black Sea countries account for the majority of world hazelnut production (FAOstat 2014): Turkey (598,158 tons, average of 2008-2012), Azerbaijan (30,030 tons), and Georgia (25,020 tons). Other important producers are Italy (104,577 tons), the USA (32,399 tons), Iran (20,832 tons), China (19,700 tons), and Spain (16,239 tons). Moreover, emerging markets such as China, India, the United Arab Emirates and Australia have increased their trade by more than 80% in the last five years (USDA, 2010) In Europe, hazelnuts account for about 16% of total world production. Italy is the first European hazelnut producer followed by Spain, France (8,137 tons), Poland (3,293 tons), and Greece (1,500 tons) (FAOstat, 2014). The per capita consumption of nuts in Europe is very high due to the importance of the crop in the Mediterranean diet (Bulló et al. 2011). Nuts are a high energy food, rich in fats and protein, and a valuable source of fibres, phytonutrients, and antioxidants (Vitamin E) (Sivakumar et al., 2005; Sivakumar and Bacchetta, 2006; Salas-Salvadó and Megías, 2005). In the past two decades, several biochemical and clinical studies have provided consistent evidence of their healthy properties (Hu et al. 1999; Fraser et al. 2009; Mullie and Clarys 2012). Nevertheless, European nut production supplies less than 40% of local demand and the rest is imported from Turkey and USA.

The conservation, characterization and survey of hazelnut genetic resources is a prerequisite to improving breeding programs, enhancing the competiveness of the European production. Breeding is in its infancy in this species, if compared to most other domesticated crops, and the high genetic diversity of hazelnut offers substantial opportunities for genetic improvement (Mehlenbacher 1991; Thompson et al. 1996; Molnar 2011). The *Corylus* germplasm collections in the world consist primarily in cultivated forms of *C. avellana* located in regions where this production occurs. An extensive inventory of hazelnut research, germplasm and references was published by Köksal (2000), supported by FAO (Food and Agriculture Organization). Major European collections are those managed by: Institut de Recerca i Technologia Agroalimentaries (IRTA) in Reus (Spain) (Rovira, 1997; Boccacci et al., 2008); University of Torino (UNITO) in Chieri, and and Centro Ricerche per l'Agricoltura (CRA) in Caserta (Italy), University of Ljubljana in Maribor (Slovenia) (Solar and Stampar, 1997; 2009) and Institut National de la Recherche Agronomique (INRA) in Villanave d'Ornon (France). A number of smaller collections and repositories have been developed as results of local and regional efforts (Mehlenbacher 1991; Berthaud et al. 1997; Solar and Stampar, 2009; Bacchetta et al. 2010).

The lack of nursery activities or certified material for the propagation in the new orchards as well as the great number of varieties, has increased the chance of mistakes or caused some confusion among local populations. The consequence is a waste of human and financial resources for conservation and duplication of useless materials. A comprehensive list of all accessions maintained in the European collections is thus essential to improve the knowledge on the existing material and to verify the trueness to type of different accessions detecting possible erroneous spelling in any of the cultivars or references and removing synonyms. As Visser and Engels (2003) pointed out, a proper documentation of genebank accessions is necessary to favor efficient and effective use of germplasm. Currently, only in three European collections a fingerprinting using molecular markers was performed (Boccacci et al. 2006, 2008). Prospects in marginal areas are therefore crucial for preserving and recovering maximum genetic diversity, mitigating the effect of genetic erosion (Bacchetta and Di Giovanni 2013). The genetic base of many commercially important crops, especially long-lived perennial tree crops, comprises only a limited number of cultivars grown as monoculture over vast areas and they are highly vulnerable to the rapid spread of insects, pests, and pathogens. Sun (1998) reported that *C. chinensis* was becoming scarce in China, leading to its threatened status. It is possible that genetic resources of other *Corylus* species are in danger of being lost, especially in highly populated countries or regions that have undergone widespread deforestation (Molnar 2011). Essentially no work has been done to investigate population structure, genetic diversity, and possible genetic erosion (loss of genetic resources) of wild hazelnut. Nearly all efforts have been focused on cultivated forms largely to better understand their origin, to fingerprint germplasm accessions, and to evaluate genetic diversity (Boccacci and Botta 2009, 2010; Boccacci et al. 2006, 2008; Gökirmak et al. 2009; Gürcan et al. 2010). Recently, a first on-farm exploration was conducted on local ecotypes and on wild accessions in northern Spain (Asturias) by Ferreira et al. (2010) and Campa et al. (2011). Finally, the long history of utilization and production by humans, probably predating the Roman era (Rosengarten 1984; Bacchetta et al., 2011; Boccacci and Botta 2006), makes this crop interesting from a social point of view, with a precious role in sustainable traditional agricultural systems.

This work summarizes the current status and potential breeding of *Corylus* genetic resources in Europe, prioritizing the need to conserve and better study the underutilized on-farm ecotypes. The aim is also to discuss the recent progresses on recovery, characterization, conservation and uses of hazelnut achieved by the 068 Agri Gen Res SAFENUT which was one of the 17 action financed by the European Commission - Directorate General for Agriculture and Rural Development. Evidence and new research directions are considered to improve the competiveness and the economical role of the European hazelnuts as a possible resourceful strategy to consider in the conservation and use of other nut or fruit crops.

Morphological evaluation of hazelnut accessions

The traditional characterization of cultivars has relied on morphological and phenological characteristics which provide the basic data for further evaluations. Morphological determinations which need to be taken by an expert in the species, could be affected by environmental factors, different developmental stages and their number is limited (De Vicente and Fulton, 2003). However one critical issue referring to morphological characterization, is the harmonization of the standard descriptors for a common characterization of germplasm. The use of a common and shared descriptors list represents and important tool to produce a universally understood 'language' for plant genetic resources data. A recent evaluation of hazelnut material was performed by Bacchetta et al. (2011) in typical cultivation areas, using a chart with specific hazelnut descriptors (Thompson et al. 1978; UPOV 1979; Biodiversity International 2008). The descriptors included: general characteristics (cultivar, synonyms, origin, and growing areas), tree traits (10 characters), flowering traits (6 characters), and nut and kernel traits (32 characters). The morphological description of 46 hazelnut European typical cultivars (13 from France;9 from Italy; 8 from Portugal;6 from Slovenia; and 10 from Spain) were published in online SAFENUT database, the 068 EU AGRI GEN RES project (http://safenut.casaccia.enea.it/). Nut traits are the most interesting traits to define the potential uses of the product. Thus among the 46 European cultivars the most representative were the medium ones (53.5%), while 37.2% had a large or very large size suitable for raw consumption. Considering nut shape, 51.2% of the cultivars had a globular shape (preferred by industry), 23.3% ovate and 14.0% long cylindrical one. The blanching index was generally moderate showing a great variability: 44.2% of cultivars presented a value ranging between 25 and 75%. Focusing on the most interesting agronomical traits, which are early nut maturity and resistance to big bud mite (*Phytoptus avellanae*, Nalepa), 64.3% of cultivars had an intermediate ripening time and only 14.3% an early or very early maturity, while about 35% were resistant to big bud mite.

The European collections exist in different countries and maintain accessions collected from different geographic areas (mainly Europe, Turkey and USA) according to each Research Institutes. In order to harmonize the various initiatives carried out at regional and national levels, Rovira et al. (2010) provided a list of 222 hazelnut clones and 58 selections from 13 European hazelnut collections (Table 1). The number and origin of the filbert accessions held in the collections were: Albania (1 cultivar), Balkan area (2 cvs.), Belgium (1 cv.), England (12 cvs.), France (8 cvs.), Germany (6 cvs.), Greece (1 cv.), Hungary (1 cv.), Italy (51 cvs.), Netherlands (1 cv.) Portugal (3 cvs.), Romania (4 cvs.), Slovenia (3 cvs.), Spain (84 cvs.), Turkey (7 cvs.), and USA (9 cvs.). Nine cultivars were of unknown origin. The main result obtained from this survey was that in many collection fields are conserved the same cultivars, indicating the need to rationalize genetic resources and that few efforts were made to include new accessions in the collections. For example, the NCGR and Oregon State University have increased efforts to collect cultivated and wild accession of *Corylus*, such as from the Balkans, Russia and Caucasus, and now their collections are more than 700 accessions, including all major *Corylus* species (Gurcan et al., 2010). However, a number of species are still lacking, especially when considering their wide geographic range, for these reasons recovering and evaluation efforts are still needed manly in Europe.

Microsatellite variability

The fingerprinting of accessions and analysis of genetic diversity in collections and natural populations are important aspects in the management and utilization of plant genetic resources. In recent years, SSRs have become the markers of choice to fingerprint accessions. Selection of loci to be used for the hazelnut DNA-typing was preliminarily carried out using 75 cultivars chosen to represent the gene pools of four hazelnut growing regions: i) Spain, composed by 33 cultivars all grown in the Province of Tarragona (Catalonia, northeastern Spain), except 'Casina' (Asturias, northern coast of Spain); ii) Italy, represented by 22 accessions cultivated in different regions: Piedmont and Liguria (North Italy), Latium (Central Italy), Campania and Sicily (South Italy); iii) Turkey, represented by 10 varieties of Turkish origin, 7 cultivated in the Black Sea coastal Provinces (northern Turkey) and 3 in Greece ('Extra Ghiaghli', 'Sivri Ghiaghli', and 'Tombul Ghiaghli'); iv) Iran, represented by 10 accessions. Cultivars originating from controlled crosses and synonyms were excluded, but those that had been found to descend from spontaneous crosses were included. True-to-type identity of the accessions was verified by Boccacci et al. (2005; 2006, 2008) and Ghanbari et al. (2005) using the following 16 SSR loci: CaT-A114, CaT-B107, CaT-B501, CaT-B502, CaT-B503, CaT-B504, CaT-B505, CaT-B507, CaT-B508, CaT-B509, CaT-B511, CaT-C001 and CaT-C504 (Boccacci et al., 2005), CaC-A102, CaC-B020 and CaC-B028 (Bassil et al., 2005).

The usefulness of these SSR loci in different hazelnut gene pools, the variability of each locus in four geographic groups and over all groups was assessed by Boccacci and Botta (2010). Tests for deviation from Hardy-Weinberg equilibrium at the 16 loci were calculated on the basis of 75 genotypes. All loci were in Hardy-

Weinberg equilibrium ($\alpha \leq 0.05$) when α was corrected according to the Bonferroni method ($\alpha T = 0.0031$). The total number of alleles (A) generated was 170 with an average of 10.6 alleles per locus. Expected heterozigosity (He) over all cultivars averaged 0.76 and ranged from 0.66 to 0.85 (Table 2). At all loci, observed heterozigosity (Ho) (mean: 0.79), averaged over samples, was slightly higher than He (mean: 0.71). The excess of heterozygotes was significant ($\alpha \leq 0.05$) at CaC-A102, CaT-B501, CaT-B505 (P=0.000), CaT-B509 (P=0.001), CaT-B504 (P=0.010), and CaT-B107 (P=0.038). On the contrary, loci CaC-B028 (P=0.002) and CaT-A114 (P=0.003) showed a significant deficit of heterozygotes. Cumulative probabilities to obtain identical genotypes from different cultivars at each of the 16 SSR loci were in order of 10^{-13} to 10^{-18}, which shows that the chosen marker set has high discriminative power in all of the investigated cultivar groups. Tests for linkage disequilibrium for all pairs of loci across samples was calculated to detect association between loci and revealed disequilibrium (Bonferroni corrected α for 120 tests, $\alpha T = 0.00042$) for eight pairs of loci: CaT-B107-CaT-C001, CaT-B501-CaT-B511, CaT-B507-CaC-A102, CaT-B507-CaTC001, CaT-B509-CaC-B020, CaT-C504-CaT-B501, CaT-C504-CaT-B511, CaT-C504-CaT-C001. In a genetic linkage map for *C. avellana* constructed by Mehlenbacher et al. (2006), an association was observed only for the loci CaT-B507-CaC-A102 and CaT-B509-CaC-B020.

The elaborations allowed to select a set of 10 SSR markers that were proposed as molecular descriptors for hazelnut (Biodiverstity International, 2008) and used for the DNA typing of all accessions of the European project SAFENUT . Loci were used to fingerprint a total of 243 accessions, 77 local landraces and 166 accessions with cultivar names planted in different European collection fields cured by: UNITO, ENEA, and CRA (Italy); IRTA of Reus (Spain), Conservatoire Végétal Régional d'Aquitanie (Montesquieu, France), Biotehniska Fakulteta of Ljubljana (Slovenia); and Universidade de Trás-os-Montes e Alto Douro (Portugal).

DNA-typing of accessions from European germplasm collections

The 10 SSR loci identified 77 unique genotypes among the 166 accessions with cultivar names, sampled in collection fields, due to several duplications and cases of synonymy or misnaming. In general, the comparison of the SSR profiles and the use of the DNA information of the existing database at UNITO were able to check and confirm the identity of most cultivars in the different collections but also to detect some mistakes presumably due to mislabelling of plants. The total probability of identity at all 10 loci was 3.73×10^{-12}, thus cultivars with identical genotypes were considered synonyms.

Analyses confirmed several synonyms reported in literature, such as ‘Nocchione’ (Latium) with ‘Montebello’ (Sicily) (Koksal, 2000) and, within the Sicilian cultivars (Mehlenbacher, 1994), ‘Comune di Sicilia’ with ‘Mansa’ and ‘Nostrale’, confirming the existence of a major Sicilian cultivar spread in the Region that we will call ‘Siciliana’ following Alberghina (1982). Furthermore, additional cultivars were found to have the same profile of ‘Siciliana’: ‘Nocchione’, ‘Barrettona’ (Latium), ‘Locale di Piazza Armerina’ (Sicily), ‘Iannusa racinante’ (Sicily), ‘Avellana speciale’ (unknown origin), confirming the results obtained by Boccacci et al. (2006). The accessions ‘Istrska okrogluplodna’ (Croatia) and ‘Lambertski beli’ (Slovenia) presented the same profile of the cultivars ‘Payrone’ (unknown origin, syn. ‘Romai’) and ‘Fructo rubro’ (Ballkans area), respectively. Two possible cases of clonal mutation were observed: a) ‘Santa Maria di Gesù’ (Sicily) appeared to be a clonal mutant of ‘Nocchione’ showing a 2 bp discrepancy at locus CaT-B501; b) ‘Negret primerenc (1-77)’ (Spain) showed the same genotype of ‘Negret’ (Spain), except for the allele 201 at locus CaT-B502.In other cases probable clonal mutations were not detected by the 10 SSR loci. An example is the cultivar ‘Tonda di Biglini’ (Piedmont, Italy) that showed the same profile of ‘Tonda Gentile delle Langhe’, although carpological and phenological differences were observed (Valentini et al. 2014).

Genetic characterization of landraces

A total of 77 landraces were surveyed in the traditional areas of hazelnut cultivation in five southern European countries (Fig. 1). Among them, 5 were collected in northern Portugal, 10 in northern Spain (Asturias), 52 in six Italian regions [6 in Piedmont (northwestern Italy), 10 in Liguria (northwestern), 1 in Marche (central Italy), 12 in Latium (central Italy), 3 in Calabria (southern Italy), and 20 in Sicily], 5 from Slovenia, and 5 from northern Greece. Farmers were contacted explaining the reasons for the project and interviewed about the presence of old endangered cultivars on their farms. Information on agronomic and qualitative traits, as well as use, local names, tradition, and social context were also collected (Boccacci et al. 2013).

Microsatellite analysis identified 42 unique genotypes while 35 accessions appeared to be synonyms. A total of 10 sets of duplicates were found between landraces and some reference cultivars. Accessions listed as duplicates were similar for nut and husk morphology. In Italy, new local genotypes were characterised in Liguria (‘Noscello’, ‘Ciasetta, ‘Tapparona’, ‘Dell’Orto’, ‘Gianchetta’, ‘Seigretta’, ‘Bardina’, ‘Del Rosso’, ‘Lunghera’, and ‘Menoia’), and in Latium (‘Itavex’, ‘Allungata’, ‘Nocciola della Madonnella’, ‘Cappello del prete’, ‘Nocciola Ada’). On the contrary, samples surveyed in Piedmont showed the same DNA profile of ‘Tonda Gentile delle Langhe’, the traditional variety cultivated in this region, in spite of differences observed at morphological traits (Valentini et al. 2014). Among the 20 accessions from Sicily, 6 showed the same genetic profile of 'Nocchione' (syn. 'Siciliana') and thus belonged to the major Sicilian cultivar. In Spain, among the 10 accessions surveyed in Asturias, 3 were classified as new genotypes ('Allande-3', 'Priero-1', Robriguedo-2'), while 6 showed the same SSR profile of 'Casina', the most common cultivar spread in this area (Rovira et al., 2005; Ferreira et al., 2009), and 1 ('Las Cuevas-1') was a possible clonal mutation of 'Casina' (allele 122 at locus CaT-B501). Some accessions from Portugal showed the same genetic profile of other known cultivars, indicating possible cases of synonymy: 'Raul' with 'Karidaty' from Turkey (syn. 'Imperiale de Trebizonde') (Manzo and Tamponi, 1982); 'Dawton', 'Purpurea' and 'Cartuxeria/Tubulosa' with 'Fructo rubro' from Balkans area (syn. 'Pellicule rouge'). 'Quinta Vila Nova Do Rego' was classified as a new genotype. Among the cultivars from Greece, three sets of synonyms were obtained. The first set was the pair 'Patem small' from Greece and 'Fructo rubro'. The second set was 'Argiroupoli' and 'Patem large' from Greece and the cultivar 'Yassi Badem' from Turkey. Finally, the third set was the pair 'Polykarpos' and 'Tombul Ghiaghli' from Greecethe latter is commonly cultivated there.

Morphological characterization revealed a wide diversity among the 42 unique landraces (Boccacci et al. 2013). These accessions should be considered original and additional local genetic diversity which needs 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 the large nut and kernel size desired by the in-shell market.

The 42 unique landrace genotypes were also analyzed by Boccacci et al. (2013) with 57 reference cultivars from different European and Turkish collections and 19 wild hazelnuts sampled in Latium and Campania (Fig 2). The study of the genetic relationships and population structure among wild forms, landraces, and cultivars in a geographic area can supply information about the putative domestication events, the evolutionary relationship, or the gene flow between them. According to several authors (Boccacci and Botta 2009, 2010; Gökirmak et al. 2009; Gürcan et al. 2010), *C. avellana* seems to have been domesticated independently in six different areas: British Islands, central Europe, Spain, Italy, Black Sea, and Iran. The results reported by Boccacci et al (2013) are in agreement with these conclusions, indicating the existence of three main germplasm groups in the

Mediterranean basin which could correspond with three domestication areas: northwestern Spain (Catalonia) and southern Italy (Campania) in the West and the Black Sea region in the East. Moreover, the data indicate the existence of secondary gene pools in the Iberian (Asturias) and Italian (Liguria and Latium) Peninsulas, 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.

Biochemical characterization of hazelnut germplasm

Nuts play an important role in the human nutrition and health, due to their very special nutritional and nutraceutical values. Hazelnuts are generally low in saturated fatty acids (SFA), and high in monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (oleic, linoleic, linolenic, palmitic, and stearic acids), where oleic is the major fatty acid. The α-tocopherol, that is the active form of vitamin E, helps to lower the risk of certain chronic diseases by protection against heart disease and has an antioxidant function (Salas-Salvadó and Megias, 2005; Di Renzo et al. 2013).

The oil fatty acid composition and the total lipid and tocopherol content were recently assessed in 75 hazelnut accessions from six European countries by Bacchetta et al. (2013) and a wide variability was found (Fig.3) . Based on Principal Conponents Analysis (PCA), the first three components are able to describe 82.5% of the sample variability. Total oil content, the oleic and linoleic were primarily responsible for the separation on PC1 (accounting for 38.395% of total variance), PC2 (accounting for 30.257% of the variance) was highly correlated to palmitic and palmitoleic acid, whereas the third component was represented by α-tocopherol (accounting for 13.897% of the variance). A part the most important widespread varieties, this work allowed to identify interesting fatty acids profile in local ecotypes present at low frequencies in the major area of cultivation and conserved *on farm*, such as 'Dal Rosso', 'Tonda di Biglini' and 'Incrocio L35', survived in Piedmont (Italy), 'Ada', 'Meloni' 'Centenaria di Ginnasi' and 'Barrettona Le Cese', survived in Latium (Italy). Thus the recovery and enhancement of ecotypes imply not only the enlargement of the basic germplasm and the availability of useful genes, but offer new economic possibilities for local market and potential industrial applications. The lipid fraction is a key factor in determining the hazelnut quality and storability, affecting the taste and the nutritional properties and numerous evidences highlighted its beneficial effect on human health (Di Renzo et al., 2014). However the enhancement of cultivars with high-quality attributes meet the demand of hazelnut confectionary industries and consumers with positive implication on the competitiveness of the European products in the international market. This aspect is of relevant importance considering that Turkish supply accounts for more than 80% of the world hazelnut trade largely determining the world export prices.

A total of 18 phenolic compounds were identified and quantitatively determined in 57 hazelnut cultivars by Solar et al. (2008) and Bacchetta et al. (2008) during the SAFENUT project (Fig. 4). The main compounds identified belong to four groups: flavan-3-ols (catechin, epicatechin, two procyanidin dimers and three procyanidin trimers); flavonols (myricetin-3-O-rhamnoside, quercetin pentoside and quercetin-3-O-rhamnoside); benzoic acids (gallic acid and protochatecuic acid); and dihydrochalcones (phloridzin). These results contribute to biochemical characterization and explain genetic variability within the European hazelnut resources, as reported by different authors (Garrone and Vacchetti, 1994; Durak et al. 1999; Alasalvar et al. 2006; Kornsteiner et al. 2006; Oliviera et al. 2008; Arcan and Yemenicioglu 2009; Bacchetta et al. 2011). The large variation in phenolic content among cultivars indicates different anti-oxidant and nutritional values for their nuts, which is of great interest to consumers and industrial users (Jakopic et al. 2011). Among dry fruits, hazelnuts show an intermediate total polyphenols content, from a major content in walnuts and pistachios to a lesser value in pine nuts and macadamia nuts (Delgrado et al. 2010). Moreover, hazelnut skin could potentially be considered as an inexpensive source of natural antioxidants (Alasalvar et al. 2009) and leaf extract also exhibited high antioxidant activity (Oliviera et al. 2008).

Mineral composition are of interest due to their proxidant activity and health benefits (Pershern et al. 1995; Alphan et al. 1996; Parcerisa et al. 1999). During the SAFENUT action, the mineral and protein components were evaluated at 92 hazelnut accessions during two years (Bacchetta et al. 2010). The results of the mineral analysis showed that potassium, calcium, phosphorus and magnesium contents varied 1.9, 2.3, 2.7 and 1.7 times from the mean values, respectively; great variation was also found in the protein content (variation of 4.1 times). Nevertheless the values of mineral nutrient and protein contents were in the range of those found by Açkurt et al. (1999) and Ozdemir et al. (2001). In order to evaluate the variation observed, principal component analysis was carried out to mean values of mineral and protein contents of seven cultivars (‘Barcelona’, ‘Gironell’, ‘Merveille de Bollwiller’, ‘Negret’, ‘Pauetet’, ‘Tonda di Giffoni’, and ‘Tonda Gentile delle Langhe’) which were collected in six different geographic sites (France, southern and northern Italy, Portugal, Slovenia and Spain). Figure 5 shows a PCA of data from samples grown at different locations, using mean values of the two harvesting years. PCA produced two components accounted for a cumulative 71.1% of variation; the most important variables integrated by the first component (50.0 % of variance). Positive values in PC1 suggests for samples with high potassium and magnesium contents, while positive values in PC2 suggest for samples with high calcium content. Samples from France tended to separate exhibiting high potassium and magnesium contents; samples from northern and southern Italy were grouped together showing high calcium and low potassium and magnesium contents. Previous works concluded that the most important factors in relation to biochemical nut composition are geographic origin and harvesting year (Parcerisa et al 1997). Ackurt et al. (1999) reported geographical region differences did not significantly affect manganese and calcium content in the hazelnut varieties cultivated in different districts of Turkey. A multivariate analysis of the nutritional and nutraceutical kernel components of the European hazelnut germplam will allow the identification of homogenous groups useful not only to discriminate the most interesting cultivars and their proper uses, but also as first step towards the definition of a reference 'core collection' (Brown 1989).

The cultural value of hazelnut genetic resources

The genetic resources represent the useful pool of genetic background utilized for different purposes in the breeding programs, but also have a precious cultural meaning related to traditional and historical uses. The safeguard of the traditional memory is indispensable for the cultural heritage of future generations. Important implications are related to the recovery of local uses which can be potentially useful for novel agro industrial applications. A survey carried out on the European festivals was summarized in a booklet on exhibitions of almond and hazelnut fruit and products published by Avanzato et al. (2009).

Traditional knowledge was recovered through the processing of 2097 questionnaires from interviews with 1115 students and their parents (506 parents and 476 grandparents) from six European countries. The inquiry showed the two crops to be well-known, both as trees and as fruits, by the people interviewed. Although they were well appreciated for their taste, but were not consumed regularly. There could be various factors behind this, including price variability and prejudices concerning dietary information (too rich in fatty acids). As for other dried fruits, consumers' preferences cannot be compared to the level achieved for fresh fruits and especially apples, which remain the favorites. However a leaflet with several traditional food uses and 65 recipes was collected.. Furthermore, a questionnaire was also addressed to farmers and provided the opportunity of comparing problems, technical practices and biodiversity status on a European level. Interesting data showed that hazelnut crop is a suitable model for forecasting studies on the relationship between agriculture and landscape ecology. An economic model for the launching and promoting of old cultivars was discussed and the crucial aspects underlined by Bacchetta and Di Giovanni (2013).

The importance of results dissemination

The management of plant genetic resources spans from collection and conservation of germplasm to its distribution and use. The development of a virtual germplasm collection using the standard descriptors makes efficient and timely the dissemination of germplasm information and therefore its utilization. The SAFENUT database (DB) represents an important tool for disseminating information on hazelnut genetic resources and their utilization. The SAFENUT database, available at hppt://www.safenut.casaccia.enea.it, was organized in order to provide users with multi-trait data based on germplasm evaluation records, by means of on-line search-queries. The core of the DB includes four sections: the data, access policy, administrative tools and outputs. The virtual inventory is coherent with other international databases, such as the *Prunus* database. The following information is accessible: passport data, morphological, biochemical (phenols, fatty acids, tocopherols, minerals contents) and molecular data (SSR loci, molecular profiles) as well as photos of 58 hazelnut accessions.

A list of new selected hazelnut ecotypes has also been included. To facilitate the utilization of the database, web-pages are dynamically interfaced with it. This approach ensures that the information derived from the database is up-to-date (Glaszmann et al. 2010). The DB allows two kinds of research: basic research, where it is possible to select the species and the list of accessions, and advanced research, in which all topics are shown. The user can apply for more than one topic; the results show a list of accessions, which links together the characteristics requested. Despite its importance, without additional regional, national, European financial support or coordinate initiatives able to promote the updating, the precious informatics tool risks to be a vain obsolete effort (Bacchetta and Di Giovanni 2012).

Conclusions

Hazelnuts are of great economic importance in Europe, playing an important role in human nutrition and health (Özdemir 2001; Rovira 2010). A better understanding of genetic diversity and its distribution is essential for its conservation and use (Ramanatha Rao and Hodgkin, 2002). This will help us to rationalise the collections, exploiting the available resources in more valuable ways. However the accessibility of collections depends largely on the information available on them. Accurate passport and characterization data are the first requirements which should be harmonized for a fluent sharing of information. The study of the intra-specific genetic variability allows the selection of ‘ecotypes’ or genotypes present in low frequency, usually well adapted to the local agro-ecological conditions, which represents a tool for crop diversification. For a few years now, the farmer becomes one of the actors of the creation and maintenance of this diversity. On farm conservation involves the maintenance of traditional crop varieties within traditional agricultural system. Recovery of nut crops, traditionally cultivated in local areas (often indicated as marginal landscape), improve local economies by revaluing cultural identities. As discussed by Molnar (2011), *Corylus* genetic wild resources are highly underutilized and underrepresented in research studies, conservation efforts and long-term breeding programs. Moreover studies focused on the hereditability of the most important biochemical nut traits as well as the analysis of quantitative traits, which are of valuable importance for breeders and stakeholders, are few. One approach to this problem is the development of core collections. Designing core collections involves an appropriate use of diversity, offering to the breeders an opportunity to work with a quite manageable number of accessions evaluated on traits of economic importance. The recent scientific progresses on molecular evaluation of hazelnut accessions and new selected genotypes maintained in the most important European collections, discussed in this paper, make this hypothesis more effective. Working not only on the unique genetic background offered by genetic resources, but also on their cultural meaning, it is possible to draw out their authentic significance straighten the genetic diversity relationship with the ecosystem (Riggs 1990) and people who preserve them on farm. Thus based on our experience, a multidisciplinary approach able to integrate competences from scientific to humanist point of view is one of the key to achieve a successful management and enhancement of European nuts germplasm.

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Figure

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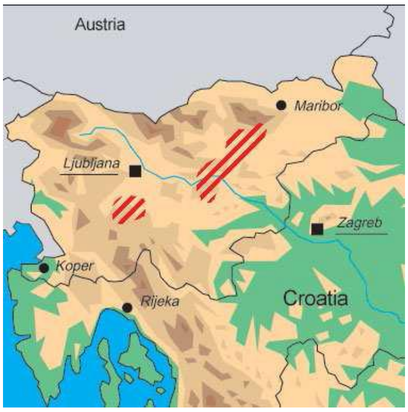
A) Hazelnut Field Surveys in Spain (Asturias Region)

B) Hazelnut Field Surveys in Greece (Drama, Sfendami and Pier Regions)

C) Hazelnut Field Surveys in Italy (Latium, Sardinia, Marche, Piedmont Regions)

D) Hazelnut Field Surveys in Portugal (Felqueiras, Viseu, Moimenta and Miho)



E) Hazelnut Field Surveys in Slovenia (Crescnjevec, Tepanje, Sentjur pri Celju and Vrhpec).

Figure 1 - Hazelnut material prospected in different European areas: Spain (A), Greece (B), Italy (C), Portugal (D) and Slovenia (E) are reported in red colour

Figure
Click here to download Figure: Fig2.docx

Figure 2 - UPGMA dendrogram based on SSR analysis of 42 unique landrace genotypes (LR), 57 cultivars (CV), and 19 wild individuals (W)

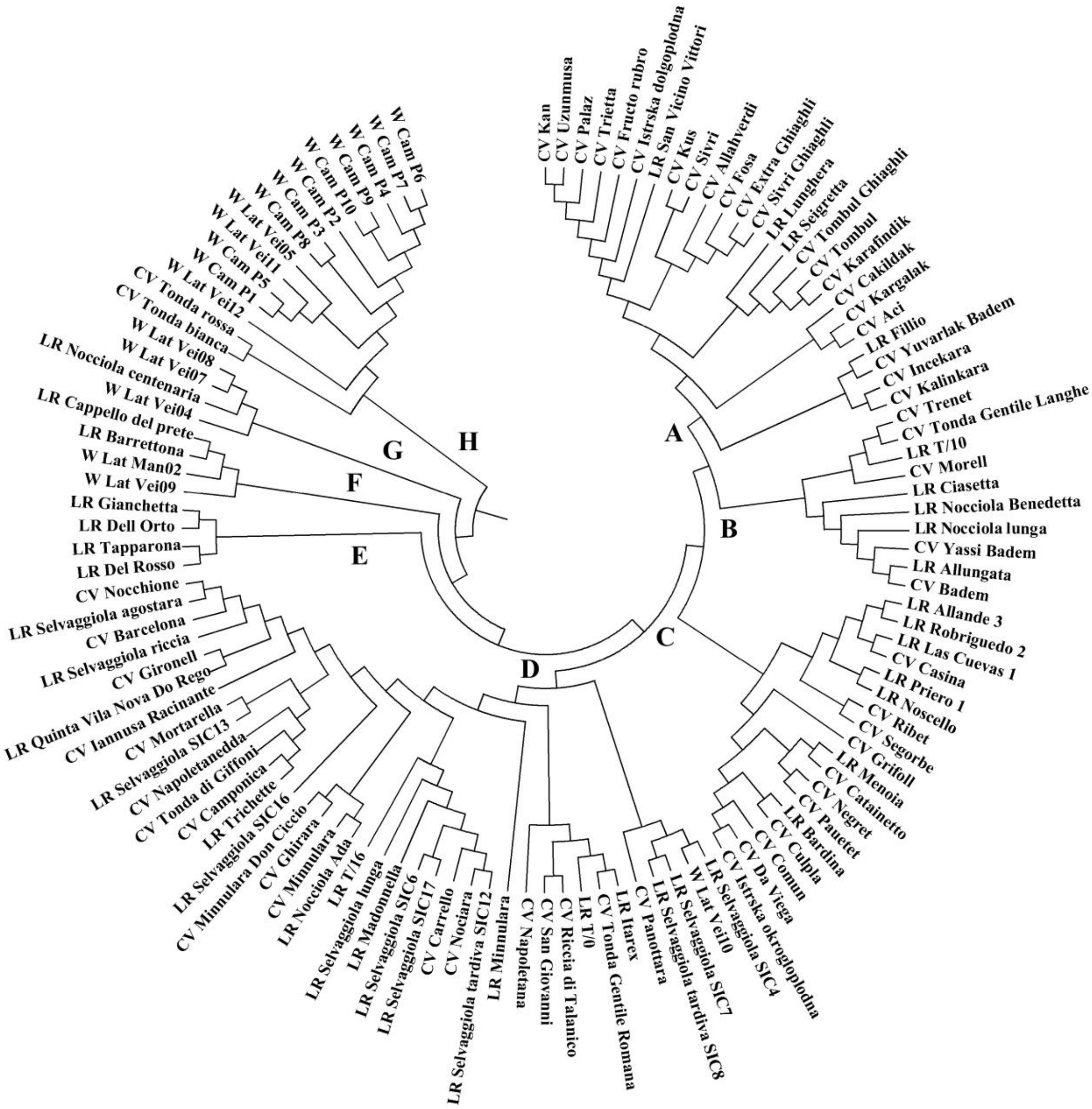
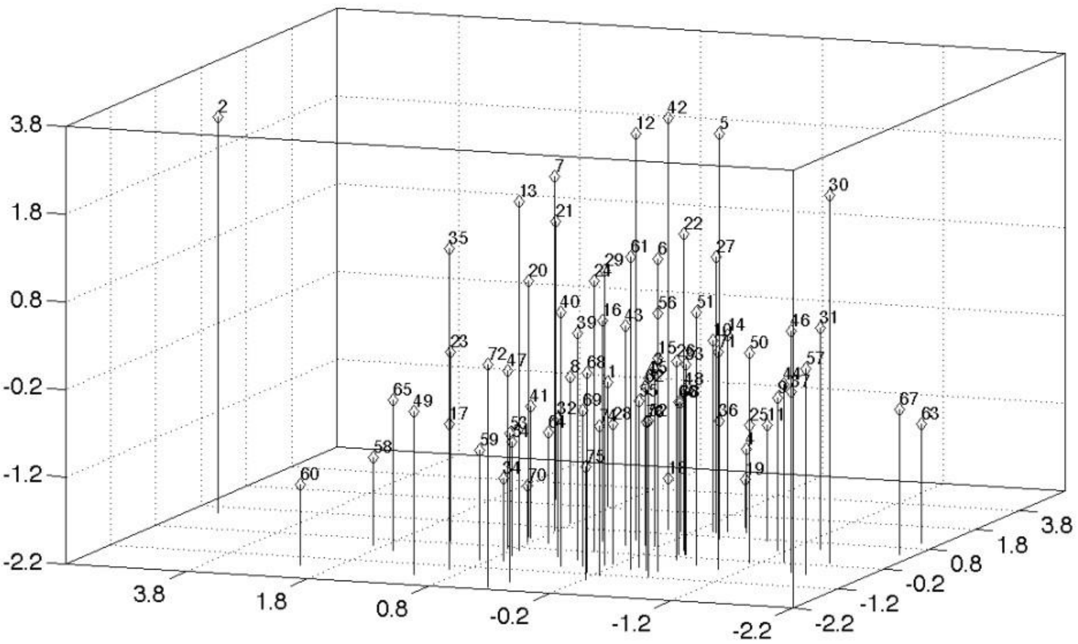


Figure 3 – Position of the principal component (PC) scores of the hazelnut kernel composition for 75 hazelnut cultivars.



Numbers refer to the clone number of the cultivar:
1C.a.9 Raul; 2 Gunslebert; 3Barcellona; 4 Hall's Giant;5 Segorbe; 6 Dal Rosso;7 C. maxima à Pellicule Blanche; 8 Negret; 9 Fertile de Coutard; 10 Bergeri;11 CV/2; 12 Rotblaftrige Lambernuss; 13 C.maxima à Pellicule Rose; 14Merveille de Bollwiller;15 Longue d'Espagne; 16 Pauetet (ref. cv); 17 Incrocio L35; 18Trenet; 19 Vermellet; 20 Corabel; 21Casina; 22 Nostrale; 23 Provence; 24 Pallagrossa; 25 CV/1; 26 Tonda di Giffoni (ref.cv); 27 Pellicola bianca; 28 San Giovanni; 29 Tonda bianca; 30 Ferwiller, 31Istrska okrogloplodna leska; 32 Tonda Gentile Langhe (ref. cv); 33 Feriale; 34 Polycarpos wild; 35 San Vicino; 36 Molar; 37 Lunga Ginnasi; 38 Gironell; 39 Tonda di Biglini; 40 TGL (clone PD); 41 C.a.5 Grada de Viseu, 42 Cosford; 43 C.a.11 Tubulosa; 44 Istrska dolgoplodna leska; 45 Nocchione; 46 Imperatrice Eugenie; 47 C.a.12Purpurea; 48 C.a.7DaVeiga; 49GR pi 03; 50 Piazza armerina ; 51 Nociara; 52 Riccia di Talanico; 53 Meloni ; 54 Karydato; 55Carrello ; 56 Tonda gentile romana; 57 Avellana Speciale; 58 Argiroupoli; 59 Patem; 60 Palaz; 61 Ada; 62 Barettona Vico; 63 C.a.8 Comum; 64 Castanyera (ref. cv); 65 Extra Giaghli; 66 Morell; 67 Grifoll; 68Camponica; 69 Daria; Tombul Giaghli; 71Comune di Sicilia ; 72 Tonda Calabrese-Caserta; 73Barettona; 74 Centenaria Ginnasi; 75 Culpla..

Eigenvectors of three principal component (PC) axes of kernel composition after principal component analysis of 75 hazelnut cultivars.

Variable	PC1	PC2	PC3
oil content %	**-0.514**	-0.329	-0.259
C16:0	-0.302	**0.833**	-0.102
C16:1	-0.16	0.597	-0.433
C18:0	-0.68	0.381	0.24
C18:1	**-0.737**	-0.649	0.068
C18:2	**0.918**	0.343	-0.095
C18:3	0.485	0.178	0.185
SFA	-0.585	**0.800**	0.058

Total MUFA	**-0.742**	-0.642	0.062
Total PUFA	**0.92**	0.343	-0.09
UFA/SFA	0.625	-0.765	-0.065
MUFA/SFA	0.362	-0.913	-0.03
PUFA/SFA	0.98	0.041	-0.125
alfa-tocoferol	0.258	-0.084	**0.91**
SI	0.087	0.259	**0.922**
Eigenvalue	5.759	4.538	2.085
Proportion of total (%)	38.395	30.257	13.897

Figure
Click here to download Figure: Fig 4.doc

Figure 4 Variability of phenolic compounds in kernel of 53 hazelnut accessions evaluated during 068 Agri Gen Res SAFENUT

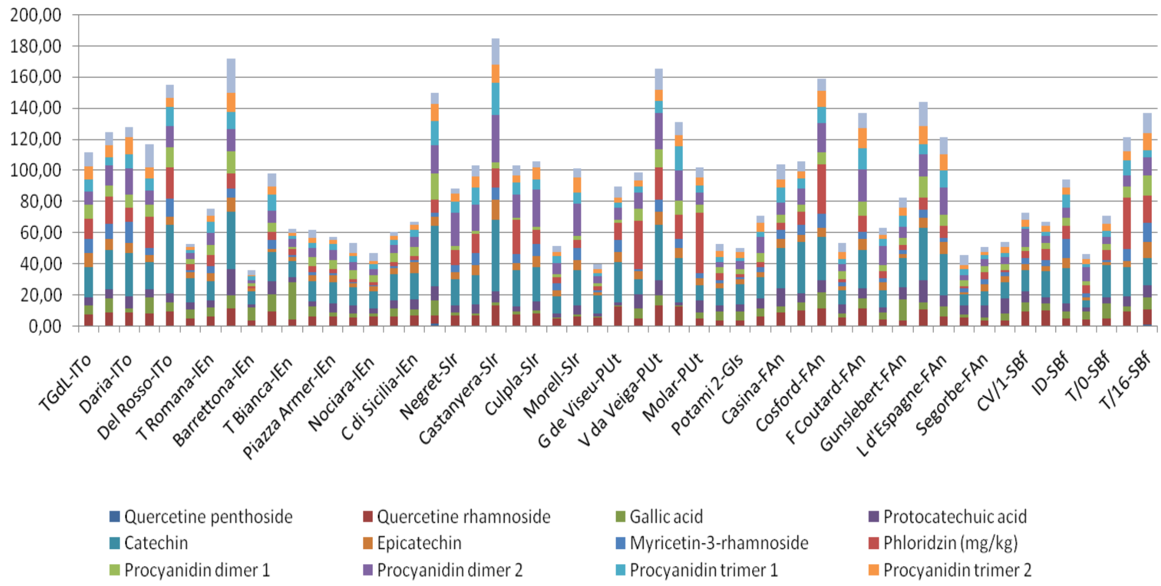
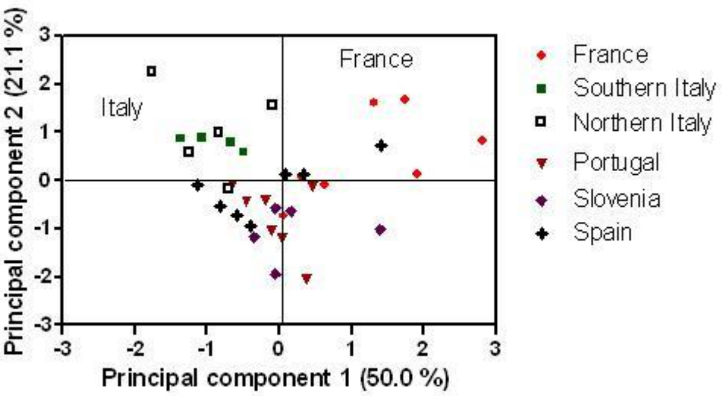


Figure
Click here to download Figure: Fig 5.docx

Figure 5 - Effect of geographic origins based of principal component analysis of kernel mineral composition for seven reference hazelnut cultivars cultivated in six European countries.



Character	PC 1	PC 2
K	0.90	-0.29
P	0.64	-0.51
Ca	0.43	0.78
Mg	0.85	0.07
Protein	0.62	0.32

Table 1 Collections of hazelnut genetic resources evaluated by Rovira et al., 2010

Country	Collections
France	Montesquieu (Conservatoire Végétal régional d'Aquitaine)
Greece	NAGREF-Pomology Institute (Naoussa)
Slovenia	National collection (Ljubljana) Ex-situ collection (Maribor)
Spain	IRTA-Constantí (Catalonia), SERIDA-Villaviciosa (Asturias)
Portugal:	CITAB - Vila Real DRAPN – Sergude, Felgueiras DRAPN - Viseu
Italy:	Cravanzana (Cuneo) Chieri (Turin) Le Cese (Viterbo) Caserta(Campania)

Legend: NAGREF National Agricultural Research Foundation; IRTA Institut de Recerca i Tecnologia Agroalimentàries CentreMas de Bover; SERIDA Institut de Recerca i Tecnologia Agroalimentàries CentreMas de Bover in Villaviciosa; CITAB and DRAPN, Centre for the Research and Technology of Agro-Environment and Biological Sciences Universidade de Trás-os-Montes.

Table 2 – Polymorphism of 10 SSR loci selected for SAFENUT projet by Boccacci and Botta (2010). A, number of alleles; H_O observed heterozygosity; H_E, expected heterozygosity; PI, probability of identity.

Locus	A	H_o	H_E	PI
CaC-B020	15	0.75	0.712	0.10
CaC-B028	11	0.70	0.78	0.08
CaT-B107	14	0.89	0.85	0.04
CaT-B501	13	0.84	0.72	0.11
CaT-B502	10	0.68	0.75	0.10
CaT-B503	12	0.75	0.73	0.10
CaT-B504	12	0.92	0.84	0.04
CaT-B505	10	0.95	0.81	0.06
CaT-B507	9	0.87	0.823	0.05
CaT-B508	12	0.70	0.69	0.11
Cumulative PI		4.3×10^{-18}		

Table 3 - Proportion of phenotypic classes of morphological descriptors of hazelnut fruits collected from landraces. 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%)