

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

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

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/156500> since 2016-07-18T16:33:20Z

Published version:

DOI:10.1007/s10722-014-0173-7

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

Genetic Resources and Crop Evolution

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

--Manuscript Draft--

| | |
|--|--|
| Manuscript Number: | |
| Full Title: | A multidisciplinary approach to enhance the conservation and use of hazelnut genetic resources |
| Article Type: | Book review |
| Keywords: | genetic resources; <i>Corylus avellanae</i> ; collections; conservation; traditional knowledge; database |
| Corresponding Author: | Loretta Bacchetta, Agrarian Science ENEA Rome, ITALY |
| Corresponding Author Secondary Information: | |
| Corresponding Author's Institution: | ENEA |
| Corresponding Author's Secondary Institution: | |
| First Author: | Loretta Bacchetta, Agrarian Science |
| First Author Secondary Information: | |
| Order of Authors: | Loretta Bacchetta, Agrarian Science Mercè Rovira Maria Aramini Pavlina Drogoudi Ana paula Silva Anita Solar Damiano Avanzato Roberto Botta Nadia Valentini Paolo Boccacci |
| Order of Authors Secondary Information: | |
| Abstract: | <p>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,</p> |

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.

1 **A multidisciplinary approach to enhance the conservation and use of**

2 **hazelnut genetic resources**

3 Bacchetta L*.¹⁾, Rovira M.²⁾, Aramini M.¹⁾, Drogoudi P.³⁾, Silva A.P.⁴⁾, Solar A.⁵⁾, Avanzato D.⁶⁾, Botta R.⁷⁾,
4 Valentini N.⁷⁾, Boccacci P.⁸⁾

5
6 ¹⁾ENEA Casaccia - UTAGRI, Agenzia Nazionale per le Nuove Tecnologie, l'Energia e lo Sviluppo Economico
7 Sostenibile, Via Anguillarese, 301. 000123, Rome – Italy

8 ²⁾ IRTA, Institut de Recerca i Tecnologia Agroalimentàries CentreMas de Bover, Ctra. Reus-El Morell, km 3.8,
9 43120, Constantí Tarragona, Spain

10 ³⁾ NAGREF, National Agricultural Research Foundation – Pomology Institute, Hellenic Agricultural
11 Organization ‘Demeter’, 38 R.R. Station, 59 035 Naoussa, Greece

12 ⁴⁾ CITAB, Centre for the Research and Technology of Agro-Environment and Biological Sciences Universidade
13 de Trás-os-Montes e Alto Douro, Apartado 1013, 5001-801 Vila Real, Portugal

14 ⁵⁾ Univerza v Ljubljani, Biotehniška Fakulteta, Biotehniška Fakulteta, Jamnikarjeva 101, 1000 Ljubljana,
15 Slovenia

16 ⁶⁾ CRA Centro Ricerca per la Frutticoltura, Via Fioranello, 52, 0013, Rome, Italy

17 ⁷⁾ Department of Agriculturae, Forestry and Food Science (DISAFA) - University of Turin, Largo Paolo
18 Braccini, 2 - 10095 Grugliasco (TO), Italy

19 ⁸⁾ Institute for Sustainable Plant Protection - National Research Council (IPSP-CNR), UOS of Grugliasco,
20 Largo Paolo Braccini, 2 - 10095 Grugliasco (TO), Italy

21

22 *Corresponding author

23 Tel +3906303483405

24 Fax+3906303486044

25 e-mail: loretta.bacchetta@enea.it

26

27

28

29

1 **Abstract**

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

22

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

1 **Introduction**

2 The European hazelnut (*Corylus avellana* L.) is one of the world's major nut crops. Total worldwide production
3 (FAOstat 2014) is sixth after that of cashew (*Anacardium occidentale* L.), walnut (*Juglans regia* L.), almond
4 [*Prunus dulcis* (Miller) D.A. Webb], chestnut (*Castanea* spp.), and pistachio (*Pistacia vera* L.). The Black Sea
5 countries account for the majority of world hazelnut production (FAOstat 2014): Turkey (598,158 tons, average
6 of 2008-2012), Azerbaijan (30,030 tons), and Georgia (25,020 tons). Other important producers are Italy
7 (104,577 tons), the USA (32,399 tons), Iran (20,832 tons), China (19,700 tons), and Spain (16,239 tons).
8 Moreover, emerging markets such as China, India, the United Arab Emirates and Australia have increased their
9 trade by more than 80% in the last five years (USDA, 2010) In Europe, hazelnuts account for about 16% of total
10 world production. Italy is the first European hazelnut producer followed by Spain, France (8,137 tons), Poland
11 (3,293 tons), and Greece (1,500 tons) (FAOstat, 2014). The per capita consumption of nuts in Europe is very
12 high due to the importance of the crop in the Mediterranean diet (Bulló et al. 2011). Nuts are a high energy food,
13 rich in fats and protein, and a valuable source of fibres, phytonutrients, and antioxidants (Vitamin E) (Sivakumar
14 et al., 2005; Sivakumar and Bacchetta, 2006; Salas-Salvadó and Megías, 2005). In the past two decades, several
15 biochemical and clinical studies have provided consistent evidence of their healthy properties (Hu et al. 1999;
16 Fraser et al. 2009; Mullie and Clarys 2012). Nevertheless, European nut production supplies less than 40% of
17 local demand and the rest is imported from Turkey and USA.

18 The conservation, characterization and survey of hazelnut genetic resources is a prerequisite to
19 improving breeding programs, enhancing the competitiveness of the European production. Breeding is in its
20 infancy in this species, if compared to most other domesticated crops, and the high genetic diversity of hazelnut
21 offers substantial opportunities for genetic improvement (Mehlenbacher 1991; Thompson et al. 1996; Molnar
22 2011). The *Corylus* germplasm collections in the world consist primarily in cultivated forms of *C. avellana*
23 located in regions where this production occurs. An extensive inventory of hazelnut research, germplasm and
24 references was published by Köksal (2000), supported by FAO (Food and Agriculture Organization). Major
25 European collections are those managed by: Institut de Recerca i Tecnologia Agroalimentaries (IRTA) in Reus
26 (Spain) (Rovira, 1997; Boccacci et al., 2008); University of Torino (UNITO) in Chieri, and and Centro Ricerche
27 per l'Agricoltura (CRA) in Caserta (Italy), University of Ljubljana in Maribor (Slovenia) (Solar and Stampar,
28 1997; 2009) and Institut National de la Recherche Agronomique (INRA) in Villanave d'Ornon (France). A
29 number of smaller collections and repositories have been developed as results of local and regional efforts
30 (Mehlenbacher 1991; Berthaud et al. 1997; Solar and Stampar, 2009; Bacchetta et al. 2010).

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

25 This work summarizes the current status and potential breeding of *Corylus* genetic resources in Europe,
26 prioritizing the need to conserve and better study the underutilized on-farm ecotypes. The aim is also to discuss
27 the recent progresses on recovery, characterization, conservation and uses of hazelnut achieved by the 068 Agri
28 Gen Res SAFENUT which was one of the 17 action financed by the European Commission - Directorate
29 General for Agriculture and Rural Development. Evidence and new research directions are considered to

1 improve the competitiveness and the economical role of the European hazelnuts as a possible resourceful
2 strategy to consider in the conservation and use of other nut or fruit crops.

3

4 **Morphological evaluation of hazelnut accessions**

5 The traditional characterization of cultivars has relied on morphological and phenological characteristics which
6 provide the basic data for further evaluations. Morphological determinations which need to be taken by an expert
7 in the species, could be affected by environmental factors, different developmental stages and their number is
8 limited (De Vicente and Fulton, 2003). However one critical issue referring to morphological characterization, is
9 the harmonization of the standard descriptors for a common characterization of germplasm. The use of a
10 common and shared descriptors list represents an important tool to produce a universally understood ‘language’
11 for plant genetic resources data. A recent evaluation of hazelnut material was performed by Bacchetta et al.
12 (2011) in typical cultivation areas, using a chart with specific hazelnut descriptors (Thompson et al. 1978; UPOV
13 1979; Biodiversity International 2008). The descriptors included: general characteristics (cultivar, synonyms,
14 origin, and growing areas), tree traits (10 characters), flowering traits (6 characters), and nut and kernel traits (32
15 characters). The morphological description of 46 hazelnut European typical cultivars (13 from France; 9 from
16 Italy; 8 from Portugal; 6 from Slovenia; and 10 from Spain) were published in online SAFENUT database, the
17 068 EU AGRI GEN RES project (<http://safenut.casaccia.enea.it/>). Nut traits are the most interesting traits to
18 define the potential uses of the product. Thus among the 46 European cultivars the most representative were the
19 medium ones (53.5%), while 37.2% had a large or very large size suitable for raw consumption. Considering nut
20 shape, 51.2% of the cultivars had a globular shape (preferred by industry), 23.3% ovate and 14.0% long
21 cylindrical one. The blanching index was generally moderate showing a great variability: 44.2% of cultivars
22 presented a value ranging between 25 and 75%. Focusing on the most interesting agronomical traits, which are
23 early nut maturity and resistance to big bud mite (*Phytoptus avellanae*, Nalepa), 64.3% of cultivars had an
24 intermediate ripening time and only 14.3% an early or very early maturity, while about 35% were resistant to big
25 bud mite.

26 The European collections exist in different countries and maintain accessions collected from different
27 geographic areas (mainly Europe, Turkey and USA) according to each Research Institutes. In order to harmonize
28 the various initiatives carried out at regional and national levels, Rovira et al. (2010) provided a list of 222
29 hazelnut clones and 58 selections from 13 European hazelnut collections (Table 1). The number and origin of the
30 filbert accessions held in the collections were: Albania (1 cultivar), Balkan area (2 cvs.), Belgium (1 cv.),

1 England (12 cvs.), France (8 cvs.), Germany (6 cvs.), Greece (1 cv.), Hungary (1 cv.), Italy (51 cvs.),
2 Netherlands (1 cv.) Portugal (3 cvs.), Romania (4 cvs.), Slovenia (3 cvs.), Spain (84 cvs.), Turkey (7 cvs.), and
3 USA (9 cvs.). Nine cultivars were of unknown origin. The main result obtained from this survey was that in
4 many collection fields are conserved the same cultivars, indicating the need to rationalize genetic resources and
5 that few efforts were made to include new accessions in the collections. For example, the NCGR and Oregon
6 State University have increased efforts to collect cultivated and wild accession of *Corylus*, such as from the
7 Balkans, Russia and Caucasus, and now their collections are more than 700 accessions, including all major
8 *Corylus* species (Gurcan et al., 2010). However, a number of species are still lacking, especially when
9 considering their wide geographic range, for these reasons recovering and evaluation efforts are still needed
10 mainly in Europe.

11

12 **Microsatellite variability**

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

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

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

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

22

23 **DNA-typing of accessions from European germplasm collections**

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

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

15

16 **Genetic characterization of landraces**

17 A total of 77 landraces were surveyed in the traditional areas of hazelnut cultivation in five southern
18 European countries (Fig. 1). Among them, 5 were collected in northern Portugal, 10 in northern Spain (Asturias),
19 52 in six Italian regions [6 in Piedmont (northwestern Italy), 10 in Liguria (northwestern), 1 in Marche (central
20 Italy), 12 in Latium (central Italy), 3 in Calabria (southern Italy), and 20 in Sicily], 5 from Slovenia, and 5 from
21 northern Greece. Farmers were contacted explaining the reasons for the project and interviewed about the
22 presence of old endangered cultivars on their farms. Information on agronomic and qualitative traits, as well as
23 use, local names, tradition, and social context were also collected (Boccacci et al. 2013).

24 Microsatellite analysis identified 42 unique genotypes while 35 accessions appeared to be synonyms. A
25 total of 10 sets of duplicates were found between landraces and some reference cultivars. Accessions listed as
26 duplicates were similar for nut and husk morphology. In Italy, new local genotypes were characterised in Liguria
27 (‘Noscello’, ‘Ciasetta’, ‘Tapparona’, ‘Dell’Orto’, ‘Gianchetta’, ‘Seigretta’, ‘Bardina’, ‘Del Rosso’, ‘Lunghera’,
28 and ‘Menoia’), and in Latium (‘Itavex’, ‘Allungata’, ‘Nocciola della Madonnella’, ‘Cappello del prete’,
29 ‘Nocciola Ada’). On the contrary, samples surveyed in Piedmont showed the same DNA profile of ‘Tonda
30 Gentile delle Langhe’, the traditional variety cultivated in this region, in spite of differences observed at

1 morphological traits (Valentini et al. 2014). Among the 20 accessions from Sicily, 6 showed the same genetic
2 profile of 'Nocchione' (syn. 'Siciliana') and thus belonged to the major Sicilian cultivar. In Spain, among the 10
3 accessions surveyed in Asturias, 3 were classified as new genotypes ('Allande-3', 'Priero-1', 'Robriguedo-2'),
4 while 6 showed the same SSR profile of 'Casina', the most common cultivar spread in this area (Rovira et al.,
5 2005; Ferreira et al., 2009), and 1 ('Las Cuevas-1') was a possible clonal mutation of 'Casina' (allele 122 at
6 locus CaT-B501). Some accessions from Portugal showed the same genetic profile of other known cultivars,
7 indicating possible cases of synonymy: 'Raul' with 'Karidaty' from Turkey (syn. 'Imperiale de Trebizonde')
8 (Manzo and Tamponi, 1982); 'Dawton', 'Purpurea' and 'Cartuxeria/Tubulosa' with 'Fructo rubro' from Balkans
9 area (syn. 'Pellicule rouge'). 'Quinta Vila Nova Do Rego' was classified as a new genotype. Among the
10 cultivars from Greece, three sets of synonyms were obtained. The first set was the pair 'Patem small' from
11 Greece and 'Fructo rubro'. The second set was 'Argiroupoli' and 'Patem large' from Greece and the cultivar
12 'Yassi Badem' from Turkey. Finally, the third set was the pair 'Polykarpos' and 'Tombul Ghiaghli' from
13 Greece the latter is commonly cultivated there.

14 Morphological characterization revealed a wide diversity among the 42 unique landraces (Bocacci et al.
15 2013). These accessions should be considered original and additional local genetic diversity which needs to be
16 conserved in situ. In addition, some landraces showed morphological and technological traits appreciated by the
17 market (Table 3). Accessions 'Robriguedo-2' (Asturias), 'Noscello' (Liguria), 'Barrettona,' 'Itavex,' 'Cappello
18 del Prete,' 'Madonnella' (Latium), and 'Selvaggiola Tardiva SIC12' (Sicily) were interesting for the food
19 industry. Nuts with globular or ovoid shape, kernels with medium size, and a caliber ≥ 12 mm are the ideal traits
20 for the industry processing (Garrone and Vacchetti 1994). On the contrary, 'Selvaggiola SIC3,' 'Trichette'
21 (Sicily), 'San Vicino Vittori' (Latium), and 'T/16' (Slovenia) showed the large nut and kernel size desired by the
22 in-shell market.

23 The 42 unique landrace genotypes were also analyzed by Bocacci et al. (2013) with 57 reference cultivars
24 from different European and Turkish collections and 19 wild hazelnuts sampled in Latium and Campania (Fig 2).
25 The study of the genetic relationships and population structure among wild forms, landraces, and cultivars in a
26 geographic area can supply information about the putative domestication events, the evolutionary relationship, or
27 the gene flow between them. According to several authors (Bocacci and Botta 2009, 2010; Gökirmak et al.
28 2009; Gürcan et al. 2010), *C. avellana* seems to have been domesticated independently in six different areas:
29 British Islands, central Europe, Spain, Italy, Black Sea, and Iran. The results reported by Bocacci et al (2013)
30 are in agreement with these conclusions, indicating the existence of three main germplasm groups in the

1 Mediterranean basin which could correspond with three domestication areas: northwestern Spain (Catalonia) and
2 southern Italy (Campania) in the West and the Black Sea region in the East. Moreover, the data indicate the
3 existence of secondary gene pools in the Iberian (Asturias) and Italian (Liguria and Latium) Peninsulas, where
4 local varieties have been domesticated in subsequent times from wild forms and/or from the introduction of
5 ancient domesticate varieties, followed by a relatively local evolution that could include crosses among them and
6 with local hazelnuts.

7 **Biochemical characterization of hazelnut germplasm**

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

14 The oil fatty acid composition and the total lipid and tocopherol content were recently assessed in 75 hazelnut
15 accessions from six European countries by Bacchetta et al. (2013) and a wide variability was found (Fig.3) .
16 Based on Principal Components Analysis (PCA), the first three components are able to describe 82.5% of the
17 sample variability. Total oil content, the oleic and linoleic were primarily responsible for the separation on PC1
18 (accounting for 38.395% of total variance), PC2 (accounting for 30.257% of the variance) was highly correlated
19 to palmitic and palmitoleic acid, whereas the third component was represented by α -tocopherol (accounting for
20 13.897% of the variance). A part the most important widespread varieties, this work allowed to identify
21 interesting fatty acids profile in local ecotypes present at low frequencies in the major area of cultivation and
22 conserved *on farm*, such as ‘Dal Rosso’, ‘Tonda di Biglini’ and ‘Incrocio L35’, survived in Piedmont (Italy),
23 ‘Ada’, ‘Meloni’ ‘Centenaria di Ginnasi’ and ‘Barrettona Le Cese’, survived in Latium (Italy). Thus the recovery
24 and enhancement of ecotypes imply not only the enlargement of the basic germplasm and the availability of
25 useful genes, but offer new economic possibilities for local market and potential industrial applications. The lipid
26 fraction is a key factor in determining the hazelnut quality and storability, affecting the taste and the nutritional
27 properties and numerous evidences highlighted its beneficial effect on human health (Di Renzo et al., 2014).
28 However the enhancement of cultivars with high-quality attributes meet the demand of hazelnut confectionary
29 industries and consumers with positive implication on the competitiveness of the European products in the

1 international market. This aspect is of relevant importance considering that Turkish supply accounts for more
2 than 80% of the world hazelnut trade largely determining the world export prices.

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

17 Mineral composition are of interest due to their prooxidant activity and health benefits (Pershern et al.
18 1995; Alphan et al. 1996; Parcerisa et al. 1999). During the SAFENUT action, the mineral and protein
19 components were evaluated at 92 hazelnut accessions during two years (Bacchetta et al. 2010). The results of the
20 mineral analysis showed that potassium, calcium, phosphorus and magnesium contents varied 1.9, 2.3, 2.7 and
21 1.7 times from the mean values, respectively; great variation was also found in the protein content (variation of
22 4.1 times). Nevertheless the values of mineral nutrient and protein contents were in the range of those found by
23 Açkurt et al. (1999) and Ozdemir et al. (2001). In order to evaluate the variation observed, principal component
24 analysis was carried out to mean values of mineral and protein contents of seven cultivars ('Barcelona',
25 'Gironell', 'Merveille de Bollwiller', 'Negret', 'Pauetet', 'Tonda di Giffoni', and 'Tonda Gentile delle Langhe')
26 which were collected in six different geographic sites (France, southern and northern Italy, Portugal, Slovenia
27 and Spain). Figure 5 shows a PCA of data from samples grown at different locations, using mean values of the
28 two harvesting years. PCA produced two components accounted for a cumulative 71.1% of variation; the most
29 important variables integrated by the first component (50.0 % of variance). Positive values in PC1 suggests for
30 samples with high potassium and magnesium contents, while positive values in PC2 suggest for samples with

1 high calcium content. Samples from France tended to separate exhibiting high potassium and magnesium
2 contents; samples from northern and southern Italy were grouped together showing high calcium and low
3 potassium and magnesium contents. Previous works concluded that the most important factors in relation to
4 biochemical nut composition are geographic origin and harvesting year (Parcerisa et al 1997). Ackurt et al.
5 (1999) reported geographical region differences did not significantly affect manganese and calcium content in
6 the hazelnut varieties cultivated in different districts of Turkey. A multivariate analysis of the nutritional and
7 nutraceutical kernel components of the European hazelnut germplasm will allow the identification of homogenous
8 groups useful not only to discriminate the most interesting cultivars and their proper uses, but also as first step
9 towards the definition of a reference 'core collection' (Brown 1989).

10 **The cultural value of hazelnut genetic resources**

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

17 Traditional knowledge was recovered through the processing of 2097 questionnaires from interviews with
18 1115 students and their parents (506 parents and 476 grandparents) from six European countries. The inquiry
19 showed the two crops to be well-known, both as trees and as fruits, by the people interviewed. Although they
20 were well appreciated for their taste, but were not consumed regularly. There could be various factors behind
21 this, including price variability and prejudices concerning dietary information (too rich in fatty acids). As for
22 other dried fruits, consumers' preferences cannot be compared to the level achieved for fresh fruits and
23 especially apples, which remain the favorites. However a leaflet with several traditional food uses and 65 recipes
24 was collected.. Furthermore, a questionnaire was also addressed to farmers and provided the opportunity of
25 comparing problems, technical practices and biodiversity status on a European level. Interesting data showed
26 that hazelnut crop is a suitable model for forecasting studies on the relationship between agriculture and
27 landscape ecology. An economic model for the launching and promoting of old cultivars was discussed and the
28 crucial aspects underlined by Bacchetta and Di Giovanni (2013).

29

30 **The importance of results dissemination**

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

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

19

20 **Conclusions**

21 Hazelnuts are of great economic importance in Europe, playing an important role in human nutrition and health
22 (Özdemir 2001; Rovira 2010). A better understanding of genetic diversity and its distribution is essential for its
23 conservation and use (Ramanatha Rao and Hodgkin, 2002). This will help us to rationalise the collections,
24 exploiting the available resources in more valuable ways. However the accessibility of collections depends
25 largely on the information available on them. Accurate passport and characterization data are the first
26 requirements which should be harmonized for a fluent sharing of information. The study of the intra-specific
27 genetic variability allows the selection of 'ecotypes' or genotypes present in low frequency, usually well adapted
28 to the local agro-ecological conditions, which represents a tool for crop diversification. For a few years now, the
29 farmer becomes one of the actors of the creation and maintenance of this diversity. On farm conservation
30 involves the maintenance of traditional crop varieties within traditional agricultural system. Recovery of nut

1 crops, traditionally cultivated in local areas (often indicated as marginal landscape), improve local economies by
2 revaluing cultural identities. As discussed by Molnar (2011), *Corylus* genetic wild resources are highly
3 underutilized and underrepresented in research studies, conservation efforts and long-term breeding programs.
4 Moreover studies focused on the heritability of the most important biochemical nut traits as well as the
5 analysis of quantitative traits, which are of valuable importance for breeders and stakeholders, are few. One
6 approach to this problem is the development of core collections. Designing core collections involves an
7 appropriate use of diversity, offering to the breeders an opportunity to work with a quite manageable number of
8 accessions evaluated on traits of economic importance. The recent scientific progresses on molecular evaluation
9 of hazelnut accessions and new selected genotypes maintained in the most important European collections,
10 discussed in this paper, make this hypothesis more effective. Working not only on the unique genetic
11 background offered by genetic resources, but also on their cultural meaning, it is possible to draw out their
12 authentic significance straighten the genetic diversity relationship with the ecosystem (Riggs 1990) and people
13 who preserve them on farm. Thus based on our experience, a multidisciplinary approach able to integrate
14 competences from scientific to humanist point of view is one of the key to achieve a successful management and
15 enhancement of European nuts germplasm.

16

17 **Acknowledgements**

18 The authors thank the AGRI GEN RES Community Program (European Commission, Directorate-General for
19 Agriculture and Rural Development, under Council Regulation (EC) No 870/2004) for its financial support.

20 Many thanks to the traditional hazelnut growers of different Countries (Spain, France, Greece, Italy, Portugal
21 and Slovenia) and also to the associations, companies, researchers and everyone who contributed with the
22 material included in the collection.

23

24 **References**

- 25 Akurt F, Ozdemirez M, Birigen G, Loker M 1999. Effect of geographical origin and variety on vitamin and
26 mineral composition of Hazelnut (*Corylus avellana* L.) varieties cultivated in Turkey. Food Chem.65:
27 309-313
- 28 Alasalvar C, Amaral JS Satir G, Shahidi F 2009. Lipid characteristics and minerals of native Turkish hazelnut
29 essential varieties (*Corylus avellana* L.). Food Chemistry113 (4): 919-925.

- 1 Alphan, E., Pala, M., Açıkturk, F., Yılmaz, T. 1997. Nutritional composition of hazelnut and its effects on glucose
2 and lipid metabolism. *Acta Hort.* 445: 305-310.
- 3 Alberghina O 1982. Indagine sulla corilicoltura siciliana. *Frutticoltura* 2: 27-30
- 4 Arcan, I., Yemenicioglu, A., 2009. Antioxidant activity and phenolic content of fresh and dry nuts with or
5 without the seed coat. *J. Food Compos. Anal.* 22, 184–188
- 6 Avanzato D, Vaccaro A, Bacchetta L, Tronci C, Drogoudi P, Duval H, Rovira M, Silva AP, Socias R, Solar A,
7 Spera D, Botta R 2009. *Festival of Almond and Hazelnut in Europe*. Edited by A.G.C. Arti Grafiche
8 Ciampino rsl.
- 9 Bacchetta L, Avanzato D, Botta R, Bellon B, Boccacci P, Drogoudi P, Metzidakis I, Rovira M, Silva AP, Solar
10 A, Spera D 2008. First results of SAFENUT: a European project for the preservation and utilization of
11 hazelnut local genetic resources. *Acta Horticulture* 845: 66-60.
- 12 Bacchetta L, Spera D, Avanzato D, Botta R, Boccacci P, Di Giovanni B, Drogoudi P, Duval H, Metzidakis I,
13 Rovira M, Silva A.P, Socias R, Solar A 2010. European Hazelnut and Almond genetic resources: results
14 and perspectives of networking SAFENUT AGRI GEN RES activities IHC Science and Horticulture for
15 people – 28th International Horticultural Congress Abstract S12.107 Vol. 2 Symposia, pag 546.
- 16 Bacchetta L; Di Giovanni B, Rovira M, Spera D, Avanzato D, Botta R, Boccacci P, Drogoudi P, Duval H,
17 Metzidakis I, Silva AP, Socias R, Solar A 2011. The AGRI GEN RES SAFENUT Action: a European
18 Strategy for the preservation and utilization of hazelnut and almond genetic resources. *NUCIS*, Num. 15:
19 30-33.
- 20 Bacchetta L, Di Giovanni B 2012. The enhancement of hazelnut and almond genetic resources through the
21 European AGRI GEN RES SAFENUT Action. The ENEA experience as project coordinator. *EAI*
22 (Energia, Ambiente e Innovazione) Magazine- 2012, Vol 1: 78–84
- 23 Bacchetta L, Aramini M, Zini A, Di Giammatteo V, Spera D, Drogoudi P, Rovira M, Silva A.P, Solar A, Botta
24 R 2013. Fatty acids and alpha-tocopherol composition in hazelnut (*Corylus avellana* L.): a chemometric
25 approach to emphasize the quality of European germplasm. *Euphytica* 2013, Vol 191(1): 57–73.
- 26 Bacchetta L. Di Giovanni B 2013. European Hazelnut and Almond Genetic Resources: Safeguard and
27 Traditional Uses. *Resources* 2013 (2): 204-212
- 28 Bassil NA, Botta R, Mehlenbacher S.A 2005. Microsatellite Markers in Hazelnut: Isolation, Characterization,
29 and Cross-species Amplification. *J. Am. Soc. Hort. Sci.* 130: 543-549

- 1 Berthaud J 1997. Strategies for conservation of genetic resources in relation with their utilization. *Euhytica* 96:
2 1-12.
- 3 Bioversity, FAO, CIHEAM 2008. Descriptors for hazelnut (*Corylus avellana* L.). Bioversity International,
4 Rome, Italy; Food and Agriculture Organization of the United Nations, Rome, Italy; International Centre
5 for Advanced Mediterranean Agronomic Studies, Zaragoza, Spain.
- 6 Brown AHD 1989. The case for core collections. In: The use of plant Genetic resources. Edited by Brown,
7 A.H.D.; Frankel, O.H.; Marshall, D.R.; Williams, J.T. Cambridge UK: Stralinger P. Cambridge University
8 Press: 135–156.
- 9 Boccacci, P., Akkak, A., Bassil, N.V., Mehlenbacher, S.A. and Botta, R. 2005. Characterization and evaluation
10 of microsatellite loci in European hazelnut (*Corylus avellana* L.) and their transferability to other *Corylus*
11 species. *Mol. Ecol. Notes* 5: 934-937
- 12 Boccacci P, Akkak A, Botta R 2006. DNA-typing and genetic relationships among European hazelnut (*Corylus*
13 *avellana* L.) cultivars using microsatellite markers. *Genome* 49: 598-611
- 14 Boccacci, P.; Botta, R.; Rovira, M. 2008. Genetic diversity of hazelnut (*Corylus avellana* L.) germplasm in
15 northeastern Spain. *HortScience*, 43(3): 667-672.
- 16 Boccacci P and Botta R. 2009. Investigating the origin of hazelnut (*Corylus avellana* L.) cultivars using
17 chloroplast microsatellites. *Genet Resour Crop Evol* 56:851–85.
- 18 Boccacci P., Botta R. 2010. Microsatellite variability and genetic structure in hazelnut (*Corylus avellana*L.)
19 cultivars from different growing regions. *Scientia Horticulturae*124:128-133
- 20 Boccacci P, Aramini M, Valentini N, Bacchetta L, Rovira M, Drogoudi P, Silva A P, Solar A, Calizzano F,
21 Erdorgàn V, Cristofari V, Ciarmiello LF, Contessa C, Ferreira JJ, Marra FP, Botta R 2013. Molecular and
22 morphological diversity of on-farm hazelnut (*Corylus avellana* L) landraces from southern Europe and
23 their role in the origin and diffusion of cultivated germplasm. *Tree Genetics & Genomes* 9: 1465-1480
- 24 Bulló M, Lamuela-Raventós R, Salas-Salvadó J 2011. Mediterranean diet and oxidation: nuts and olive oil as
25 important sources of fat and antioxidants. *Curr Top Med Chem*. 11(14):1797-1810.
- 26 Campa, A.; Trabanco, N., Pérez-Vega, E.; Rovira, M.; Ferreira, J.J. 2011. Genetic relationship between cultivated
27 and wild hazelnuts (*Corylus avellana* L.) collected in northern Spain. *Plant Breeding*, doi:10.1111/j.1439-
28 0523.2010.01835.x.
- 29 Delgado, T., Malheiro, R., Pereira, J.A., Ramalhosa, E. 2010. Hazelnut (*Corylus avellana* L.) kernel as source of
30 antioxidants and their potential in relation to other nuts. (*Industrial crops and products*)32, 621-626.

1 De Vicente, C., T. Fulton, 2003. Molecular Marker Learning Modules – Vol. 1. IPGRI, Rome, Italy and Institute
2 for Genetic Diversity, Ithaca, New York, USA.

3 Di Renzo L, Carraro A, Minella D, Botta R, Contessa C, Sartor C, Iacopino AM, De Lorenzo A, 2014. Nutrient
4 Analysis Critical Control Point (NACCP): Hazelnut as a Prototype of Nutrigenomic Study . Food and
5 Nutrition Sciences 5: 79-88

6 Durak, I., Köksal, I., Kacmaz, M., Büyükkocak, S., Çimen, B.M.Y., Öztürk, H.S., 1999. Hazelnut
7 supplementation enhances plasma antioxidant potential and lowers plasma cholesterol levels. Clin. Chim.
8 Acta 284: 113–115.

9 FAOstat 2014. Agriculture data. Available from: <http://faostat.fao.org/site/339/default.aspx>. Accessed 01 August
10 2014

11 Ferreira, J.J., Garcia, C.; Tous, J., Rovira, M. 2009. Structure and genetic diversity of local hazelnut collected in
12 Asturias (Northern Spain) revealed by ISSR markers. Acta Hort., 845:163-168.

13 Ferreira, J.J., García González, C., Tous, J., Rovira, M. 2010. Genetic diversity revealed by morphological traits
14 and ISSR markers in hazelnut germplasm from northern Spain. Plant Breeding, 129:435-441

15 Fraser GE 2009. Vegetarian diets: what do we know of their effects on common chronic diseases?. Am J Clin
16 Nutr 89:1607S-1612S.

17 Garrone W, Vacchetti M 1994. Hazelnut quality in relation with the requirements of confectionary industry.
18 Acta Hort 351:641–648

19 Ghanbari, A., Akkac, A., Boccacci, P., Talaie, A., Vezvaie, A. and Botta, R. 2005. Characterization of hazelnut
20 (*Corylus avellana* L.) cultivars using microsatellite markers. Acta Hort. 686: 111-115

21 Glaszmann JC, Kilian B., Upadhyaya HD, Varshney RK(2010) Accessing genetic diversity for crop
22 improvement. Current opinion in Plant Biology 13: 167-173.

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

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

27 Hu FB and Stamper MJ 1999. Nut consumption and risk of coronary heart disease: A review of epidemiologic
28 evidence. Curr. Atheroscler. Rep. 3: 204–209.

29 Jakopic, J.; Mikulic-Petkovsek, M.; Likozar, A.; Solar, A.; Stampar, F.; Veberic, R. 2010. HPLC-MS
30 identification of phenols in hazelnut (*Corylus avellana* L.) kernels. Food Chem., 124:1100-1106.

- 1 Koksai, A.I. 2000. Inventory of hazelnut research, germplasm and references. REU Technical series. FAO-
2 CIHEAM. 129p.
- 3 Kornsteiner, M., Wagner, K.H., Elmadfa, I. 2006. Tocopherols and total phenolics in 10 different nut types. Food
4 Chem. 98: 381–387
- 5 Mehlenbacher SA (1991) Hazelnuts (*Corylus*). Genetic resources of temperate fruit and nut crops. Acta Hort.
6 290: 791–836.
- 7 Mehlenbacher S A, 1994. Genetic improvement of the hazelnut. Acta Horticulturae, 351: 23-38.
- 8 Mehlenbacher S A, Brown R N, Nouhra R E, Gökirmak T, Bassil N V, Kubisiak T L, 2006. A genetic linkage
9 map for hazelnut (*Corylus avellana* L.) based on RAPD and SSR markers. Genome 49(2): 122-133
- 10 Mullie P Clarys P 2012. Nut Consumption Is Associated with a Healthy Dietary Pattern in Military Men. Food
11 and Nutrition Sciences 3: 1048-1054
- 12 Molnar J T 2011. *Corylus*. In C. Kole (ed.), Wild Crop Relatives: Genomic and Breeding Resources, Forest
13 Trees. Springer-Verlag Berlin Heidelberg 2011
- 14 Özdemir M, Filiz Açkurt F, Kaplan M, Yıldız M, Löker M, Gürcan T, Biringen G., Okay A, Seyhan FG (2001)
15 Evaluation of new Turkish hybrid hazelnut (*Corylus avellana* L.) varieties: fatty acid composition, α -
16 tocopherol content, mineral composition and stability. Food Chemistry 73 (4): 411-415.
- 17 Oliveira, I., Sousa, A., SáMorais J. Ferreira I.C.F.R., Bento A., Estevinho L.M., Pereira J.A., 2008. Chemical
18 composition, and anti-oxidant and antimicrobial activities of three hazelnut (*Corylus avellana* L.) cultivars.
19 Food Chem. Toxicol. 46: 1801–1807.
- 20 Parcerisa, J., Richardson, D. G., Rafecas, M., Codony, R. and Boatella, J. 1997.. Fatty acid distribution in polar
21 and nonpolar lipid classes of hazelnut oil (*Corylus avellana* L.). Journal of Agricultural and Food
22 Chemistry 45: 3887–3890
- 23 Parcerisa, J., Rafecas, M., Codony, R., Boatella, J. 1999. Triacylglycerol and Phospholipid Composition of
24 Hazelnut (*Corylus avellana* L.) lipid fraction during Development. J. Agric. Food Chemistry 47, 1410-
25 1415.
- 26 Pershern, A. S., Breene, W. M. and Lulai, E. C. 1995. Analysis of factors influencing lipidoxidation in hazelnuts
27 (*Corylus* sp.). Journal of Food Processing and Preservation 19: 9–25.
- 28 Ramanatha Rao V and Hodgkin T, 2002. Genetic diversity and conservation and utilization of plant genetic
29 resources. Plant Cell, Tissue and Organ Culture 68: 1–19,
- 30 Riggs LA 1990. Conserving genetic resources on-site in forest ecosystems. Forest Ecol. Manag. 35: 45–68

- 1 Rovira, M. 1997. Genetic variability among hazelnut (*Corylus avellana L.*) cultivars. *Acta Hort.*, 445: 45-50.
- 2 Rovira, M.; Tous, J.; Ferreira, J.J.; Ciordia, M. 2005. Hazelnut diversity in Asturias (Northern Spain). *Acta*
3 *Hort.*, 686:41-45
- 4 Rovira M, Avanzato D, Bacchetta L, Botta R, Drogoudi P, Ferriera JJ, Sarraquigne JP, Silva AP, Solar A (2010)
5 European *Corylus avellana* germplasm collections. IHC Science and Horticulture for people – 28th
6 International Horticultural Congress, Lisboa (Portugal). Abstract S12.354 Vol. 2 Symposia: 581.
- 7 Rosengarten F (1984) The book of edible nuts. ISBN 0802707696. Walker (New York) eds: 1-384
- 8 Salas-Salvadó, J. and Megias, I. 2005. Health and tree nuts: scientific evidence of disease prevention. *Acta*
9 *Hort.*, 686:507-513
- 10 Sivakumar G, Bacchetta L, Gatti R, Zappa G 2005. HPLC screening of natural vitamin E from mediterranean
11 plant biofactories a basic tool for pilot-scale bioreactors production of α -tocopherol. *Journal of Plant*
12 *Physiology* 162 :1280-1283
- 13 Sivakumar G and Bacchetta L 2006. α -Tocopherol from Italian hazelnut germoplasm. *Chemistry of Natural*
14 *Compounds*, Vol. 42, No. 1: 1120-123.
- 15 Solar, A. and Stampar, F. 1997. First experiences with some foreign hazelnut cultivars (*Corylus avellana L.*) in
16 Slovenia. *Acta Hort.*, 445:83-89.
- 17 Solar A, Bacchetta L, R, Botta R, Drogoudi P, Metzidakis I, Rovira M, Serrraquigne, J P, Silva AP 2008.
18 Phenolic characterization of some hazelnut cultivars from different European germplasm collection.
19 Seventh International Congress on hazelnut “Università della Tuscia” Viterbo, from June *Acta Horticulturae*
20 845: 130-132.
- 21 Solar, A.; Stampar, F. 2009. Inter-year variability of pomological traits evaluated in different hazelnut cultivars
22 in Slovenia. *Acta Hort.*, 845: 169-174.
- 23 Sun W 1998. *Corylus chinensis*. In: IUCN 2010. IUCN Red List of Threatened Species. Version 2010.2: [www.](http://www.iucnredlist.org)
24 [iucnredlist.org](http://www.iucnredlist.org). Downloaded 13 July 2010
- 25 Thompson MM, Romisondo P, Germain E, Vidal-Barraquer R, and Tacias Valls J 1978. An evaluation system
26 for Filberts (*Corylus avellana L.*). *HortScience* 13: 514—517.
- 27 Thompson, M.M.; Lagerstedt, H.B.; Mehlenbacher, S. 1996. Hazelnuts. In: *Fruit Breeding. Vol. III. Nuts. J.*
28 *Janick and J.N. Moore eds.:* 125-184.
- 29 UPOV 1979. Guidelines for the conduct of test for distinctness, homogeneity and stability (hazelnut). UPOV,
30 Genève, Switzerland. TG/71/3.

- 1 Valentini N., Calizzano F., Boccacci P., Botta R. 2014. Investigation on clonal variants within the hazelnut
2 (*Corylus avellana* L.) cultivar ‘Tonda Gentile delle Langhe’. *Scientia Horticulturae* 165: 303–310
- 3 Visser B. Engels J. 2003. Setting objectives for genebanks. In: A guide to effective management of germplasm
4 collections. (J.M. M. Engels and L. Visser, eds.) IPGRI: 20-42p. Rome, 2003.

5



A) Hazelnut Field Surveys in Spain (Asturias Region)



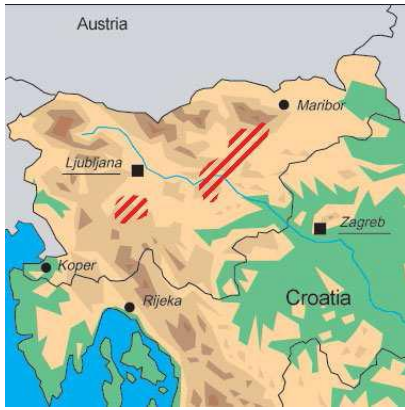
B) Hazelnut Field Surveys in Greece (Drama, Sfendami and Pier Regions)



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



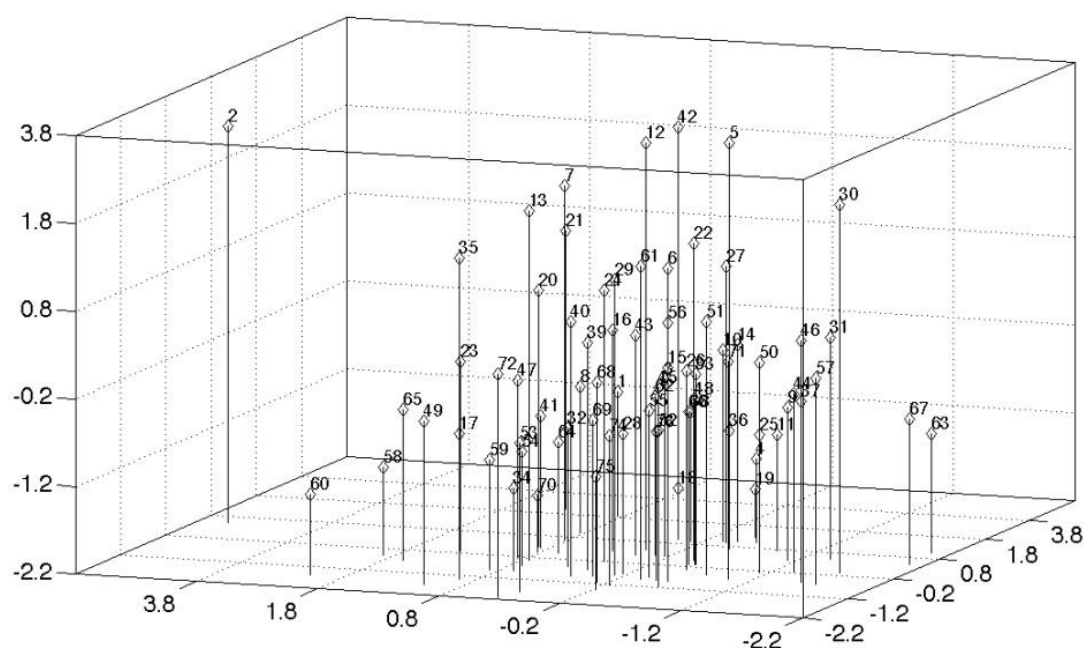
D) Hazelnut Field Surveys in Portugal (Felqueiras, Viseu, Moimenta and Miho)



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

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



Et:

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 Rotblafrige Lambernuss; 13 C.maxima à Pellicule Rose; 14Merveille de Bollwiller;15 Longue d'Espagne; 16 Pautet (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 Polycarpus 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; 28 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 | -0.742 | -0.642 | 0.062 |
| MUFA | | | |
| 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- | 0.258 | -0.084 | 0.91 |
| tocoferol | | | |
| SI | 0.087 | 0.259 | 0.922 |
| Eigenvalue | 5.759 | 4.538 | 2.085 |
| Proportion | 38.395 | 30.257 | 13.897 |
| of total (%) | | | |

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

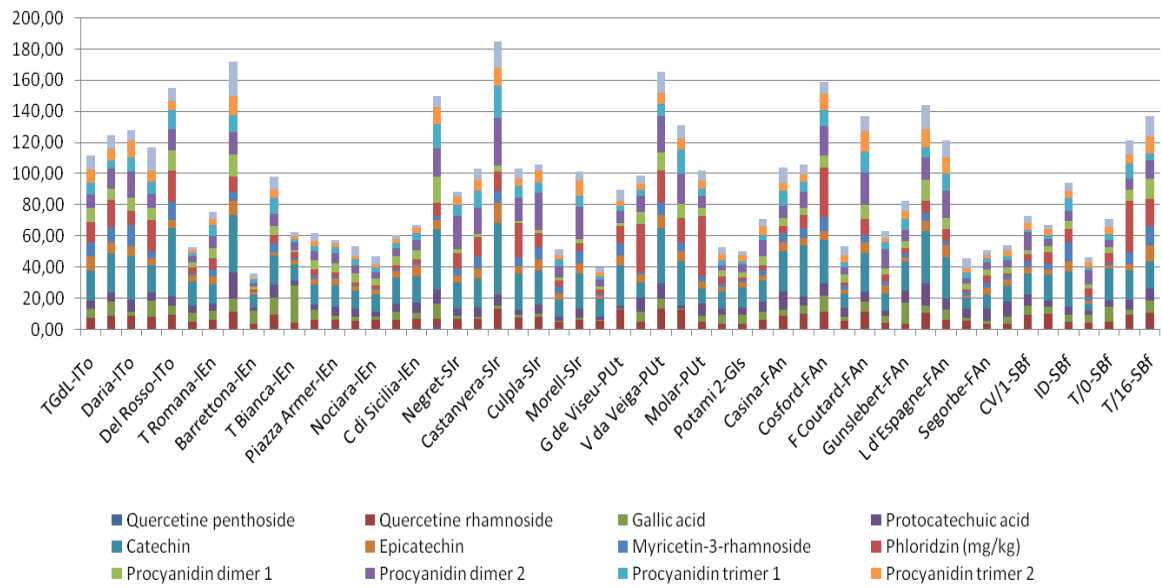
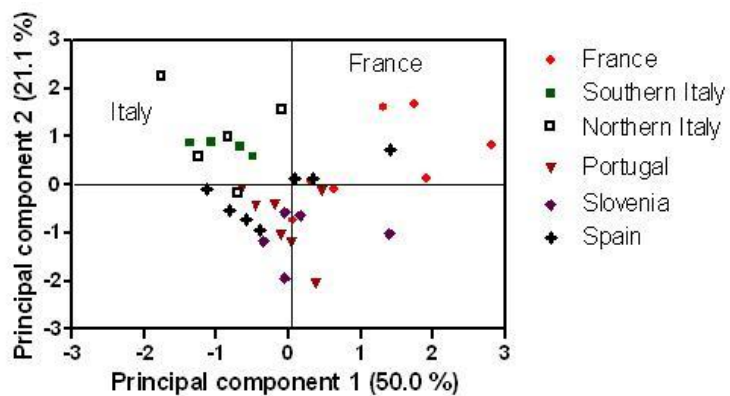


Figure 5 - Effect of geographic origins based of principal component analysis of kernel mineral composition for seven reference hazelnut cultivars cultivated in six European countries.



| 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 |
| Italy: | DRAPN - Viseu |
| | 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} | | |

1 **Table 3** - Proportion of phenotypic classes of morphological descriptors of hazelnut fruits collected from landraces. N: number of landraces characterized; H:
 2 Shannon-Weaver diversity index.
 3

| 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 |
| Appearance 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 |

4 (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 size 1= 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)
 5 Percentage of kernel by weight 1=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-
 6 50%) 3= Medium (50,1-75%) 4= High (75,1-100%)

