FIRST RESULTS OF 'SAFENUT': A EUROPEAN PROJECT FOR THE PRESERVATION AND UTILIZATION OF HAZELNUT LOCAL GENETIC RESOURCES.

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Abstract

The project SAFENUT: 'Safeguard of almond and hazelnut genetic resources from traditional uses to modern agro industrial opportunities' has been elaborated within the Council Regulation (EC) N. 870/2004 AGRI GEN RES which established a Community programme on the conservation, characterisation and utilization of genetic resources in agriculture. The project represents a resourceful strategy for re-organizing and sharing, in a more efficient manner, the genetic resources by upgrading the knowledge on their value and uses. One of the main objectives of the project is the centralization of available hazelnut germplasm by harmonizing the standard descriptors for a common characterization of cultivars. SAFENUT could represent an important step towards the characterization, documentation and management of *Corylus avellana* genetic resources in the Mediterranean Basin by addressing genetic resources collected in typical areas of cultivation, as well as less known local varieties or underutilized genotypes present in marginal areas. Among its objectives, the creation of a core collection and gene banks. The final goal is to set up a European virtual inventory linked with other important databases in order to share and spread all the information that will also be used to promote a wider application of traditional knowledge, agricultural practices, as well as to raise stakeholder awareness on the values of biodiversity components from the biological, economical and socio-cultural perspective. The project benefits from the participation of 11 partners from 6 European Countries, including the ONG Lega Ambiente and a Farmers' Association. The present work summarizes the main actions and the first-year-results which focused on the centralization and harmonization of hazelnut germplasm with the definition of specific morphological descriptors. A survey was performed in different areas of traditional cultivation and novel ecotypes were pre-selected. In the frame of the biochemical evaluation, 60 varieties were analysed for oil, tocopherol, phenolic and mineral content of the kernel. These results showed great health and technological interest in defining the use of nuts. SSR markers were performed to avoid synonymous and homonymous. With regard to traditional knowledge, a review of the existing hazelnut exhibitions was carried out and questionnaires were designed with the aim to recover historical memories from students and to compare agricultural

techniques and importance of crops in landscape among the partner Countries.

Introduction

The general interest for genetic resources is based on the opportunities offered by their utilization (Maxet *et al.*, 2002). Genetic resources not only provide the required raw material for sustainable genetic improvement of crops, but offer a unique gene combination, as naturally occurring co-adapted gene constellations, to ensure adaptability and productivity. Some trees cultivation is also agro-ecosystem of good environmental and landscape quality. Therefore, their conservation is of paramount importance to achieve sustainable production and food security for future generations (Kassar & Lasserre, 2004). This is reflected in the objectives of the Convention on Biological Biodiversity (CDB) and the FAO International Treaty on Plant Genetic Resources for Food and Agriculture (PGRFA). However, the conservation of agro-diversity is of critical importance, because of the direct benefits to humanity that can rise from its exploitation in improving agricultural and horticultural crops as well as the potential for developing new medicinal and other products. The problem of continuously expanding the number of accessions in gene banks introduces the concept of 'core collection': a selected and smaller collection representative of species' diversity (Brown 1989). Designing of core collections involves an appropriate use of diversity, offering to the breeders an opportunity to work with a quite manageable number of accessions. Another interesting aspect concerning the genetic resources is their precious cultural meaning in the traditional and historical uses. The European hazelnut represents an interesting case study being its utilization strongly intertwined with human civilization. About 5000 years before Christ a Chinese manuscript highlighted the medical properties of *Corylus avellana,* while Catone (234-149 a.C), the most ancient Latin writer, spurred the cultivation of this specie indicated as '*….nuces, calvas, avellanas, praenestinas et graecas'.* Evidences of the hazelnut use were shown by the discovery of its fruits in the funeral equipment of the Etruscan tombs. Both Columella (I sec. after C.) in his *De re rustica* and Plinio il Vecchio (23-79 after C.) in his *Naturalis Historia* mentioned '*avellana*'. The Romans used to give hazelnuts as symbol of fertility and happiness to young married couples (Ciabbattani,1996) and this is well documented, for example in the fresco "Deer's Houses" of Ercolano, Naples,. This plant is also very important for its symbology in Celtic people. Looking for heraldry which express the strong link between family, people or army and the territory, *Corylus avellana* is present in the Escudo del Baix Camp. Terragona Spain (for timber), in the Coat of arms of Volano Trento Italy as well as of Lohja Swedish. Artists as Carlo Crivelli ('400) included hazelnut in his painting on 'Madonna di Locri' for the Christian meaning of nuts and Giovanna Garzoni (1600-1670) chose this fruit to realize a beautiful still-life (Gallery of Uffizi, Florence). Different poets from Virgilio (*Georgiche* II, 299) to Gabriele D'Annunzio ('Settembre' in Alcyone) dedicated some versus to the hazelnut and its wood and fruits.

Besides the above mentioned considerations, the present work describes the main results of the first year of the SAFENUT project, a European efforts for reorganizing, evaluating and presenting hazelnut genetic resources.

Material and Methods

The project benefits from the participation of 11 Research Institutions in 6 European Countries (Italy, Spain, Portugal, France, Slovenia, Greece) and from the involvement of some of the most important hazelnut producers, directly engaged in breeding activities and conservation of genetic resources.

The following activities draw attention to the material and methods that have been used during the fist year of the SAFENUT project:

1. ***Survey of local, national and European "Corylus avellana" collections and farm recovery of "ecotypes"***. Each partner has produced a list of its own germplasm to centralize the available genetic materials and to avoid mislabelling. On the basis of Thompson et al.,1978 and UPOV, 1979, taking into account the experience of the researchers involved in the project, a list of specific descriptors was discussed and elaborated for harmonizing the morphological characterization. In this first year, areas from different Countries have been prospected with the aim to select new interesting hazelnut material: Drama, Sfendami and Pieria regions in Greece; Latium, Basilicata, Marche and Piemonte in Italy; Felqueiras, Viseu, Moimenta and Miho in Portugal and Crescnjevec, Tepanje, Sentjur pri Celju and Vrhpec in Slovenia; and finally, Asturias in Spain.
2. ***Characterization of "Corylus avellana" cultivars***. Harmonisation of descriptive data of cultivars in collection started in 2007 and was carried out on major cultivars, relevant for each Country. Each cultivar was studied in its typical area of cultivation, whenever possible. Existing information was harmonised using the specific hazelnut descriptors defined within the project. Nut samples were collected from hazelnut germplasm, including major and endangered cultivars and newly rescued plants in Spain, France, Greece, Portugal, Slovenia, and Italy. The identity of the plants used for sampling was checked using DNA typing techniques. The nut collection was carried out from the same single plants that were DNA typed harvesting all of the production. Nuts were stored in shell in a dry (UR<70%) and cool (10-15°C) room to maintain kernel moisture <6%. Random samples from 64 genotypes were analysed for fatty acid and tocopherol using the method illustrated by Kodak et al., 2006; the same genotypes were evaluated for ash and mineral content as well as phenolic composition (data showed in the specific paper of section 5: A. Solar et al). For the determination of the ash content, a sample of finely ground nuts was placed into a pre-weighed porcelain crucible, weighed and burned in a temperature of 580°C. The crucible was then cooled to room temperature and weighed. The ash content was expressed in % weight on dry base. For the determination of the K and Ca contents, about 0.5 g of finely ground nuts were dried at 150°C for 8 hours and then at 580 °C for another 8 hours. 10 ml 2N HCl was added and the sample was heated at 80 °C for 30 min. The digest was diluted with 50 ml of deionised water prior to analyses. potassium was measured using flame-photometer method and calcium volumetrically using EDTA. DNA was extracted from 70 samples (0.2 g of vegetal tissues) using the modified procedure described by Thomas et al. (1993) in a Tris-EDTA-NaCl buffer containing 0.25 M NaCl, 0.2 M Tris pH 7.6, 2.5% PVP 40,000, 0.05 M Na2EDTA, and 0.1% β-mercaptoethanol. After purification the DNA was finally suspended in 50 µl Tris-EDTA buffer. A total of 10 SSR loci, fully characterized in recent studies, were used: CaT-B107, CaT-B501, CaT-B502, CaT-B503, CaT-B504, CaT-B505, CaT-B507, CaT-B508 (Boccacci et al. 2005) and CaC-B020 and CaC-B028 (Bassil et al., 2005) Analyses were carried out as described by Boccacci et al., 2006. Results of the run were then analyzed with GeneMapper software and alleles were designated by their size in base pairs (bp). Results obtained from the 10 SSR loci were compared with the SSR database.
3. ***Traditional knowledge and social ecological actions***. The survey on existing exhibitions of hazelnut was carried out looking for the opportunities to expose nuts (pomologic exhibitions) and/or their products (processed nuts) as well as celebrating hazelnut as trees and testing fruits

and fruits products. The methods mainly used were Internet, and inquiry data from Municipalities (office devoted to organize the local agriculture exhibitions) or Professional Organizations (for example, Nut Associations).

4. ***Development of centralized, permanent and widely accessible European database***. The first step was the definition of the database characteristics and the conceptual model of the SAFENUT data-base. At the same time, queries and descriptors were defined to be included for each record of the database.

Results and Discussion

1. **Survey of hazelnut collections**. Data have been collected from 12 different collections: Montesquieu (Conservatoire Végétal Régional d'Aquinatanie) (France), NAGREF-Athens (Greece); Arsial-Rome, Cravanzana, Chieri and Caserta (Italy), Vila Real, Viseu and Felgueiras (Portugal): Nat. Collection and ex-*situ* collection in Maribor (Slovenia), IRTA-Constantí, SERIDA-Asturias (Spain). Material from USA is not included in the list. In total, 222 cultivars and 42 selections exist in different collections. In the Excel file that elaborates this material it was observed the erroneous spelling of some cultivars. These errors must be corrected and the spelling of each cultivar must be harmonised among all the partners. Each Country partner provided to the leader of this Work Package some pictures of their own hazelnut collections.
2. **Standardization of hazelnut descriptors**. Three charts with the specific descriptors for hazelnut have been harmonised and standardised among different partners. For hazelnut material in collection one chart was approved, including: general characteristics, tree traits, flowering traits and nut and kernel traits. At the same time, two charts with the specific descriptors for hazelnut have been harmonised and standardised among the partners, to characterise hazelnut from survey. In this case, descriptors were less specific than those referred to material in collection. As for the prospected material, one chart was elaborated for hazelnut characterisation *in situ* (zone, tree and fruit characters) and another to characterise fruit and kernel in laboratory .
3. **Harmonisation of data for cultivars in collections**. The harmonizing of cultivar descriptive data in collection was started and a first group of accessions were characterized using the common set of specific hazelnut descriptors.
4. **Recovery and prospect for ecotypes**. An hazelnut survey has been carried out in various European regions of Greece, Italy, Portugal, Slovenia and Spain. Some interesting hazelnut clones have been pre-selected. A first tree and fruit characterisation has been done following the descriptors charts elaborated and approved for this purpose.
5. **Nut biochemical analysis**. The oil content varied widely among cultivars and ranged from 51 to 64%. The main fatty acids found in hazelnut were oleic (mean 46.4%) and linoleic (mean 5.8%), in agreement with literature. Oil was poor in saturated fatty acids, represented by palmitic (mean 3.3%) and stearic (mean 1.4%). Linoleic acid ranged between 3.54 in the Slovenian cultivar 'Istrska dolgoplodna leska' to 10.15% in 'Casina'. Oleic acid varied between 35,2% in 'Vermellet' to 54% in the Italian accessions 'Lunga Ginnasi' and 'Meloni'. Tocopherol analysis is in progress but data from 37 samples showed that alfa-tocopherol, the main form of tocopherol found in hazelnut, ranged between 55.5 ppm ('Barrettona') and 258.7 ppm ('Pellicola bianca'). The highest α-tocopherol contents (> 200ppm) were detected in cultivars 'Morel', 'Segorbe', 'Gunslebert', 'Rotβlaftrige Lambernuss', 'Lambertski beli', 'Corabel', 'Pellicola bianca'. The variation found among accessions indicates the possibility of selecting genotypes for different nut uses and with different technological and nutritional

properties. The ash, K and Ca contents varied among the different hazelnut genotypes and areas of origin with values ranging from 1.96% to 3.38%, 0.38 to 1.06 %, and 0.27 to 0.655 %, respectively. The greater ash content was found in Cv/2 (Slovenia), K content in 'Karydato' (Greece) and Ca content in 'Vermellet' (Spain). Although large variation was found among different genotypes from a studied area, there was also variation among the different areas of origin. For example, higher ash and K contents were found in the genotypes coming from France and higher Ca content was found in the genotypes coming from Spain.

6. **Hazelnut DNA typing** The 10 SSR loci identified 47 unique genotypes among the 70 accessions analysed, because several possible cases of synonymy or misnaming were observed. The genetic identity of several accessions, such as 'Camponica', 'Ghirara', 'Mortarella', 'Nocchione', 'Pallagrossa', 'Rotβlaftrige Lambernuss', 'San Giovanni', 'Tonda di Giffoni', 'Tonda Gentile Romana', 'Tonda Gentile delle Langhe' and its clones PD6 and MT5 was confirmed. Synonymy cases reported in literature were confirmed by the analysis: 'Nocchione' with 'Montebello', and 'Nocchione' with a group of Sicilian cultivars: 'Comune di Sicilia', 'Mansa', 'Nostrale', 'Santa Maria del Gesù'. The accessions 'Istrska okrogloplodna' and 'Lambertski beli' presented an identical profile with the cultivars Payrone (syn. Romai) and 'Fructo rubro', respectively. Two possible cases of clonal mutation were observed: a) 'Santa Maria del Gesù' appeared to be a clonal mutant of Nocchione b) 'Negret primerenc 1-77' showed the same genotype of 'Negret', except for one allele at locus CaT-B502
7. **Survey on existing hazelnut European exhibitions**. The exhibitions recorded are 34 for Hazelnut - 24 in Italy, 5 in Spain, 4 in Slovenia and 1 in Portugal. By the collected information it is possible to understand the importance of the hazelnut greatly linked to the territory, its traditions and uses (not only culinary). Some exhibition is circumscribed as local event, but the renown of many others has gone beyond the provincial and regional borders and sometimes also the international ones. Every year these exhibitions attracts many tourists and visitors (gardeners, professional growers, consumers, researches, old and young people). Hazelnut does not have only an agronomic value but it is also symbol of history, art, folklore and food, strongly linked to the territory and the culture of the population and in the same way needs to be kept and transferred to the next generations
8. **Organisation of the SAFENUT data-base**. The SAFENUT database was organized on the basis of the a national database (Atlante multimediale dei prodotti tipici). The new database takes into account the most important International databases and provides users driven on-line interrogation of search-queries, across multi-trait data based on germplasm evaluation traits. At the time of writing the present report, the SAFENUT DBMS is ready for data input via the web interface available at the address: http://www.safenut.net.

Conclusion

Following the first year of the project activities, the above mentioned results are only preliminary and partial. A final and more comprehensive outcome will be achieved on completion of the SAFENUT project, in April 2010.

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Table 1. Hazelnut survey in different European regions

Country	pre-selected Material	Surveyed regions
Greece	10	Drama, Sfendami Pieria
Italy	6	Basilicata, Latium, Marche, Piemonte
Portugal	6	Felgueiras, Viseu, Moimenta, Miho
Slovenia	14	Cresnjevec, Tepanje Sentjur pri Celju, Vrhpec
Spain*	16	Asturias