The enhancement of hazelnut and almond genetic resources through the European AGRI GEN RES SAFENUT Action. The ENEA experience as project coordinator

The European project SAFENUT "Safeguard of hazelnut and almond genetic resources: from traditional uses to modern agro-industrial opportunities" aims at enhancing the characterization, preservation and utilization of the hazelnut and almond germplasm, through the recovery and valorisation of local endangered varieties in the traditional productive areas of the Mediterranean basin. The project, financed by the European Commission, Directorate-General for Agriculture and Rural Development, involves eleven partners from six countries and is coordinated by ENEA

Loretta Bacchetta, Barbara Di Giovanni

Il miglioramento delle risorse genetiche di nocciolo e mandorlo con l'Azione europea AGRI GEN RES SAFENUT. L'esperienza ENEA come coordinatore del progetto

Il progetto europeo SAFENUT "Salvaguardia delle risorse genetiche di nocciolo e mandorlo: dagli usi tradizionali alle moderne opportunità agro-industriali", intende migliorare la caratterizzazione, la conservazione e l'utilizzazione del germoplasma di nocciolo e mandorlo attraverso il recupero e la valorizzazione delle varietà locali in pericolo nelle tradizionali aree produttive del bacino mediterraneo.
Il progetto, finanziato dalla Commissione Europea, coinvolge 11 partner di sei Paesi ed è coordinato da ENEA

Loretta Bacchetta, Barbara Di Giovanni
ENEA, Technical Unit for Sustainable Development and Agro-Industrial Innovation

Introduction

The progressive impoverishment of agrobiodiversity, due to overdevelopment in many regions, has determined a reduction of local varieties that were selected over millennia in traditional agricultural systems and that are now seriously endangered. Many scientists came to agree that genetic erosion is affecting different crops all over Europe (Trommetter, 2002). Often presented as an ecological problem, genetic erosion is usually the result of social, economic and agricultural changes (the Agenda 21, agreed to at the United Nations Conference on Environment and Development – UNCED, stated that "the current decline in biodiversity is largely the result of human activity and represents a serious threat to human development").

Following the Strategy adopted by the European Community on biological diversity, the European programme AGRI GEN RES was established in 1998 with the aim of fulfilling the objectives of the 2003 Common Agricultural Policy (PAC) reform as well as integrating and promoting all initiatives on conservation, collection and utilization of plant genetic resources in agriculture.

The European Union is one of the world's leading producers and consumers of almonds and hazelnuts, two traditional crops of the Mediterranean Basin. In fact, Italy and Spain are the second largest world producers, of hazelnut and almond respectively, following the United States and Turkey, which contribute to more than 80% of the total world supply. Therefore, almonds cultivation in Europe, with its progressive abandonment, is turning out to be unprofitable due to the strong competition from the Californian almond production, traded at low prices. Driven by low labour costs, also Turkish hazelnuts are price competitive, heavily influencing the international market. Moreover, the lower quality of the imported supplies affects the taste and nutritional properties of the European traditional products, which is worsened by the increase of the maximum level of aflatoxin in nuts based on the recent regulation of the Commission.

In the light of the above, the preservation, study and utilization of *Corylus avellana* and *Prunus dulci* genetic resources are of fundamental importance to enhancing the competiveness of the European almonds and hazelnuts (Sivakumar et al., 2005), to improving their breeding efficiency, and to meeting future requirements and demands.

The SAFENUT 068 Action (2007-2011) was carried out under the aegis of the AGRI GEN RES Community Program to enhance the characterization, preservation and utilization of the European hazelnut and almond germplasm through the recovery and valorisation of local endangered varieties in the traditional productive areas of the Mediterranean basin (Bacchetta *et al.*, 2008a; Bacchetta *et al.*, 2010a; Bacchetta *et al.*, 2010 b). This paper aims at describing the project's main objectives, its strategy and the main achievements, highlighting the ENEA experience as project coordinator.

The SAFENUT objectives

The project's main objectives are: (i) improving the knowledge of the European hazelnut and almond germplasm to enhance their characterisation, preservation and utilization; (ii) recovering and valorising local endangered germplasm in the traditional productive areas of the Mediterranean basin; (iii) increasing the utilization of the germplams through the creation of core collections; (iv) strengthening the traditional knowledge on genetic resources to raise stakeholders' awareness on the values of biodiversity in the framework of sustainable development; (v) setting up an interactive web database linked with the main thematic international databases and creating a strong European network.

The most recent trends show that there has been a remarkable increase in the number of consumers requiring food products for dietary, health, organoleptic or environmentally-friendly reasons (Sivakumar and Bacchetta, 2006). On the basis of the consumer's demand and the distinctive properties of nuts, the project focused on characterisation, emphasising the nutritional and 'nutriceutical' aspects that can reinforce the economic competitiveness of the products. It is envisaged that the best opportunities of development for agriculture arise from policies aimed at exploiting qualities and characteristics linked to the products' geographical origin and to the traditional processes which feature the European agricultural and agro-industrial productions.

The project also strengthened the importance of harmonising the standard descriptors for a common

characterization of accessions. With regard to hazelnut, the UPOV guidelines (1979) are usually followed, although there are also other descriptors (Thompson *et al.*, 1978, Bioversity International *et al.*, 2008). Therefore, the SAFENUT Partners gathered all the information with the aim to decide which characters are useful for the authentication of the hazelnut material.
The enhancement of national and local efforts in safeguarding traditional germplasm as well as the recovery of endagered genetic heritage is of inestimable importance for *Corylus avellana* and *P. Dulcis* genetic resources, their management and use for breeders, growers and consumers.
The development of a database meets the recommendations of the Commission to virtually maintaining the germplasm and facilitating the exchange of material and information (among European and extra-European Countries).

The strategy of the SAFENUT Action

The management of the Action has been organised in the activities which have been summarized in Figure 1 on the basis of two main thematic areas - the acquisition and characterization of plant material and the conservation and management of genetic resources – from which six Working Packages (WP) originated.
The activities performed in WP1 and WP2 aimed at increasing the knowledge of the *ex situ* and *in situ* collections of hazelnut and almond material among partner Countries by harmonizing the morphological descriptors (Rovira *et al.*, 2009). For this purpose, in spite of local genetic resources maintained in national collections, a survey has been carried out in Spain, Slovenia, Greece and Italy with the objective to gather the maximum useful genetic diversity.
The molecular and biochemical characterization of the germplasm was realized in WP2 and WP3. The availability of reliable data on biochemical and molecular traits of the germplasm allowed to define the suitable use of the nut products, as required by industries, row consumers, etc. (Solar *et al.*, 2008). This information is also very useful for breeding programs. Furthermore, results in DNA analysis can show the true-to-type identification as well as problems of germplasm's misnaming or homonymies and synonymies present in the conserved germplasm. Therefore, the SAFENUT partners published and shared methodologies and protocols on the project's website (www.safenut.enea.it), developed by ENEA, providing the standardization and endorsement of the analytical procedures among the different laboratories involved in the characterization.
References cultivars, in the six partner Countries, were chosen to monitorate the effect of the enviromental factors on morphological and biochemical traits. The Action, by turning its attention on genetic resources as a pool of useful genes as well as gathering information on traditions and local uses, provides an added value for future generations and historical memory (Bacchetta et al., 2010a).
WP4 aimed at recovering and enhancing the 'cultural meaning' of genetic resources for people that use, conserve and, in same cases, improve them. This approach usually builds a strong link between genetic resources and the eco-geographical areas they belong to.
Within the second task (conservation and management of genetic resources) the enhancement of the utilization of germplasm was highlited in WP5 through the establishment of a European core collection, namely a sampling of the base collection which represents most of the genetic diversity. On this basis, traits of economic importance - such as agronomic data - which often show high genotypes per environmental interactions can be evaluated in an easier and more efficient way. As a consequence, there will be a better use of genetic resources as information builds up. Fur-

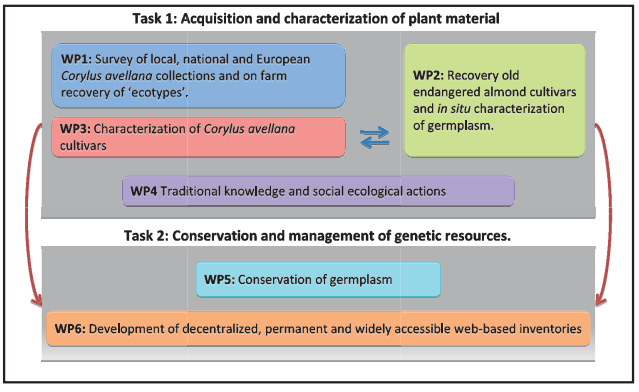

FIGURE 1 Chart of the SAFENUT strategy and WPs' interaction

thermore, the second target of this workpakage was to set up a DNA bank.
Finally, the development of the SAFENUT database, as an efficient and timely device for the dissemination of the collected germplasm information, was the main activity of WP6.
The project, coordinated by ENEA (the Italian National Agency for New Technologies, Energy and Sustainable Economic Development), benefited from the participation of 11 partners from 6 European Countries (Italy, France, Greece, Slovenia, Spain, Portugal) representing 95% of the hazelnut and 87% of the almond total European production (Bacchetta *et al.*, 2008b).
The partnership was characterized by a long-term strategic interest in the field and was composed of two Universities and nine Research Organisations. One NGO and Producers Associations were also involved for their relevant role in sharing and promoting the results to stakeholders and growers. In view of the size and scope of the project, the management was structured by assigning clear responsibilities to each partner in its specific areas. To this aim, tasks, responsibilities and work distribution were clearly defined and an appropriate communication system was established by the parties. Among others, main criteria were excellence in the field, reliability, experience and commitment. The key element of the ENEA coordination was the organisation of a cross-party team, with different skills but interchangeable at the scientific and administrative levels. In fact, the team was involved throughout the Action to guarantee a prompt feedback, meet deadlines and overcome difficulties and emergencies. This approach made the coordination activity feasible and efficient.

Main activities and related results

During the past years many efforts were made on Plant Genetic Resources (PGR) conservation, using different techniques and approaches (Berthaud et al., 1997). The SAFENUT Action aimed at harmonising the various initiatives carried out at the regional and national level through the re-organisation and conservation of genetic resources. This has been achieved, firstly, through data acquisition on *in situ* and *ex situ* partners' collections and, secondly, through the recovery and the exploitation of *on farm* conserved ecotypes to preserve the maximum genetic diversity. A list of about 222 hazelnut clones and 58 selections from 13 hazelnut collections have been completed and shared to verify synonymous and misnaming. On the basis of almond achievements in the 061*Prunus* AGRI GEN RES (1996-1998), 180 almond cultivars were centralised from the reference collections in Spain, France and Greece, and the European Prunus database, established at the "National Institute for Agronomical Research" (INRA) in Bordeaux, France (http://cbi.labri.fr/outils/EPDB/index.html) was updated. Furthermore, a widespread survey was carried out in Spain, Greece, Italy and Portugal in order to recover the 'on farm' conserved ecotypes at risk of genetic erosion: about 121 hazelnut accessions have been pre-selected, more than 30% of which have already been indentified as new genotypes by SSR markers. In addition, a useful survey was carried out in Slovenia, Italy and France to retrieve and restore numerous old endangered almond clones.
With the aim to harmonise the morphological evaluations, specific descriptors were elaborated for the genetic materials' characterization, both in the permanent collection and in new selections. More than 150 almond and 305 hazelnut accessions were analyzed over three years at 20 and 10 SSR loci respectively, in order to verify their genetic authenticity (Botta *et al.*, 2011). Two hundred and seventy five genotypes were analysed for incompatibility genes and 110 accessions of the two species were evaluated for fatty acids, tocopherols, phenolic compounds, mineral and protein contents during the three years of the Action. Seven reference hazelnut cultivars were identified and monitored each year to investigate the environmental effect on the biochemical nut properties.
In the light of the above, a multivariate analysis carried out on all the data allowed to identify the core collections focusing on the nutritional and nutraceutical properties. DNA from 283 almond accessions was collected and conserved in the DNA Bank of the "Centro de Investigación y Tecnología Aplicada" (CITA) (Socias i Company *et al.*, 2011). The traditional knowledge was recovered through different activities: 2097 questionnaires were elaborated following the interviews of students and their parents in all partner Countries; a survey was carried out on festi-

vals related to the two Mediterranean species and a publication was released as a result (Avanzato et al., 2009). Furthermore, two questionnaires – one on hazelnut and one on almond – were addressed to farmers and provided the opportunity to compare problems, technical practices and the status of biodiversity at the European level. Interesting data were collected and elaborated by the above mentioned questionnaires, drafted by the ENEA team, therefore proving that hazelnut and almond crops are suitable models for forecasting studies in the field of relationship between agriculture and landscape ecology (see the SAFENUT website at: http://safenut.casaccia.enea.it/).

The SAFENUT Database (DB), an important tool to disseminate information on hazelnut and almond genetic resources and their utilization (Agrawal *et al.*, 2007; Bacchetta *et al.*, 2011), has been implemented on the basis of the Scrigno Database framework, a virtual Atlas developed by ENEA - Casaccia at the National level and related to the Italian traditional food crops. The SAFENUT DB has the following tools: the DB management system is MDB Access, the application programming interface is Asp 3.0, web server program: IIS.

FIGURE 2 SAFENUT database: the home page

Currently, the host of the DB is Aruba (AR-Italy), later on it will be ENEA Casaccia (Italy). The core of the DB includes four sections: the data, the policy of access, the administrative tools and the outputs. The SAFENUT DB, developed by ENEA in collaboration with Spazio VERDE SRL, is a web interface - available at the address: hppt://www.safenut.net (figure 2) - which has been organised in order to provide users driven online interrogation of search-queries, across multi-trait data based on germplasm evaluation data.

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) as well as photos for 58 hazelnut and 248 almond accessions. A list of new selected hazelnut ecotypes has also been included. Following the indications of the European Cooperative Programme for Plant Genetic Resources (ECPGR), each accession is described by Passport data and Specific Primary descriptors. To facilitate its utilization, web-pages are dynamically interfaced with the database. This approach ensures that the information derived from the database is up-to-date (Glaszmann et al., 2010). The SAFENUT Database allows two kinds of research: the basic research, where selecting the specie is possible to entry the list of accessions, and the 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 the requested characteristics together.

Conclusions

In times when agriculture is becoming more and more intensified and plant breeding objectives are becoming more complex, the importance of conservation and use of germplasm diversity is substantial for desired sustainable agriculture in the world.

In the frame of the AGRI GEN RES programme, the SAFENUT Action provided a relevant contribution to re-organising and sharing the European hazelnut germplasm, particularly in view of its genetic erosion and eventual wipe-out. The survey of hazelnut ecotypes carried out in those European Countries involved in the project made available numerous acces-

sions conserved *on farm* and selected for traits of interest, such as non-suckering growth habits, extreme precocity, early maturing and peculiar nut traits. These accessions, precious for local economies, have also a crucial importance as donor parents for breeding programs (Molar, 2011). As for almonds, the survey which was carried out in traditional areas of cultivation where crops were progressively abandoned, has led to the identification and preservation of numerous cultivars from few specimen conserved *on farm*. Furthermore, based on the recent epidemiological and clinical studies which provided the strong evidence of a benefic effect on human health of tree nuts consumption (Alasalvar and Shahidi, 2009), the SAFENUT Action has enhanced the quality and the proper utilization of the European varieties, providing indications for the definition of European core collections. The long history of almonds and hazelnuts' utilization and production by man (Bacchetta *et al.*, 2008) has produced a great heritage of traditions and local uses which were emphasized during the project's activity. Therefore, sharing the SAFENUT achievements through a freely available database is a great opportunity to preserve genetic resources, also being the primary input to decide on current resources' utilization and to reduce duplication of efforts.

Besides its scientific achievements, one of the main strengths of the SAFENUT project is a strong partnership among European institutes playing an outstanding role in the hazelnut and almond domain.

As project coordinator, ENEA guaranteed the management of the Action providing actual support and exerting formal control in order to ensure efficient feedback loops and a close interrelation among different partners. Not only has this allowed to perform a series of activities and researches whose results have a considerable impact on future studies, but also, and particularly, to create an active network to deal with various issues and future projects.

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