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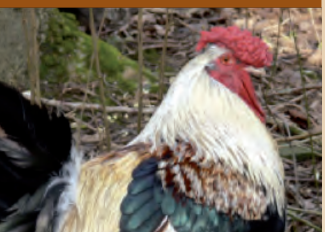
GUIDELINES
FOR THE CONSERVATION AND CHARACTERISATION OF PLANT, ANIMAL AND MICROBIAL GENETIC RESOURCES FOR FOOD AND AGRICULTURE



NATIONAL PLAN FOR AGRICULTURAL BIODIVERSITY

EXECUTIVE SUMMARY

Gruppo di lavoro Biodiversità in Agricoltura

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ISTITUTO NAZIONALE DI ECONOMIA AGRARIA

GUIDELINES

FOR THE CONSERVATION AND CHARACTERISATION OF PLANT ANIMAL AND MICROBIAL GENETIC RESOURCES FOR FOOD AND AGRICULTURE

National plan for agricultural biodiversity

EXECUTIVE SUMMARY

OCTOBER 2012

This study, carried out under the supervision of the Standing Committee on Genetic Resources in Agriculture, is financed by the Ministry of Agricultural Food and Forestry Policies within the framework of the National Plan for Agricultural Biodiversity's activities (Ministerial Decree 28672 of 14/12/2009).

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Conclusions provided in these Guidelines were considered appropriate at the time of the preparation of the work. These may be subject to modification should further knowledge be manifested, and/or if new methodologies are acquired in subsequent stages.

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Views and opinions expressed in this publication are those of the authors, and do not necessarily reflect those upheld by the institutions for which they work.

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Foreword

Brief overview of the international setting

Since 1992, year in which the Convention on Biological Diversity (CBD) was adopted, a series of major international events kept the focus on discussing protection and preservation of genetic resources for food and agriculture.

Three very significant international agreements directly related to the CBD have been negotiated from 2000 to present. These instruments focus on issues of global importance such as bio-safety and access to genetic resources. They are the Cartagena Protocol (CBD, 2000); the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO, 2004) ; and the recent Nagoya Protocol (CBD, 2010) on access and sharing of benefits resulting from the use of biodiversity.

These instruments, though different in their provisions, share a common goal: the fair and equitable sharing of benefits arising from the use of genetic resources.

The increased awareness at global level that the loss of genetic resources is not per se just the loss of genetic material, comes with the recognition that loss of biodiversity is above all the slow extinction of an immense wealth of information related to typical and traditional crops associated to knowledge and local flavours

National framework and recent actions related to Biodiversity

a) *The National Strategy for Biodiversity (NSB)*

The Ministry for the Environment, Land and Sea has suggested, through the development of the National Strategy for Biodiversity, several lines of action in respect of environmentally friendly agricultural policies for the management and conservation of biodiversity. This goal of environmental protection is also geared with the European “Common Agricultural Policy” (CAP). This is a very important tool adopted by the State- Regions Permanent Conference on 7 October 2010 in order to ensure in the coming years a true integration of “development objectives of the country and the protection of its biodiversity”.

The NSB is organized around three key themes:

- biodiversity and ecosystem services
- biodiversity and climate change
- biodiversity and economic policies

The conservation of biological diversity is one of its most important goals, both in terms of species and genes, of ecosystems and communities, for the sustainable use of its components and the fair and equitable sharing of benefits arising from the utilization of genetic resources and the transfer of technologies related to it.

With respect to activities related to food and agriculture, the NSB highlights some problems of the agricultural sector and focuses on specific objectives, such as "to promote the conservation and sustainable use of agricultural biodiversity, and the preservation and dissemination of forestry and agricultural systems with high natural value; to preserve and restore ecosystem services in the agricultural environment; to promote the safeguard of the territory (particularly in marginal areas) through integrated policies that promote sustainable agriculture and benefit biodiversity by contrasting abandon and marginalization of agricultural areas".

b) The National Plan for Agricultural Biodiversity (NPAB)

A first tentative was made in 1999 by the Ministry of Agriculture and Forestry, which funded the first national programme aimed at safeguarding the agricultural biodiversity of plant, animal and microbial genetic resources in Italian Regions and Autonomous Provinces (AAPP). However, nine years had to go past before a proper National Plan for Biodiversity of interest to Agriculture was adopted.

In fact, the Ministry of Agriculture and Forestry with the active collaboration of Regions and AAPP had prepared the NPAB more than two years ahead of the NSB, which was approved on 14 February 2008 by the State-Regions Permanent Conference.

The plan actually initiated a new stage of concerted long-term discussions by which the State and local governments undertake, each according to their skills and mandate, to preserve and enhance the wealth of genetic resources for food and agriculture.

The Plan ensures the great local relevance of all actions undertaken to protect biodiversity. For this reason, and in order to ensure the connection among the various scientific stakeholders with the Regions and the AAPP , the establishment of a Standing Committee on Genetic Resources (SCGR) was envisaged, under the coordination of the Ministry of Agriculture and Forestry.

This implies the prominence of a long-term strategy aimed at coordinating the actions to be implemented at local level, with the aim of transferring the information needed to protect local resources typical of our agriculture to local operators and all interested stakeholders.

c) The Working Group on Agricultural Biodiversity (WGAB)

According to the contents of the NPAB, planned initiatives are divided into three phases:

- Phase A (national level): definition of operational requirements and shared tools and establishment of a Working Group on Agricultural Biodiversity;
- Phase B (local level): possible interregional projects;
- Phase C: activation of the National Register of varieties and local species/populations.

In 2009, the SCGR focused its attention on the first phase, while developing programmes for the later stages and approved the launch of a specific project for the establishment and operation of a WGAB. Obviously, the implementation of the first phase is a prerequisite for the completion of the other two.Herewith the composition of the WGAB:

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Project manager: Antonella Trisorio – National Institute of Agricultural Economics (INEA)

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Francesco Sottile	University of Palermo
Concetta Vazzana	University of Florence

The WGAB – Coordination meeting, Rome, 1 March 2013 (photo by O. Porfiri)

The WGAB was given the task of defining:

a) the descriptors for the characterization of plant varieties, animal breeds/local populations and micro-organisms;

b) a common and shared methodology for research and characterization of varieties, breeds and populations in order to allow comparison of data in the various Italian territories;

c) guidelines for proper *in situ* conservation, on farm and *ex situ* conservation of plant varieties and animal breeds/populations;

d) guidelines for the proper storage of microorganisms *in situ* and *ex situ*;

e) the definition of risk of extinction and genetic erosion through thresholds and criteria for the main species of plants, animals and microbes for food and agriculture.

The Group has prepared, in less than a year, three separate manuals with guidelines for the *in situ* conservation, on farm and *ex situ* conservation of animal, microbial and plant biodiversity of agricultural interest. Each manual will be available separately.

In this respect it should be noted that:

- The guidelines are addressed to the Regions / AAPP and their technicians, which in turn will use them to guide farmers and other stakeholders in conservation strategies through common, standardized and shared methodologies;
- Each book is scientifically rigorous, but at the same time easy to read and clearly outlining all actions that an operator must carry out to achieve the conservation of biodiversity of agricultural interest.

To facilitate reading, texts were standardized as much as possible, although keeping some fundamental differences as in the case of concept of "species" for microorga nisms or "species" and "pure breed" for animals.

For microorganisms a widespread and shared concept of species was used, the so-called biological species concept (BSC), which is based on sexuality as the only means of reproduction. In fact, the vast majority of microorganisms are not known under this condition thus another species concept was to be found, different from the one used for animals and plants (see Guidelines Microbial).

For animals, to date there is still no single, agreed definition for the terms of species and pure breed. In this case it was decided to employ the definition proposed by FAO (see Guidelines Animals).

The manuals provide a framework for scientific and technical reference, consistent with both national and international principles, and with the specific objective of promoting, in the case of plant genetic resources for food and agriculture, the implementation by Regions and AAPP of the FAO International Treaty on Plant Genetic Resources for Food and Agriculture (Law no. 01/2004). The chapters developed in each manual include:

a) a brief introduction to the concept of species / variety / breed in reference to the field in question and the definition of agro-biodiversity as accurate as possible;

b) the definition of risk of genetic erosion;

c) a reasoned glossary;

d) the identification of protocols for characterization and conservation, with the indications of the various operational phases for each specific sector (animal, microbial and plant);

e) some characterization studies for the protection and exploitation of typical local species;

f) the referenced bibliography

d) The NPAB and the concept of local variety

The NPAB identified the concept of local variety as a priority, at the same time underlining its high socio-cultural value. The term "local varieties" derives from the translation of the English term "landraces".

Although different definitions of local variety already existed in the Guidelines for the proper *in situ*, on farm and *ex situ* conservation of plant varieties, the Working Group on Agricultural Biodiversity (WGAB, 2010) adopted the following: "A variety or local crop that reproduces by seed or by vegetative process is a variable population, which is identifiable and usually has a local name. It lacks "formal" genetic improvement and is characterized by specific adaptation to the environmental conditions of the area of cultivation (tolerant to the biotic and the abiotic stresses of that area) and is closely associated with the traditional use, knowledge, habits, dialects and celebrations of the people who developed and continue to grow it."

As reported in the NPAB, this definition is supplemented by those provided by the various regional Italian laws on the protection of indigenous genetic resources (actual local breeds and varieties) which, in summary, are referred to as species, breeds, varieties, cultivars, populations, ecotypes and clones originating in a region or from external sources if introduced at least for 50 years and integrated into traditional farming and management of that territory.

This scenario also includes local varieties that have actually disappeared from the region, but are preserved in botanical gardens, farms or research centres in other regions.

It is quite evident that the local variety cannot and should not, in my opinion, be separated from the territory of origin (bio-territory) as it is understood that this is a place in which, thanks to farmers, these varieties have adjusted to that environment over time.

e) The concepts of bio-territory and characterization and conservation of local varieties

Varieties and local breeds must be correctly identified, starting with research of historical documents demonstrating the link with the country of origin.

Conservation of local varieties can only happen in bio-territories with agronomic techniques dictated by local rural tradition, in very close relationship and mutual dependence among those who conserve *ex situ* (in gene banks) and those who protect and promote conservation on farm (farmers / breeders / keepers).

The possibility of recovery and reintroduction of a traditionally recognized local variety in its bio-territory is closely related to the enhancement of production by the same farmers / livestock keepers. Financial support from the local government agencies would encourage present and future efforts of farmers towards cultivation and conservation of local varieties at risk of extinction, which are generally not valued within the current commercial circuits.

Conclusions and recommendations

The SCGR has approved the guidelines proposed by the Working Group and the Conference of State ratified the agreement on the Guidelines, pursuant to Article 8, paragraph 6, of the Law of 5 June 2003, n. 131.

On 24 July 2012, a Decree of the Minister of Agriculture, Food and Forestry on the official adoption of national guidelines for the *in situ*, on farm and *ex situ* conservation of plant, animal and microbial biodiversity of agricultural interest was published in the Official Gazette no. 171.

The guidelines are a necessary standard tool for the conservation and characterization of species, local varieties and breeds providing capacity to fully implement the NPAB. This is the first significant work in which operational guidelines for the protection of plant and animal biodiversity for food and agriculture, as well as food-related microbial and soil genetic resources are addressed. It is a practical response to the needs of operators/stakeholders who rightly demand equal scientific dignity of microbial versus animal and plant genetic resources.Therefore, a considerable effort has been made to produce operational guidelines in the three areas mentioned and it is not excluded that in the future other sectors will be considered, such as forestry, fisheries, pathological and entomological genetic resources.

The WGAB - Official presentation of the Guidelenes – Bologna, 21 November 2012 (Photo by F. Dell'Aquila, Diateca Agricoltura of Emilia-Romagna region)

At this stage, it could be useful for the regions and the AAPP, in consultation with the Ministry of Agriculture and Forestry, to start developing as soon as possible the subsequent phases outlined in the aforesaid Plan through interregional projects consultation and the activation of the Register for National varieties, breeds and populations.

Rome, 30 September 2012

Mario Marino
(*FAO – Agriculture Department International Treaty on Plant Genetic Resources for Food and Agriculture*)

Acronyms

CBD	Convention on Biological Diversity
FAO	Food and Agriculture Organization of the United Nations
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
SCGR	Standing Committee on Genetic Resources
WGAB	Working Group on Agricultural Biodiversity
NPAB	National Plan for Agricultural Biodiversity
NSB	National Strategy for Biodiversity

CHAPTER 1

Summary of the guidelines for conservation of plant genetic resources for food and agriculture

1.1 Outline

This work contains the guidelines for the conservation of Plant Genetic Resources for Food and Agriculture (PGRFA). As for the ones on livestock and microbial genetic resources, this work is scientifically sound and provides a streamlined and schematic structure for the easy consultation of useful operational tools by all involved at various levels in PGRFA management. In order to respond to the requests of the Standing Committee on Genetic Resources (SCGR), the Working Group on Agricultural Biodiversity (WGAB) has produced a volume in two parts divided into six chapters, and a series of appendixes designed to provide an in depth analysis on the various topics.

The first part provides general information ranging from the definition of biodiversity and PGRFA (Chapter 1) to the assessment of the risks of extinction and genetic erosion (Chapter 2), and concludes with the regulatory and operational framework for the conservation and valorisation of PGRFA in Italy (Chapter 3). The second part offers detailed operational procedures, which provide standard guidelines for the protection of PGRFA. Practical case studies on conservation are included, which have been adopted by some regions (Chapter 4 and related Appendix).

A typical Italian agricultural landscape, characterized by high "diversity" (photo by M. Fontana)

Thereafter, the methods characterising both morpho-physiological and molecular resources are discussed (Chapter 5). In conclusion, a series of case studies covering the widest possible contexts are outlined (Chapter 6).

Despite the multitude of situations relating to biodiversity of agricultural interest in Italy and the difficulties relating to schematization, the WGAB has attempted to "typify" the various possible contexts and to describe the implementation of the various interventions adopted. This is achieved by referring to issues that were previously addressed and were positively concluded. Various "typologies" have been proposed, each highlighting the respective strengths and opportunities, as well as their weaknesses and potential threats. Some of the known actions for each "typology" are reported and specific case studies are then explained in detail and outlined as examples.

The Appendixes have the dual purpose of streamlining the various chapters, thereby allowing easy reading even by non-specialist users and providing a more in depth analysis of certain topics, in particular with regard to methods, laws and other specific competences of experts in the field. The following Appendixes are integrated: a detailed glossary of the many technical terms in the study, which are widely debated and shared (Appendix 1); the translation of the Standard Material Transfer Agreement (SMTA) for plant genetic resources for food and agriculture (Appendix 2); the proposal of a simplified agreement for the transfer of vegetal material of plant species not belonging to the list of crops under the Treaty, and their direct use in the field (Appendix 3); a series of methodological details of techniques to be applied (Appendix 4); a framework of EU and Italian legislation for the marketing of seed material (Appendix 5); different guiding principles used for the description of material (Appendix 6); a plan for the reproduction and multiplication of seeds (Appendix 7) and finally, a case study for the genetic characterization of specific resources in the Region of Lazio (Appendix 8).

All topics discussed are supported by an extensive bibliography (both cited and referenced), which includes the most recent publications from links to network connections and numerous references relating to case studies and initiatives present throughout Italy.

1.2 Agricultural biodiversity: from past to present

The PGRFA or phytogenetic resources are defined as "any genetic material of plant origin that has a present or potential value for food and agriculture". This definition has also been adopted by the International Treaty on Plant Genetic Resources for Food and Agriculture. This includes all material under cultivation, the wild progenitors of cultivated material, the progenitors of those species not cultivated, and wild species not cultivated but used by mankind for specific purposes (medicinal plants, plants for dyes, etc.).

Over the last fifty years, several international agreements have been negotiated to ensure the conservation and sustainable use of PGRFA. This was a necessary response to reports received from different continents, documenting a rapid loss of genetic diversity in crops. In 1967, during the Technical Conference on Analysis, Use and Conservation of Plant Genetic Resources, organized by FAO and the *International Biological Programme* (IBP), the term "**genetic erosion**" was used for the first time. This term then became synonym with loss of variability within crops.

It was necessary to wait until 2002 to have a more precise definition of genetic ero-

Landrace (local variety)

Of all the definitions included in this document certainly that of “**landrace**” is the most important (and most controversial) because it allows to determine exactly the areas of intervention of the NPAB, i.e. to establish "what" and "how" must be identified and, therefore, "what" must be safeguarded and how.

Among all the numerous definitions of "landrace" available in literature, the one proposed at the second meeting of “On-Farm Conservation and Management Taskforce of the European Cooperative Programme on Plant Genetic Resources (ECPGR)”, held in Stegelitz in 2006 can be considered quite complete and appropriate: “**A landrace of a seed-propagated crop is a variable population, which is identifiable and usually has a local name. It lacks ‘formal’ crop improvement, is characterized by a specific adaptation to the environmental conditions of the area of cultivation (tolerant to the biotic and abiotic stresses of that area) and is closely associated with the traditional uses, knowledge, habits, dialects, and celebrations of the people who developed and continue to grow it**.”

This definition highlights that one of the distinguishing features is the strong bond of the landrace with a specific socio-economic context. However, among the many Italian case studies there are many examples of varieties historically present in a given area and subsequently introduced into another. If a resource is no longer present in the area of origin, but it’s present in the introduction area, it is obvious that in this new environment the historical connection with the local socio-economic element is less strong than the one existing in the area of origin. Nevertheless, the resource may have found there important elements of context and therefore, in this case, it is defined as a landrace.

sion, which was formulated during the Ninth meeting of the FAO Commission on Genetic Resources for Food and Agriculture (CGRFA). This definition referred to genetic erosion as “the loss of genetic diversity, in a particular area over a given period of time, including the loss of single genes or combinations of genes that can be found in either landraces or varieties “.

The causes of genetic erosion throughout the period of agricultural modernization are ecological, socio-cultural, agricultural, commercial. In general, such erosion goes through a phase of under-utilization of a given species or variety, which in turn is accompanied by the loss of knowledge regarding the traditional use of these crops. In other words, the under-utilization of a crop plant leads to a cultural impoverishment, since the elderly, guardians of our local agro-food culture, are increasingly incapable of passing this information on to subsequent generations. Besides the loss of species, the growing awareness of the loss of cultural heritage in the agricultural world revealed the need for appropriate international policies. There is also a requirement for research programs on the evaluation, use, development and conservation of plant genetic resources that are at risk of extinction and that in turn would tend to preserve local knowledge on crops.

The dog-rose (Rosa Canina) is one of the most widespread and utilized wild species in Italy (photo by O. Porfiri)

At international level, the focus on agricultural biodiversity has resulted in two crucial negotiating processes. These are the Convention on Biological Diversity (CBD), which entered into force in 1994, and the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA, International **Treaty**, or simply referred to as the Treaty), which has been in operation since 2004.

The CBD establishes three fundamental points:

1. The ending of open access to genetic resources as Common Heritage of Humanity, since these resources become the "goods" on which governments apply sovereignty of the respective States in which these resources originate and are located.
2. Conservation is closely related to the sustainable use of resources.
3. Access to resources (not only the actual material, but also intangible resources such as traditional knowledge) must be established by the Prior Informed Consent (PIC) of the community holders and by an agreement for the equal sharing of any benefits arising from the use of these resources (benefit-sharing).

The Treaty, adopted in 2001 by the FAO Conference and subsequently adopted by Italy in 2004, has the following objectives: the conservation, the sustainable use of plant genetic resources, the fair and equitable sharing of benefits arising from their use in harmony with the provisions of the CBD and the creation of a multilateral mechanism of facilitated access to PGRFA. To reach this objective, member States decided to create an ad hoc space, managed on a multilateral level (the **Multilateral System**, MLS), which favours the exchange and sharing of PGRFA through a **Standard Material Transfer Agreement** (SMTA) (Appendix 2). However, at present, this Multilateral System only applies to the 64 crops listed in Annex I of the Treaty.

The above mentioned international agreements reflect the ongoing scientific debate about the most suitable conservation conditions. This debate is still very much alive, as the choice of the optimal conservation techniques is not only based on purely scientific examinations, but also on social, and especially economic considerations. It is useful to outline the process starting in the early 60's to understand the reason for the choices made today.

Generally, in order to identify germplasm conservation techniques, reference is made to two classes of genetic resources: namely wild and domesticated species. The former are best preserved in their natural habitats and plant communities where they belong. In cases where these are in danger, it is necessary to resort to specific forms of protection. This may occur in forest reserves, protected areas, in special genetic reserves or *ex situ*, for example in genebanks. On the contrary, all cultivated species, require active measures for their conservation. The ***ex situ* conservation** is distinguished from the ***in situ*** because the plant material is stored in areas other than those of origin. The *ex situ* may be a dynamic system if the populations of domesticated or wild species are kept in *habitats* where they are still exposed to selective pressure. However, the *ex situ* is considered a static system when recombination with external material is prevented and the erosion of each genetic accession is minimized, as well as selective pressure.

For many years *ex situ* conservation was adopted as main strategy. PGRFA were maintained in controlled environment far from their area of origin, and hence they were removed from a logical evolutionary process over time, as well as from the selective pressure of environmental and anthropogenic factors. In so doing, the role farmers might have played in performing this important function of conserving diversity of agricultural interest in their fields was neglected. In the rapid process of modernization, to keep cultivating old, often unproductive traditional varieties was seen by younger farmers as a sort of link to a rural community belonging to a past from which they wanted to break away. Due to this, Frankel was prompted to comment that "*in situ* conservation of local varieties is socially and economically impossible." As it turned out, much diversity has been lost, but much has also been preserved *in situ* thanks to local families continuing to cultivate old varieties to sustain their own food needs. Cultivation of old varieties also occurs in rural communities often located in marginal areas, where tradition is important. As for rural communities accepting the cultivation of modern varieties it is important to reflect on the studies carried out during the 80s in some countries of the Southern hemisphere. Anthropologists and sociologists showed that in certain rural areas in marginal social, cultural and economic contexts, modern varieties were not used by farmers because the performance of these varieties did not guarantee consistency in yield and production, which was the primary objective of those farmers. Following these studies, possible *in situ* conservation linked to agricultural systems and their development was initiated. It was only in the 90s, when the discussion turned to industrialized countries, that the central role biodiversity can play in sustainable agricultural systems was highlighted, even in the context of modern agriculture.

Tracing the historical path up until present, the question of which conservation model is to be adopted evolves over time, showing greater linkages to the more general question of which agricultural model is to be sustained. Pistorius and van Wijk wrote in 2000: "The debate on the strategies of farm conservation must be extended to include the antagonism between, on the one hand, globally organized industrialized agriculture and on the other hand, locally organised, traditional, non-industrialized productive strategies". It is thus obvious that for the non-industrialized agricultural systems the use of different crops (on an inter- and intra-specific level) is not aimed at conservation, but is an essential element of the system to cope with a variable production.

The grapevine "married" to the maple, one of the most traditional mixed cropping systems in Italy, in the past (photo by O. Porfiri)

In 2001, M.S. Swaminathan began to speak about the integrated approach to conservation, which includes mutual supportive strategies of *ex situ*, *in situ* and on farm. In agriculture, the *in situ* concept became wider over time, delineating a specific dynamic storage system, which is presently applied by farmers in their agricultural systems, namely the so-called **on farm conservation**. This strategy guarantees greater biodiversity and the safeguard of crops adaptability to the environment in a complementary manner to *ex situ* conservation, which has the advantage of keeping the resources in protected areas and making them easily accessible for wider use, but has the limit to conserve these resources in a static manner. Over the past ten years a lot of scientific literature has been published on the subject. Among various approaches, the one proposed by Maxted et al. in 2002 attempted to establish a common methodology for on farm conservation. The authors outlined two possible strategies to be pursued:

1. Actual on **farm conservation**, based on the conservation of genetic diversity of a particular resource within a well-defined farming system;
2. **On farm management**, where the *focus* is on the safeguard of the agricultural system as a whole, and not just the genetic diversity *per se*.

An example of the difference between the two approaches is given by different opinions that arise regarding the introduction of modern varieties into a given agricultural system. These varieties can be integrated by farmers in their fields and also crossed with local varieties. This ensures the continuity of the agricultural system, but produces a certain degree of genetic erosion of traditional varieties initially present. This process, if analysed from the point of view of conservation is negative because genes and varieties are lost. However, if analysed from the point of view of on farm management, the process is valuable because it helps to maintain a high level of diversity within the system. The rationale is that something will be lost, but at the same time new diversity is created. In this context, it is certainly very useful to preserve the evolutionary processes that normally occur in agro-ecosystems, making sure to either facilitate or support agricultural practices where diversity plays a central role.

In Europe, farmers who proved to be more interested in on farm conservation / management were those involved in organic farming. Organic cultivation differs substantially from conventional cultivation with regard to the heterogeneity of culture conditions and technical itineraries, as well as the different requirements of farmers for crop varieties, the lack of varieties on the market bred specifically for organic farming, and specific demand by consumers. These characteristics generally favour the use of traditional, local varieties and their conservation.

1.3 The Italian framework

To understand both the role and the importance of biodiversity in Italian agricultural systems, it is interesting to read the statistics that describe this role. Italy appears to be a country caught in between tradition and modernity, where agricultural activities – an insignificant percentage of GDP - still retain great value for a large part of the population. In fact, despite the decline in recent years, Italy is the third largest agricultural country in Europe after Poland and Romania, with more than one million people involved in agricultural activities. After Romania and Poland, Italy also holds the third place for number of farms.

In this framework, agro-biodiversity plays a dual role: on the one hand, it is still strongly linked to farmers who manage farms defined as "enterprises" and on the other hand quality production and labels attesting products' geographical origin (PDO, PGI and TSG) represent excellence worldwide. Regarding the latter, Italy is the queen of Europe with more than 200 certified products, which represent more than 20% of the European range. Geographic indication products are a demonstration of the link between territory, culture and agriculture. Their strong presence in Italy testifies to the importance that this trio still has in shaping the economic development of agriculture. It should be noted, however, that most of the cultivated biodiversity and associated traditional knowledge is maintained in a class of farms generally managed by people over 65 year-old.

It is therefore necessary to adopt policies coping with this situation, in order to avoid the loss of knowledge and the loss of local varieties due to the change in generation, and to create economic, social and cultural rights to ensure that these farms continue producing agricultural products. In fact, the global market and international competition are targets that are unattainable for those farmers who, without adequate forms of protection or development, would disappear taking with them the specific varieties and traditional knowledge passed on through generations.

In this context **agricultural policies**, in particular those for **rural development,** play a pivotal role. If correctly set up, these policies would narrow the gap between tradition and modernity, avoiding interruptions and using agricultural diversity as an incremental factor for local development. In this regard, the objective is not simply to deal with implementing conservation policies for PGRFA, but changing the perspective by moving to a system of safeguarding which would guarantee interaction and complementarities between *ex situ* and *in situ*/on farm conservation strategies.

The Regions and the Autonomous Provinces (AAPP) are public bodies which, by their knowledge of the territory and their legislative autonomy in the field of agriculture, are in a privileged position to synthesize and coordinate the principle actions for the conservation and valorisation of biodiversity. In fact, there are many regions that fund and promote similar actions in their territories. In some cases, these activities have resulted in the implementation of specific regional legislation with the aim of protecting local breeds and varieties. Tuscany was the first region to enact a law in 1997 on the protection of agricultural biodiversity and was followed in subsequent years by Lazio, Umbria, Friuli Venezia-Giulia, Marche, Emilia-Romagna and Basilicata. At present, similar laws are being discussed in other regions.

Regional Italian legislation can be considered one of the few operating examples for the protection and exploitation of PGRFA in Europe. These bodies have advanced laws at both national and European level, while keeping in line with the objectives of the Treaty.

In addition to the regions, in Italy there are several bodies which interact at various levels, depending on territorial dynamics, towards the creation of a virtuous circle for plant genetic resources for food and agriculture (**from conservation to valorisation**). There are three categories of stakeholders: scientific institutions, local authorities and people or institutions that are not included in the former categories, defined as the "non-governmental sector". The three categories ought to work in complete synergy . In general, it can be stated that:

- Scientific institutions deal with the collection, inventory, material characterization, eventual rehabilitation and *ex situ* conservation, as well as the dissemination of information collected;

- Regions, AAPP and other local institutions (Provinces, Municipalities, Mountain Communities, Local Action Groups, etc.) coordinate and promote such actions, often supporting them with dedicated lines of credit (e.g. regional laws for the protection of cultivated biodiversity) or through funds for agricultural research and regional Rural Development Plans or others;
- The non-governmental sector (all subjects not included in the previous two categories, such as farmers as individuals and groups, associations, foundations, various organizations, etc.) stimulate and/or plan, based on the needs of local communities and farmers and their history, processes for safeguard and valorization of specific local varieties or particular territories.

In this setting **the role of farmers** is crucial. Farmers have always been involved in the safeguard of genetic resources and this central role is also reflected in all activities outlined in these guidelines. Farmers are involved both in their capacity as cultivators (using local varieties within the management of their farm) and as "custodians of biodiversity", either as individual producers or as participants in organized programs enhancing and promoting specific PGRFA.

Consumers appear to be particularly attentive and interested in local varieties, to such extent that a vibrant market of typical and/or local products has developed. A typical local product is categorised as such on the basis that a local variety, its product and any process of transformation are closely linked to the territory in which the genetic resource evolved over time. It is hardly necessary to point out that the term "territory" is construed in the broadest sense, indicating both the physical space (geographical demarcation, orographic, geo-soil type, and climate) and anthropogenic attributes (typical elements relating to the mode of human settlement), as well as the set of values, history and culture that characterize the area. This term also encompasses the dynamics and stratification over time of the presence of man, including the concept

Corn is the species of agricultural interest with the highest number of landraces still cultivated in Italy for the production of flour, a well-known typical product (photo by O. Porfiri) of “technological-productive culture”, bearing in mind that the recovery and development of “local values” or “territory” is only achievable through a comprehensive evaluation of all aspects that contribute to its definition. In recent years there have been many experiences, either completed or still in progress, on conservation and valorisation of old varieties by individuals, both farmers and non-farmers, that have provided funding for projects on a voluntary basis. Such practices have often been linked to the promotion of a particular territory and products connected to it. These initiatives are dispersed throughout the country (fairs, markets, awareness raising events, promotion and valorisation, consortia of producers, development of product specifications, small projects for local products) and it has become evident over time that they are highly fragmented, poorly coordinated and frequently overlapping.

In particular, most initiatives failed to adequately convey the appropriate “know-how”. However, it must be said that dissemination activities, including publications produced in recent years, have contributed substantially to the current available knowledge relating to the heritage of local Italian varieties, which often were not adequately described in the official documents. In addition, the collection of information derived from cookbooks and traditional knowledge (which allows for the adequate cultivation and use of old local varieties) should not be underestimated. The material heritage and traditional knowledge developed through the ages and the objective experience of farmers of the past are a precious heritage that must be preserved for the benefit of humankind

1.4 Guidelines for the Protection of Plant Genetic Resources

In the preparation of these Guidelines, the recommendations made by international treaties and guidelines of the NPAB have been taken into account.

In summary, it is important to recall the characteristics of the conservation systems ***ex situ* and *in situ*/on farm.** The first is conservation of PGRFA in specific structures and by different means depending on the species. With the exception of collection fields, it is a virtually static system, at least during the storage phase. The onset of changes or loss of genetic diversity in the regeneration phase of field material becomes possible, when standards are not respected. The *in situ* conservation is conservation of PGRFA in their ecosystems and natural habitat, as well as the maintenance of populations and species, both wild and domesticated, within environments where, in accordance with the definitions given by the CBD, these have developed specific characteristics. This is a dynamic system of safeguard. Different populations are adapted continuously to both biotic (including human pressure) and abiotic pressures. The *in situ* conservation of cultivated species is generally defined as on farm.

The two systems - *ex situ* and *in situ*/on farm - should not be seen as alternatives, but as possible complementary actions to safeguard diversity. In fact, when it is not possible to implement the *in situ*/on farm of a particular genetic resource, at least the *ex situ* may guarantee its survival. In particular, the *in situ*/on farm conservation practices are considered the better options for local varieties, which have been selected and preserved for hundreds of years by farmers and represent a biological, cultural and territorial “system” and not only a biological entity. Given that **the farmer** is the central figure of this particular system, s/he can be identified as the **main player in this conservation activity.** The central role of farmers must, therefore, be properly taken into account in all on farm conservation projects. In certain contexts, it is appropriate to emphasize their contribution to conservation. Thus it is important to support initiatives present in all regions, also in order to develop responsibility and awareness in local resources holders.

***Ex situ* conservation**. The WGAB refers to Article 9 of the CBD, which emphasizes the importance of integrating *in situ* conservation with *ex situ* actions and calls on States that are Contracting Parties to take measures for *ex situ* safeguard, while trying to give preference to *ex situ* collections located in the country of origin of genetic resources. Ultimately, *ex situ* conservation programs are not only complementary to those *in situ*, but sometimes, as we shall see later, the only viable option in some instances.

As already mentioned, from a genetic point of view the *ex situ* maintains a static genetic situation, whilst the *in situ* conservation allows for evolution. Evolution can be described as change in the wealth of genetic variants. However, it is not possible to understand in advance in what direction (either increasing or decreasing) the change will occur. For small populations, the trend is generally towards a reduction of genetic diversity, which could culminate in the final extinction of the population. In this case, the *ex situ* conservation practice is able to ensure a higher level of diversity compared to the *in situ*. In addition, for species of interest to the agricultural and food industry, where the intensity of erosion/extinction can dramatically change even within a very short period of time, the *ex situ* conservation ensures the maintenance of specific genotypes, populations, varieties, breeds, strains, etc. It can also ensure their reintroduction into cultivation if they are lost.

In summary, the *ex situ* conservation becomes a compulsory tool of conservation when:

- The *sensu lato* populations are subject to heavy impact due to human activities, such as the replacement of local breeds and varieties with other alien species in the territory (for instance the introduction of modern varieties);
- Changes in either environmental or socio-economic conditions radically alter the structure and the vocation of a territory, causing the abandonment of agriculture activities;
- The area of cultivation of a given population decreases steadily for various reasons, thus exposing it to high risk of extinction.

To identify the most appropriate and effective conservation techniques, it is important to be familiar with the biology of the species (in particular, reproductive biology) as well as the genetic structure of their populations. This can be obtained in different ways which can be grouped as follows:

- Collection of plants in the field, in pots, and in a greenhouse;
- Seed collections maintained in either seed banks or germplasm banks (a widespread practice);
- Collections of propagation material, seedlings, and other tissues, maintained in vitro or in cryopreservation.

All material preserved *ex situ* should be managed so as to minimize risks in the event of natural disasters, technical problems, biological damage, socio-economic problems, etc. The procedures for protection, then, must provide for a continuous monitoring of the material. In particular, germplasm should be conserved in duplicates held in different locations. Moreover, the management of *ex situ* populations must carefully avoid any actions that may undermine the genetic integrity and viability of the material (reduction in genetic diversity, artificial selection, transmission of pathogens, uncontrolled hybridization, etc.). Additionally, particular attention must be paid to the collection of the minimum number of genotypes necessary to guarantee the maximum diversity of the population, within the scope of logistical and financial limits.

The ***in situ*/on farm conservation.** This mode of safeguard is certainly one that should be given widespread recognition. For this reason, the WGAB has focused its attention on this mode of safeguard. The *in situ*/on farm is a dynamic form of conservation, where populations are constantly changing in response to both the selective pressures to which they are exposed and to the soil-climatic environment in which they are located. In so doing, this process allows for possible adaptation of species or populations, as well as the co-evolution between different life forms. Therefore, it is more appropriate to speak of "safeguard" instead than "conservation", as the latter has a more static connotation.

From this viewpoint, the *in situ*/on farm appears to have a holistic approach to agro-ecosystem biodiversity conservation, which is intended to safeguard all life forms, whether cultivated or wild. Great importance is given to the maintenance of the complex relationship among them, which is not neglected but rather strengthened. Local varieties then integrate this framework. These have existed for a long period of time and have been cultivated by specific communities in specific locations. As such, these varieties can be defined as "native" in the sense that they have "always existed". The terminology "always existed" should be further clarified. For annual species propagated by seed, the continued maintenance of a given population in a defined area over fifty reproductive cycles (50 years) can be considered a sufficient period of time for a variety to develop both the characteristics of adaptation and the appropriate relationship with the environment (including the anthropogenic environment) for it to be defined as "local". As it is not easy to define the precise framework of time required in order to consider a variety "adapted", a time frame of 50 years has been set. However, for most trees and shrub species (perennial), 50 years (which cover just one or few generations) are considered insufficient for a species to be considered adapted to a certain area, and therefore "local".

The calloused hands of elderly farmers in the region Umbria, careful guardians of a precious landrace of cowpea (the "fagiolina" of Lake Trasimeno) (photo by O. Porfiri)

As far as time is concerned, it follows that both the **actions of reintroduction** of local varieties in an area and the development / selection of new populations from local varieties (actions that also help maintain diversity that is useful to man) should not be covered by the term "on farm conservation". When referring to *ex situ* populations preserved for decades, "reintroduction" (a particularly topical subject), can lead to the cultivation of plant forms that are not adapted to the physical, biological and cultural conditions of the area of re-introduction which characterizes the local varieties. In other words, from the time of re-entry a new process of adaptation starts, which will result over time in these populations becoming real local varieties, while being different from the original populations.

It is true that the margin between the reintroduction and exchange of material propagated in an area (especially in large areas with variable climatic conditions) is often quite thin. It is equally true that inducing the evolution of genetic material not perfectly adapted to a specific environment is still useful to conservation (e.g. resulting in the movement in the frequencies of rare or under-represented alleles in the original environment, etc.). In addition, reintroduction (including into similar areas) is sometimes necessary when the variety has completely disappeared from cultivation and it is not possible to reintroduce it into the same area because of changes either to the environment or the social context.

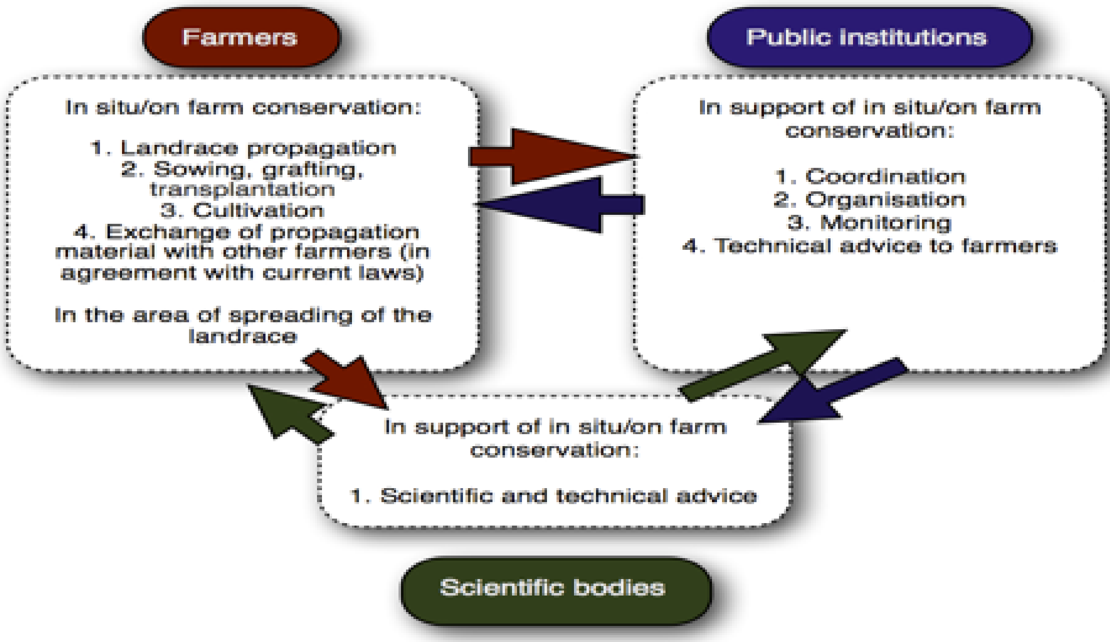
The *in situ*/on farm conservation must be carried out in such a manner as to permit the population/local variety to maintain the variability that distinguishes it. Moreover, it is important that this process is in balance with the environment in which the population/local variety evolved distinctive characteristics, to ensure that the latter are not lost. For this purpose, it is particularly important to plan the production activity for the propagation of material, which must take place in the area of origin and under conditions that avoid both mechanical (pollution during the sowing, harvesting, storage) and genetic pollution. The former is easier to control. In contrast, the latter can be more problematic and depends on the following: the species (if autogamous or allogamous, and in the latter case if pollination is anemophilous or performed by insects), the orographic conditions within the area of multiplication, the surface area, the weather conditions, etc.Regarding measures to develop **new populations/varieties** either by crossing with other varieties or by selection activities aimed at identifying, maintaining and propagating only certain genotypes, it is evident that these actions can distort the genetic constitution and therefore, the characteristics of local varieties. Genetic variation is the basis of any genetic improvement programs. In the past, local varieties represented the raw material from which scientific research, starting from the beginning of the twentieth century, produced improved or "modern" varieties. Even at present, many vegetable and fodder crop (grasses and legumes) varieties are obtained by selection using local varieties. Every selection process leads to a reduction in diversity (when compared with the original material) because the specific choices dictated by the objectives of the improvement program are implemented. Recently, an interesting approach to the use of such variability in genetic improvement is offered by **participatory breeding** (*Participatory Plant Breeding*). The purpose, similar to that of classical breeding, is to obtain improved varieties, but with the participation of farmers in the selection process. The objective is the attainment of a variety with a large genetic basis.

At this point, at least two fundamental considerations must be highlighted, on which the guidelines for the *in situ*/on farm conservation are based. The **first consideration** is that the objective of *in situ*/on farm conservation, apart from protecting farmers' rights, is similar to that of any other conservation action, which is to maintain the current and potential utility of PGRFA for the needs of both current and future generations. Since it is

Technical visit of the cereal's field within the initiatives of the participatory plant breeding promoted by the SOLIBAM EU-project (www.solibam. eu) (photo by O. Porfiri)

not possible to predict future needs, in other words to know which genes and gene structures in populations will be needed, it is necessary to adopt conservation strategies that maintain the highest diversity. Contrary to the general practice for the conservation of wild species and populations in protected areas where conservation is generally implemented directly by public bodies, the implementation of on farm conservation of crop plants is the responsibility of farmers. It is them who, year after year, continue to cultivate and maintain local varieties. The public sector can (and always should) promote, organize, coordinate and monitor activities on conservation, by providing financial and technical support to farmers and promoting their activities through appropriate public policies. The role of scientific institutions in conservation is also important.

They serve as junction between farmers and public institutions, as schematically shown in the following **image**.



From this, it follows that if circumstances don't make it possible to guarantee conservations activities by farmers over time (for various reasons), it is important to formulate *ex situ* conservation alternatives in order to at least ensure the survival of populations. With regard to the role of the public sector, support activities provided can be managed in different ways. These include promoting increased awareness on: the importance of PGRFA Food Safety; the well-being of present and future generations; the financial support to create the knowledge needed to exploit in the market a product obtained from a local variety. In any case, these activities must always be oriented towards ensuring that cultivation of local varieties is maintained, or even increased, over time.

The **second consideration** is that local varieties (both autogamous and allogamous species propagated by seed, as well as some vegetatively propagated species) are different populations, therefore distinguishable from each other. Moreover, these local varieties must be populations with a certain level of internal diversity, being composed of different genotypes. These varieties are subject to evolutionary change over time in response to changes in the physical/agronomic environment and changes within the biotic community, both in terms of genotypes present and the numerical relationships amongst them. This evolution is obviously much faster for the annual species. The genotypes that better adapt to a different environment perform better at the expense of others, while new genotypes, due to mutational change, may also appear. It is this inherent characteristic in local varieties that renders the populations adaptable to the physical, biological and cultural diversity, thereby making them useful to agriculture. Therefore, in the preparation of these Guidelines, the inherent variability of local populations and their ability to change over time were taken into consideration as positive features. These same positive features must be safeguarded. In other words, given that local varieties retain their usefulness in the various stages of the on farm conservation activity, they should be given the freedom to mutate or change over time.

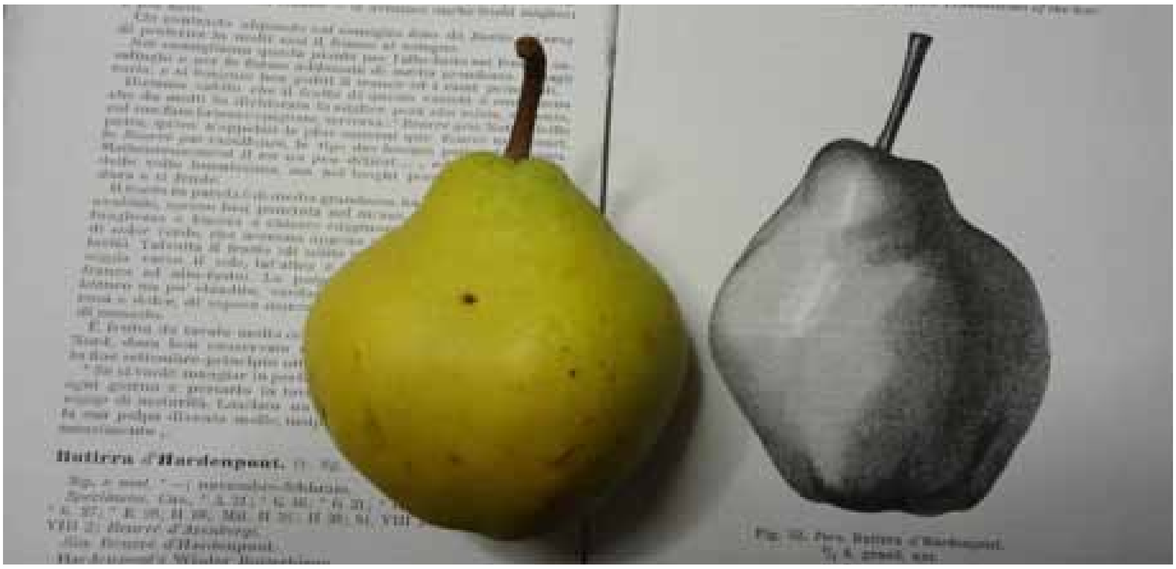
Finally, a reference describing **the complexity of the situations** is required. This complexity includes the conditions under which local varieties are maintained and the lack of scientific data on the subject. Therefore, it is difficult to propose guidelines based on solid practices that can comfortably be applied everywhere. The current situation in which local varieties are maintained on farm both in Italy and Europe, has never been so complex (especially for annual plants). This complexity is due to the multiplicity of variables involved which include the species, the number of local varieties, as well as the physical, climatic, ethno-anthropological, social and economic situation. There is very little scientific evidence (based on distinct analysis of the results obtained by applying a particular strategy) to provide precise directions on how to implement on farm conservation. There are also limited data relating to the conservation of *in situ* wild populations, even if some progress have already been made. This is particularly true with regard to the potential of maintaining an adequate level of genetic diversity over time, whilst avoiding the phenomena of genetic erosion due to mixing with similar commercial varieties.

Fortunately, sound practical experiences are there, particularly those that have been developed in different regions in Italy. These regions are either already equipped with a law for the protection of PGRFA or have already provided funding for those activities. These may serve as reference in providing operational guidance. In the Volume, case studies in the regions of Tuscany and Lazio are reported (Appendix to Chapter 4). These regions were the first to adopt a law on this issue. In the first case study the valuable role of the "farmers-custodians" is shown, as well as the operational validity of the storage and security network, in particular with regard to supporting the exchange of resources between farmers. The second case study shows the importance of conducting a detailed investigation in the territory reaching every single "holder", in order to collect as much information as possible. This is a prerequisite in understanding the various issues and dynamics within each farming community.

The organizational and monitoring activity of *in situ*/on farm conservation is accomplished according to the following phases:

1. **Collection of information on existing local varieties (inventory)** and collection of propagation material for *ex situ* conservation and for characterization;
2. **Identification of the priority areas to be allocated for in situ/on farm conservation** (choice of areas to implement this activity, with priority on the promotion, organization and monitoring of activities);
3. **Characterization and assessment of the distinctiveness of local varieties;**
4. **Assessment of population size and genetic structure of local varieties maintained in situ/on farm;**
5. **Monitoring the effectiveness of in situ/on farm conservation** (periodic assessment of the maintenance of an adequate level of genetic diversity and absence of genetic erosion);
6. **Set up and operation of an information system for work related to in situ/on farm conservation.**

The proposed steps must not necessarily be in sequence, since certain interventions may proceed in parallel, whilst others transverse all stages, such as the setting up and management of the information system.



The consultation of archival documents is a fundamental step in the research on the local varieties (photo M. Fontana)

The outline of the proposed activities is calibrated primarily on herbaceous species, but provides a useful model for the *in situ* conservation of tree species. It is to be noted that the steps listed above are also important in the planning of actions for *ex situ* conservation. **Coordinated activity among the various stakeholders** involved (government agencies, research organizations, farmers, technicians) is obviously necessary to achieve the best results.

Step 1. Gathering information about existing local varieties (inventory). This phase is supported by a series of instruction sheets developed by the WGAB, based on the analysis of existing experiences. The process is initiated with the indication factsheet, followed by the morpho-physiological characterization factsheet, and by the synthetic varietal factsheet. To support this activity, a historical investigation based on both written documents and oral testimony is a key factor in the genetic resource inventory process. The historical investigation makes it possible to verify the strong connection of genetic resources with the territory.

Unfortunately, much of the farmers' traditional knowledge has been passed down orally and a lot of information about use, production techniques and utilization of local agro-food products has been lost. Recently, we have become aware that this oral culture may be very important to drive protection policies and strategies for the enhancement of biodiversity. Therefore, at this stage an anthropological approach is of great help since some of these practices have already been implemented in some Italian regions. This method developed in Italy represents a novelty in the conservation of PGRFA and has recently been taken into consideration by other European and international standards.

This initial phase must be accompanied by the cultivation of propagation material for *ex situ* conservation and characterization.

Step 2. Identification of areas to be devoted primarily to *in situ*/on farm conservation. The conservation of PGRFA in the greatest possible number of environments and involving the greatest number of farmers is certainly the most effective. However, given the limited resources, it is often necessary to select and give priority to specific areas in order to promote, organize and monitor the conservation activities. To this end, guidelines based on scientific theories have not been fully developed and standardized yet. Nevertheless, it is possible to refer to some research studies funded by the European Commission, which suggest that priority be given to those areas (defined as "appropriate areas") with greater wealth in terms of agro-biodiversity. These are areas that are rich in local varieties, diversity and agro-ecosystems, and that have already been targeted for measures to protect nature (e.g. in parks and protected areas). On the other hand, a completely different approach may be taken, based on alternative objectives, by giving priority to areas less rich in biodiversity in order to safeguard existing genetic material and to increase the existing level of biodiversity.

The area designated for conservation would be the same as the one where seeds multiplication of conserved varieties occur.

Step 3. Characterization and assessment of the distinctiveness of local varieties. These actions are of considerable importance because they allow for:

- The identification of those populations that are really representative of local varieties and that must be protected for their unique characteristics and genetic diversity, for their link with the customs and traditions of the people who developed them, and for the possible risk of their erosion/extinction;
- The promotion of the product to be obtained from local varieties based on their uniqueness, authenticity, characteristic features and link with the territory;
- The listing of varieties, that forms the basis for planning conservation actions on both farm and territory level, the implementation of potential marketing initiatives for seeds, and the assessment of the risk of extinction.

At this stage, it is necessary to distinguish between local and commercial varieties. Morpho-physiological characterization is essential. Alternative forms of characterization (including genetic) can certainly be implemented to resolve specific problems (e.g. the genetic identity of a particular variety or the study of genetic relationships between populations). The type of characterization chosen is inherently related to the availability of financial resources.

Step 4. Assessment of population size and the genetic structure of local varieties maintained *in situ*/on farm. This aspect, together with the correct identification of a PGRFA, is of major importance for the appropriate planning of conservation actions. In the case of small sized populations, these are at risk of losing (in a random and unpredictable manner) the genetic variability that characterizes and determines their adaptation. If in a given area a local variety exists in genetically distinct populations, it is necessary to conserve diversity in order to maintain all the different populations. On the contrary, if the different populations are essentially similar, conservation may be limited to only one population within a given farm. Although intra-variety variability is more limited in species propagated by vegetative means than for crops that reproduce by seed, this variability still exists and should be preserved as much as possible. In fact, this forms the basis for either mass or clone selection. From a safety perspective, this makes it possible to recover individuals that are exempt from diseases transmitted by grafting. In essence, the more accessions of a single PGRFA are conserved (in larger and more varied populations) thus involving in the process more areas and more farmers, the greater the guarantee in achieving an effective and efficient system of conservation. This understandably depends on the availability of resources (both human, structural and financial) within the cultivation areas.

Step 5. Monitoring the effectiveness of on farm conservation. This is the cornerstone of all actions put in place for conservation because the aim is to assess whether the objectives are delivered on time and in the appropriate manner using both the human and financial resources provided. The monitoring also assess whether over time there is any erosion of the diversity that ought to be preserved.

In other words, this step allows for the evaluation of the effectiveness and efficiency of the conservation actions implemented. To achieve this goal, the monitoring activity should be initiated from the start of the *in situ*/on farm conservation process. This is because often, when dealing with fragile and complex situations, it is necessary to have full information on the "status ante" (farm information and genetic features of both populations and subpopulations). Thereafter, it is necessary to repeat the collecting of information at regular time intervals and to compare the initial data with the data gathered after the start of activities involved in the promotion, organization and management of *in situ*/ on farm conservation.

Step 6. Establishment and management of an information system for *in situ*/on farm conservation. *In situ*/on farm conservation provides in each phase for a series of activities that either gather information or generate information, which is necessary for the understanding and better management of the local variety conserved. Therefore, it is important that all data is maintained and organized in a rational and functional manner, possibly in a computerized system. The objective of this phase is to collect full information on the activities carried out on *in situ* conservation for easy control and management. Moreover, the use of a database featuring both rapid access and rapid data processing makes it possible to compare many factors. These include different case study experiences, the development of improved conservation practices, the compilation of inventories on a larger scale (e.g. national register) and, in general, the promotion of an ever more extensive conservation activity.

1.5 Commercial aspects of the multiplication and dissemination of propagation material of local varieties.

This is a topical aspect in the management of PGR conservation, both for the large and complex regulatory framework that characterizes it and for the growing number of requests by farmers for multiplication material.

Here attention is focused on two specific elements. The first is the introduction of the concept of "conservation variety" in European seed legislation. The second involves implementation of provisions for plant nurseries and pest control for fruit trees and grapevine.

Seed propagated species. A recent and interesting new element was the introduction of the concept of **varieties for conservation** (forged at first at EU level and then at national level). This was followed by the subsequent establishment of a specific section for agricultural species in the National Register of Varieties, with specific access rules. Only in this area is it possible to establish appropriate methods of marketing and distribution of local varieties (landraces), while highlighting the fact that the varieties for conservation are a subset of those local varieties. In fact, only a fraction of these can be included in the Register. For others, it is possible to envisage a limited circulation at the local level, defined by regional law as "**Conservation and Security Networks**".

A variety for conservation to be entered in the Register must meet the following requirements: be of interest to a conservation program; be accompanied by information derived from an official examination or by inscriptions, characterizations, knowledge and other details obtained from competent authorities or organizations; not be entered in the Community Catalogue for at least two years; not be protected by either community or national property rights; be identified by the area of origin, and meet the limited DUS requirements (DUS stands for Distinctness, Uniformity and Stability) for those characteristics determined by technical questionnaires (Community Plant Variety Office - CPVO or International Union for the Protection of New Plant Varieties - UPOV). Precise requirements must also be met for the production of seeds. These include the seed reproduction area, the phyto-sanitary quality and the marketable quantities. On the regional level, the registration dossier is "filtered" and then the request for inclusion of the variety for conservation is sent to the MiPAAF (Ministry of Policies for Agriculture, Food and Forestry Resources) where the dossier is verified for its compliance with the requirements. Entry is free, unless it is necessary to perform supplementary investigations in order to establish variety distinctness.

Fagiolo a carne" (meat bean), local variety still cultivated in the small gardens in the Umbria Region, in the border area with the closed Region Marche and Tuscany (photo by O. Porfiri)

Fruit trees. Among the large number of current regulations governing the production and marketing of plants and plant products, the Legislative Decree n. 124-25/06/2010 can be recalled. According to this decree, in order to produce and market local fruit/olive variety, the variety needs to be registered in the variety Register (maintained by the MiPAAF) and the producer must have obtained the required phyto-sanitary certification. These certificates can be provided by an appropriately authorized nursery. In specific cases, as defined by law, Plant Protection Services can award certificates to small producers.

Vine. As in the case of fruit trees, the law has not taken into account grape varieties for conservation. This means that conservation and valorisation of local germplasm are operations that are neither immediate nor simple. Given the current legislation, it is clear that old grape varieties not registered in the national Register can only be cultivated for family use and only by a farmer who does not possess another vineyard. A further obstacle to the cultivation of grape varieties unlisted in the Register is nursery legislation. In fact, vine propagation material can only be marketed if subject to certification control.

Collection of fruit species diffused in Central Italy (photo I. Dalla Ragione)

However, only material from the varieties registered in the National Register are admitted to official controls and certification. On the other hand, the law's definition of "marketing" opens the possibility of propagating unregistered grape varieties that are destined for experimentation and for internal farm use. In other words, it is possible to transfer propagating material to a nursery for the production of grafted rootling for farm use, but not for selling purpose.

All of the above shows that there are limitations not only for the *in situ* conservation of vines, but also for the rapid reintroduction of an old grape variety into cultivation, a prerequisite for the valorisation of any wine produced. For the purpose of conserving and exploiting old grape varieties, it is appropriate to proceed very rapidly towards their propagation (better if controlled and on a small-scale). This should be undertaken without waiting to register the variety in the National Register and without considering any possible sanitation (to prevent virus transmission), since during the long period required the material could risk extinction. Obviously, virus and other pathogens control is important in preventing the spread of diseases transferable by grafting. Up to present some grape germplasm has been preserved, thanks only to the care of old farmers. This dedication is something that has been handed down in families through generations, including the art of grafting and the ability to propagate material for personal use. It can be concluded, therefore, that at present the safeguard of an old grape variety may be entrusted only to *ex situ* collections linked to research institutions (therefore exempt from the regulations for reasons relating to research or trial purposes). Safeguard can also be entrusted to farmers owing the varieties in danger of extinction, providing these varieties are intended for the exclusive family use. In any other case, the process leading to the registration of the variety in the National Register must be undertaken. Most of the above mentioned information for vines also applies to fruit and olive trees.

Grape of the local variety "Centesimino", an ancient grapevine grown in the Emilia-Romagna Region (photo by M. Fontana)

1.6 The characterization of Plant Genetic Resources

As mentioned above, characterization is finalised to a precise identification of PGR-FA. In this study the WGAB presents the most effective **markers** divided into categories and illustrating the guidelines for their utilization.

The proposed work starts from the analysis of individual accessions to the establishment (if possible) of a varietal factsheet summarizing the morpho-physiological profile of the variety, starting from the observation of individual accessions. It is important to reiterate that sometimes local varieties (especially herbaceous varieties) are characterized by a degree of internal diversity. During their evolution in time and space (under both environmental and anthropogenic pressure) this diversity renders the varieties unstable.

When these characteristics are particularly accentuated, it is not possible to fully utilize the characterization guideline tools (descriptors) developed for identifying improved varieties (typically uniform and stable). In these cases, an evaluation of single plants must be performed so as to identify sub-populations or varietal typologies through the attribution of frequency classes. The data collected is then statistically analyzed. In contrast, when the level of internal variability shown by a local variety is low, it is possible to apply the characterization systems developed for DUS evaluation. These criteria, although more flexible, are indispensable for the eventual registration in the National Register of varieties for conservation.

Collection of information on existing local varieties. An initial description of PGRFA collected in the territory represents the first step on the path towards conservation. A more precise *in situ*/on farm or *ex situ* characterization according to the conservation model is then carried out. After the elaboration of existing models, the WGAB produced a group of crop descriptors for the collection of information and PGR characterization (available in the appendixes to this Volume). The series starts with an indication factsheet, followed by a factsheet to describe the single accession, and then another containing the passport or identification descriptors. This is then followed by a factsheet for markers, allowing a detailed description of the morpho-physiological characteristics of accessions, which vary from species to species (species-specific descriptors). In conclusion, the varietal factsheet summarizes the characteristics of the different potential accessions in a variety.

Taken as a whole, the proposed methodology for the collection of information through the use of factsheets permits the characterization, organization, coordination and monitoring of the previously described conservation activities. However, it must be noted that based on different necessities as well as on financial and human resources, single parts of the general scheme may also be carried out, for instance by using specific conservation methods but not others, or collecting information using just some of the factsheets (the most important in the specific context) and not others.

Some important aspects for the proper use of these tools are provided herewith, while for details on the factsheets reference should be made to the Volume.

Passport descriptors (or those identifying PGRFA in relation to precise collecting conditions) are fundamental in exactly identifying and distinguishing each accession, including those propagated or transferred. These passport descriptors are also currently in use by international data banks (MCPD and EURISCO), which have a common coding system allowing the comparison with materials kept in other countries. Besides the passport descriptors shared at international level, the WGAB (following advice provided by regional delegates) is proposing four additional and complementary identifying descriptors, that may be useful in providing interesting information on both local and national level for more detailed accession identification. Finally, two more specific descriptors have been identified for accessions of crops contained in Annex I of the International Treaty and/or as components of European collections, as defined by the European Integrated Genebank System.

Morpho-physiological markers. The description of the plant phenotype represents one of the most important instruments to investigate biodiversity. This description, based on the measuring of morpho-physiological parameters, allows for characterization and identification of varieties by specific comparative methodologies. In general, the descriptors refer to highly inheritable and stable characters which often represent the basic elements for plant classification. Characterization must be carried out following shared and objective criteria within a scientific framework and where possible according to common procedures in harmony with relevant national and international procedures.

On these basis, the WGAB proposed a descriptive factsheet (defined as species-specific) to describe a local variety or accessions of a local variety within the framework of the species considered in the present Volume. If the characterization is finalized towards variety identification, then generally all the characters described by the factsheet must be used and systematically collected according to the recommended procedures. However, to provide users with easy-to-understand factsheets which can be rapidly compiled, some descriptors (marked with the acronym WGAB) were highlighted. **These descriptors are considered indispensable and therefore highly "recommended" for the characterization/identification of PGRFA in accordance with the objectives of the present Guidelines.**

Different systems focussing on variety characterization were developed at international level. These are specifically designed for description, documentation, exchange and management of genetic resources (Bioversity International, USDA-GRIN) or to evaluate the distinctness, homogeneity, stability and uniqueness of those resources, a requirement in order to obtain certification for varietal protection (by the CPVO, Community Plant Variety Office). In line with the objectives of the present guidelines, for most of the species the UPOV international system was found appropriate. Therefore, this system is generally quoted in the varietal characterization methodologies listed in the Volume. The basic criteria of the UPOV international system are coherent with both national and European systems for the official registration of varieties. These are well known and already in use in different regions for many species and they correspond almost completely to the IPGRI/Bioversity International system descriptors for characterization. For some species, including vines, other organisations such as l'*Organisation Nationale de la Vigne e du Vin* (OIV) worked together with UPOV and Bioversity to create a system of common descriptors for the genus *Vitis*. Given that it represents the most utilized system for vines at regional, national and international level, the morfo-physiological characterization factsheet for *Vitis vinifera* refers to these descriptors.

In some factsheets describing fruit species, the WGAB used other descriptors, such as those published by the Tuscany Region. In the case of emmer (*Triticum dicoccum* and *Triticum monococcum*) due to lack of UPOV/CPVO descriptors, national descriptors were employed and a completely original factsheet was prepared. Finally, based on the experiences of the WGAB components, other descriptors were elaborated and introduced in the factsheets. In species propagated by seed, it is important to recall that local varieties do not possess the same characteristics of the improved varieties, on which both UPOV and CPVO criteria were set. In fact, these often show high internal variability and therefore some of the procedures foreseen and proposed by these Organisations may not apply (for example those relative to the evaluation of "homogeneity"). To evaluate the level of homogeneity in a local variety, it is frequently necessary to evaluate the characteristics on single individuals and then apply the appropriate statistical analyses.

Molecular markers. From the time of their first application in the field of plant science approximately twenty years ago, molecular markers have proved to be useful investigation tools for the study of genetic diversity. These are proving to be ever more promising, owing to the increasing knowledge on the genomes of organisms and to the subsequent development of more efficient and less expensive analytical techniques. Each individual contains small differences in the DNA that render that specific individual different from the other individuals belonging to the same species or population. These polymorphisms can be identified if homologous DNA traits among individuals are compared. Therein “lies” the analysis of so-called molecular markers, that is of DNA fragments positioned at various points of the chromosome (thus inheritable). Their presence distinguishes (“marks”) the portion of DNA in which they are located in a univocal manner.

If compared with the phenotypic type of morphological description, genotype characterization using molecular markers is definitely more advantageous. These advantages include a greater reliability as there is no environmental interference on the expression of the characteristics. Moreover, there is no subjective bias, which may occur when conducting a morphological analysis. Genotype analysis is also the most reliable from a legal aspect. Furthermore, DNA analysis may also be used to detect differences among individuals that are genetically very similar (often indistinguishable phenotypically). Because of marker inheritance, it is possible to obtain objective information on the genetic proximity among individuals or populations and on parental identification (pedigree) to establish/ validate the genetic origin of a variety. Further advantages of the method are that DNA can be extracted from many parts of the plant (stem, leaves, fruits, seeds, roots), during the vegetative cycle or during winter dormancy. Moreover extracted DNA is fairly stable and can, therefore, be stored.

The aforementioned positive aspects, combined with the development of analytical techniques and more sustainable instrumentation costs, have resulted in the **increasingly popular use of molecular markers**. In so doing, these do not replace but complement the morpho-physiological characterization of PGRFA. Knowledge relating to the phenotypic variability within a species is always necessary, both during the sampling of material and in the interpretation of the results obtained by genetic analysis. For certain species, molecular markers have proved highly effective in distinguishing differences between individuals, in the identification of varieties and in the study of genetic relationships among individuals and varieties (databanks containing reference genetic profiles have become available to operators). However, for other species that have not received the same research attention by the scientific community, the available methods are either poor or not particularly informative, or simply nonexistent. Among the crops of the first type, the vine can undoubtedly be recalled. Some of the most widely used microsatellite markers have been adopted as genetic descriptors. After the development of a system for encoding results in order to standardize data from different laboratories, these have been added to the official list of morpho-physiological markers for international use in the characterization of Vitis species and varieties. Databases of the genetic profiles of grape varieties are now available online, and are regularly updated.

In summary, both practical and field skills relating to the study of the morphology and physiology of the species under characterization are irreplaceable, whilst genetic methods are useful for the objective confirmation of varietal identity on the basis of a specific genetic reference profile. This has been demonstrated for example in the case of errors made when rendering the denomination of a particular variety, or in synonyms between cultivars in distant places. Molecular markers may ultimately provide scientific information of great importance for the management and study of PGRFA, such as in the establishment of core collections (collections which contain in an individual limited number the widest genetic diversity) and in defining genetic variability of a population and its structure This information is also important in assessing the risk of genetic erosion and in monitoring the effectiveness of conservation activities.

1.7 Concluding remarks

The present Guidelines aim to make operating tools available to all stakeholders in order to provide effective and coordinated actions in the territory, with emphasis on a systematic approach. Various reasons reflected the need of standardised operating tools. In Italy there is no centralized coordination entity acting as a reference point for PGRFA. Moreover, there are numerous public and private initiatives, all dedicated to safeguarding PGRFA.

So the first step is to circulate this instrument over the entire national territory. The Study wishes to provide all operators with the basic regulations relating to PGRFA, as well as common methodologies for PGRFA description and management. Additionally it provides, through case studies, experiences that can serve as examples towards either investigation or towards ascertaining the value of PGRFA.

The next immediate step will involve the activation of a **National Register** for local varieties and breeds, among other things, provided for by phase C of the NPAB. This represents an effective action to improve knowledge of biodiversity heritage of interest to Italian agriculture, in order to fully protect and enhance it. This Register may provide various levels of detail, necessary in defining the specific morpho-physiological and genetic profile of each local variety under conservation. This will facilitate the comparison among material originating from different areas or regions (identifying synonyms, distinguishing homonyms). Hence, the Register will serve as a precision tool for the identification, the correct denomination and knowledge of PGRFA. In addition, the Register would serve to improve relations with other European and non-European countries for the exchange and development of materials, as well as to provide the tools to create *ex situ* core collections with less financial resources.

Strengthening national coordination that can play as reference is important. This would permit a more widespread circulation of knowledge, experiences and resources. It would also facilitate relations at international level through cooperation within the wealth of our scientific and administrative bodies, in particular with the view to full implementation of the International Treaty on Plant Genetic Resources for Food and Agriculture.

CHAPTER 2

SUMMARY OF THE GUIDELINES FOR CONSERVATION OF ANIMAL GENETIC RESOURCES FOR FOOD AND AGRICULTURE

2.1 Outline

The main objective of this work is to provide guidelines for the conservation of biodiversity for animal genetic resources. This text has been designed to meet the operational needs of technical stakeholders involved in the implementation of measures for the conservation of animal genetic resources and has been written thanks to the contribution of experts and the consultation of numerous articles and scientific papers.

The document emphasizes the intrinsic value of native Italian breeds as irreplaceable and unrepeatable national heritage. It also highlights the need for further work on the current and future economic assessment of the domestic breeds, and the scientific, cultural and environmental services they can provide to society.

This evaluation is a prerequisite to advance and evolve the overall strategies and individual activities for the conservation of biodiversity in agriculture - and breeds in particular - put in place up to now. Strategies and activities that, in many cases, helped to avoid or slow down the extinction of native breeds, but in other instances proved ineffective in halting the genetic erosion process that began with the establishment of intensive breeding systems, currently more and more unsustainable from an economic or environmental point of view.

The document provides concepts, tools and operating protocols for the conservation of animal genetic resources through an innovative approach that takes into account the multifunctional role of local breeds; it also lists some examples of application of the suggested protocols.

The text is divided into 2 parts: the first describes the general concepts of biodiversity and animal genetic resources, provides some data on the loss of biodiversity and the genetic erosion in the world and in Italy, as well as legislation and breeds conservation initiatives put in place at global and local level. In the second part, after the chapters dedicated to the nomenclature, the definition of species and breed, and the use of morphological and molecular markers for characterization of the breeds, the study proposes tools and operating protocols for the protection and enhancement of native breeds threatened by genetic erosion or at risk of extinction.

In addition to the conservation strategy currently implemented in countries of the European Union, the guidelines propose an innovative approach that provides the concept of "priority" of a certain breed to achieve a specific conservation objective.

Finally, the report includes some case studies that help to understand the concepts and protocols described in the text, as well as a glossary of terms and extensive scientific literature.

2.2 Nomenclature and Definitions

The document reports a concise description and the evolution of the concepts of species and breed, together with the various definitions and revisions that have taken place over time with the objective of establishing an appropriate classificatory order.

For both terms of species and breed, not a single shared and accepted definition exists so far. With regard to breed, the evolution of genetics population and the gradual understanding of the mechanisms of speciation have nowadays introduced the use of concepts and new terms which, although not yet completely integrated in the common language, appear more appropriate to distinguish between individuals of the same species with clear dimorphisms.

The concept that a breed is not a static but an evolving entity, with morphological and functional characteristics that are subject to change under the influence of environmental conditions and selection is widely accepted. Domestic breeds, although genetically pure for a certain number of characters, are actually "populations" with a significant degree of genetic variability and therefore different genotypes, although similar for the manifestation of characters. In general, therefore, different phenotypes may correspond to the same genotype, and vice versa.

Population defines a group of animals (called "biotypes"), more or less dissimilar to each other, that always show a certain morphological and physiological variability. The population, such as the breed and other sub-specific groups, is composed of interbreeding animals who are more or less different, but characterized by spatial continuity over long periods of time.

Varzese-Tortonese-Ottonese cattle breed

From the previous concepts, it is easy to see that among the domestic species there are no “pure breeds” from a genetic point of view. In the common language, pure breeds are a group of animals with a number of characters that are consistent, replicable and predictable. When these characters depend from single genes the similarity will be high; on the contrary, a degree of variability will be more evident for quantitative or polygenic traits (e.g. size, productions, etc.).

Blue Barberia duck (Cairina moscata)

Due to the difficulties of determining a single and shared definition of breed, these guidelines accept the definition proposed by the FAO: “A breed is either a homogenous, sub-specific group of domestic livestock with definable and identifiable external characteristics that enable it to be separated by visual appraisal from other similarly defined groups within the same species; or, it is a homogeneous group for which geographical separation from pheno-typically similar groups has led to general acceptance of its separate identity”. This definition allows to link under a single descriptive framework both selected and native breeds.

2.3 Morphological and molecular characterization of breeds

The description and characterization of breeds and populations, both morphologically and genetically, is essential and necessary for the subsequent choice of strategies and techniques of conservation.

Regarding the already recognized and classified breeds, the description and identification tools are the standards reported in the Herd Book and Anagraphical Records.

For the recognition and description of populations not yet ascribed to defined breeds, the guidelines propose the use of both morphological and molecular markers. For the use of morphological markers, the guidelines suggest a rather innovative methodology, easy to apply in the field and, in some ways, similar to that used in the plant sector.

For each species there is a specific list of "primary" markers and, in some cases, of "secondary" or "other" markers (to be used in unclear cases). The use of morphological markers is cheaper and faster than a complete morphological study, and allows a fast and very reliable assessment of the genetic resource. Due to their versatility, morphological markers can describe comprehensively not only populations with high degree of variability, such as polychrome breeds, but also breeds or population spread in areas where the selection is only partially addressed by man, or it does not respond to precise encoding schemes.

In addition to morphological markers, the guidelines also suggest the search for other information to complete the description of genetic resources. This first "field" phase is always followed by a genetic characterization of the animals.

Thanks to the development of new molecular biology techniques, it is now possible to describe and quantify accurately the genetic variability (approximately 50% of the genetic variability within species depends on the genetic diversity among breeds or populations, and it is statistically described in terms of genetic variance between and within breeds), and to determine the similarity between animals within and between breeds or populations.

The guidelines report the main types of molecular markers used in genetic studies (RFLP - Restriction Fragment Length Polymorphisms, VNTR - Variable Number of Tandem Repeats, microsatellite or STR - Short Tandem Repeats or SSR - Simple Sequence Repeats, and minisatellites, the AFLPs - Amplified Fragment Length Polymorphisms, the STS - Sequence Tagged Site, SNPs - Single Nucleotide Polymorphisms, and polymorphisms of the mitochondrial DNA (mtDNA) in the region D-loop or control as well as the major databases currently available.

The use of molecular markers allow to estimate parameters of diversity and mixture within and between breeds or populations, to outline the geographical habitat of the breeds, to obtain phylo-genetic information on the evolutionary relationships and centers of origin, modes of domestication and migration routes. In addition, molecular markers can be used for practical purposes, including measurement of the degree of relationship among animals and survey of the kinship (especially in the absence of pedigree information), support to selection assisted by markers, definition and development of the concept of molecular or genetic traceability. From the latter objective, the issue of the attribution of a certain animal to a specific breed or population arises. This attribution can be traced back to the principles of kinship; for the identification of animals, microsatellites are the markers for which there are now the first applications, but in the future it is likely that SNPs, that have characteristics that can be exploited for a complete automation of DNA analysis, will be used.

In general, the guidelines emphasize that at the present state of knowledge, the use of molecular markers refers to phylogenetic studies tending to define the degree of genetic variability within and between breeds and to the identification of genetic distances, and therefore to quantify biodiversity between breeds and/or populations within species. It is in this context that the information contained in the document should be used.

2.4 Conservation strategies

Until today, the European approach to the necessity and duty to safeguard domestic breeds is a conservation strategy mainly based on their risk status. This strategy, often called “risk strategy”, has been applied through the allocation of financial funds to breeders. Even though easy and intuitive, this strategy has been criticized because it does not clearly state the conservation objectives and the role of local breeds; moreover, the financial efforts to maintain the endangered breeds may not represent a cost-efficient contribution to biodiversity.

The guidelines suggest a new approach for conservation of local breeds. Basic conditions for starting a new conservation policy of animal genetic resources and/or new conservation projects are: 1) the creation of a database of local breeds, identified and classified using the criteria and procedures previously described, and 2) the accurate definition of one or more specific conservation objectives for each breed, according to their risk status and characteristics.

The primary objectives of any conservation effort are the safeguard of sufficient genetic diversity to be able to cope with future possible changes in production or market environment, and to reduce or to avoid the risk of extinction of all breeds. However, in the meantime it is necessary to improve or maximize the utility of local breeds and to reach an economic and productive self-sustainable role.

Due to the different traits and characteristics of breeds, these results can be achieved only by defining one or more specific conservation objectives.

The new strategy proposed in this document (called “maximum-utility-strategy”) involves a cost function for the optimal allocation of financial funds. The strategy assumes that marginal costs and marginal returns of conservation activities must be maximized not only in terms of diversity, but also in terms of their economic, environmental, scientific, social or cultural utility.

From a conceptual and systematic point of view, the maximum-utility-strategy is the most appropriate method for the future selection of breeds to be saved. The use of this strategy requires the definition of specific conservation objectives for each breed threatened with genetic erosion or extinction. To do this, as much information as possible must be acquired; in many cases, the future scenarios where the breeds can play a profitable role must be hypothesized. Today these information are often incomplete or unknown, and this may be a constraint to the use of this strategy. But at the same time, these deficiencies are also a stimulus to further research on all those local breeds that are still poorly studied.

2.5 Conservation objectives

The “maximum utility strategy” is certainly the most effective response to the problem of conservation of animal biodiversity in agriculture, not only to achieve specific conservation objectives, but also in terms of allocation of human and financial resources, whose availability will be increasingly scarce in the future.

Risk status will continue to be the first and most important parameter to consider in the choice of breeds to be protected, but - as mentioned above – the selection of breeds for conservation should be made according to the defined objective of conservation efforts and according to the characteristics of each breed.

Having this in mind, the “prioritization” of breeds should be done in accordance with the respective objective of the conservation plan.

The guidelines report a non-exhaustive and expandable list of conservation objectives for the various breeds at risk of extinction.

The main objectives are:

1) Meeting the current and future demands of the market. Today it’s the first objective that justifies the safeguard of a local breed, and it is particularly urgent in Europe and in Italy, where the efforts for the conservation of animal genetic diversity are primarily addressed to satisfy a growing and diversified demand of animal products. Moreover, in Europe the demand is also highly variable over time depending on changes in markets and consumer tastes.
2) Coping with changes in production processes. Since modern production systems are based on very high input/output - that show obvious symptoms of environmental and/or economic unsustainability - the maintenance of a broad base of genetic variability guarantees farmers a sort of “insurance” against unfavorable situations to the breeds used today, and it allows them to adapt to changes in production systems.
3) Providing opportunities for scientific research. The knowledge of characteristics of local breeds provides a valuable opportunity for scientific community; this important conservation objective allows to realize, for example, new crossbreds or to isolate qualitative and quantitative traits of economic interest.
4) Enhancing the present and future socio-economic role of breeds. Often the local breeds are able to support local micro-economies thanks to the value of their products, and to ensure the presence of man in marginal areas.

Istriana sheep breed

5) Safeguarding the historical and cultural values. Many breeds reflect a long history of symbiosis with humans and are part of local traditions, often abandoned. Although difficult to quantify, the historical and cultural role of a breed has recently become an important objective of conservation, especially in countries rich in traditions.

6) Safeguarding the ecological and environmental values. The maintenance of breeding techniques of many local breeds is essential for the preservation and maintenance of landscapes and semi-natural environments. As with the previous objective, this may be difficult to quantify, but it is now considered of great importance in many countries around the world.

2.6 Prioritizing breeds for conservation

The knowledge of the risk status of a breed is the first parameter to consider for any conservation program; it is understood that, as a matter of fact, the ultimate goal of any conservation program is to stop extinction.

There are several criteria for assessing the degree of risk of a breed. These guidelines refer to the classification proposed by FAO ("Secondary guidelines for development of National farm animal genetic resources management plans", 2003), which identifies seven categories: extinct, critical, critically preserved, endangered, endangered preserved, not at risk , and unknown status.

The classification is based on the overall size of the population, the number of reproductive females and the population trends (increasing, stable or decreasing).

With the implementation of the maximum-utility-strategy, each breed at risk is a "priority" for the achievement of one or a few conservation goals, but not for others.

The identification of these breed ("priority breeds") depends on their characters and characteristics.

This document lists the main ones, but others may be considered.

1) **Adaptability to the environment.** The preservation of breeds that are adapted to specific environments is a priority if the objective of conservation is, for example, to have animals that can cope with future production systems that provide uncontrolled environmental conditions for animal breeding; or to have animals with an "ecological" function or for the maintenance of agricultural landscapes. Though difficult to express in purely economic terms, the adaptability to the environment plays an important role to satisfy the growing demand for "sustainability" of livestock systems.

2) **Economic importance**. It's the most common parameter used today to justify the conservation of a local breed. It depends on the characteristics of current importance (for example: high fertility, high rate of feed conversion, high quality of products, disease resistance, etc.) and/or on characteristics of future importance. As the demand for animal products is expected to increase, in the future there will be undoubtedly an increasing competitiveness of some breeds that today are not common. The estimate of the future economic value of a breed, however, is more difficult than assessing the current economic value; this estimate can only be done through the simulation of different production scenarios between 10, 50 or more years. The document gives some examples from the scientific literature that illustrate methods of

economic evaluation of breeds based on hypothetical future markets.

3) **Uniqueness of one or more characters**. Some breeds may be given priority in order to achieve specific conservation objectives due to their behavioral, phenotypic or physiological characteristics. These characteristics may depend on a single gene or on polygenic effect. Moreover, in addition to having current or future economic importance, these characteristics may be of great scientific interest: saving these breeds actually means to have material for future research, whose results will find application in various economic sectors.

4) **Historical and cultural value**. This value is difficult to quantify, but is particularly important in societies where agriculture and animal husbandry have radically changed. This value can generate income if properly exploited as a tourist resource; more often, however, its preservation requires a financial support which only high-income countries can generally afford. The document provides a methodology that estimates the historical and cultural value of a breed.

5) **Genetic uniqueness**. Saving genetically distant breeds is important to preserve the different alleles and gene combinations that characterize them, and which are manifested through characters that could prove useful in the future. The "genetic history" of most Italian breeds can be estimated by studies based on microsatellites or other techniques illustrated in the document.

Valgerola or Orobica goat breed

2.7 Conservation techniques

After the definition of the conservation objectives and the prioritization of breeds, the conservation programs are started with the selection of the most suitable techniques; the available human and financial resources should always be considered in this phase.

Techniques for the conservation of animal genetic resources are divided into two categories: *in situ* and *ex situ*.

When *in situ* conservation is possible, a local population is maintained and bred for production in its agro-ecosystem of origin or evolution, or in the areas of current breeding. With this technique it is possible to start selective plans targeted to increase the total number of animals and to improve their productivity while maintaining the genetic variability of the breed.

The effective population size is a parameter to be considered with care; the guidelines explain the calculation of the effective population size.

In the document there are some examples of *in situ* conserved breeds, both in Italy and abroad; as far as Italy is concerned, the guidelines list some examples of *in situ* conservation programs based on the Anagraphic register. In the document, a list of websites for detailed and updated description of current conservation activities is provided.

In this chapter the major role played so far by farmers and shepherds in conservation of local breeds is also underlined; thanks to their involvement in conservation programs, many breeds have survived until today and their role will be even more important in the future.

The *ex situ* conservation is divided into *ex situ in vivo* and (*ex situ*) cryo-conservation technique. The *ex situ in vivo* conservation is defined as conservation through maintenance of live populations not kept under normal farm conditions (including zoos, agricultural parks, etc.) and/or outside the area in which they evolved or are now normally found.

The differences between *in situ* and *ex situ in vivo* are rather vague and, in many cases, poorly defined.However, in order to achieve the conservation objectives, the two techniques differ for their effectiveness.

The (*ex situ*) *cryo-conservation* technique is defined as the storage of gametes of embryos in liquid nitrogen. Developments have been made in freezing techniques for oocytes and for all animal species DNA-storage and storage of somatic cells is a well-known technology. Although there is now a broad consensus on the *in situ* technique, the *ex situ* or cryo-conservation is in many cases a powerful and safe tool to preserve animal genetic resources. Therefore, the integration of *in situ* and *ex situ* methods can provide a powerful conservation technique. The last one alone, in fact, does not offer opportunities for socio-economic development of the farmers, because: 1) it requires the removal of animals from the areas of origin, 2) the populations bred *ex situ* are generally small compared to those bred *in situ* and more likely exposed to genetic erosion, and 3) cryo-conservation may "freeze" the natural evolutionary processes of a breed.

Whatever the chosen technique may be (*in situ*, *ex situ* or a combination of both), it is necessary to ensure the maintenance of the greatest genetic variability within the breed; this is particularly true for small populations with high risk of inbreeding and loss of genetic variability.

In an attempt to slow down as far as possible the negative effects of inbreeding, the document refers to some models of genetic management, aimed to maximize the actual number of the population, to minimize the relationship between animals, and to plan the mating. Also some schemes of calculation of inbreeding and mating for small populations are reported.

2.8 Towards a National Bank of animal germplasm

The document provides some general guidelines and technical and scientific support for policy-makers in view of a future national plan for cryo-conservation of animal genetic resources according to the "Guidelines for cryo-conservation of animal genetic resources" (FAO, 2011).

The general objectives of an animal germplasm bank are: 1) the "back up" of in vivo preserved populations in case of genetic problems, 2) the reconstruction of extinct or relic breeds, 3) the creation of new lines/breeds in case of risk of extinction, 4) the reorientation of evolution or selection of a population, and 5) research and experimentation.

The document underlines the need to involve all interested stakeholders in the creation of a national bank of animal germplasm, such as breeders associations, NGOs, public and private companies and organizations dealing with animal breeding, universities, research institutes and schools.

Nero of Nebrodi or Nero Siciliano pig breed

The general policies, priorities and conservation strategies must be defined by a national committee that works within the national plan of cryo-conservation of animal genetic resources. Its implementation should be assigned to a specific management committee of the germplasm bank, which also provides for the coordination of stakeholders, the definition of conservation objectives, the prioritization of breeds, the development of a database of donors, a cost/benefit analysis of the planned actions and all the activities necessary to achieve the conservation objectives.

With regard to funding, the study suggests to perform an actual cost/future benefits analysis to justify the creation of the bank from an economic point of view.

Finally, on a practical level, the document lists a number of operational steps, such as: the identification of type and quantity of material to stock (semen, embryos, oocytes, somatic cells, etc.); the need for integration in an international animal germplasm bank in order to avoid the conservation of identical material in different countries; the priorities within and between the breeds; the possibility of having more storage sites; the temporal parameters.

2.9 Practical guidance

In the final part of the document two flow diagrams report the decision-making procedures to be applied to: 1) both groups of animals or populations in "critical" or "endangered" status according to the classification FAO, but not yet recognized as breeds, and 2) both groups of animals or populations in "critical" or "endangered" status but already recognized as breeds.

The first phase of the flow diagram consists in evaluating the morphological and functional characteristics of animals through the use of morphological markers listed in the guidelines, and in the search for historical information, illustrations, management, etc. through the consultation of all available documents.

The outcome of this first phase will determine whether or not the population matches the definition of "breed" adopted in these guidelines, or if it already belongs to a local or national breed.

The second step is a genetic analysis using molecular markers (microsatellite markers and/or SNPs) to determine whether the population is discriminated from others. If the results show a single cluster, the preservation of the population has no scientific justification. Otherwise, an investigation of the territorial distribution of the population is needed: parameters such as numerical and geographical distribution of animals are useful not only for the subsequent choice of conservation techniques, but also to involve the breeders (the so-called "livestock savers") in a conservation program.

The next steps concern the choice of the most appropriate strategy and conservation technique. For breeds / populations in "critical" numerical status and/or whose main characteristics are not yet known, the primary objective of conservation is to increase the number of animals; the best strategy to be applied is the "risk strategy". The switch to the "maximum-utility-strategy" and the identification of specific conservation objectives will occur only when the population has reached a suitable number of animals (passing from the "critical" to the "endangered" category) and all – or most – of its characteristics are known.

The choice of the conservation technique for "critical" populations will depend on the number of animals and their territorial distribution, as well as the possibility to involve farmers in the conservation program.

The document suggests to use, where possible, the combination of *in situ* and *ex situ* techniques, especially if animals are bred in only one or in few farms. For "endangered" breeds or populations, in addition to a desirable increase in the number of animals, it is possible to immediately identify one or more specific conservation objectives according to their characteristics and number, thereby applying immediately the “maximum-utility-strategy”.

For these populations, the *in situ* conservation technique will be chosen; the final objective is to reach rapidly the conservation objective and a self-sustainable breeding. With the application of the maximum-utility-strategy, breed must be prioritized according to their characteristics for one or more specific conservation objectives. This step is achieved by solving a simple matrix “conservation objectives x characteristics of the breed”.

The document shows the example of a 6 x 5 matrix, with 6 objectives and 5 characteristics of the breeds. The solved matrix shows that a breed can have useful characteristics to achieve one or more conservation objectives, or that its characteristics are of little or no interest to others.The accuracy of the matrix can be improved using, for each breed, a numeric index (or percentage) to define the “weight” of each characteristic for each objective.

Applying the maximum-utility-strategy and solving the matrix for every breed, it is possible to avoid the financing of general conservation projects targeted to all threatened breeds in a given territory. Thanks to this strategy, only projects with a specific conservation objective and targeted to few “priority” breeds will be financed. Moreover, the better allocation of the available human and financial resources will increase the possibility of a successful result.

2.10 Case studies and glossary

To facilitate the implementation of strategies and operational protocols outlined in the guidelines, and for a better understanding of the concepts and terms used in the text the document contains several case studies and a glossary.

The first case study illustrates an example of the difficulties in defining the concept of “breed” through the results of a survey of sheep breeds in the Tuscany and Emilia Romagna regions. The text shows how the different denominations of “breed” used in the past could differ from a region to another, as well as numerous examples of synonymy.

The second case study shows how to reduce inbreeding and to control the genetic drift of a breed. The results of a project on the Girgentana goat breed illustrates that the most suitable solutions to these problems were the use of basic biotechnologies, such as artificial insemination, and the expansion of the inter-generation interval.

An example of genetic distances measurement is shown in the third case study, which reports the results of a survey carried out on 5 different Sicilian sheep breeds with a common and known origin. This case study demonstrates the effectiveness of the discriminating parameters and methods described in the guidelines.

In the fourth case study, the recovery of the Varzese-Tortonese-Ottonese cattle breed is taken as an example of a successful transition from the “risk strategy” to the “maximum utility strategy”. The text summarizes the recent initiatives undertaken by a group of farmers aimed at increasing the number of animals of this local breed through the sale of milk and meat in two areas characterized by a distinct and differentiated demand.

In the last case study, some recovery projects of local cattle breeds undertaken in France are reported. These projects were based on the morphology (Bordeaux), the ethnic base (Béarnaise), the productive aptitude (Blue du Nord), the numerical consistency (Lourdian) and the territory (Saônoise).

CHAPTER 3

Summary of the guidelines for conservation of microbial genetic resources for food and agriculture

3.1 Outline

The main objective of this work is to provide guidelines for the conservation of microbial biodiversity of agricultural interest for the two main sub-sectors related to food and soil fertility. Following decision of the WGAB (Working Group on Agricultural Biodiversity) the work shall focus exclusively on microorganisms, completely excluding all plant pests and diseases for which separate guidelines are to be developed. It is a document of scientific rigor, while providing operational guidance to allow experts to plan for actions in the territory for conservation of microbial genetic resources. In agriculture, microbial genetic resources conservation involves fundamental aspects such as soil fertility, without which nothing could be cultivated and preserved. Soil fertility is the core value to preserve biodiversity and life on the planet.

Preserving microorganisms relevant to food and agriculture means protecting typical national products as well as the Italian gastronomic traditions. The document provides operational hierarchical tools such as morphological markers or practical, phenotypic and molecular markers. Operational protocols for *in situ* and in factory conservation are provided, as well as for the sampling and *ex situ* conservation.

The Volume then presents case studies as an example of application of the suggested protocols. It consists of six chapters: (i) definition of the concept of microbial species for the sector, (ii) description of morphological, molecular and objective markers, (iii) description of the analytical methods to be used in the characterization and isolation of microorganisms, (IV) protocols for *in situ*, *ex situ*, on farm, in factory conservation; (V) definition of the risk of genetic erosion, (VI) concluding remarks and recommendations

3.2 Importance of microbial genetic resources

Microorganisms of agricultural interest play a key role both in food production (soil fertility, crop nutrition, bio-control, bio-fertilizers) and with regard to conservation of foodstuffs (toxins and pathogens) and in the production of processed foods (milk and cheese, wine, oil, etc.), so their presence and their biodiversity are instrumental for the maintenance of living organisms on earth. In the study of biological diversity (biodiversity) ecological theories have been developed essentially for ecosystems present on the surface of soil, neglecting for a long time all those forms of life that are present in it, in particular microorganisms, which represent a huge amount of “invisible life” of fundamental importance to all living forms on earth (Wardle and Giller, 1996). In fact, the microbial population represents the most relevant part of soil biomass and is the one that most affect its biological properties, adjusting all biochemical processes that determine the nutritional functions.

It is difficult to define and specifically to "measure" the microbial diversity of the soil for many reasons, while the classic definition of biological diversity and its subdivision in "ecosystem, species and genetic" diversity attributed to animals and plants, can also be extended to soil microorganisms, with the exception however of the definition of diversity of species as this is not applicable to organisms such as bacteria and viruses that reproduce asexually. Microbial diversity is therefore commonly defined in terms of richness, i.e. the number of individuals belonging to different "groups" denominated taxa, and evenness i.e. their distribution within the same taxa. The composition of communities (that is, the set of microbial species present in a given environment) can vary over time as a result of changes that occur in the microenvironment or by the action of microorganisms that are part of it (or those that are placed in it) and/or because of climate, topological, biochemical and anthropological changes. In addition, many microorganisms can maintain the same composition within a community, but change certain metabolic processes with functional and ecological consequences. This view implies also a correlation of individuals to their function, associating the study of single cell with those genomic and proteomic functions. The most modern evolutionary theories that relate variability to adaptive performance are therefore also applied to microorganisms. This adaptive performance allows the genetic heritage of each species to evolve gradually and then survive the changes that may occur in the environment.

In the case of microbial biodiversity linked to food production, the above observations for soil microorganisms could also apply, except that the substrate on which they are to develop microbial communities is essentially much simpler than soil and acts as a growing medium although, especially with processes of transformations such as the production of cheese or wine, it is possible to run into problems similar to the non-cultivability and cultivability of soil microorganisms.

As far as food is concerned, the study of microbial biodiversity must be carried out for each specific foodstuff and never be derived from food categories. This obviously leads to an objective difficulty related to the heavy workload for characterization that is going to be faced when testing a product linked to a specific territory.

3.3 Concept of species

The definition of species is one of the most debated in biology. At present, there are over twenty definitions based on quite different criteria and concepts. The choice of a definition leads to major differences in the distribution and classification of biodiversity and requires analytical and statistical tools or different phylogenetic depending on the adopted criteria for the species.

In particular, with regard to microorganisms:

- Biodiversity is defined according to the number of species and the species is therefore rightly regarded as the basic unit of biodiversity.
- Risks of extinction are normally associated to species and not to their components,

thus it is essential to correctly define (or at least to adopt a shared definition) a concept of species that allows to understand if erosion and extinction relate to specific or sub-specific groups.

- The definition of species implies concepts such as evolution, which must be as consistent as possible with existing biological and general knowledge.
- In the case of microorganisms the definition of species is often associated to a technique or a strategy for identification. Speed, reproducibility and cost-effectiveness of these procedures are critical to meet the dual need of performing accurate identification within the set timeframe.
- Particularly important for microbiology is the possibility that the concept of species and the corresponding analytical technique may allow the identification of viable non-cultivable microorganisms (VNC). The fact that isolated biodiversity is estimated between 1% and 10% suggests that the majority of biodiversity is precisely not cultivable according to current laboratory procedures. Hence the need for the identification technique, consistent with the concept of species, be also applicable to the DNA of species whose strains prove to be VNC.

Because of all mentioned above it is clear that the **concept of microbial species** to be employed must be the result of a conscious and motivated decision shared by the scientific community, easily applicable and understandable to operators and as close as possible to the current biological knowledge.

The problem with the concept of microbiological species arises from the fact that the most common and shared concept of species is the so-called **biological species concept (BSC)**, which is based on sexuality as sole reproduction system. In actual fact, the vast majority of known microorganisms do not belong to this condition. Thus, a different concept of species needs to be found, different from the one used for animals and plants. The choice of a particular criterion will be presented with the appropriate theoretical and practical reasons, designed specifically to enable biodiversity studies in a fast and accurate way, possibly disconnected from the need of cultivating microorganisms in a laboratory.

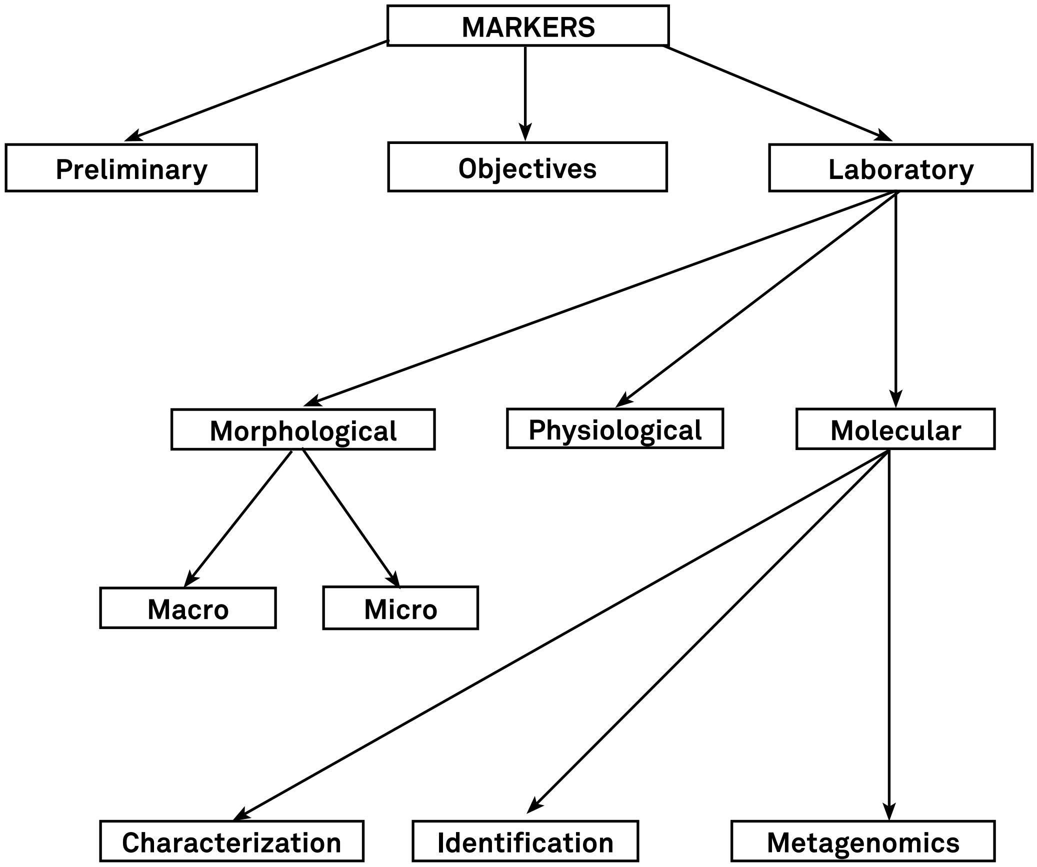
Bapteste and Boucher propose that the microbial taxonomy is to classify the *composite evolutionary units*: integrated associations of replication elements of low rank held together by cohesive biological mechanisms. These evolutionary units are composite because they consist of different genes philo-genetically diverse. Furthermore, these evolutionary units operate at different levels of organization. Some may be parts of organisms, others represent the entire body, and others consist of entire microbial syntrophic communities. The composite evolutionary units are not species in the usual sense, because the individuals are not necessarily organisms, but genes, groups of genes and microbial communities.

3.4 Markers

The guidelines propose possible markers that can be used to describe the microbial diversity of agricultural and environmental interest. These markers are presented according to the principle "from simple to more complex" in order to provide a picture as complete as possible and to allow sequence analysis, more and more complex, of microbial diversity.

The observation of these markers should allow operating personnel in the agricultural, environmental and food sectors to perform through rapid and simple systems preliminary estimates of the present level of variability and diversity, so as to direct analysis of samples collected and actions for safeguard or valorization.

Image 1 - Hierarchical representation of markers used in conservation of microbial genetic resources



For ease of reference, a general classification of markers is given herewith:

A. **Preliminary markers**: are those markers allowing to identify a site or situation that is potentially interesting for the characterization and subsequent conservation of biodiversity of agricultural interest (eg, a typical product, DOP etc, specific means of cultivation, uncommon traditional rotations etc);

B. **Objective markers**: are all parameters allowing to show a status or characteristics that link a product or environment to essential microbial metabolic processes so as to determine unique and characteristic results (for instance: quality of foodstuff, soil fertility, typical product with particular characteristics, uncommon environmental conditions, etc);

C. **Laboratory markers**: are those only detectable through accurate analysis in the laboratory and are in turn divided into:

Objective markers: quality of foodstuff (caciottina senese) (photo by Anna Benedetti)

1) ***Macro- and micro-morphological markers*** (shape and size of the colony or of the cell). In particular, the Macro-morphological are those that can give information on the visible level (eg. colonies, patinas etc.), while the micro-morphological ones relate to the size and shape of cells and cell aggregates observable only under the microscope.In the instance of soil microorganisms it is difficult, if not impossible, to provide the morphological markers on the microorganisms at the field level, due to both the size of microorganisms themselves, which cannot be seen on a naked eye, and to the fact that it is very difficult to isolate and cultivate them. Thus for the observation in the field, the definition of soils "morphological markers" can be applied to everything that can be seen on the naked eye and which is correlated to soil life and to functions of microorganisms, but these will never be real microorganisms.

Objective markers:soil fertility (photo by Bruno Pennelli)

The field sheet (photo by Bruno Pennelli)

Conservation of biodiversity at ground level is a practice that cannot be separated from good agricultural practices and farming. Microorganisms are the main actors of the nutrient cycle and soil fertility, therefore, at the field level objective markers can be identified which correlate to the functions of soil and its fertility. Conservative or destructive agricultural practices are known to affect the fertility of the soil and its biodiversity and a first analysis of the field must refer to this, while later, if appropriate, activities to preserve biodiversity can be put in effect. Objective markers targets at ground level can be considered those specific environmental conditions that cause a specialization in the microbial population of the soil due to endogenous natural causes. Many objective markers at ground level may be identified by operators. These markers can easily be identified in the analysis of the field-sheet. In fact the person who carries out the sampling should take note of any interesting element .

2) ***Physiological markers*** (assimilation, fermentation, resistance to stressful conditions, etc.)

3) ***Molecular Markers*** divided into markers for the definition of species and markers used for characterization

Molecular markers play a key role in the study of microbial biodiversity. They can be divided into those used at the level of identification (determination of species), characterization (description of strains within species) or meta genomic (analysis of populations of genomes). Also in this instance it is essential to define the markers on the basis of microbial groups and choose (or classify them) on the basis of their effectiveness.

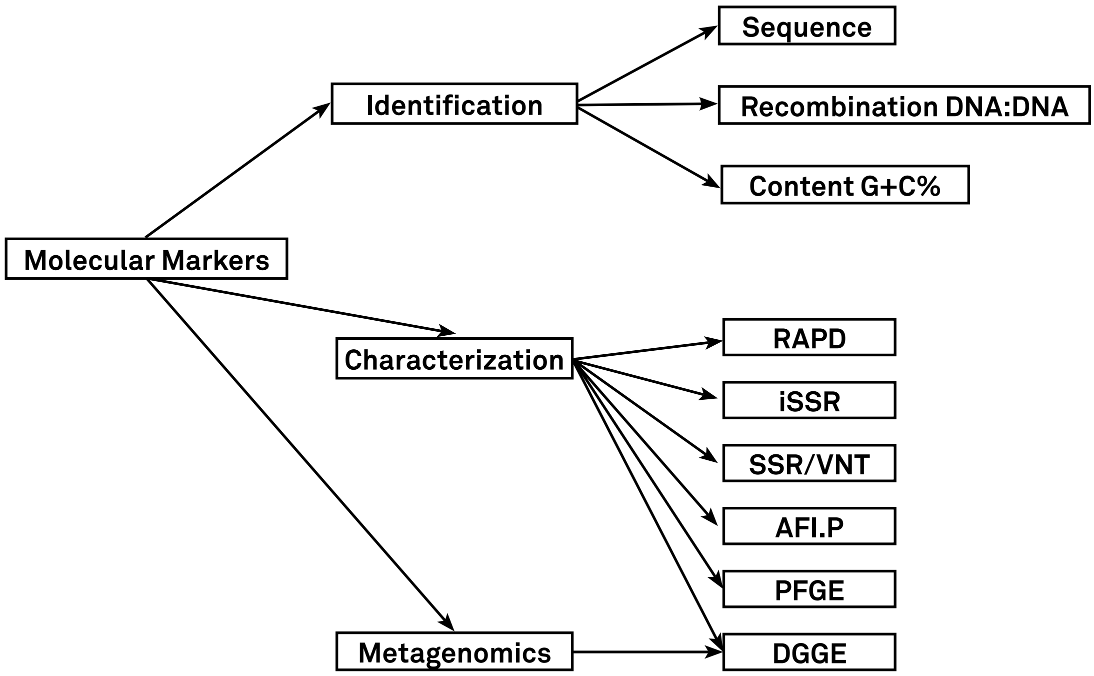
3.4.1 Classification of molecular markers according to their function

Laboratory markers have been described according to their functions, in short:

- **Identification markers**; they determine the species to which the strains are associiated. These markers are based on identification of specific sequence such as for eukaryotic microbes the sequence for domain D1/D2 of gene 26S coding the ribosomal RNA.

- **Characterization markers**: they describe strains in details. They allow to look inside the genetic difference among strains. In the guidelines several analytical techniques are described followed by short operational comments.
- **Meta genomics markers**: they can be used to describe microbial communities, independently from the possibility to cultivate the strains in a laboratory.

Image 2



3.5 Methodologies (standard protocols)

In these guidelines different methodologies are proposed, microbiological and molecular, for the study of microbial biodiversity in various areas and depending on the level of precision required. Methodologies and choices that are common in environmental and food microbiology were treated together, while more specific methodologies were presented separately. This part of the study makes assumptions on the average technical microbiological knowledge, usually provided by the various graduation studies in biological, agricultural and biotechnology issues. Methods that were exhaustively discussed in appropriate manuals and in collections of official methods will not be presented, but sometime they are mentioned when deemed necessary for a better understanding of the methodologies themselves.

3.6 Guidelines for the conservation of microorganisms

This part is the core of the guidelines. In fact, here are provided the criteria for the three basic strategies for conservation of microbiological, food and environmental biodiversity together with a critical analysis of the reasons for adopting them. Particular attention has been paid to the effects of various forms of conservation on the genetic structure of the stored materials

3.7 Critical analysis of systems for microbial conservation

Like any other organism, microbes can be stored in the same place where they live and from where they would be isolated (*in situ*), or in special collections (*ex situ*). For microorganisms to be used by the agro-industrial sector an intermediate form of conservation may be applied, that could be called "in factory", similar to "on farm" for plants and animals.

Each system has associated advantages and disadvantages, and in addition specific types of microorganisms may react differently to the different types of conservation methods. The following are some considerations on the three storage systems.

Collection of microorganisms in laboratory (photo by Anna Benedetti)

3.7.1.a Ex situ conservation

Ex situ conservation was the first form of preservation of microbial diversity long before the treaties on biodiversity and the development of specific sensitivity in this regard. From the second half of the nineteenth century microbiologists have started to keep the strains that they obtained, trying to conserve them. Only after the Second World War refrigeration and even freeze-drying spread around. Freezing at very low temperatures in liquid nitrogen or at -80 ° C became available only towards the end of the last century and these conservation methods are now considered among the most valuable and widespread.

This brief history of the maintenance of strains is functional in two aspects: to understand the different types of conservation and to understand that the related technologies are still under development

3.7.1.b Collections

Microbial collections allow the *ex situ* maintenance of microbial genetic resources isolated in the form of distinct strains and possibly well described.

There are different types of collections according to their purpose:

- **Taxonomic collections**. These collect strains of species and strains of various origins, yet unequivocally identified at species level according to current standards.
- **Patented collections**. These collect natural strains, engineered and subject to genetic improvement on which there are patent claims.
- **Working collections**. Each laboratory tends to keep the strains isolated and studied throughout the period of their use. The advent of cryopreservation systems has encouraged the maintenance of such strains also for longer times.
- **Collections of service**. These are taxonomic collections available to provide maintenance services, identifications, etc.
- **Collections and application areas**. These collections are for specific sectors (plant pathology, food, environmental, etc.) and possibly aimed at maintaining biodiversity and its reintroduction. Such collections should minimize selection induced by isolation and by keeping in the laboratory and in the collection. These collections are particularly aimed at the combination of "conservation and valorization."

3.7.2 Conservation "in factory"

Microbial biodiversity for food and agriculture has grown almost certainly within sites of transformation, even primitive in which the first rudimentary steps to transform and preserve foodstuffs were made. The fact that a lot of literature emphasizes the presence, but at low density, of *S. cerevisiae* in nature, while this is found in high concentrations in cellar environment, is one among many evidence to suggest that the development of yeast is strongly linked to its unconscious use in the transformation of sugar fermented juices. In general, it should be noted that the transformation, even in its simplest forms, involves masses of product kept somehow isolated from the environment in which microbial strains at very high density often exceeding 108 cells mL-1 may develop. Compared to a few thousands of cells per gram of substrate in natural environment, the density of food fermentations are about 4 or 5 times higher in magnitude. It is actually this difference in concentration that emphasizes the importance of processing facilities in the development and preservation of biodiversity. In actual fact the single manufacturing facility does not necessarily have an extremely high biodiversity at the level of strains because a whole series of operations have led to an unconscious mass selection out of which few jambs emerged. These operations are the reuse of “mothers” who have given good products (like many traditional wines and vinegars), reuse of “good barrel”, or those in which the best wine was produced, but also accurate washing, decontamination and, ultimately, the dismantling of the barrels from which a bad product was obtained.

The fact that in specific processing sites high biodiversity is not necessarily present, may also be due in some cases to the limited means by which this biodiversity has been detected and then characterized. In any case, the key aspect to be noted is the fact that in typical production areas biodiversity is high, especially considering the many small and varied production facilities, such as the many wineries that literally carpet the wine producing areas or small dairies scattered all over. The complex orography of our territory has fueled the development of specific structures, to be found possibly in adjacent valleys, with obvious enhancement of biodiversity. From these geographical, socio-political and traditional aspects (including the much-abused “parochialism”) originated microbial biodiversity for food and agriculture. Much of this biodiversity has remained in individual plants and could still be kept there.

At present, to maintain biodiversity in one’s own plant basically means to put in place all the activities to avoid mass contamination by contaminants strains without preventing, however, the possible evolution of the microbial wealth in the plant. As that evolution is not necessarily positive in terms of quality, strategies should be put in place to save at the same time biodiversity together with the producers whose work depend on it.

3.7.3 In situ conservation of microorganisms

As for soil microorganisms *ex situ* conservation, i.e. in the laboratory, represents only a small part of the environmental reality. In fact, it is well known, that only 1% of the microbial population of the soil, the population that actively contributes to the main

Soil Samples collected in the red garlic terroir (Castelliri – Frosinone – Italy) (photo by Paola Taviani) tenance of soil functions and fertility, may actually be cultivated. This means that only 1% of the principal architects of life in the soil can be isolated and stored in *ex situ* collections. This does not mean that conservation techniques described may not be useful and interesting to conserve and study *ex situ* those organisms recognized as architects of a particular process or action. For biodiversity related to soil functions and its fertility it is important instead to apply *in situ* conservation, as described in the relevant chapter. Moreover, in the case of this work, ecosystem conservation may prove more suitable, i.e. analyzing and monitoring microorganisms in relation to crops/strains.

What is the conservation of biodiversity at ecosystem level? It is the study and the subsequent evaluation of the microbial diversity associated with a particular strain or plant species. It is known from literature that each plant species in the soil releases some root exudates also as a result of climatic and environmental characteristics, which will attract a specific microbial population. This will create the edaphic microenvironments that constitute food webs associated with specific plant. This means that especially in the case of typical plant species or those risking erosion, the sole *ex situ* conservation of plant germplasm may not provide the desired result.

The microbial diversity is largely related to land management, so the bacterial communities being the most represented are also the most studied in order to predict the fertility of agricultural soils; the soil fungi are less studied, although they represent a large part of the microbial mass, they are involved in fundamental processes such as the degradation of organic residues and have a primary role in the ‘C sequestration. The main obstacle that limited research in this area has always been the difficulty of growing soil microorganisms in vitro, making it impossible to study them. The possibility of studying soil microbial communities starting from nucleic acids is given by new molecular techniques that allow characterization of even the non-cultivable organisms.

This allows to overcome difficulties associated with traditional microbiological reco gnition and it makes possible the characterization from the point of view of quality and quantity of microbial communities, thus allowing to calculate, with greater ease than in the past, the indices of microbial diversity of agricultural systems so for them to be also applied for the monitoring of biodiversity.

3.8 Optimization of storage protocols in *ex situ*, in factories and *in situ* collections

3.8.1 Optimization of ex situ conservation

Ex situ conservation, or microbial collections, present the advantages and limitations listed above. The optimization of storage protocols must consider the following principles:

1. **To limit selection of populations in complex phase of isolation**. This can be obtained using isolation media as universal as possible and avoiding enrichment. In case interest is directed towards forms of biodiversity not prevalent in the land, it may be appropriate to use specific land or selective conditions. The use of liquid culture media should be avoided, to reduce competition among crops with different fitness in specific cultural conditions.

2. **To limit changes induced by a culture medium necessarily different from environmental conditions of the substrate from which the microbe was isolated**. This is achieved by maintaining cultures within isolation media only for the time strictly necessary to the operations of isolation
3. **To give priority to conservation rather than to full characterization of the culture.** This consists in freezing strains at -80 ° C immediately after the second re-isolation, or even just after the first isolation, then proceed to the re-isolation and possibly

   freeze the strains from second isolation . Certain features such as the presence of capsule or the ability to sporulate are lost massively after a few generations in land laboratory.
4. **Assessment of microbiological purity of the strain**. This is carried out through careful macroscopic and microscopic observation. In case of doubt, there shall be a new insulation. On the other hand, the natural polymorphism of many species must not be undervalued.
5. **Maintaining the culture under conditions that minimize change**. Maintenance should be carried out directly in liquid nitrogen or at -80 ° C, avoiding prolonged keeping in culture.
6. **Provide for reliable identification**. Depending on the prevailing technique it is appropriate to proceed with unequivocal identification systems, even though these are standard name-and-type lists.
7. **Perform de-replication so as to limit redundancy of collections**. De-replication is the operation that allows to put identical strains in a single group. Usually one or few blocks per group are kept , while the others are discarded as they are regarded as identical copies. Techniques of de-replication include all systems for molecular characterization described in Objective 2 and techniques of metabolic fingerprint such as FTIR (Fourier Transform InfraRed spectroscopy).
8. **Registration of all the information gathered in a special electronic database**. With regard to information, reference is made to pick slips previously mentioned.

3.8.2 Optimization of conservation "in factory"

The conservation of crops in factory means keeping them in normal conditions of use. Two different ways of conservation can be implemented: *dynamic and static conservation.*

Dynamic conservation does not impose significant restrictions on the use of strains, except for the introduction or mixing with cultures of different origin. This kind of conservation may maintain biodiversity neither at community level nor at the level of individual components and it somehow reflects an evolutionary trend which is open to problems of various kinds (e.g. contamination from raw materials) and to selection within the micro biota.

Static conservation is very restrictive and tries to maintain strains under conditions such as to avoid any kind of changes. In static conservation the following must be avoided: contamination (especially if massive) by the micro biota of raw materials: introduction or mixing with other crops, even if they come from the same area; changes in technology;

and environmental contamination of any kind. In traditional conservation too two different approaches are observed, which somehow recall these two schools of thought. For example, in the conservation of bread-making sourdough two different qualities have been passed down called "the peasant's way" and " the baker's way".

In the first one, all the sourdough is used to form the dough from which, before cooking or before the loaves are formed, an aliquot is taken to be retained. In the baker's way only a part of the sourdough is used in the formation of the dough. The remainder is mixed with flour and water and separately left to rise.

Obviously, the two ways are different and the baker's allows a more accurate maintenance of the sourdough, in addition to freedom of integrating the dough to bake bread with other ingredients, perhaps incompatible with sourdough itself.

The attention to devote to this type of conservation can be outlined in the following principles:

1. Avoid introduction of allochthonous microorganisms.
2. Avoid mixing the inoculums with components having a high microbial level.
3. Avoid environmental contamination (for static conservation).
4. Avoid contamination from raw materials (for static conservation).
5. Avoid technological changes that could destabilize the inoculums.
6. Maintain different inoculation rates in *ex situ* collections.
7. Periodically check the quality of products processed with the preserved inoculums.
8. Promote the dissemination of the inoculums among different processors, possibly within confined areas.
9. Promotion of quality products: the politics of quality for agricultural products may have positive impacts on biodiversity. The protection of "traditional" products typical of Italian regions is indirectly useful for the conservation of biodiversity as it is associated with the environmental system and cultural heritage, to crafts and local art.

Soil Characterization: analysis of the profile (photo by Bruno Pennelli)

3.8.3 Optimization of in situ conservation

In Italy, *in situ* conservation is possible in areas where traditional agriculture is practiced, especially if placed within protected areas, not only for the binding regime derived from it, which is useful to ensure continuity in land use and management of agro-systems in co-evolution with the biodiversity present in them, but also because it provides easier access to support schemes for production.

Conservation of microorganisms *in situ*, or better said on farm can be practiced in conjunction with conservation of plant germplasm. Anywhere conservation actions are conducted for plant germplasm, there it will be necessary to keep soil microorganisms. The conservation protocol is very simple: the soil and its fertility must be preserved according to the actions outlined herewith.

This analysis should be conducted according to an in-depth study by" level" of hierarchy. Level 1 will have to be considered indispensable and must provide the basic characterization of the soil in relation to the physio-chemical and biological properties. One of the most important phases is the measurement of biological fertility (IBF) according to the procedure described in detail in the guidelines. The second and third levels are recommended for districts of great ecological and economical importance, or where there are species threatened by high genetic erosion.

Level 4 instead should always be practiced. So levels 0 - 1 - 4 will always be performed, while levels 2-3 in the case of ecological district of great value or at risk of genetic erosion.

Level	Action
0	Matrical analysis
1	Evaluation of the Conservation IFB of soil *ex situ*
2	Analysis of genetic and functional composition of the microbial community
3	Sequencing and characterization of specific species and possible conservation *ex situ*
4	Space-time bound monitoring. Spatial monitoring may be voluntary , while time-bound monitoring is compulsory.

Level 0

The farmer-custodian must be helped to fill in the matrix and guided in its interpretation. S/He should be explained what macroscopic elements characteristics (morphological) must be observed over time and how to interpret the changes. The farmer -custodian will have to contact the regional technical services when s/he might observe changes perceived as non-standard. The farmer should note any changes in land management with respect to time 0 (fertilization, irrigation, processing, any rotating crop, etc.). S/He should also be asked to keep 1 kg of air-dried soil in dark glass bottles, sealed and labeled. Every 5 years 1 kg of air-dried soil will need to be collected on the same site and sampled.

Should heavy changes or natural disasters intervene in land management a new 1 kg of air-dried soil will need to be collected and stored, noting on the label the reason for such new sampling. In the instance of different sequences it will prove necessary to get a sample of all species if they are subject to conservation of germplasm.

Level 1

The analysis of the biological fertility IFB (index of biological fertility) must be carried out in laboratory soil corresponding to the crop germplasm subject to conservation of and for each species retained. The methods of soil sampling are the same as those described for soil conservation *ex situ*. This analysis will have to be carried out every five years for soil conservation *ex situ*. This analysis will need to be repeated if heavy changes in culture management become evident. This analysis could provide important information about the effectiveness of any corrective actions that affect fertility.

Level 2

It consists of proper identification of the real biodiversity of microbial communities in the soil. In fact, through the extraction of DNA from soil, abundance and richness of biodiversity can be observed. It's a measure that can only be undertaken in the laboratory. This should be carried out wherever plant germplasm is preserved, and certainly wherever the microbial diversity of soils need to be known. Given the cost of this analysis, however, it is advisable to perform it in places of special importance. In this instance too, level 4 should be repeated over time according to what reported in the appendix.

Level 3

More information on "who does what" or "who am I" can only result from this level of detail. It still deals with molecular analysis to be conducted in the laboratory to characterize the specific organism and the specific community. It should be aimed at screening that may emerge from the analysis of level 2. If in an ecological district showing molecular abundance and wealth were to emerge typical bands associated with a particular plant species in that particular area, soil and climate, characterization will be needed because that particular community might be bi-univocally related to the plant species and then be crucial to its survival. All the samplings of soil for the analysis of 2nd and 3rd level should be carried out as previously reported. If this microorganism appears as cultivable, then it may be isolated and preserved in collections.

Interview with farmer–custodian (photo by Paola Taviani)

Level 4

It consists solely of the timing and spatial actions. Regions will have to establish on how many sites biodiversity of soil should be monitored.

1. At this point some campaigns for monitoring over time may be organized: always on the same site through surveys every 5 years.
2. On the same strains, but on different sites in the region: one-off, but should different organisms be found a new data collection should be organized.
3. At random, without correlation to crops, but following the example of the European standard “LUCAS” within squares of 9 km x 9 km.

3.9 Definition of risk of extinction and of genetic erosion

In this chapter the risks of erosion, extinction and replacement which may threaten microbial biodiversity of agricultural interest were analyzed. This analysis took also into account the specific nature of microbes such as their number, their reproductive speed and their extreme adaptability.

a. **Extinction**: the disappearance of entire microbial species is very unlikely, so microbial diversity is hardly at risk

b. **Substitution**: Substitution of strains with others of the same species or other species is very likely and frequent, so microbial variability is at risk and above all, the specific variability that might have improved territories and food

c. **Erosion**: the loss of microbial and overall biodiversity is more than likely, especially where good agricultural practices and processing are not followed. This aspect will be discussed in the following paragraphs, especially in relation to the soil. On the contrary, erosion and replacement in food systems are likely to take place (especially with technologies that excessively reduce the microbial component).

Concept of microbial genetic erosion

Microbial genetic erosion is defined as the loss of genetic diversity in a specific area and in a given period of time, associated with the concept of loss of a function. The concept of erosion is directly linked to the concept of genetic diversity and functional diversity.

In an ecosystem composed by numerous metabolic and energetic pathways such as the soil, the altering of a species determines a minor effect on other present species than what the same alteration could cause to the species of an ecosystem having low energy network

3.10 Systems for assessing the risk of extinction, erosion and replacement

3.10.1 Monitoring microbial biodiversity in general

Monitoring of microbial biodiversity is largely depending on the technologies described in the section on shared methods for identification and characterization.

To synthesize and recall the concepts described above, it is useful to remember that:

1. **Diversity** concerns the number of species in an environment, food or *habitat*.
2. Species are defined by means of the **identification** process.
3. **Variability** concerns the strains, that is to say the variants of each species present in a given environment, food or habitat.
4. Variability is defined by **characterization**.
5. **Biodiversity** is the integration of diversity and variability.
6. Not all biodiversity can be studied by microbiological methods, but this can be done through **metagenomic** molecular strategies that can analyze the DNA (or RNA) of an environment, food or *habitat*.

Assessment of the risks of loss or change of biodiversity must be carried out as appropriate by analysis of identification or characterization depending on whether one is more interested in the level of species or strain. Typically, in the environment we focus more on the composition at the species level within extraordinarily complex communities. At dietary level, vice versa, the concept prevails that particular strains imprint flavors or peculiar characteristics to various foodstuff, so much so that maintenance or improvement at the level of strain prevails on considerations at the species level. For example, there is no doubt that wine, as defined by tradition, by product category and by law, is the product of fermentation of grape provoked by the yeast *Saccharomyces cerevisiae* and not by other species.

On the other hand there is evidence that various strains produce different wines from the same grape (MUST), and each particular strain has its peculiar characteristics which can be variously appreciated, but that place the focus on the level of taxonomic variability among strains. Possibly in the near future environmental microbiology will turn more and more to the size of microbial communities, as well as to cross-domain yeast bacteria in food, which represent the reality developed in the traditional preparation of food and beverages transformed by microorganisms.

3.10.2 Monitoring agri-environmental microbial biodiversity

From what discussed so far, it is clear that, albeit with some difficulty and with a certain degree of approximation, it is possible to define the microbial diversity of soil and give a time-bound characterization in terms of natural or pathological fluctuations. Characterization of microbial diversity of a soil and its biodiversity in general, must be built by levels of approximation and using the proposed markers according to suggested hierarchy.

Appropriate biological methods to study soil combined with physical-chemical properties may serve as indicators of changes in soil quality and provide early indications of whether there has been an alteration or modification of the “soil biota”. However, Kennedy and Papendiek (1995) pointed out that although tools to characterize the soil are numerous, there are few strategies to integrate these tools to determine the quality of the soil and its biodiversity in a way that is unique and indisputable for all situations; indicators that could characterize a given situation are yet to be found for each instance.

It is important to consider the standardization of every aspect of the method, from sampling through storage and pre-treatment of samples to the current analytical procedures, to interpretation and presentation of results.

3.11 Conclusions

The flow chart introduced by image 3 summarizes all the material presented in the guidelines taking into account the following.

1. **Biodiversity** is constituted by the diversity and variability having both **environmental** and **food** origin. It would be appropriate to consider other sources, not strictly for food and agriculture from which to draw biodiversity to be used in agriculture. In the environmental field sensu lato there are a whole series of realities to be considered, whose biodiversity is could be used in the agricultural field. To be taken in consideration, for example, is biodiversity that is forming in landfills, plants for the production

of methane and contaminated sites. All this micro-flora can be used for example for the enhancement of *in situ* biomass waste from agricultural production.

2. **Monitoring** has been widely described and discussed as well as monitoring procedures to prevent or at least to measure erosion and replacement. Monitoring occurs twice in the flow chart. The first (prospective) should be a quick and inexpensive reconnaissance based mostly on preliminary and objectives parameters (markers), with some detailed analysis through laboratory markers. Control monitoring (in the lower part of the graph) is a specific monitoring functional to controlling risk situations and to keeping the operation of *in situ* and in-factory. Monitoring therefore arises as the key measure for the conservation of biodiversity. It must be as flexible as possible and ideally reducible to a single technique.

3. **Risk factors** of biodiversity can be condensed into four:

   a. Production or technological change

   b. Intrinsic importance of the food or the environment to be preserved

   c. Replacement

   d. Erosion

   All factors are conceivable on the basis of preliminary and objective markers. The laboratory markers (and in particular the meta-genomic techniques such as the DGGE) must be used either as a more in depth prospective monitoring or as monophasic system in the monitoring of conservation.

4. **Conservation**. The three modes *in situ*, *ex situ* and in-factory were explained and several conservation techniques described.

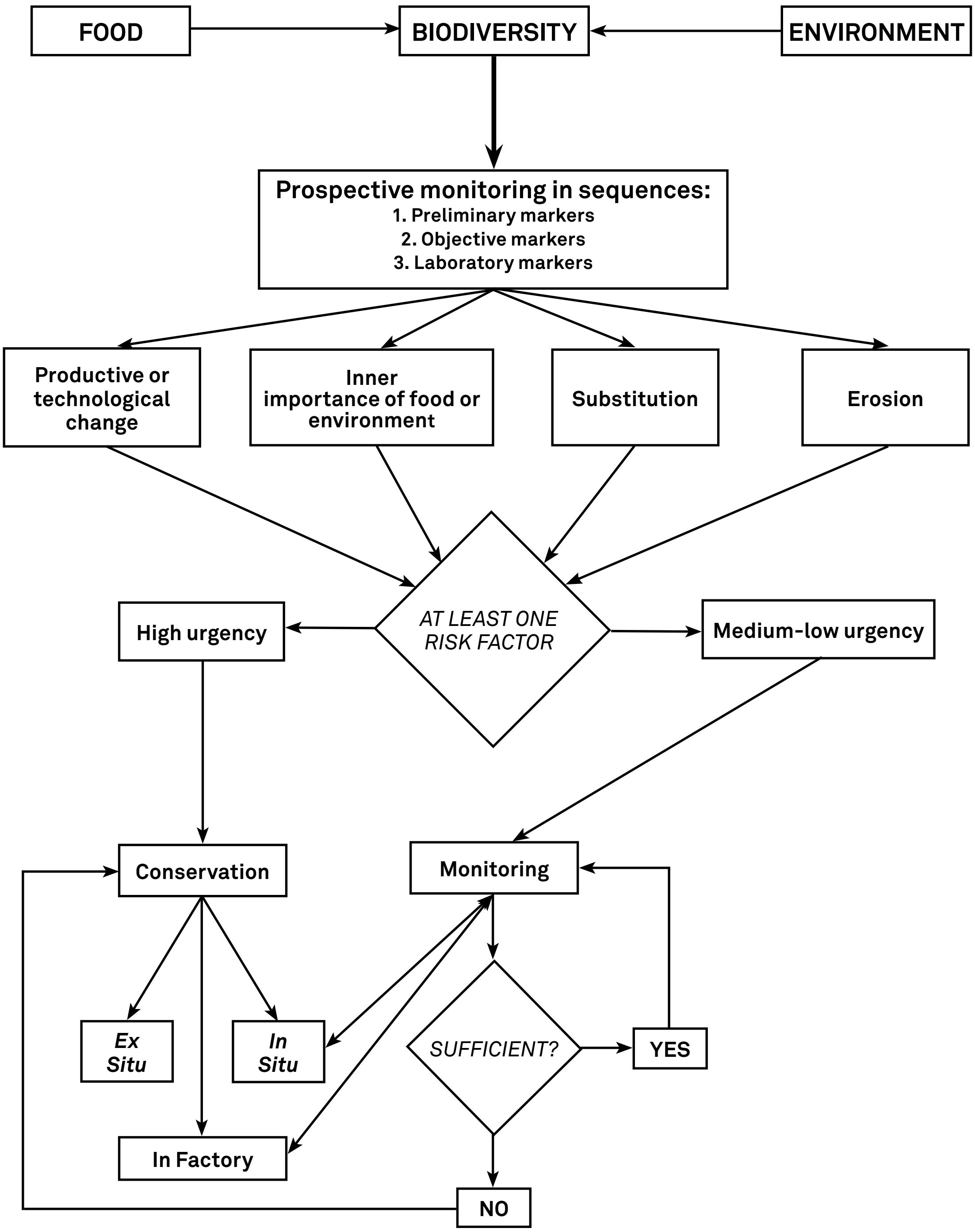
5. **The technical and political choices** are highlighted and italicized, while set between two diamond signs.

The first choice concerns the assessment in presence of at least one single risk factor (diamond sign at the top). The complexity of choice lies in the evaluation of monitoring data describing the situation of microbial biodiversity, especially when standards for assessment and comparable conditions are missing, as it is the case at present. The smaller the number of these standards, the greater the weight of discretion and subjectivity of choice. Given that this discretion cannot and should not be abolished, especially on the political phase of making a choice, greater standardization and normalization of the data can only have highly beneficial effects. In this regard, there are two possible measures to be taken, one of technical and scientific nature, the other logistical and organizational, still considering that the two possibilities should be integrated and not considered as a substitute.

a. **Technical and scientific solution**. It consists in comparing situations at different levels of risk with one or very few techniques. The level of risk may be expressed as an index that vary between 0 and 1 by means of the following formula where Rn is the normalized risk, Ri the risk involved in a given situation, Rmax and Rmin the minimum and maximum risk found in the course of these studies:

$$Rn = (Ri-Rmin) / (Rmax-Rmin)$$

b. **Logistical and organizational solution**. It would involve to systematize the agencies and stakeholders interested in the protection of biodiversity in order to promote standardized monitoring giving results to be merged into a single database and organize it in order to provide real-time values of Rmin and Rmax of each food and environmental instance.The decision on the appropriateness of monitoring (diamond at the bottom of the diagram) as the former is subject to subjectivity and discretion. Clearly, the choice must take into account the sustainability of choice, knowing that *ex situ* conservation is severely limiting in terms of the maintenance of effective microbial genetic resources and that is the most expensive, especially in terms of energy. On the other hand, preservation in the form of collection is the first choice in case of change of technology or high risk of erosion or replacement.



It is then important to consider the overall costs of the form of conservation and the actual resources available. In some instances it might be difficult to perform even *in situ* or on farm conservation, so it is strongly recommended to develop effective monitoring systems that will:

a. Inform about the structure of microbial diversity in various typological, geographical and temporal areas and during its development.

b. Avoid initiating procedures for conservation where it is not essential.

c. Encourage less expensive forms of conservation (*in situ* or on farm), highlighting the level of risk to which microbial diversity is subject during these methods of conservation.

d. Act as a support tool to isolation to quantify the portion of microbial biodiversity effectively insulated and included in the collection.

Image 3 - Flowchart on decisional pattern for sustainable conservation of microbial biodiversity for food and agriculture

From these considerations it is clear that sustainable conservation of biodiversity requires a different approach from that taken so far with the setting up of collections left to the sensitivity of the individual, but often highly redundant in many cases where the same source of biodiversity has been repeatedly isolated. The proposed way forward is therefore to promote awareness of biodiversity and prefer forms of conservation that allow free evolution of biodiversity in natural environments or in those affected by agricultural activity.

Finally, it is clear that to make a choice requires such skills and sensitivity that should be supported by relevant information and especially enhanced by specific training which could be directed to all stakeholders involved in the maintenance and development of microbial biodiversity.

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