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Identification, collection and agro-morphological characterization of lentil (*Lens culinaris* M.) landraces of Molise

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To Mike Ambrose
and Dalila Trupiano
the real best friends

CHAPTER I

Introduction

1.1 Biodiversity definition

In 1992 the United Nations Conference on Environment and Development held in Rio de Janeiro approved the Convention on Biological Diversity (CBD), signed by 168 countries, including Italy. The text states that the ecological, genetic, social, economic, scientific, educational, cultural, recreational and aesthetic of biodiversity which art. 2 defines as: "*The variability among living organisms from all sources including inter alia terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part: this includes diversity within species, between species and diversity of ecosystems*". Since then, the definition remains unchanged in its fundamental concept.

The term Biodiversity, probably, was used for the first time in the second half of the 80s by W. G. Rosen in a forum on Bio-Diversity organized in Washington by the National Academy of Science and the Smithsonian Institution. In reality in 1980 Thomas Lovejoy invented the expression *biological diversity* and a couple of years later M. A. Wilcox had used the term Biological Diversity to indicate the variety of life forms, the ecological role that they have and the genetic diversity that they include. E.O. Wilson (1988) defines biodiversity as: "*the diversity of life on earth, in all its forms and varieties*." The current definition of biodiversity is that of the United Nations Conference, which is enriched with specific definitions at three levels:

- genetic diversity (*intra-species diversity*);
- diversity of species (species, breeds or varieties of wild and domestic, *inter-species diversity*);
- diversity of ecosystems (natural environments such as oceans, lakes, rivers, forests, mountains, hills, deserts, grasslands, etc.).

Thus, the term biodiversity, having a broader meaning, is used to indicate, in addition to the diversity of living things (micro and macro organisms), the diversity of natural environments, with their distinctive features and the richness of the complexity of the relations are established between the environment and life forms that occupy (D. De Long, 1996; L. Gustafsson, 2000).

1.1.1 Genetic diversity

The diversity within a species is most frequently referred to as "genetic diversity" and is related to differences in genes and their recombination. Alternative forms of a gene are called "alleles". The populations are made up of individuals who for the most part are able to inter-cross freely, influencing the genetic variability that is closely linked to the reproduction system of the species. The set of genes in a population in all the different allelic forms is defined as the gene pool, while the particular combination of alleles that characterizes each individual represents the "genotype" (F. Lorenzetti, et al., 1996; 1994). The phenotype of an individual is the set of morphological, physiological and biochemical changes resulting from the expression of its genotype in a given environment.

Random mutations that occur at the level of DNA nucleotide sequence (point mutations, loss or acquisition of genes or portions of DNA), are a primary source of genetic diversity. Of genetic variation provided by mutation, acts another important factor related to sexual reproduction: recombination, which generates new gene combinations.

The genetic diversity among individuals within a population is the intra-population diversity. Populations of the same species can differ in the relative frequencies of alleles and in the type of allelic forms, and this diversity is defined genetic variability interpopulation or genetic divergence

From the evolutionary point of view, genetic variability is an advantage for the survival of the species, because it allows populations to adapt to environmental changes through the process of natural selection on a large number of genetically different individuals often over many generations.

The knowledge and assessment of genetic diversity is of fundamental importance in any plan of conservation of the species (F. Grassi, et al., 2006), especially in reference to those at risk of extinction and/or threatened.

1.1.2 Species diversity

Often diversity at the species level is associated with the term biodiversity as it relates directly to the diversity of life forms that inhabit the Earth, a particular ecosystem or a particular area.

The diversity of species can be expressed as the number of species present in a specific area (species richness), as the relative abundance of different species or under the evolutionary relationships between species that share the same habitat (phylogenetic diversity) (Grassi F., et al., 2006; Campbell, N. A., et al., 2008).

The level of species richness is not evenly distributed on our planet, it is usually more prevalent in the equatorial regions, and gradually decreases towards the poles or to particular areas where the density of individuals of each species per unit area is very high (dominant species).

Most experts believed that the biodiversity of species diversity is a key factor in maintaining the health of the ecosystem and the entire biosphere.

1.1.3 Ecosystem diversity

Ecosystem diversity includes the large differences between the types of ecosystems, habitat diversity and ecological processes that occur within each type of ecosystem (Commonwealth dell'Australia, 1993). As one might guess, the concept of ecosystem diversity is enclosed, and in reality is expressed in the two concepts previously exposed (genetic diversity and species diversity). Ecosystem diversity is a difficult concept to define materially due to a lack of clear boundaries between the different communities (a combination of species) and ecosystem. While the genetic diversity and species are easily locatable in an individual and / or a population, the diversity of ecosystem boundaries are more fluid "sliding" and so are difficult to define unequivocally. The same concept of ecosystem is very dynamic, as the only element that remains a common point between ecologists and the natural state is that of ecological processes, such as energy flows in which cycles of elements between the biotic and abiotic, are "conserved".

1.2 Value of Biodiversity

In the popular imagination the word "value" is immediately associated with the quantitative relationship of exchange that an object has with other property or money (economic value), or the utility that it represents for those who have it (utility value). Biodiversity has a complex value that cuts across the various environmental, economic, socio-cultural and medical pharmacology levels. The combination of all these aspects makes the total value of biodiversity.

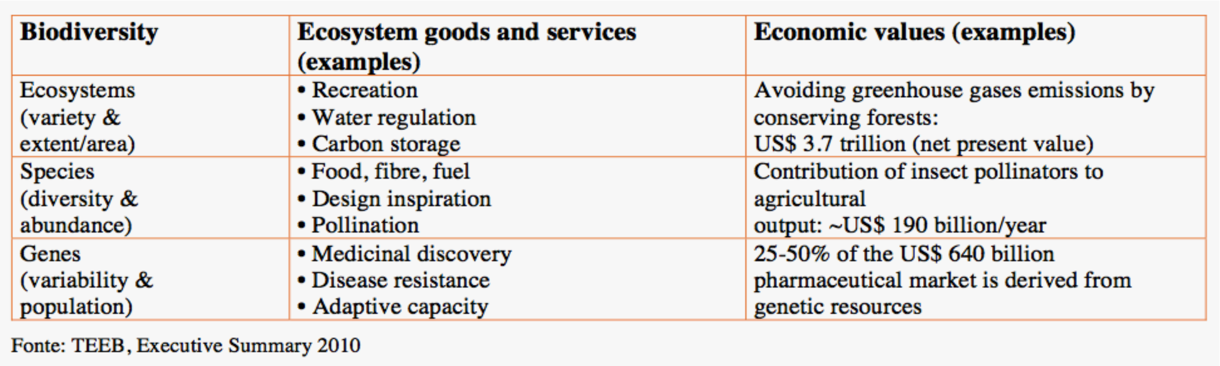
Pearce (2001) emphasizes the difference between biological resources and biodiversity: the biological source is only one component of biodiversity, mostly associated with a form of *direct use*, while biodiversity as a whole is much more than the sum of its components and this complexity is also due to the difficulty in quantifying its economic values of *non-use*.

In particular, the values of biodiversity are divided into: environmental, economic and cultural value.

Environmental value is characterized by the complexity (not to be confused with the amount) of species that have evolved and adapted in a given environment (including humans). This value increases in direct proportion to the threat of extinction of the ecosystem in question.

Economic value is characterized by all economic benefits generated by biodiversity. Conceptually, total economic value of a biological resource consists of its use value (this might be the use of a forest for timber or of a wetland for recreation) and not use value. Use values are further divided into direct use values, which refer to actual uses such as fishing; indirect use values, which refer to the benefits deriving from ecosystem functions such as a forest's function in protecting the watershed. There is finally an optional values which is a value approximating an individual's willingness to pay to safeguard an asset for the option of using it at a future date. In some cases the economic value is easily estimated and the models used are well established in other cases it is difficult to quantify the economic benefits generated by biodiversity, because it is closely connected with the other values that characterize it. For example, a tree or ancient woodland has a market value as firewood which is easily calculated, while its environmental and cultural value are much more complex to estimate, if it's possible. The fact remains that man depends on biodiversity for food, fiber, medicines and other renewable resources, not least the sources of renewable energy produced with modern technology. An idea of how biodiversity can be influence the economy is given by the following table, which summarizes the relationships between biodiversity, ecosystems goods and services and economic values.

Tabella 1. Relationships between biodiversity, ecosystems goods and services and economic values.



Biodiversity	Ecosystem goods and services (examples)	Economic values (examples)
Ecosystems (variety & extent/area)	• Recreation • Water regulation • Carbon storage	Avoiding greenhouse gases emissions by conserving forests: US$ 3.7 trillion (net present value)
Species (diversity & abundance)	• Food, fibre, fuel • Design inspiration • Pollination	Contribution of insect pollinators to agricultural output: ~US$ 190 billion/year
Genes (variability & population)	• Medicinal discovery • Disease resistance • Adaptive capacity	25-50% of the US$ 640 billion pharmaceutical market is derived from genetic resources

Fonte: TEEB, Executive Summary 2010

Recognition of a broader total economic valuation of natural assets can be instrumental in altering decisions about their use, particularly in investment and land-use decisions which present a clear choice between destruction or conservation.

Many political decisions made to protect biodiversity and have created new markets between nations and even between continents. For example, since 1980, WWF has used more than 400 million dollars in trust funds for **debt for nature swap** (safeguarding the biodiversity of developing countries in exchange for zeroing the debt to creditor countries) between which include:

- In 2002, between France and Cameroon signed the first agreement was debt-for-nature swap worth **$ 25 million** in 5 years to protect the second largest tropical forest in the world.
- In 2008, WWF contributed to the construction of the largest contract debt-for-nature swap in Madagascar for a value of about **$ 20 million** in exchange for the conservation of island biodiversity.
- 2002 and 2008 the Peruvian government and the U.S. reached an agreement for about **$ 40 million**, according to which Peru is committed to preserve for 12 years, over 11 million hectares of forest with all the species that it hosts.

Other examples of Global political choices are: PES (Payments for Environmental Services), GEF (Global Environmental Facility); Wetland Banking, Coalition of Rainforest Nations, International Network for Bamboo and Rattan, NTFP trade, etc.. (Marino, D., et al, 2010; The Economics of Ecosystems and Biodiversity - TEEB, 2010).

Examples of markets generated by the policies for the protection of biodiversity more easily understood by consumers are:

- organic farming, the market has tripled between 1999 and 2007;
- ecotourism, since the 90s has grown by 20-30% per year;
- certified products (such as those that are forest quadrupled since 2005 to 2007);
- the energy sector (wind, photovoltaic, solar and hydro);
- Ecolabel with fish products increased 50% worldwide between 2008 and 2009 (TEEB report, 2010).

One particular market that is invaluable to biodiversity is the medical-pharmacological. The use of plant biodiversity for the production of medicines has been used since ancient times. Most of the active ingredients (drugs) that are used in medical, pharmaceutical, herbal and cosmetic products are extracted from plants and animals or products with the help of microorganisms. Among the most famous is penicillin (antibiotic), acetyl salicylic acid (aspirin, pain reliever, etc..) Or the even more natural as propolis (antibacterial), etc. The *cultural value* of biodiversity is closely linked to two factors of a society: the habits and customs. The loss of even one species, varieties or new technologies, will have, and still affect, loss of use of knowledge and skills accumulated. This type of effect is easily noticeable in rural civilization, where, in the last century, constantly witnessed the replacement of the traditional varieties grown with technological innovation. Often these substitutions were very sudden leaving no time to understand whether these actions were, in their holistic aspect, right or counterproductive.

To conclude, the increasing scarcity of natural resource is no longer a hypothesis at the basis of a scientific exercise, but is becoming a tragic reality and the science economy (or community) is called to give answers about its efficient allocation. Paradoxically, the economy, which has never considered it useful or necessary to “take account” of the environment and biodiversity, today is the linchpin on which to place all hopes of saving what remains of natural ecosystems (Marino et al. 2010).

1.3 Measurements of biodiversity

A better understanding of biodiversity can be obtained assess biological diversity. Measure of biodiversity cost-effectiveness used guide investments in conservation must have some index or set of indices of biodiversity change able to distinguish the components of biodiversity (genetic diversity, species diversity and ecosystem diversity).

1.3.1 Measurement of genetic diversity

The assessment of genetic diversity represents the analysis and conceptualization of genetic differences within and among populations (collection of individuals, geographical race, subspecies, species, or higher taxonomic group). These differences can be measured in terms of phenotypic traits, allelic frequencies or DNA sequence differences.

Phenetic diversity is based on measures of phenotypes (morphological and physiological characteristics) of individuals which share the same characteristics. This method avoids examination of the underlying allelic structure. It is usually concerned with measurement of the variance of particular morphological traits and can be easily measured. However, their genetic basis is often difficult to assess.

Measures of allelic diversity require knowledge of the allelic composition (number of variants and these variants of a gene) at individual loci and it may be measured at the individual level, or at the population level. In general, the greater the genetic diversity results from more alleles and their frequencies, and the more loci that are polymorphic. Average expected of heterozygosis (the probability that two alleles sampled at random will be different) is commonly used as an overall measure.

Finally, genetic diversity might be characterised at DNA level by a range of techniques: by observation of inherited genetic traits, by studying the chromosomes and their species specific karyotype, and by analysing the DNA information using molecular technology.

1.3.2 Measurement of species and communities diversity

Species diversity is a function of the distribution and abundance of species within a region or given area. In its ideal form, species diversity would consist of a complete catalogue of all species occurring in the area under consideration, but this is not usually possible unless it is a very small area.

Alternative measures being developed augment species richness with measures of the degree of genealogical difference derived from cladistic methods, such as close-to-root species, higher-taxon richness, spanning-tree length and taxonomic dispersion (Williams et al, 1991).

Finally, it is important include generic measures of community level diversity. These include biogeographical realms or provinces, based on the distribution of species, and ecoregions or ecozones, based on physical attributes such as soils and climate.

1.4 Causes of Loss of Biodiversity

The major source of the recent interest in diversity of life on earth arises from the feeling of a rapid decline in biodiversity. Extinction of species is part of an evolutionary process. However, during recent times, extinction rates are ten to a hundred times higher than during pre-human times (Sinclair, 2000). Most of the authors (Millennium Ecosystem assessment, 2005; Campbell, N. A, et al., 2008; Padovani, L. M., et al., 2009) believes that the main causes of the loss of biodiversity are:

- Reduction, processing and / or degradation of habitats;
- excessive predation, or collection;
- spread of exotic species;
- climate change.

Excluding the last point and analysing more carefully the causes listed on one realises that all can be traced to the expansion of human society coupled with economic development. This view holds that the expansion of human society needs to be stopped and reversed to stop biodiversity loss.

Economists and biodiversity conservationists tend to agree on one central point regarding the relationship between the economy and biodiversity: damage to biodiversity increases dramatically during the course of economic development until, at a certain level of wealth, opportunities for biodiversity conservation can potentially be improved, but there is always the risk of irreversible damage to biodiversity maintaining and regenerating ecosystem processes. There is also strong agreement about the central importance of development for the eradication of poverty.

The increasing world population and the necessary economic growth in developing countries are the causes of anthropogenic environmental change, which together with the natural changes cause **genetic erosion**. The genetic erosion is defined as the more or less rapid decline in genetic variability of populations or entire communities, which, if it persists can lead to the extinction of the species (or communities) in question.

For example, DIVERSITAS[1] has warned of an alarming increase in the extinction of species because of threats to biodiversity and ecosystems caused in particular by pollution, climate change and urban spread. In addition they have found that the extinction rates of species are much higher than had been predicted only a few years ago.

One of the main causes of biodiversity loss is that many environmental components are not bargaining chips, and thus have no price, the environment is rather the final destination of the remains of the "journey productive" in the sense that it ends up with all the negative effects arising by human activities. For example the production wastes which are buried, the exhaust gas and liquid chemical products that have an impact on air, soil and water are not taxed.

1.5 Effects of biodiversity loss

The possibility to predict to what extent and by what mechanisms the loss of biodiversity will impact on human welfare is limited due to our incomplete and fragmentary understanding of the complex processes involving ecosystems and communities in which we live. However, conservation biologists argue that in light of current knowledge gives clear evidence that the welfare of human society will be greatly reduced by the loss of species and ecosystem services (Balmford and Bond 2005).

Biodiversity directly provides or is a major factor in maintaining the critical "ecosystem services" on which development depends (figure 1), including air and water purification, soil conservation, disease control and reduced vulnerability to natural disasters such as floods, droughts and landslides.

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[1] Second DIVERSITAS Open Science Conference: Biodiversity and society: understanding connections, adapting to change. 13-16 October 2009 - Cape Town, South Africa; DIVERSITAS is an international, multidisciplinary network of scientists that addresses the linkages between biodiversity and human activity. In recent research DIVERSITAS has released statements to coincide with the meeting of the DIVERSITAS group of global experts on biodiversity in Cape Town October 2009.

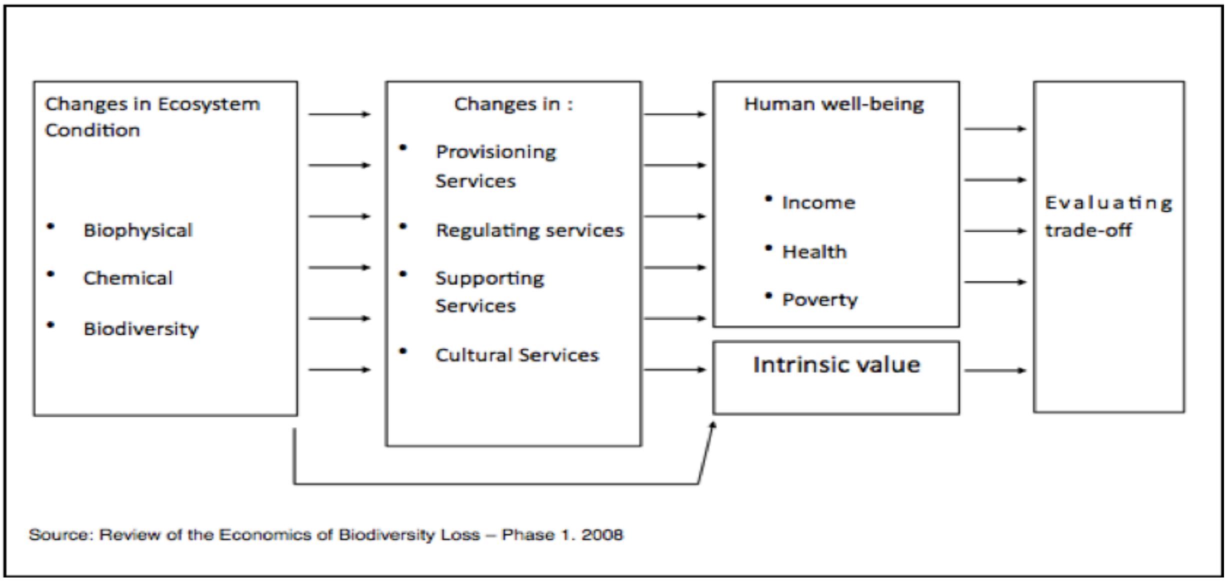


Figure 1. Mapping the link between biodiversity, ecosystem services, and human wellbeing (Review of the Economics of Biodiversity Loss – Phase 1. 2008).

In some cases the services provided by ecosystems are particularly important to the livelihoods of local populations, as in the case of semi-arid areas. In other cases, the importance of ecosystems is also expressed on a more global scale, as are the polar ecosystems, which are particularly sensitive to climate change and the potential to trigger feedback mechanisms on climate control at the global scale. In further cases, it is necessary to deepen some functions to reflect the complexity of a given ecosystem, regardless of its bio-geographical characteristics: for example, a change in ecosystem structure, accompanied by a loss of biodiversity, can result in an increase in the spread of some pathogens and in the manifestation of some diseases with large scale implications to human health.

The report of the Millennium Ecosystem Assessment (2005) clearly identifies biodiversity and ecosystem services as the prerequisite for social-economic development and supports the evidence that their loss is a source of food insecurity, deterioration of social relations, higher vulnerability economic and in some cases decrease or total loss of freedom of choice and action.

To conclude, it is not yet possible to estimate the loss of landscape due to the visual impacts of processes such as open dumps, which are eventually covered with a layer of soil and the area is “RE-NATURALIZED.” For now we can estimate the costs of re-naturalization, but we are not able to estimate the damage that may arise in future years due to loss of ecosystem services. Thus, natural capital (biodiversity and Pharmaceutical and Bio-organic), although it is becoming scarce, is not allocated efficiently, due to the lack of markets for this type of goods and well-defined property rights.

1.6 Loss of biodiversity in agriculture

Some 10 000 different plant species have been used by humans for food and fodder production since the dawn of agriculture 10 000 years ago.

Yet today just 150 crops feed most human beings on the planet, and just 12 crops provide 80% of food energy, wheat, rice, maize and potato alone provide 60% (FAO 2004).

In countries that are so-called "developed", the loss of biodiversity in agriculture was fast and unconscious of the resulting consequences. The processes generated are essentially two: the euphoria of farmers and geneticists taken by the high yields of new varieties, triggered processes of genetic erosion which were often followed by extinction of traditional cultures and local ecotypes; the abandonment of fields and the transition from a rural to an industrialized civilization, which triggered both the loss of landraces is the degradation of the landscape and rural culture. A good example is what has happened in Italy after the Second World War until today. As well as experiencing the depopulation of less fertile and less industrialized areas, the population became more concentrated in the industrialized cities of the north.

There have been rare cases in which some researchers included, in advance, the danger of loss of this genetic variability of crops, with the remedy of the construction of the first seed banks.

Today we are witnessing the same events in developing countries, where the primary sector is a major source of income of the population and the replacement of local cultivars with cultivars more profitable purchased from third countries makes them poorer in terms of genetic variability especially dependent on international lobbying. For instance, according to one study, North America is completely dependent upon species originating in other regions of the world for its major food and industrial crops, while sub-Saharan Africa is estimated to be 87% dependent on other parts of the world for the plant genetic resources it needs.

Recognizing, safeguarding and using the potential and diversity of nature are critical points for food security and sustainable agriculture (FAO 2004).

1.7 Global activities for conservation and restoration of Biodiversity

The first real global step in defence of biodiversity was achieved in 1992 in Rio de Janeiro, with the Convention on Biological Diversity (CBD). At that time it was signed, by representatives of different nations, a regulation (not binding at the time but internationally binding at the time of ratification in December 2004) which provided for the application of policies in defence of biodiversity and sustainable development, in addition to financing structures applying *in situ* and *ex situ* conservation of biodiversity: parks, botanical gardens, seed banks and germplasm banks, nature reserves, etc.

After the Convention of 1992 many global organizations, continental associations through to small locals (both governmental and none) were formed for the preservation, restoration and maintenance of natural areas and all the functions that these involve.

A topic that has recently caught the attention of economists and policy makers concerns the damage caused to biodiversity and the loss of benefits associated with it, both from wrong policy choices but also by the absence of political action. For this reason, and based on previous experience, an approach increasingly being tested is to assess the damage caused to the environment and humans if biodiversity is compromised by incorrect policy choices or lack of policies for management and protection.

These important aspects are analyzed in the report The Cost of Policy Inaction (Braat and ten Brink, 2008).

There are a considerable number of international instruments concerned with protection and biodiversity conservation, mostly in the form of conventions and agreements, to create the required legislative framework of laws and regulations.

The current policies for biodiversity have varying degrees of efficiency. In general the more effective policies are those that are backed up by legal instruments such as, for example, those in Europe regulate the establishment of protected areas (http://europa.eu/legislation_summaries/environment/index_it.htm).

One of the problems hampering the effectiveness of conservation policies is the lack of sufficient funding in the medium - long term, resulting in policy implementation being both patchy and discontinuous in time, especially in countries in the developing world.

The limited effectiveness of conservation policies are often further reduced by the negative effects of determined policies from other sectors. It is the case, for example, of the Common Agricultural Policy (CAP), which historically has produced no appreciable effect on the conservation of biodiversity and ecological management as proper agricultural ecosystems. In fact, in the past it has promoted intense agricultural models with a high degree of mechanization, with significant damage to the species, breeds and varieties of agricultural interest, as well as for agricultural landscapes across Europe.

Today, in an attempt to remedy the damage of the past, the CAP is essentially aimed at reducing agricultural intensification, but its policies do not seem sufficiently adequate to address and solve the problem of land.

The awareness of the importance of the role of politics in the protection of biodiversity is increasing more and more as a result of the awareness of the economic importance of natural resources. This has developed through long and persistent lobbying by groups of people interested in conserving biodiversity, research centres and institutions for monitoring and conservation of biodiversity and ecosystems.

There are many examples globally and locally level. WWF's *Conservation Finance Program* has been pursuing for several years a series of initiatives aimed at long-term funding for conservation programs *(i.*e. *debt-for-nature swap).*

PES (Payments for Environmental Services) and GEF (Global Environmental Facility): the PES are payments for ecosystem services, in other words*for particular forms of subsidy paid to the owners or manager of the areas that wa*nt to preserve the ecosystem services that originate from them continue to be produced, while the PES refer to the local level, the GEF are the same as a subsidy on a global level.

Wetland Banking: Essentially a form of economic incentive for the restoration, creation and preservation of wetlands. The mechanism is similar to a bank account.

The Economics of Ecosystems and Biodiversity (TEEB 2009, 2010) is a study sponsored by the European Commission, which has produced reports articulated and well supported by data and case studies around the world.

Beyond *in-situ* conservation has also developed *ex-situ* conservation that began on a large scale about three decades ago in most countries as a means of preserving the diversity of plant species for future generations. The *ex-situ* conservation method is particularly suitable for the gene pool concept and can be achieved through propagation/maintenance of plant genetic resource centres, botanical gardens and seed banks (OECD 1999).

In addition to the seed-banks, other conservation strategies such as in vitro and cryopreservation are used to maintain collections based on different genetic materials (seeds, vegetative organs, propagules, fragments of tissue, cells) for species which are not suited to seed banking in the traditional sense. The use of *ex-situ* conservation techniques has become increasingly important at the international and national level for storing a wide variety of genetic resources and making them available for use, for example, in the improvement of crops or reforestation (FAO, FLD, IPGRI, 2004).

To achieve the objectives of *ex-situ* conservation of threatened plants, however, it is suggested by several parties to create regional networks of national and international coordination (Global Strategy for Plant Conservation, GSPC, target 16), to exchange knowledge and technology, develop synergistic actions of priorities set by mutual agreement while avoiding waste and duplication. With this in mind, in Europe there have arisen a series of national coordination structures (networks). Spain has made the Association *RED de Bancos de Germoplasma* (REDBAG), which coordinates the *ex-situ* conservation of the Botanical Gardens; operates in France network of governmental Conservatoires botaniques; in Italy there is: Italian Network of Germplasm Banks for *ex-situ* Conservation of Native Flora (RIBES).

RIBES is part of The European Native Seed Conservation Network (ENSCONET) began as an EU project which ran from 2004 to 2009. This is now a Consortium of some 30 institutes, European seed banks, botanical gardens and other institutes interested in seed conservation, from 18 European countries who wish to continue and spread the momentum for seed conservation in Europe generated by the ENSCONET project. The ENSCONET Consortium is coordinated from Kew’s Millennium Seed Bank. The ENSCONET Consortium maintains ENSCOBASE, the European Native Seed Banks' database, and the ENSCONET website. It also provides a platform for discussion and exchange of experiences between members.

The “Global Strategy for the Conservation of Biological Resources of the Planet” (The World Conservation Strategy, IUCN, Gland - Switzerland) identified in the botanical gardens as the most suitable tools for the protection and conservation of biodiversity. These structures allow the creation of collections of living plants, grown in a greenhouse or flowerbed with the purpose of reintroduction in the wild.

At the regional level there are two different but closely related approaches to the conservation of biodiversity. There is the practical approach that develops through the cultivation of local varieties *in situ*: Association "Arca Sannita" that has recovered hundreds of species and varieties of Molise crops and promotes the cultivation and distribution in the region. Another example is the farm "Melise" in the village Castel del Giudice, in "Alto Molise", where apples are grown and marketed along with other products like truffles and honey (all organic products).

Besides these examples of agricultural production are also the National Parks of Abruzzo Lazio and Molise; two reserves MAB (Man and Biosphere; UNESCO), Monte di Mezzo e Colle Meluccio, which joined a consortium that includes 7 municipalities for an extension of 25.758 hectares of Reserve of Biosphere, and a Oasi WWF in the municipalities of Guardiaregia and Campochiaro.

While the other approach involves scientific and technical researchers, universities and regional institutions for agriculture using techniques of *ex situ* conservation germplasm. In fact, there are two germplasm banks: the agency for regional development in agriculture and the University seed bank of Molise, with some related projects coordinated by the University. The most important project is Life Dynamo managed by the Biodiversity of Molise. The goal of Life DINAMO project is to increase local biodiversity through conservation action in production areas and state-owned, produced in collaboration with both public and private entities. The conservation actions are implementing measures which enhance the naturalness of the areas next to the SCI (Sites of Community Importance) and SPAs (Special Protection Areas), preserving some species of birds and amphibians and re-naturalization areas degraded by the plant species native shrubs and trees. The Model DINAMO has been calibrated on the Basso Molise territorial reality, characterized by a high incidence of rural areas and the presence of areas of high biodiversity value (Carraba P. et Al. 2011).

From what has been stated above, it follows that the situation for the natural environment (biodiversity, ecosystems, etc.) globally is critical, furthermore there is the large increase in population size, which will cause an increase in the demand for food and the bringing into cultivation of currently non-agricultural land. This will cause a contraction of the woods, forests and reclamation of wetlands. In addition, the increase of the population size will also involve urbanization at the expense of fertile land, which historically has always been adjacent to human settlements. All these causes lead to the transformation of the landscape and habitats, especially in developing countries, and decreased well-being in general.

1.8 From Global to local

Many traditional and local cultivars that have been under local non-intensive agricultural production up until today are in danger of disappearing completely because of the slow and inexorable replacement with other newly created (genetic erosion). These new varieties are much more likely to have been bred for use across a range of environments and are often much more suited to a modern and mechanized agriculture: plants adapted to mechanical harvesting, easier seed cleaning, easy removal of the commercial product, high value of harvest index, improved disease and pest resistance.

To achieve these objectives and express the full genetic potential of these new varieties, it is necessary to intervene with massive support chemicals (fertilizers, herbicides, insecticides, and hormonal stimulators parahormonal, etc.) together with irrigation, while as a consequence water resources become increasingly polluted.

The consequences of these changes is that agriculture becomes increasingly demanding and out of balance with the more ‘traditional’ practices required in certain non-mainstream or niche farming regions, This is disruptive from an ecological point of view as new areas of land are “reclaimed” for agricultural use from other habitats that provide a balance to the region as a whole. Also from a sociological point of view there are strong frictions in areas of Africa where many local farmers have used ancestral land inherited for generations, but without written records. The international companies are taking advantage of this by bribing local politicians and are buying up large tracts of land to exploit and produce biofuel (UN: http://www.un.org/ecosocdev/geninfo/afrec/vol23no3/233-land.html). There are many strategies to remedy this continuous depletion of biodiversity and ecosystems, and some examples for global and local level have already been explained above.

The time evolution of the loss and recovery of biodiversity can be divided into 4 phases:

phase 1 - loss of biodiversity due to various causes (the strongest is the increase in population);
phase 2 - awareness of biodiversity loss and the first steps to remedy it;
phase 3 - conservation multiplication and characterization of species most at risk;

phase 4 - commercial use of the species recovered.

The works planned in the first and second phase are perpetual because of continuing threats, especially anthropic, to the survival of biodiversity.

The 4th stage is a hypothesis explored in this thesis, which has already been implemented for both Italian and cultures in other parts of the world;

A case study is the situation in Molise, where, over the last sixty years, there has been the large scale abandonment of farmland both marginal and fertile, because of the mass transfer of labor to other regions or even to other nations. This triggered an ecological succession on abandoned fields leading to the expansion of forests at low altitudes and high. This is also due to the reduction in the number of grazing animals in the wild. In this situation there is still loss of genetic diversity both animal and agro-food and along with the consequence of the loss of biodiversity is the loss of traditional rites, folk customs and culture of rural civilization of Molise.

Many food crops had, fortunately, already been collected and preserved in germplasm banks of international importance (Bari, Portici, Kew Garden (UK), Norwich (UK), etc.). But until now, none of the institutions listed above has proceeded to plan projects for the characterization of the material collected.

Fortunately in the University of Molise (member of RIBES), as reported above, there is a new Germplasm Bank (BGMOL) which is engaged in the collection, storage and agro-morpho-genetic characterization of the agronomic and wild plants.

Over the past three years, germplasm of several agricultural plants typical of the province of Isernia in Molise have been collected. Among these were very interesting crops belonging to the legume tribe owing to their large use in the past as food for human as a meat substitute, as food for animals and as an organic fertilizer for soil (green manure) or in crop rotations.

The aim of the collection of germplasm made around the world and for many years (in some cases and for some plants more than 2 centuries) is the preservation of the genetic variation representing the “genetic potential” in the various animal and plant species. Regarding the material collected in the province of Molise, the next appropriate step is the sustainable use of agricultural and food resources. To achieve this it is necessary to examine and explain many aspects (economic, ecological, biological, agronomic, nutritional and market risk) related to each culture.

1.9 An important resource: the legume

In the certainty of ever increasing reduction of biodiversity due to the increasing world population, which in turn generates increased demand for food, growing urbanization, increasing pollution and scarcity of drinking water, comes the need to concentrate all efforts, political and economic *in primis*, of more sustainable development in all sectors. In this situation, agriculture can have two opposing roles: use all the resources intensively, contributing heavily to the increase of soil and water pollution, erosion and genetic sterilization and desertification of large areas of fertile planet currently. Agriculture can act as a “buffer” for all the negative effects listed so far, and even support the increase in biodiversity, ecosystem and related services, to maintain customs and traditions of many rural and actually play a role in social - economic importance (Finucane, M. L., 2002; Marino, D., 2010) also landscaped appearance of many rural areas, especially European (Vos, W., Meekes, H., 1999).

The lentil crop has the added advantage (as will be seen below) that they can be cultivated on marginal land and are very fertile, with zero impact (sustainable agriculture and organic).

Legumes are part of the Order of Fabales, which is an Order of enormous economic importance for the large number of plant species used as food for humans and animals. The plants belonging to this Order make many interesting “secondary” compounds: coloring, medicinal principles, etc.

The Order of the Families of Fabales includes: *Fabaceae*, *Mimosaceae* and *Caesalpiniaceae*. Many authors consider the Order to the rank of a single family called *Leguminosae*, according to the character of the fruit is a legume (Gerola, M. F., 1997).

The Order of Fabales belong many plants trees and shrubs (many *Mimosaceae*, which can be bushy, herbaceous or liane which are rich in tannins, alkaloids and cyanogenic glucosides. It is believed that the family *Fabaceae* is the most advanced of the Order, while *Cesalpiniaceae* occupy an intermediate position. For the two other botanical families have ties phyletic (Gerola, M. F., 1997).

The family *Fabaceae* is called by some authors *Phaseolaceae* of the genus *Phaseolus*, which includes both woody and herbaceous plants, as well known as food crops and other plants of industrial and agronomic-zootechnics interest. For example, clover (*Trifolium* genus), vetches (*Vicia*), the birdsfoot trefoil (*Lotus*), alfalfa (*Medicago*), beans (*Phaseulus*), the broad bean (*Vicia*), lentils (*Lens*), the chickpea

(*Cicer*), peas (*Pisum*), the soybean (*Glycine*), peanut (*Arachis*), etc. Other species are of interest as medicinal plants and wood (Gerola, M. F., 1997).

Among the many interesting characteristics of the "legumes" the most important is the ability to sustain a symbiosis with two micro-organisms, the first is with Rhizobium leguminosarum which are hosted in specific organs developed on the roots, namely nodules (figure 2). The bactierium within the nodules have the ability to 'fix' atmospheric nitrogen and make it available to the plant. The second is an association with arbuscular mycrorhiza which effectively extends the root system of the plant and thus makes further soil nutrients available for plant growth In agronomy, legumes are considered "improvements" in the sense that they leave in the soil residual organic nitrogen and simultaneously, due to their capillary roots, help to structure the ground, so that the crop that follows can make the most of the capabilities of the soil fertilizer.

The plant-microorganism relationship is species-specific, so each plant species corresponds to a different species of microorganism symbiont. For lentils the organism symbiont is *Rhizobium leguminosarum* and it has been calculated that in one year one hectare of lentils is able to produces kg 20 of nitrogen (N), but much depends on the type of soil, climate, water availability, culture technology and Rhizobium concentration in the soil (Yadav SS, et al. 2007).

1.10 Lentil Morphology and market: the strange couple

The plant varies from 15 to 45 centimeters in height, and has many long ascending branches. The leaves are alternate, with six pairs of oblong-linear, obtuse, mucronate leaflets. The flowers, two to four in number, are of a pale blue colour, and are borne in the axils of the leaves, on a slender peduncle nearly equaling the leaves in length. The pods are about 1.5 cm long, broadly oblong, slightly inflated, and contain two seeds, which are of the shape of doubly convex lens, and about 0.5 cm in diameter (Yadav S. S. et al. 2007; Muehlbauer, F. J., et al., 1996) (figure 2).

Figure 2. Illustration of the Lentil plant, font it.wikipedia.org

Lentil is consumed mainly as seeds (after cooking) for human consumption, while the remaining part of the plant varieties or wild, are used in some developing countries for animal feed. Popular in parts of Europe and a staple throughout much of the Middle East and India, this tiny, lens-shaped pulse has long been used as a meat substitute. They can be used as a side dish (puréed, whole and combined with vegetables), in salads, soups and stews. One of the most notable showcases for the lentil is the spicy Indian dhal.

There are many cultivars of lentils grown commercially throughout the world which can be distinguished by the appearance of the seed according to the tastes of consumers down to the level of small regions. It was found that some consumers prefer very small seeds, because they are easier to cook and less consistent than larger seeds (Molise), but it is an exception, because most of the varieties marketed in the Mediterranean, as claimed by Muehlbauer et al. (1985) seeds are big, in fact both the lentils from Turkey as well as those Italian (Sicily and Umbria) and Spain seeds are large.

In other continents (especially oriental) the small seeds can be peeled (hulled) and used to create lentil flour. Also the color of the flour varies, depending on the color of the cotyledons, which can be yellow, orange or brown to bronze. Brown lentils are the most popular with Occidental markets, while yellow and orange in oriental. In Italy are found on different types of large distribution desks. This is due, perhaps, from the geographical position of Italy, which has allowed for centuries to have commercial exchanges with both the West and the East. Also for this, most of the lentils are not marketed "pure line" but are mixture of several varieties or forms that have adapted to the environment and the growing local demand.

The shape of the seed of the cultivar is also strongly influenced by market demands. There are markets that require very convex seeds, while others prefer flat seeds.

Finally, the color of the coating of the seeds (testa) is a characteristic influenced by the market (especially in Canada), which leads many biologists to elucidate the genetic mechanisms of coloration of the seed which still remains unknown, and to obtain pure lines with color and homogeneous seed shape.

1.11 Origin of Lentil and distribution

There are several theories on the origin of lentils, and their place with respect to their phylogeny with other legume. The discussion has been generated because of the discoveries that have followed over time and era which dates back to the archaeological site. An important archaeological site is to the North of Israel (6800 BC), where for the first time heaps of lentil seeds were found. This suggests that from that moment the culture is an integral part of rural life (reference needed).

While, until proven otherwise, in all other findings of archaeological sites dated antecedently, the remains Carbonized lentil seeds (Tell Mureybit in Syria 8500-7500 BC) show the coexistence of different species of lentils (Van Zeist, 1971; Zohary, 1972; Hansen et al., 1978).

The fact remains that the first traces of lentils close to human settlements date back to 11,000 years BC (Greece's Franchthi caves).

Many authors support the theory that the real genesis of agriculture and the domestication of lentils in the human diet first originated within the Near East.

Other finds have been made Hacilar in Turkey (5800-5000 BC) and Tepe Sabz in Iran (5500-5000 BC). In conclusion, according to the dates of the archaeological finds, we can say that between 11000 and 6800 BC lentils were already part of man Food and were then distributed from Greece, Syria and then into northern Israel. Here they were selected and domesticated, and is distributed in Turkey and Iran, to arrive in North-East Pakistan (Harappan) (3300-1300 BC). For now these are very approximate theories.

The predominant species that is now cultivated is *Lens culinaris* M. and over the years there have been several scientific approaches to demonstrate what have been, phylogenetically, the progenitors of this species. The approaches used are: the crossings between related species, the electrophoretic study of seed proteins, cytoplasmic studies, cytogenetic, and the comparison between the morphology of plants and pollen grains, as well as archaeological discoveries in the fields of lentils grown in the Middle East of two or more species of the genus *Lens*.

Landizinsky in 1979, studied the electrophoresis proteins profiles of the seed of 11 varieties of *L. culinaris*, 6 *L. orientalis,* 4 *L. ervoides* and 1 of *L. nigricans* and showed that *L. culinaris*, *L. nigricans* and *L. orientalis* are highly correlated with each other, and *L. ervoides* is different.

Renfrew (1973) suggested that *L. nigricans* is the progenitor of the cultivated species L. culinaris based on his belief of the domestication of lentil in southern Europe.

Other authors, from 1930 to 1979 (Barulina 1930, Zohary 1972 and Williams et al. 1974, Ladizinsky 1979), have argued that the progenitor of the crop would be *L.*

orientalis, basing their claims on studies of crosses between *L. culinaris* x *L. orientalis* and for analyzing the F1 and F2 generations. The same conclusions are arrived at with the comparative of the cytoplasmic study. In contrast, cytogenetic analysis on interspecific hybrids has shown that there are more similarities between the three chromosomes of *L. culinaris* and of *L. nigricans* .

More recently, Muehlbauer et al. (2006) claimed that *L. orientalis* is the progenitor of *L. culinaris* and after the crossing between the two species were fully fertile descendants.

In Italy Piergiovanni (2000) published data on the distribution of crops of lentils in Italy and heterogeneity in seed size and cotyledon colour in different ecotypes. She obtained the following figure, where she reported on the lentil from Capracotta, but was not collected.

Figure 3. Sites of Italian lentil populations. ▲ indicates populations heterogeneous in seed size; ■ indicates populations heterogeneous in cotyledon colour; ● Ecotypes marked. (font Piergiovanni, 2000)

1.12 Botanical descriptor

Lentil plants are herbaceous annuals with slender stems and branches. Plant height usually ranges from 25 to 30 cm for the majority of genotypes, but may vary from extremes of 15 to 75 cm depending on genotype and environmental conditions. Plants have a slender taproot with fibrous lateral roots. Several rooting patterns, which range from a much-branched shallow root system to intermediate types that are less branched and more deeply rooted, have been observed on similar soil types (Muehlbauer et al. 2006). The taproot and lateral roots in surface layers of the soil have numerous, indeterminate nodules that vary in shape from round to elongate (Saxena and Hawtin, 1981). The herbaceous stems of lentil plants are square and ribbed and usually thin and weak. The color of the stem turns from light green to reddish, depending on the presence or absence of red pigment in the basal part of the stem.

The habitus of the plant of lentil varies from a single stem very high and consistent, more false stems (shrubby habitus) less consistent, lower and branched The bushy habitus, in fact, consists of primary branches that form the base of the main stem (cotyledon node) that takes a similar size to the primary branches (Wilson and Teare, 1972; Malhotra et al., 1974).

The leaves of lentil are composed and small (1 to 3 cm length) and are described as pinnate or imparipinnate and comprise as many as 14 sessile, ovate or elliptic, abovate or lanceolate leaflets. The leaf may terminate in tendril or no, each leaf is subtended by two small stipules. The entire stipules are oblong-lanceolate and unappendaged (Davis and Plitmann, 1970; Summerfield at al., 1982).

The flower are borne singly or in multiples on peduncles that originate from the upper nodes of the plant. Each Peduncle normally bears from one to or three, but rarely four flowers, although seven flowers per peduncle have been reported for plants grown in a glasshouse (Hawtin, 1977).

The individual flower is complete and has a typical papilionaceous (butterfly like) structure (Photo 1). Flowers are small (4 to 3 mm long) and white pale purple, or purple-blue.

The flower has a calyx comprising five, equally (elongated sepals that equal or exceed the length of the corolla of the unopened flower. The corolla has a standard, two wings, and two lower petals that lie internal to the wings and are united at their lower margin to form the keel (Summerfield et al., 1981).

Photo 1. Flower of lentil (by G. Pelino)

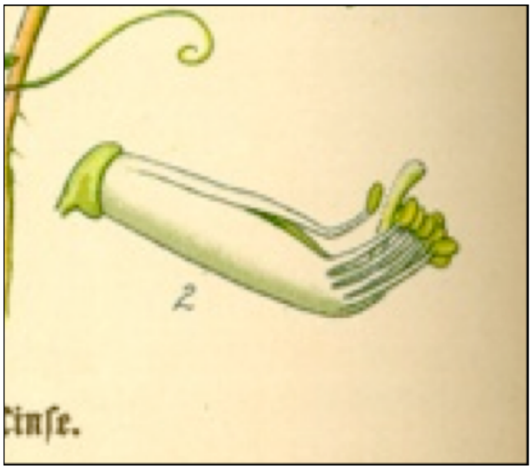


Figure 4. Stamen of lentil flower

The stamens are diadelphous (9 + 1) with the upper vexillary stamen free (Figure 4). The ovary is flat and glabrous; it normally contains one or two ovules that alternate along the margin and terminate in a short curved style. The style is pilose on the inner side; it usually develops at a right angle to the ovary, and is flattened on the outer side (Muehlbauer et al., 1980; Saxena et al., 1981; Summerfield et al., 1985).

The fruits (referred to as pods) are oblong, laterally compressed, 6-20 mm long and 3.5-11 mm wide, and usually contain one or two, but rarely three seeds (Saxena and Hawtin, 1981).

Seeds are lens-shaped and weigh between 20 and 80 mg. Their diameter ranges from 2 to 9 mm and the testa may be light red, green or greenish-red, grey, brown, or black. Purple and black mottling and speckling of seeds is also common in some cultivars and accessions (Duke, 1981; Saxena and Hawtin, 1981). The seeds are divided into *macrosperma*, when the seed diameter is greater than 6 mm, and *microsperma*, when the diameter is below 6 mm, as was proposed by Barulina (1930).

In literature it is found that the distribution of macrosperma seeds is widespread in the Mediterranean basin, while the microsperma are more prevalent towards the Middleeast and India (Muehlbauer et al. 1985).

1.13 Taxonomy

The genus *Lens* comprises six species (Ferguson 1998; Ferguson et al., 2000) as *Lens orientalis* is generally now classified as *Lens culinaris subsp orientalis*. Only one species, *L. culinaris* Medikus is cultivated. The other lentil wild species are namely *L. montbretii* (Fisch & Mey.) Davis and Plitman; *L. ervoides* (Brign.) Grande; *L. nigricans*

(Bieb.) Godr., and *L. orientalis* (Boiss.) M. Popov., the latter two species possess morphological similarities to the cultivated lentil (Ladizinsky 1979b). However, Ladizinsky and Sakar (1982) suggested that *L. montbretii* should be placed in the genus Vicia and named as *Vicia montbretii* (Fisch & Mey.) based on morphological and cytological data and breeding experiments. The cultivated lentil originated from *L. orientalis* (Barulina 1930) and chromosome number of cultivated lentil, its progenitor species, *L. orientalis* and *L. nigricans* are same, i.e. 2n = 14. The cultivated species, *L. culinaris* has been divided into two sub-species (Barulina 1930).

Williams et al. (1974) classified *L. culinaris* in the order Rosales, sub-order Rosineae, family Leguminosae and sub-family Papilionaceae. The genus Lens occupies an intermediate position between *Vicia* and *Lathyrus*, the two other members of papilionaceae.

On the taxonomy of the genus *Lens* and overlaps with genres *Vicia* and *Lathyrus* there are discussions going on and still not entirely clear that going on for years. The classical taxonomy is based (or based) only on the morphology of plants and it does not necessarily represent biological entities. Hybridization within the genus indicates the classification of species based on morphology is not valid and accessions based on morphology classified in the same species sometimes turn out to be cross-incompatible (Sandhu, J. S., 2007)

In the last thirty years the techniques of laboratory and genetic investigation and proteomics, have greatly improved and, for example, have allowed Ladizinskv and Sakar (1982) to distinguish that *L. montbretii* has 2n = 12 chromosomes whereas the other *Lens* species have 2n = 14, attraverso used morphological and karyological information to show considerable differences between the two species. Based on that information, it is clear that *L. montbretii* is more appropriately reclassified as *V. montbretii*.

1.14 Genetics of quantitative traits

Many researchers have conducted analysis on heritability of quantitative traits of the species *L. culinaris* M. at the same time evaluating the *genetic divergences* between different varieties of lentils and *genetic correlations* between characters. For *genetic divergence* means is measured the genetic distance among the cultivars or germplasm lines. This divergence may be due to geographic barriers or any other reasons, which may restrict the gene flow, resulting in the formation of distinct groups (Mishra S. K., et al., 2007).

To set up a breeding program it is very important for geneticists to know the genetic divergences between the parental varieties. Many investigations are therefore conducted to measure the genetic divergences which are summarized in the Table 2 on next page.

For genetic correlation means the direct or indirect association between the events of two or more traits. Of course this type of research has been taken to improve the yield of the crop. Some examples, for lentils, of researches on this issue are presented in Table 3.

The heritability of a character is a very precise and predictable genetic parameter, with values between 0 and 1. This coefficient has two main uses in the genetic improvement. Used to estimate the value additive genetic (A) of the parents and to predict, depending on the chosen strategy of improving the genetic progress expected in the population or genetic increasing (ΔG).

Differences in heritability in the broad sense (H^2), describes the relative contribution of the total genotypic effects (VG) to the total phenotypic variance of the character (VP), and heritability in the narrow sense (h^2), which instead describes the relative contribution of reproductive effects, or additive genetic (VA) to the total phenotypic variance of the character (VP).

The need to refer at the variance is due to the fact that it is the parameter most commonly used to measure the degree of differentiation between individuals.

Table 4 shows heritability index calculated by some of the most respected experts of lentils.

Table 2. Genotype convergences and divergences in *Lens* (source Shyam et al. 2007)

Biswas and Das (1985) estimated genetic diversity over two environments for ten characters in lentil accessions collected from Bangladesh and India and reported that the population from the two countries were divergent from each other.
Balyan and Singh (1986) grouped 48 genotypes of lentil into 12 clusters based on the analysis of nine characters.
Harvey and Muehlbauer (1989) established that the wild lentils (*L. orientalis* and *L. odemensis*) had greater variability and were more close to the cultivated lentil (*L. culinaris*).
Chahota et al. (1994) classified 40 genotypes of small seeded lentil (microsperma) into six clusters, they considered 15 traits.
Ferguson and Robertson (1996) found low level of diversity in the cultivated taxon as compared to the wild species was reported in lentil based on 11 loci in 439 accessions.
Rathi et al. (1998) reported that the number of primary branches per plant contributed most towards the total genetic divergence followed by yield per plant. The clustering pattern predicted that the genetic diversity is not necessarily parallel to the geographical diversity.
Singh et al. (2001) reported eight clusters based on the multivariate analysis of 58 diverse strains of lentil.
Jeena and Singh (2001) analysed 30 genotypes of lentil (28 wild accessions and 2 cultivated) for qualitative, quantitative and both qualitative and quantitative traits. The results indicated wide genetic divergence as each analysis yielded four, three and three clusters, respectively.
Jeena and Singh (2002) evaluated 61 lentil accessions representing four wild species viz., L. nigricans (2), L. odemensis (16), L. ervoides (24) and L. orientalis (19). Based on the analysis of data on 20 quantitative traits, all the accessions could be grouped into four clusters. Interestingly, 58 accessions could be grouped into Cluster 1 while the rest of the clusters were mono-genotypic. **The study clearly indicated that the genetic diversity was not related to the geographical diversity and species differences**.
Solanki et al. (2002) reported 72 genotypes to be classified into 8 and 9 clusters under normal and late sown conditions, respectively.
Singh et al. (2002) reported the genotype x environment interaction on determination of clustering pattern. They analyzed 40 genotypes for two consecutive years and grouped them into six and seven clusters.
Rakesh et al. (2005) grouped 44 genotypes in to five clusters based on the analysis of data on 15 important characters at two locations.
Yadav et al. (2005) worked-out genetic divergence based on analysis of data on 50 genotypes under two environments and **reported that the genetic diversity was not paralleled to the geographical diversity**.
Poonam (2006) grouped 100 lentil genotypes of diverse origin into 10 clusters based on analysis of 12 quantitative traits. These genotypes could be grouped into 10 close-knit clusters, there was no parallelism between the two types of the clustering pattern.

Table 3 .Correlation between traits (source Shyam et al. 2007)

A positive association between seed size and pod size reported by Sharma and Sharma (1978) can be useful in selecting the variability for seed size.
Erskine (1983) found significantly positive genotypic and phenotypic correlations between seed and straw yields have been recorded both in small seeded (microsperma) and bold seeded (macrosperma) accessions
Sarwar et al. (1984) reported positive correlation of seed yield with number of pods per plant, number of primary and secondary branches per plant in the Indigenous as well as in the exotic germplasm.
Erskine et al. (1985) recorded negative genetic correlation between seed yield and protein content.
Hamdi et al. (1991) reported that there was positive association between cooking time and seed size .
Shahi et al. (1986)seed impermeability and germination were negatively correlated.
Murari et al. (1988) Positive correlations of seed yield per plant with number of primary branches per plant, plant height, number of seeds per plant and 100-seed weight were observed in lentil.
Zaman et al. (1989) Profuse branching and number of pods per plant were positively correlated with seed yield.
Nigam et al. (1990) A positive and highly significant correlation coefficient was found between seed yield per plant and number of pods per plant.
Hamdi et al. (1991) reported positive correlation between seed yield and straw yield. Multiple correlation and regression analysis revealed that the combination of two or three variables such as plant height, number of branches per plant, number of pods per plant was the best method for improving the seed yield.
Singh and Singh (1991) observed that plant height was always correlated positively with seed yield per plant in both microsperma and macrosperma lentils.
Pandey et al., (1992) Seed yield per plant was positively correlated with all the yield components except 100-seed weight in Indigenous lentil germplasm.
Esmail et al. (1994) reported that seed yield was positively and significantly correlated with plant height, number of branches per plant, number of seeds per pod and number of pods per plant, however, it was negatively correlated with flowering duration.
Kumar et al., (1995) Both genotypic and phenotypic positive correlations of seed yield with plant height, number of primary branches per plant, number of pods per plant, protein and methionine contents were observed in 13 parents and their 31 F1s.
Chauhan and Singh, (2001) Seed yield per plant was positively correlated with harvest index.
Rakesh et al. (2005) reported the correlation among yield and yield components (15 characters) in 44 germplasm accessions of lentil. The analysis indicated that the values of genotypic correlations were slightly higher, in general, than the phenotypic correlations.

Table 4. Coeficient of heritability traits in Lens (source Shyam et al. in Yadav, 2007)

Dixit and Dubey (1985) reported the highest heritability estimate for days to flowering. However, moderate heritability (59.7%) coupled with highest genetic advance in percent of mean (72.9%) was observed for seed yield.
Erskine et al. (1985) reported highest heritability estimate for average seed weight (98%) followed by cooking time (82%).
Lakshmi et al. (1986) recorded higher heritability coupled with high genetic advance for germination percentage, hard seed percentage and 100-seed weight.
Ali and Johnson (2000) worked-out heritability estimates for winter hardiness under natural and controlled condition. The estimates of narrow sense heritability estimates ranged from 0.32 to 0.71 under field conditions whereas under controlled condition it was maximized at 1.00.
Omvir and Gupta (2000) studied heritability in microsperma x macrosperma derived lines. They reported low heritability estimates in poor environments, however, it was higher in the best environment.
Rathi et al. (2002) reported high heritability estimates along with higher genetic advance for 1000-grain weight.
Dayachand (2007) reported higher estimates of broadsense heritability in combination with high genetic advance for days to maturity.

1.15 Nutritional component and food uses

Lentil is an important dietary source of energy, protein, carbohydrates, fiber, minerals, vitamins and antioxidant compounds, as well as diverse non-nutritional components like protease inhibitors, tannins, α-galactoside, oligosaccharides and phytic acid (Table 5,6,7 and 8; Appendix I; Urbano, G., et al. 2007).

Lentils are consumed in different ways, both as whole seed cooked in soups, both hulled (dhal) or as flour for preparing purees or biscuits. In India the very popular red lentils are used for dhal prepared as the basis for many dishes, but also used uncooked; either soaked crushed and moulded to make cakes or sprouted as an ingredient in salads in some parts of India and as such provide better nutrient value (Shyam, S., 2007).

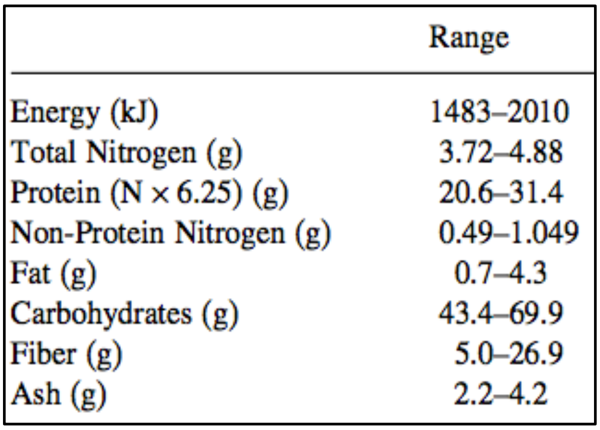
Besides being highly nutritious lentil seeds also contain anti-nutritional factors such as protease inhibitors, lectins or phytohaemoglutins and oligosaccharides that cause flatulence. These anti-nutritional factors can be minimized by heating, water soaking and germination (Wang N., et al. 2009).

Table 5. Amino acids (g) in 100 g of dry seeds

Amino acids	
asparti acid	2.855
glutamic acid	4.002
alanine	1.078
arginine	1.994
cystine	338
phenylalanine	1.273
glycine	1.05
isoleucine	1.116
histidine	727
leucine	1.871
lysine	1.802
methionine	0.22
proline	1.078
serine	1.19
tyrosine	689
threonine	924
tryptophan	232
valine	1.281

Font: http://www.valori-alimenti.com/nutrizionali/tabella16069.php

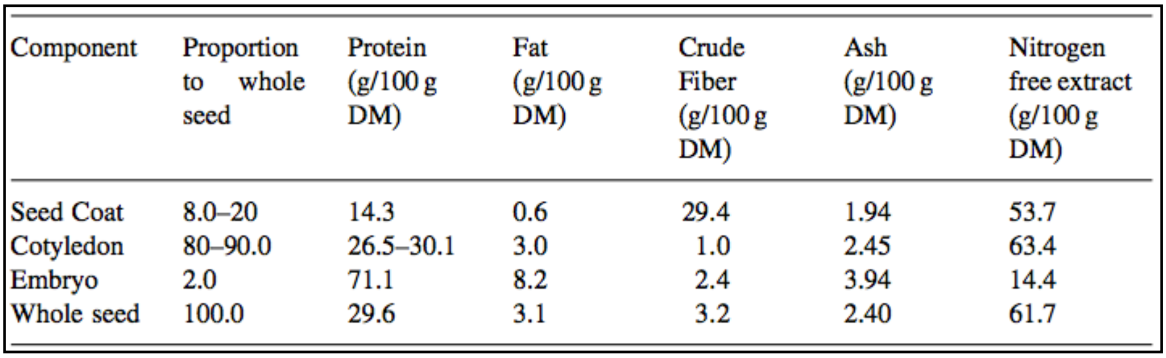
Table 6. Chemical composition of row lentil (per 100 grams of dry matter)



	Range
Energy (kJ)	1483–2010
Total Nitrogen (g)	3.72–4.88
Protein (N × 6.25) (g)	20.6–31.4
Non-Protein Nitrogen (g)	0.49–1.049
Fat (g)	0.7–4.3
Carbohydrates (g)	43.4–69.9
Fiber (g)	5.0–26.9
Ash (g)	2.2–4.2

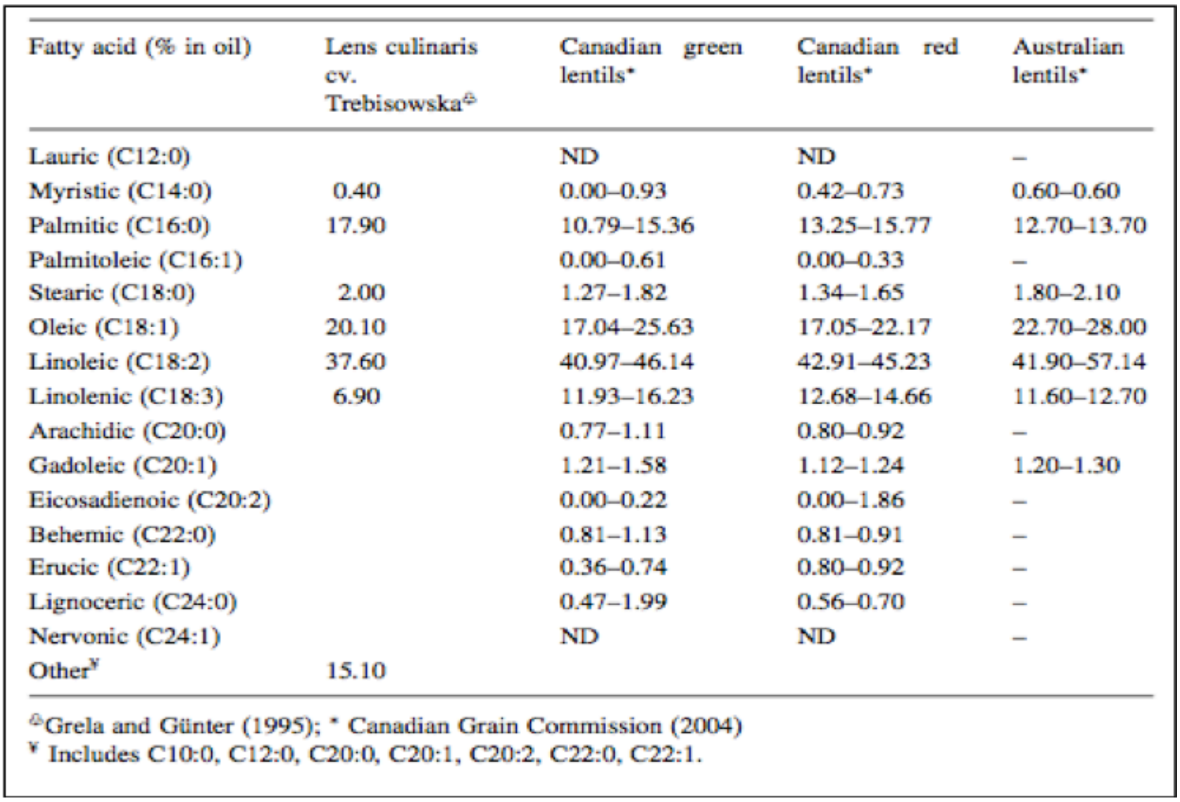
Font: AA.VV. in Nutritional value, Urbano, G., 2007

Table 7. Distribution of chemical constitutes in the different anatomical part of lentil seeds



Component	Proportion to whole seed	Protein (g/100 g DM)	Fat (g/100 g DM)	Crude Fiber (g/100 g DM)	Ash (g/100 g DM)	Nitrogen free extract (g/100 g DM)
Seed Coat	8.0–20	14.3	0.6	29.4	1.94	53.7
Cotyledon	80–90.0	26.5–30.1	3.0	1.0	2.45	63.4
Embryo	2.0	71.1	8.2	2.4	3.94	14.4
Whole seed	100.0	29.6	3.1	3.2	2.40	61.7

Table 8. Fatty acid composition of lentil



Fatty acid (% in oil)	Lens culinaris cv. Trebisowska♧	Canadian green lentils*	Canadian red lentils*	Australian lentils*
Lauric (C12:0)		ND	ND	–
Myristic (C14:0)	0.40	0.00–0.93	0.42–0.73	0.60–0.60
Palmitic (C16:0)	17.90	10.79–15.36	13.25–15.77	12.70–13.70
Palmitoleic (C16:1)		0.00–0.61	0.00–0.33	–
Stearic (C18:0)	2.00	1.27–1.82	1.34–1.65	1.80–2.10
Oleic (C18:1)	20.10	17.04–25.63	17.05–22.17	22.70–28.00
Linoleic (C18:2)	37.60	40.97–46.14	42.91–45.23	41.90–57.14
Linolenic (C18:3)	6.90	11.93–16.23	12.68–14.66	11.60–12.70
Arachidic (C20:0)		0.77–1.11	0.80–0.92	–
Gadoleic (C20:1)		1.21–1.58	1.12–1.24	1.20–1.30
Eicosadienoic (C20:2)		0.00–0.22	0.00–1.86	–
Behemic (C22:0)		0.81–1.13	0.81–0.91	–
Erucic (C22:1)		0.36–0.74	0.80–0.92	–
Lignoceric (C24:0)		0.47–1.99	0.56–0.70	–
Nervonic (C24:1)		ND	ND	–
Other¥	15.10			

♧Grela and Günter (1995); * Canadian Grain Commission (2004)
¥ Includes C10:0, C12:0, C20:0, C20:1, C20:2, C22:0, C22:1.

1.16 Lentils area, production and yield trends

The lentil is a crop grown all over the world thanks to its adaptability to different environments. Since the 1990s all over the world there has been an increase (6%) of land sown to lentils, from 3,4 million ha in 1994 to 3,75 million ha in 2008 (FAOSTAT, 2010)[2]. While at the same time, total world yields have increased by 26%, from 2,79 million tons to 3,54 million tons (FAOSTAT, 2010).

The nation that produces the highest amount of lentils is Canada (1.043.200 t) (data 2008) while in India there is the greatest expansion of cultivated land (1.469.969 ha) compared to Canada which grown 700.185 ha. The general trend around the world is positive, both as cultivated land both as the quantity produced, the greatest impact is in developing countries, only in Turkey was there a decrease both production and cultivated land lentil (FAOSTAT, 2010). Finally, Italy changed from 1012 ha in 1994 to 1815 ha in 2008, while production has increased from 887 t to 1322 t for the same period (FAOSTAT, 2010).

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[2] http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1578

Another data source from the first half of the 20th Century shows Italy was among the major lentil producers of the Mediterranean basin. In 1936 the hectares planted with lentil were 22.000, in 2009 (ISTAT, 2012)[3].

In Italy lentils have been cultivated and appreciated since ancient times, providing a cheap source of dietary proteins to rural and urban families. (Piergiovanni A. R., 2000).

The easy cultivation of the crop and the ability to adapt to different environments, from the mountains to the lowlands, has resulted, in all these centuries, many ecotypes in different areas of Italy, from north to south, including the islands (Piergiovanni A. R., 2000; Torricelli, R., 2011).

Lentils were grown in both intensive and subsistence agricultural systems, although the types utilized in the two systems were different (Piergiovanni A. R., 2000). *Macrosperma* was used for intensive agriculture while *microsperma* was used for home consumption.

Currently the lentil crop is restricted to marginal land in the mountains and hills for home consumption (Torricelli, R., 2001), while only in the areas of Castelluccio di Norcia, Colfiorito, Altamura, Villalba and Pantelleria is there industrial production, which supplies almost all the Italian national markets. There are also other varieties but these are sold in niche markets.

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[3] http://www.istat.it/it/censimento-agricoltura

Appendix Chapter I

Table 9. Composition of lentil (http://www.valori-limenti.com/nutrizionali/tabella16069.php)

fats	g	1.06	Lycopene	mcg	0
carbohydrates	g	60.08	Lutein + zeaxanthin	mcg	0
proteins	g	25.8	tocopherol beta	mg	0
fibers	g	30.5	gamma tocopherol	mg	4.23
sugars	g	2.03	delta tocopherol	mg	0
water	g	10.4	**Sugars**		
ashes	g	2.67	Dextrose	g	0
Minerals			Fructose	g	0.27
calcium	mg	56	Galactose	g	0
sodium	mg	6	Lactose	g	0
phosphorus	mg	451	Maltose	g	0.3
potassium	mg	955	Sucrose	g	1.47
iron	mg	7.54	**Lipid**		
magnesium	mg	122	Fatty acids, monounsaturated	g	189
zinc	mg	4.78	Fatty acids, polyunsaturated	g	516
copper	mg	519	Fatty acids, saturated	g	156
manganese	mg	1.33	Cholesterol	mg	0
selenium	mcg	8.3	**saturated fat**		
Vitamins			4:0	g	0
Retinol (Vitamin A)	mcg	0	6:0	g	0
Vitamin A, IU	IU	39	8:0	g	0
Vitamin A, RAE	mcg_RAE	2	10:0	g	0
Thiamine (Vitamin B1)	mg	873	12:0	g	0
Riboflavin (Vitamin B2)	mg	211	14:0	g	3
Niacin (Vitamin B3)	mg	2.605	16:0	g	133
Pantothenic Acid (Vitamin B5)	mg	2.14	18:0	g	15
Pyridoxine (Vitamin B6)	mg	0.54	**monounsaturated fat**		
Folic acid (Vitamin B9 or folacin or M)	mcg	0	16:1 undifferentiated	g	3
dietary folate	mcg	479	18:1 undifferentiated	g	0.18
Folate, DFE	mcg_DFE	479	20:1	g	6
Folate, total	mcg	479	22:1 undifferentiated	g	0
Cobalamin (vitamin B12)	mcg	0	**polyunsaturated fatty**		
Vitamin B-12, adding	mcg	0	18:2 undifferentiated	g	404
Ascorbic acid (Vitamin C)	mg	4.4	18:3 undifferentiated	g	109
Vitamin D (D2 and D3)	mcg	0	18:4	g	0
Colecalcifenolo (Vitamin D)	IU	0	20:4 undifferentiated	g	0
Alpha-tocopherol (vitamin E)	mg	0.49	20:5 n-3	g	0
Vitamin E, added	mg	0	22:5 n-3	g	0
Phylloquinone (vitamin K)	mcg	5	22:6 n-3	g	0
Total choline (Vitamin J)	mg	96.4			
Carotene, beta	mcg	23			
Carotene, alpha	mcg	0			
Cryptoxanthin, beta	mcg	0			

CHAPTER II

2.1 Lentils (Lens culinaris M.) in Italy

The lentil crop has been and is still practiced throughout Italy, from North to South including the islands. The spread of the crop, much appreciated by the market, has prompted researchers to address the characterization in different areas of Italy and with different techniques of investigation. The following is a chronological order of the work done on Italian lentil since 2000.

In 2000, Piergiovanni A. R. of the Germplasm Institute-CNR published a work on morphological characters of lentil (*Lens culinaris* M.) and the evolution of culture in Italy during the last century. In particular it showed the importance of the crop especially during the early 1900. During the following years, lentil suffered a decline in cultivated area and became relegated to increasingly to the marginal land of the South Centre and small islands of Italy. Several indigenous people and the lentils they cultivated have disappeared following the abandonment of fields. To avoid the total loss of these ecotypes the Germplasm Institute of the National Research Council (Bari, Italy) and the Institut für und Pflanzengenetik Kulturpflanzenforschung (Gatersleben, Germany) were involved in the collection of 63 Italian varieties, providing for their ex situ conservation and characterization, hoping to promote policies to grow in situ for the maintenance and development of indigenous varieties more adapted to the cultivation site.

In 2008, Sidari M, et al., (Mediterranean University of Reggio Calabria) assessed the strength of lentil seeds to salinity of soil cultivation. Soil salinity is a growing problem exacerbated in recent years due to desertification and the use of salt water in irrigation. In particular, the germination rate was compared with 4 varieties of lentils: Eston, Red Castelluccio, Pantelleria and Ustica at different salt concentrations (NaCl). The concentrations of osmolytes and enzymatic activities of amylase-and-glucosidase enzymes involved in seed germination were also measured. The research showed that the varieties Eston and Red Castelluccio had low resistance to the presence of salt in the solution and difficulties in producing proline, soluble sugars and decreased the activity of enzymes involved in the process of germination. By contrast Pantelleria and Ustica lentils showed good resistance to salinity, producing proline, soluble sugars and a good enzymatic activity required forgermination.

Another 2008 work on the characterization of lentils in the Molise region, through an integrated approach, was performed by Scippa G. S., et al. the University of Molise. The work involved an analytical approach using morphological analysis, DNA and proteins of the seed of two varieties of Molise (from Capracotta and Conca Casale) and some of the varieties grown and marketed in Italy. The study demonstrated that combining morphological, genomic and proteomic approaches is a powerful tool for integrating the analysis of biodiversity in ecotypes of this species.

In 2009, a work was published by F. Fiocchetti et al., the NRC of Lecce, which shows the results of the analysis to quantify the genetic diversity of three Italian varieties of lentils macrosperma (Onano, Altamura and Vilalba), using the technique of fluorescent AFLP markers. The levels of investigation were expressed as indices: the total genetic diversity (HT), the genetic diversity within population (HS), the extent of differentiation between populations (DST), the fixation index (GST) and the gene flow estimate (Nm). The results obtained showed that about 78% of the observed total genetic variation can be attributed to within population differences and around 22% is due to differences among populations. The gene flow estimate (N m=1.774) and the mean genetic distance value (0.077) suggested narrow genetic base among the analyzed populations, confirming the tendency of Italian lentil landraces to group together. In addition it was shown that the AFLP technique is an investigative tool that allows biotechnology to provide important insights for research.

In 2010, Scippa et al. identified specific markers of different lentil landraces [a local ecotype (Capracotta) and five commercial varieties (Turca Rossa, Canadese, Castelluccio di Norcia, Rascino and Colfiorito)] analyzing the 2-D gel electrophoretic maps of mature seeds. 2-DE analysis resolved hundreds of protein in each lentil sample, among which 122 were further identified by MALDI-TOF PMF and/or nanoLC-ESI-LIT-MS/MS. A comparison of these maps revealed that 103 protein spots were differentially expressed within and between populations. The multivariate statistical analyses carried out on these variably expressed spots showed that 24 protein species were essential for population discrimination, thus determining their proposition as landrace markers. Besides providing the first reference map of mature lentil seeds, data confirm previous studies based on morphological/genetic observations (Scippa et al., 2008) and further support the valuable use of proteomic techniques as phylogenetic tool in plant studies.

In 2011, Zaccardelli M. et al., CRA (Research Centre for Horticulture) of Pontecagnano (SA) in collaboration with the RNC of Bari and RNC of Milano published research results of investigations of genetic relationships, agronomic, nutritional and technological characteristics, of 10 Italian landraces, two improved lines and two cultivars of lentil (*Lens culinaris* Medik). The research used a multi-disciplinary approach: seed storage proteins, used as biochemical markers and microsatellite (SSR) molecular markers provided very useful information on genetic variation and relationships among landraces. From the investigations it was shown that lentils has high levels of genetic variability, while for the technological and nutritional investigation data was worth considering for the variety of lentils Linosa and San Gerardo.

Finally in 2011, Torricelli R. et al. (University of Perugia), in collaboration with the ARSSA (Agenzia Regionale per i Servizi di Sviluppo Agricolo) of Avezzano (AQ) and the Experimental Station of Granicoltura for Sicily Caltagirone (CT), published results concerning the characterization of certain varieties of lentils from Abruzzo, in particular the different varieties collected in S. Stefano di Sessanio. The analysis focused on the agronomic traits, genotypic and phenotypic lentils. The results showed that lentils sold in local markets that are not of the place. Therefore we propose methods to prevent fraud and that can undermine conservation of ecotypes of the area as well as the local economy.

2.2 Aim of the thesis

Following the awareness of the continuing loss of biodiversity, both globally and locally and, as already said, the negative consequences resulting from multiple fields (social, food, economic, industrial, cosmetic, pharmaceutical, etc.), the work have tried to add new measures and values to a complex recovery package, namely characterization leading to a revaluation of native crops of Molise.

In detail, the aim of the thesis is the agro-morpho-physiological characterization of lentil (*Lens culinaris* M.) of Molise, in particular of three ecotypes from the municipalities of Capracotta, Conca Casale and Castelverrino. These local landraces are highly threatened by genetic erosion and neglect of the culture with the real risk of extinction.

On these bases, before the agro-morpho-physiological characterization of landraces of Molise, steps were taken to recover through collection the germplasm of lentil still extant in the Molise. The analysis and conceptualization of differences within and among lentil populations is measured in terms of phenotypic traits variation. In detail, agro-morpho-physiological analysis is carried out measuring of the variance of particular morphological, agronomic and physiological international descriptors.

Following the collection and characterization of lentils can be planned programs for the conservation of crop cultivation, in these villages. Taking a cue from other parts of Italy, where after cultivation for conservation of germplasm recovered, has developed a production system (in some cases even organic), which led to the formation of niche markets and, in the case of Castelluccio di Norcia, national and international markets. Hopefully, even in the region of Molise where presently farmers are unable to achieve sustainable socio-agro-economic development of areas interested and adjoining, including through the use of other traditional crops. These objectives are now possible thanks to the use of information technology which allow fast exchange of news and consequently of products on a large scale. Therefore the main objective of the thesis is the agro-morphological characterization of germplasm and its revaluation based on the data collected within the wider objectives aiming at sustainable development for the recovery and conservation of biodiversity of Molise.

2.3 Summary of the PhD

The studies undertaken for this thesis have focused on collecting, characterizing and conserving the agricultural and food resources from Molise and to use the resulting information to inform and directly encourage their future production in an environmentally sustainable economy.

The first year was focused on undertaking a collecting mission of many local varieties from the region as possible for preservation in the Germplasm Bank of Molise (BGMOL) at the village of Pesche (IS) of the University of Molise. In doing so, took note from the farmers about the uses of the material and its key characteristics. For example, was collected the seeds of cereals like wheat “Marzuolo” in Vastogirardi and “Solina” of Indiprete, barley and oats in Pietrabbondante, corn “Agostinello” of Carovilli and corn in S. Pietro Avellana, tomatoes of mountain in S. Pietro Avellana (with a characteristic strawberry flavor), potato “turchesca” in the municipality of

Carovilli and red potatoes in Roccasicura, red and white onion of Isernia and the very characteristic “slices onion” in Conca Casale, the tomato for salad in Isernia, two varieties of broccoli, one in Indiprete and one in S. P. Avellana, peppers in S. Agapito.

Many other plants were also noted but not collected including fruit trees, for example: the apple “limoncella” in the municipalities of Roccamandolfi and Isernia, pears, plums and cherries in Carovilli; pears “trentatreonce” and “risciole” in Roccamandolfi; the “sorb” in Macchiagodena, the grapes “Moscatellona” of Isernia and almost all villages of Molise, there is at least one farmer who owns an old grape vine “Tintilia” typical of Molise, as found in common of Chiauci and S. Pietro Avellana.

The botanical family most cultivated in the province of Isernia (actually throughout the Molise region) appears to be that of legumes.

All the species are cultivated, Beans with over 20 varieties found (by far the most widely cultivated species and almost all pure lines) and lupine of Peche communities; chickpeas of Roccasicura and Conca Casale, lentils from Pietrabbondante Capracotta, Conca Casale and Castelverrino.

These legumes are all considered to be “landraces,” local varieties exhibiting high genetic and phenotypic variability, with strong adaptability to the place of cultivation (Camacho Villa, CT, 2006)

The second year included a 6 month study period undertaken at the John Innes Centre in Norwich (UK) was focused on the exploration of the genetic variation of landrace populations from an agro-morphological point of view informed by genetic characterization for three local varieties of lentils from Molise, grown in standard environmental conditions (in a glasshouse at a constant temperature and photoperiod adjusted with a constant alternating day and night, in the absence of biotic and abiotic stress at the same time).

Initially, the seed samples of each population collected from the farmers was examined carefully to try to identify potential sub-accessions on the basis of the phenotype of the seeds. Small samples of each identified sub-population were grown in a glasshouse to study the variation within and between macro-population, population and sub-populations with particular attention being focused on the phenological phases of plants and variation in the descriptor states for a set of characters determined to be of high heritability. The data obtained were statistically analyzed to clarify the structure of each macro-population and population that make up a landraces.

The technologies and methodological approaches used in this work for the description and characterization of each sub-population, forms the basis for the discovery and development of defined single plant stocks that will form the basis of material for both future applied and strategic research from the detailed description of local varieties and how they can be better managed and potentially enhanced for niche markets of organic food products to the more precise starting materials for looking for novel compounds. The establishment of pure breeding single plant lines that differ for fixed genetic characteristics from within the local populations enables more specific questions to be addressed in investigations that could range from agronomy; comparing the field performance and buffering capacity of the overall population to bulked up single plant lines with rare or in frequent alleles that might open up the opportunity for developing superior lines, through mixing of specific single plant lines to more detailed biochemical or proteomics approaches where again, the variation of single plant lines developed from within a population might provide more powerful resources than trying to work with the average of what are heterogeneous populations.

The second half of year 2 and the third year of the PhD have been focused on completing the morphological characterization of the seeds and plant phenology and focused still further on varieties of lentils from the municipalities Conca Casale, Capracotta and Castelverrino.

CHAPTER III

Materials and Methods

3.1 Lentils

Lentils (*Lens culinaris* M.) used in this study come from three villages of Molise (Conca Casale, Capracotta and Castelverrino) and one of Umbria (Castelluccio di Norcia, marketed under the European PGI). Lentils from Molise are typical crop of mountain and cold areas and they are grown on marginal land of low fertility and rich in gravel (photo 2 and 3). Along with this material, a number of other lentil accessions from different sources were made available by the Germplasm resources Unit of the John Innes Centre to act as controls. These originated from Spanish (Salamaca), Syrian (Syrian local large, named Syrian), Argentine (Precoz) and Indian (Pant-L-406).

Photo 2. Typical field for the cultivation of lentils in Conca Casale

Photo 3. Soil rich of grail, Conca Casale

3.2 Description of the environment of local origin and cultivation techniques

Conca Casale has 218 inhabitants and is situated at 657 meters above sea level; Castelverrino is the smallest among the three considered and has 138 inhabitants and is situated at a altitude of 600 m; while Capracotta is the most populous village with 959 inhabitants and situated at an altitude of 1421 meters above sea level.

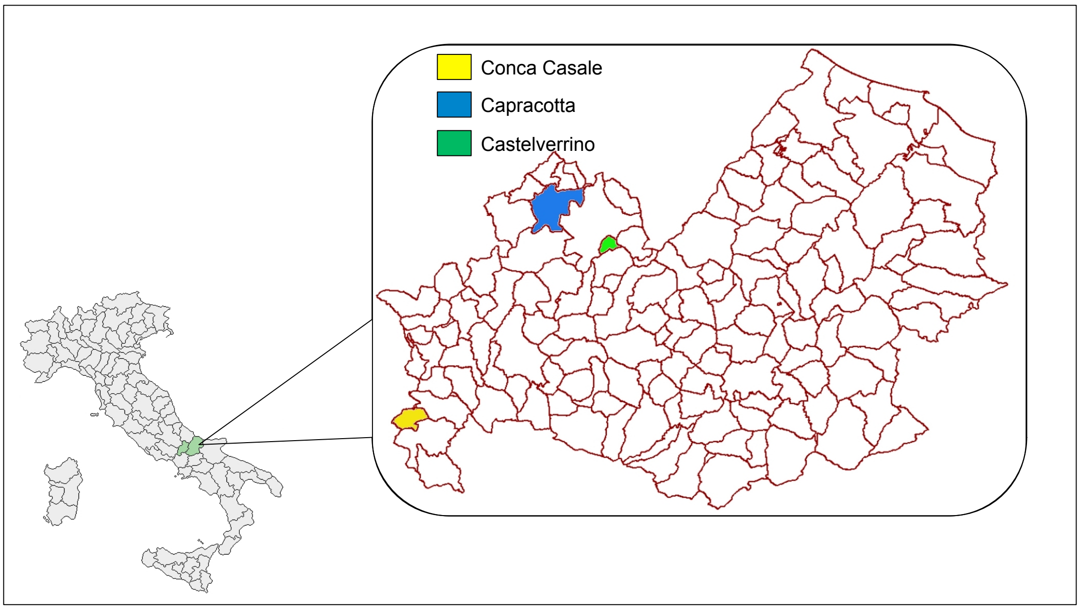


Figure 5. Position of three small municipalities of Molise

In Capracotta there were two typical lentils and their vernacular name are: “Miccula” (small seed, currently present) and “Micculona” (large seed, currently absent).

Generally the sowing of lentils in Molise begins immediately after the melting of snow, on the ground prepared in September or October the previous year. The area is usually dug by hand in small portion of land (40/50 square meters). As the snow melts, the ground is muddy and full of water in the deeper layers. The lentil is a traditional dry crop, capable of exploiting deep soil water reserves. Sowing is done by hand and during the growth it's need to removal manually the weeds in one or two occasions. At the end of August the plants are uprooted and left to dry under the sun. When the plants are dry, the seeds are sieved and cleaned of leaves and other impurities. Finally the seeds are stored in clean glass jars with cloves of garlic to keep away the insects (*Bruchus ervi* F.) because it feed on seeds of lentils.

For the lentils harvested in the province of Isernia, any amount of seed relating to a farmer was referred to as population.

Four farmers in the village of Conca Casale donated a modest amount of lentil seeds (Farm Store). The same was been obtained in Capracotta, where one farmer supplied the seeds, while two populations came from the “Giardino della Flora

Appenninica" (institute store), one was bought in a local market and sold as a 'local product' (commercial market) and one other was already available at the collection of the Germplasm Bank of Molise (BGMOL) (institute store).

The population of lentils from the village of Castelverrino was kept at the BGMOL (seed store institute). We can consider all these varieties of lentils as primitive cultivars or, more precisely, landraces (IBPGR/ICARDA, 1985; Zeven, 1998; Camacho Villa 2006).

In the three small towns in Molise region (Fig. 5) many elderly people still preserve the traditions of rural life, often still using non-mechanized cultivation techniques and according to the lunar calendar.

Along with this material, a number of other lentil accessions from different sources were made available by the Germplasm Resources Unit of the John Innes Centre (JIC) to act as controls. These originated from Spanish (Salamaca), Syrian (Syrian local large, named Syrian), Argentine (Precoz) and Indian (Pant-L-406).

The following table 10 summarizes the origin and the alpha-numeric identification code of the populations.

Table 10. Origin of populations of *Lens culinaris* M.

Nation	Council	population
Italia	Capracotta	PD03
Italia	Capracotta	PD04
Italia	Capracotta	PD05
Italia	Capracotta	PD06
Italia	Capracotta	PD07
Italia	Conca Casale	PD08
Italia	Conca Casale	PD09
Italia	Conca Casale	PD10
Italia	Conca Casale	PD11
Italia	Castelluccio di Norcia	PD12
Italia	Castelverrino	PD13
Argentine	JIC	Precoz
Canada	JIC	Laird
Spain	JIC	Salamaca
Syria	JIC	Syrian local large
India	JIC	Pant-L-406
Chile	JIC	Chilean

3.3 In laboratory

The work on observation and selection of sub-populations from the different local populations of lentils from Molise was performed following the theoretical schematic shown in Figure 6 (the proposed model has been developed for use with self-pollinating crops, but with some modifications can be applied to cross-pollinating crops).

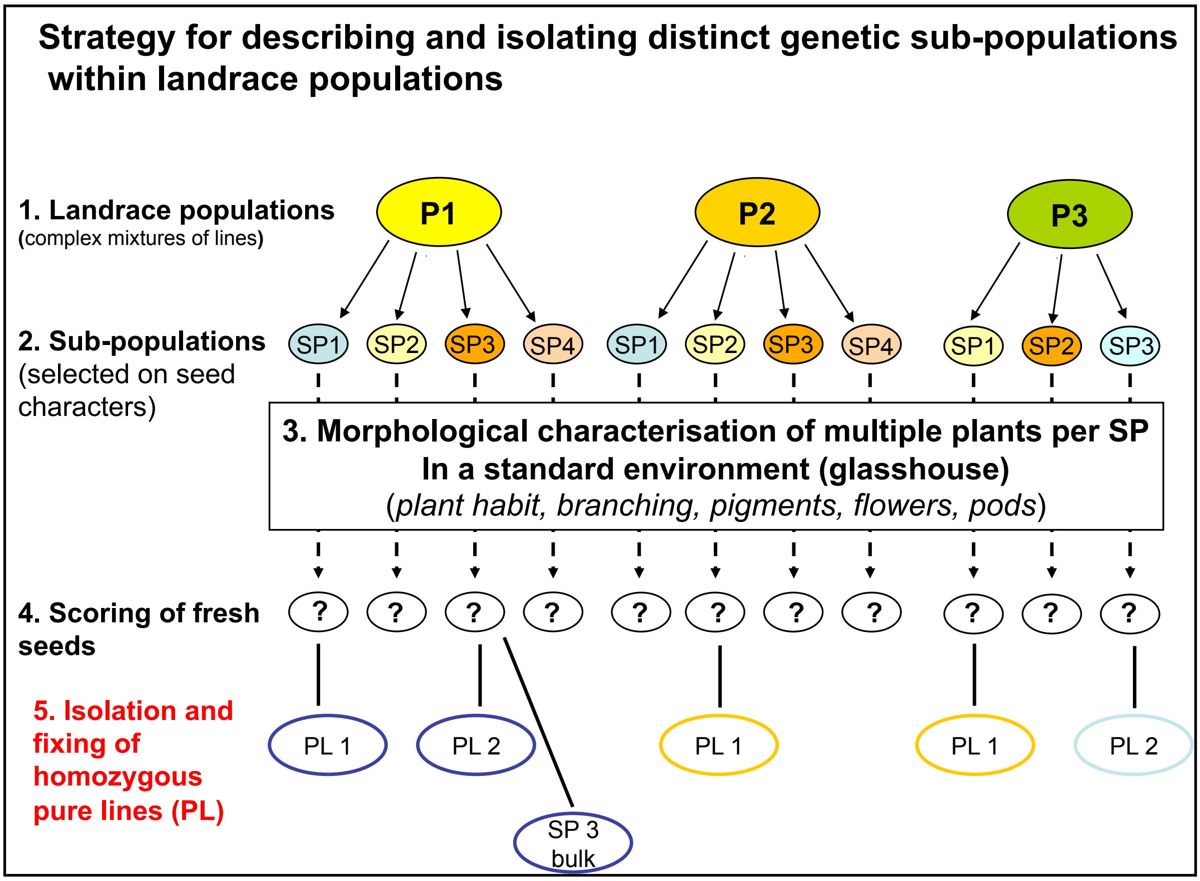


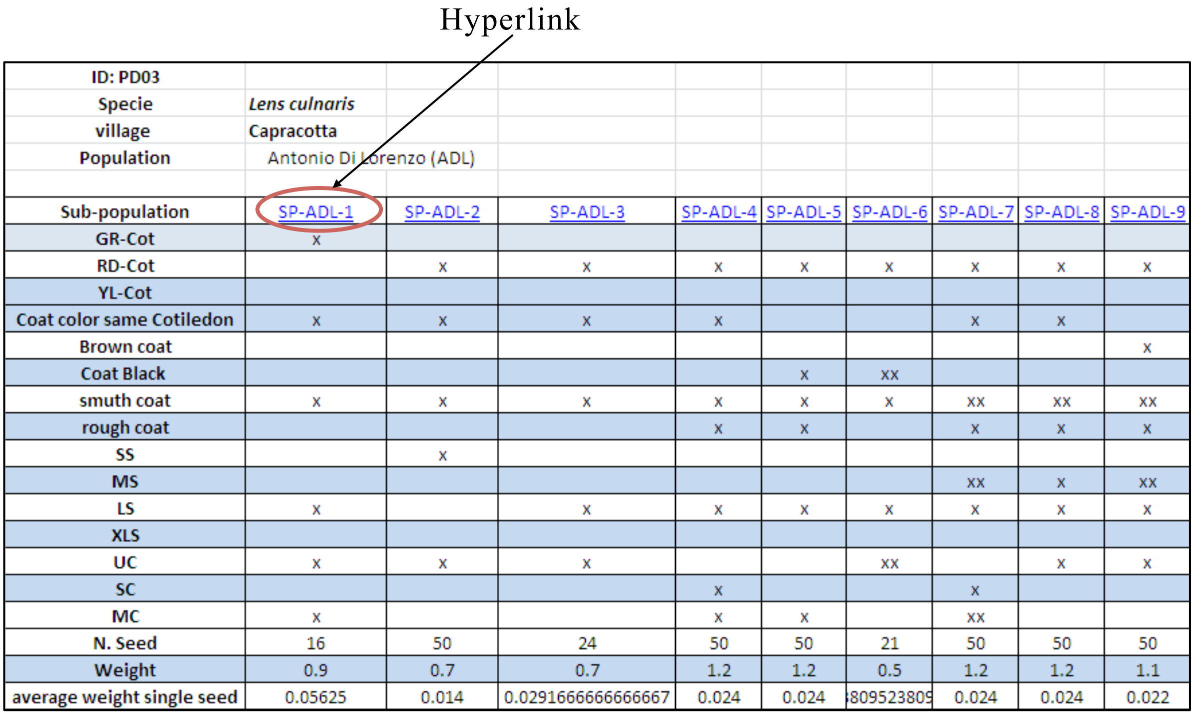
Figure 6. Working scheme followed for the characterization of self-pollinated landraces

From the landraces of Molise and the variety of Castelluccio di Norcia (PD12) various numbers of sub-populations were isolated by phenotypic traits of the seeds known to be of high heritability. The characteristics taken into consideration to differentiate the sub-populations were: color of the cotyledons, color of the coating of the seed (testa) and size of the seed (area, perimeter, diameter and 100 seeds weight) (Cubero, 1984; Vandeberg et al., 1990).

The extreme smallness of the seed features as a discrete sub-population over and above any other character, so the small seeds have different colors but the same average size. The average seed weight of each sub-population and the size of the seeds, obtained from processing the images with the free software *Image Tool* were also recorded.

Each character had the following characteristics discriminating: color of the cotyledons [orange-red (RD-Cot), yellow (YL-Cot) or green (GR-Cot)], color of the coat of the suit (testa) equal to the cotyledons (color coat examination cotyledons) and brown (brown coat). Of these colors can also be other spots more or less marked, indicated as a spot coat (SC) and marbled (MC), to give a completely black (black coat). In addition, seeds were also selected with only one spot because the character is dominant over the absence of spots (IBPGR / ICARDA, 2009, Erskine et al. 1993; Farouk et al., 2009).

All these data were recorded on a spreadsheet as shown in Figure 7 and each sub-population was done with a hyperlink on the image.



ID: PD03									
Specie	***Lens culnaris***								
village	**Capracotta**								
Population	Antonio Di Lorenzo (ADL)								
Sub-population	SP-ADL-1	SP-ADL-2	SP-ADL-3	SP-ADL-4	SP-ADL-5	SP-ADL-6	SP-ADL-7	SP-ADL-8	SP-ADL-9
GR-Cot	x								
RD-Cot		x	x	x	x	x	x	x	x
YL-Cot									
Coat color same Cotiledon	x	x	x	x			x	x	
Brown coat									x
Coat Black					x	xx			
smuth coat	x	x	x	x	x	x	xx	xx	xx
rough coat				x	x		x	x	x
SS		x							
MS							xx	x	xx
LS	x		x	x	x	x	x	x	x
XLS									
UC	x	x	x			xx		x	x
SC				x			x		
MC	x			x	x		xx		
N. Seed	16	50	24	50	50	21	50	50	50
Weight	0.9	0.7	0.7	1.2	1.2	0.5	1.2	1.2	1.1
average weight single seed	0.05625	0.014	0.0291666666666667	0.024	0.024	809523809	0.024	0.024	0.022

Figure 7. Example of Excel spreadsheet to store data on sub-populations of lentils: GR-Cot, cotyledons green, RD-Cot, cotyledons orange, YL-Cot, yellow cotyledons, Brown and Black coat, brown or black coating; smuth and rough coat, rough or smooth coating, SS, MS, LS and XLS indicate the size small, medium, large and extragrande seed; N. Seeding the number of seeds weighed and the total weight. Finally, the average weight of the seed.

At the end of the selection was obtained 106 sub-populations divided as reported in Table 11.

Table 11. Population and sub-population of Lens culinaris M

Council	**Population**	**Sub-population tot.**
Capracotta	PD03	9
Capracotta	PD04	7
Capracotta	PD05	13
Capracotta	PD06	12
Capracotta	PD07	11
Conca Casale	PD08	8
Conca Casale	PD09	7
Conca Casale	PD10	7
Conca Casale	PD11	7
Castelluccio di Norcia	PD12	13
Castelverrino	PD13	12

Each population of lentil was photographed before and after selection, to frame each step, in the second picture the sub-populations were represented by three seeds, Photo 4 and 5.

Photo 4. Population PD06

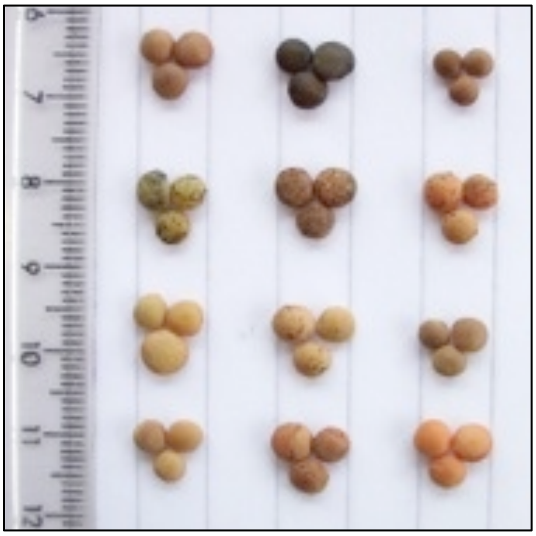


Photo 5. Sub-population comes from PD06

After selection, each sub-population was scanned with a 2D HP scanner and the images were first processed with the free software GIMP 2 to eliminate the defects of images that could distort the data and then processed with free software *Image Tool* (UTHSCSA, v 3.0)[4].

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[4] http://compdent.uthscsa.edu/dig/download.html

3.4 Characterization of plants under controlled environment in greenhouse.

After processing the images from each sub-population, 10 seeds were collected (minimum number of seeds for a crop like lentil self-pollinating) and placed to germinate in small pot in the greenhouse on 28/10/2010, while the PD12 and PD13 sub-populations were sown on 01/11/2010. Each row represented a sub-population and the first plant was marked with a label. On the label was written the code of the population, the sub-population (Photo 6 and 7).

The technique of splitting the population into sub-populations allows every single plant to be followed and to monitor better and faster the internal variability in populations.

The pots had been filled with a mixture of loam, peat, composted green material, limestone, sand and nutrients including potassium nitrate, ammonium sulfate, ammonium mono-phosphate and trace elements; and the pH of the substrate ranged between 6 and 7 (the name commercial substrate: J Arthur Bower's John Innes No.1 Compost), (Photo 7).

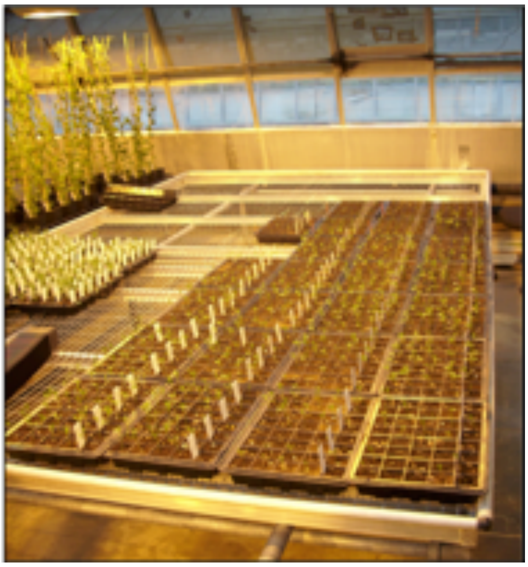

Photo 6. Pots in glasshouse

Photo 7. Individual label and the substrate

The temperature in the greenhouse was maintained at 15 °C with a photoperiod of 15 hours of light per day, since 1:00 a.m. to 16:00 p.m. Irrigation took place regularly at intervals of two days.

The first recorded data in the greenhouse was the number of seeds germinated out of a total of 10 for each sub-population. In some cases, however, some sub-populations consisted of fewer than 10 seeds. In addition, some were sown a few days late due to logistical problems of the technicians in the greenhouse, while three were lost during the various steps.

The number of germinated seeds was checked on 04/11/2010 on seeds that were sown in a greenhouse on 28/10/2010. On 08/11/2010 were recorded the seeds that were placed in a greenhouse on 01/11/2010. On the same day data on the pigment of the epicotyl was scored. For greater accuracy the pigmentation character was also rechecked 10/11/2010.

Twenty days after sowing 5 representative plants of the particular sub-population were selected and transplanted into larger pots, while the others were discarded. (All groups of discarded plants were photographed as bare roots and data recorded relative to the length and weight of the whole plant, stem and roots, but these data have not been used for the characterization).

For each sub-population of 5 plants that were selected, each was marked with a label on which was written the population, sub-population and the number of the plant. This method allowed the clear and immediate identification of discrepancies within the sub-population and population. In photo 8, are evident sticky traps for insects (sciarid fly).

The plants changed in habit during growth and tended to grow into each other, to prevent the intergrowing between neighboring plants it was decided to tie each plant to a short cane placed next to a plastic label (Photo 8 and 9).

Photo 8. Plants in big pots whit tutor

Photo 9. Individual label for each plant

On these selected plants were carried out checks on other characters which were recorded on a spreadsheet. The descriptor states for these characters were based largely on the descriptors published and continually reviewed by the International Board for Plant Genetic resorces (IBPGR) and International Centre for Agricultural Research in the Dry Areaa (ICARDA). These two international institutions publish and update, in collaboration with other institutions of world renown, the descriptors of many crops and characters are considered to have high heritability and the characteristic to be recognizable on sight. The traits considered in this study are listed in Table 12.

In table 12 the first 10 characters are qualitative while the other 13 are quantitative. Quantitative traits were subject to in-depth statistical analysis that revealed the differences between the populations studied and into populations of lentil. Furthermore, quantitative traits were divided in two categories: agronomic and morphological.

Table 12 Traits used for the morphological and agronomic characterization from 'Lentil Descriptors' by IBPGR (1985) (G. Laghetti et al, 2008).Seed colour character was analyzed considering three principal descriptor: groung colour of testa, pattern of testa and colour of pattern on testa.

Trait	**Type of item**	**Subtrait/Score**
Epycotil pigmentation	qualitative	absent (0), present (1)
Leaf pubescence	qualitative	absent (0), slight (3), dense (7)
Leaflet size	qualitative	small (3), medium (5), large (7)
Tendril length	qualitative	absent (0), rudimentary (1), prominent (2)
Flower colour	qualitative	white (1), white with blue veins (2), blue (3), violet (4), pink (5), other (6)
Pod colour	qualitative	absent (0), present (1)
Seed colour (groung colour of testa*; pattern of testa**; colour of pattern on testa***)	qualitative	*green (1), grey (2), brown (3), black (4), pink (5) **absent (0), dotted (1), spotted (2), marbled (3), complex (4) ***absent (0), olive (1), grey (3), brown (4), black (5)
Cotyledon colour	qualitative	yellow (1), orange/red (2), olive–green (3)
Pod shape	qualitative	truncate (1), intermediate(2), pointed (3)
Days to germinate	quantitative	days
Germination ability	quantitative	% of number of seed germinated
Plant habit	qualitative	None(0), low (3), medium (5), high (7)
Plant height	quantitative	cm
100 seeds weight	quantitative	g
First pod height	quantitative	cm
Seed Area	quantitative	cm^2
Seed Diameter	quantitative	cm
Seed Perimeter	quantitative	cm
Time to flowering	quantitative	days from sowing
Time to maturity	quantitative	days from sowing
Time to pod	quantitative	days from flowering
Number of seeds per pod	quantitative	no.
Number of pods per plant	quantitative	no.

At the end of the biological cycle pods and seeds were collected from each plant and placed in paper bags on which was recorded: the date of collection, the population, the sub-population and the identification number of the plant and the number of pods produced by the plant.

The material was transferred to the laboratory where, after the rough cleaning, were recorded the color of the coating of the seed and cotyledons and, finally, 20 seeds of each plant were photographed and the images analyzed with the free software "Image Tool" which calculated the major and minor axes of each seed, the area and perimeter.

This first part of the data collection for agro-morphological characterization of Molise's lentils was performed during time spent at the John Innes Centre in Norwich (UK).

The second phase of the research, which involved the statistical analysis, was performed at the Laboratory of Germplasm Bank of Molise (BGMOL).

The software used was Microsoft Excel with the addition of an application (XLSTAT 2011, Addinsoft and Multibase[5]) to increase the ability to investigate the recorded material.

For statistical analysis only quantitative agronomic and morphological traits were considered. In detail, for agronomic trait were considered:

- pod per plant
- seeds per pod
- days to flowering
- days to pod
- days to maturity

while for morphological trait were anlyzed:

- seed area
- seed perimeter
- seed diameter
- 100 seeds weight
- plant height
- first pod height.

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[5] http://www.numericaldynamics.com/

Different landraces of each village (Capracotta, Conca Casale and Castelverrino) were considered as belonging to a single large group (population) and compared, statistically, to the characteristics of the other control populations (Castelluccio di Norcia, Precoz, Syrian, Salamaca and Pant-L-406).

A clearer idea of the differences between the populations was provided by ANOVA (analysis of variance), but even more significant was the analysis of *Pairwise Comparisons*[6], which generated matrices with results that highlight the differences as "significant" (P value was <0,05) or "highly significant" (P value was < 0,01) for each traits considered among the different populations.

A clearer idea of how landraces of Molise are similar or different from each other and the control variety is obtained from the Principal Component Analysis (PCA) and the Hierarchical Ascending Classification (HAC).

PCA is an analysis that allows the characters to discriminate on the basis of their importance as visualized in multidimensional space. The traits are represented by a vector, the most important of which have a greater distance from the origin[7].

In this way the populations of lentil are grouped according to similarities and close to the vector (traits) more influential. The result is shown in one graph quick and easy to interpret.

Finally, the populations were also represented in a "Cluster Analysis" (Ascending Hierarchical Classification), a Multivariate Analysis technique that organizes different variables in groups with similar characteristics. These groups that originate (or cluster), possess intrinsic similarities from those in adjacent clusters for the characters used in the analysis.

Scatter Plots of the AHC were organized in such a way that: two variables that have similar characteristics will be evident that are closest to within an n-dimensional space that wants to represent, they originate a "condensation nucleus" (proto clusters) whose

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[6] Pairwise comparison generally refers to any process of comparing entities in pairs to judge which of each entity is preferred, or has a greater amount of some quantitative property. The method of pairwise comparison is used in the scientific study of preferences, attitudes, voting systems, social choice, public choice and multiagent AI system.

[7] Beauty consulting principal component (PCA or also known as CPA) is a technique used in multivariate statistics for the simplification of the original data. The primary purpose of this technique is the reduction of a varying number of variables (representing many features of the phenomenon) in some latent variables. This is done through a linear transformation that projects the original variables into a new Cartesian system in which the variables are sorted in descending order of variance: thus, the variable with higher variance is projected on the first axis, the second on the second axis, etc. . The complexity reduction is limited to analyze the main (for variance) between the new variables.

midpoint joining the two variables is called a "centroid". The more the centroid is found close to the point of origin of the proto-cluster the more the two populations (in this case) can be said to have a high degree of similarity. This is defined mathematically by a coefficient of similarity that turns out to be closer to zero when the two cluster are more similar. The process of generating new proto-clusters and centroids between variables belonging to the same space is repeated until the assimilation of all groups into a single entity.

CHAPTER IV

Results

4.1 Analysis of Agro/Morpho/Physiological Lentil Traits

The results shown in this chapter were obtained taking into account the list of characters descriptors published and continually reviewed by the International Board for Plant Genetic Resources (IBPGR) and International Centre for Agricultural Research in the Dry Areaa (ICARDA). IBPGR and ICARDA (in this case lentil descriptor)[8] adopted to share data and information at globally level on some crops, using standard models. This strategic set of qualitative and quantitative descriptors, together with passport data, is the basis for the global accession level information data.

For this study, 23 descriptors (13 quantitative and 10 qualitative characters) were used to characterize three local lentil populations (Capracotta, Conca Casale, Castelverrino), one Italian commercial lentil landraces (Castelluccio di Norcia) and four International commercial pure line (Precoz, Salamaca, Syrian Local Large and Pant-L-406).

To simplify the exposure data, the descriptors were divided into: morphological, physiological and agronomic descriptors.

In detail, for morphological analysis a total of 16 (10 qualitative and 6 quantitative) descriptors were used while for the physiological and agronomic analysis a total of 2 and 5 quantitative descriptors were used, respectively.

4.2 Mophological traits

As reported above, for the morphological analysis, were examined 10 qualitative and 6 quantitative different seed and plant morphological characters/descriptors (see ‘Materials and methods’). The quantitative seed characters were evaluated by use of Image tool software (UTHSCSA, v 3.0) while plant characters were evaluated by manual measurements; each of them was analyzed on 20 seeds and 60 plants and successively was calculated average and standard deviation (Table 13).

The quantitative morphological descriptors were: area (understood as a projection on a plane), perimeter and diameter of seed, weight of 100 seeds, plant height and first pod

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[8] High heritability characters, distinguishable on sight in any environment.

height. The qualitative descriptors were: the colour of the cotyledons and the coat (testa) of the seed, epycotil pigmentation, leaves pubescence, leaflets size, tendril lenght, plant habit, flower colour, pod colour and shape.

4.2.1 Seed morphological characters

4.2.1.a. Colour of seed coating and cotyledon

The landraces of Molise presented almost all the combinations for the colour of the covering of the seed and the cotyledons. Lentil seeds showed all the colour combinations for both cotyledons (yellow, orange-red and olive-green) and coat (gray, black, brown, pink and green; with or without green, olive, brown and black colour spots). They were all expressed in the landraces of Capracotta and Castelverrino while in the Conca Casale lentil always presented red cotyledons and coloration of the tegument both with more or less extensive and no spots. Examples of different seed coating colour combinations are represented in the following picture (Photo 10).

Photo 10. Colour of seed coating of A) Capracotta, B) Castelverrino and C) Conca Casale lentil seeds. Bar represent 1 cm of length.

4.2.1.b Area, Perimeter and Diameter of seed.

Among the landraces of Molise, Castelverrino lentils were found to have seeds with area, perimeter and diameter greater than the other two landraces analyzed. Lentils of Conca Casale are those that appear to be smaller for the same characters, while seeds from Capracotta were intermediate for both on the perimeter, and diameter. All three landraces of Molise can be considered to belong to the category of microsperme, since none had a seed diameter greater than 6 mm (Table 13).

The highest values among all populations, was manifested by the "pure line" (pl)

Salamanca, which together with the lentils indicated as Syrian, can be considered macrosperma, since the value of the diameter is 0,72 cm and 0,69 respectively (Table 13). The smaller absolute values showed them the variety Pant-L-406, very similar to the values of lentils Conca Casale. Castelluccio di Norcia, Italian variety used as a control, showed very similar values to those of the landrace of Capracotta, with 0,15 cm^2 area of the seed, 1,45 cm of perimeter and 0,443 cm in diameter (Table 13).

4.2.1.c Seed weight

The seed weight (100 seeds) of lentil landraces from Molise were found to cover a range from g. 2,36 Pant-L-406 lentil to g. 8,89 of Salamaca lentil (Table 13).

With regard to the populations coming from Molise, have that the landrace of Castelverrino reaches a weight of 5.64 g per 100 seeds, followed by the landrace of Capracotta with g 3.47, and the last landrace of Conca Casale reached a weight of 2.74 g, equal to about half of the seeds of Castelverrino (Table 13).

The variety of Castelluccio di Norcia showed an intermediate value between the varieties of Conca Casale and Capracotta, with a weight of 2.746 g per 100 seeds (Table 13).

4.2.2 Plant morphological character

4.2.2.a Plant height

The character "plant height" was recorded in the days between flowering and pod formation. The leguminous plants generally grow in height at a constant speed after the early exponential seedling stage until the flowering stage, and then slow down very sharply (M. Ambrose, personal communication).

The highest average for this character was recorded for the landrace of Castelverrino with 69.3 cm, while the lower was the variety Pant-L-406, with a height of 46 cm (Table 13). Among the ecotypes of Molise the smallest was for Capracotta, with a population mean of 51.3 cm, a value equal to that of the variety Precoz, while Conca Casale showed an average height of 53.4 cm. Finally, Castelluccio di Norcia showed an average height value of 64.2 cm which was slightly higher than that of the Syrian variety (Table 13). An outstanding figure was shown from a plant of the variety of Castelluccio di Norcia, which reached the height of 99 cm, followed by a plant from

Castelverrino with a height of 81 cm (data not showed).

4.2.2.b First pod height

For this character, the variety that has reached the greater height was from Castelverrino with 51.8 cm, the lower was the variety Pant-L-406 with 22.2 cm (Table 13).

Among the varieties of lentils, of the Molise, Capracotta was found to have the lowest average with 29.3 cm, slightly higher than the variety Precoz, while Conca Casale reached an average height to the first pod of 33.4 cm. Castelluccio di Norcia reached a height of 40.7 cm, equal to the Syrian variety (40.2 cm) (Table 13).

4.2.2.c Epicotyl pigmentation

About Epicotyl pigmentation character, the lowest absolute value (0%) ie. Complete absence, was recorded for the Syrian variety, while the highest value was expressed by the population of Precoz and Pant-L-406 (100%) (Table 13). Among the varieties of Molise, Conca Casale showed the character on 97% of the plants, Casteverrino on 78% and Capracotta on about half of the plants germinated (49%). Castelluccio di Norcia reached a value of 63% of pigmented plants. Salamanca is the only pure line that did not deliver a net character (presence or absence of pigment) but also showed 44% of pigmented plants (Table 13).

Table 13. Morphological measurements of seed and plant characters of the Capracotta, Conca Casale and Castelverrino lentil populations and of the commercial lentil *cultivars* (Castelluccio di Norcia, Precoz, Salamaca, Pant-L-406 and Syrian). For each character average and standard deviation was calculated. Seed character average was calculated on 100 seeds while plant character average was calculated on 60 plants.

Lentil populations and cultivar	**Area (cm^2)**	**Perimeter (cm)**	**Weight seeds (g) (100 seeds)**	**Diameter (cm)**	**Plant height (cm)**	**First pod height (cm)**	**Epicotyl pigmentatio (%)**
Capracotta	0,159 ± 0,02	1,448 ± 0,1	3,478 ± 0,264	0,447 ± 0,0523	51,3 ± 7,69	29,3 ± 5,62	49%
Conca Casale	0,115 ± 0,02	1,251 ± 0,085	2,746 ± 0,064	0,387 ± 0,005	53,4 ± 5,87	33,4 ± 4,59	97%
Castelverrino	0,24 ± 0,04	1,85 ± 0,15	5,614 ±16,310	0,559 ± 0,052	69,3 ± 5,45	51,8 ± 6,59	78%
Castelluccio di Norcia	0,15 ± 0,02	1,45 ± 0,09	2,921 ±17,176	0,443 ± 0,04	64,2 ± 14,63	40,7 ± 14,16	63%
Precoz	0,23 ± 0,08	1,75 ± 0,58	5,660 ± 0,099	0,594 ± 0,038	51,6 ± 4,15	28 ± 3,02	100%
Salamaca	0,29 ± 0,0,14	1,88 ± 0,88	8,890 ± 0,097	0,726 ± 0,012	68,2 ± 7,04	44,2 ± 10,28	44%
Pant-L-406	0,11 ± 0,01	1,22 ± 0,04	2,360 ± 0,013	0,38 ± 0,012	46 ± 3,31	22,2 ± 1,98	0%
Syrian	0,25± 0,19	1,58 ± 1,15	7,938 ± 0,192	0,695 ± 0,032	62 ± 1,91	40,2 ± 2,33	100%

4.2.2.d Leaves pubescence.

The pubescence of the leaves was estimated as dense, medium and slight pubescence grade (Photo11A, B and C, respectively) of each plant compared to the leaves pubescence of lentil Precoz variety, which was given a mean value pubescence. Figure 8 shows the percent of plants per population that have the character slight, Medium or Dense pubescence for each population. Lentil landraces from Molise all exhibited average slight and medium pubescence of leaves. Dense pubescence not always performed, it was expressed in low percentages.

Photo 11. Character Dense (A), Medium (B) and Slight (C) leaf pubescence considered for each lentils plant.

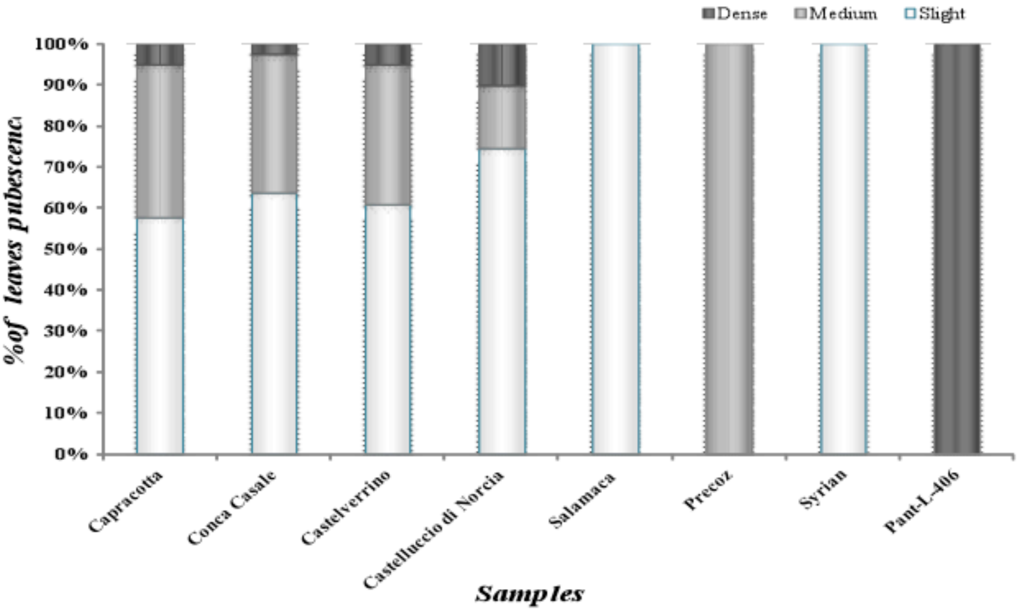

Figure 8 % of Dense, Medium and Slight pubescence leaves in each lentil populations. Dense pubescence leaves are reported in black, Medium pubescence leaves are reported in grey and Slight pubescence leaves are reported in white.

4.2.2.e Size of leaflets.

Leaflets size character was recorded using the codes suggested ICARDA and IBPGR (small, medium and large leaves) estimated at sight, for each plant (Table 14).

The majority of the populations have shown have a size of the leaves medium, while only Conca Casale Syrian and were shown to have small leaves (Photo 12A,B). Within the population, there were some individual plants have shown that large values of leaves, but had little result on the average population.

Table 14 and **Photo 12 a and B.** Leaflets size was reported as index value : “S” indicated small leaflets size and “M” indicated medium leaflets size. Two examples of Medium (A) and Small (B) leaflet are reported in photo 12.

LEAFLETS SIZE	
Population	**leaflets size**
Capracotta	**M**
Conca Casale	**S**
Castelverrino	**M**
Castelluccio di Norcia	**M**
Precoz	**M**
Salamaca	**M**
Pant-L-406	**M**
Syrian	**S**

4.2.2. f Flower colour and pod shape

The flower colour and shape of pods were the same for all analyzed samples. Petals were coloured white with purple streaks (Photo 13 A) while shape of the pods (Photo 13 B) was truncated. For this reason, these two traits were not considered discriminating between analyzed population or/and cultivar.

Photo 13 A) Lentil flowers colour: white petals with purple streak. B) Shape of lentil pod. These two morphological characters were the same in each lentil populations and cultivars.

4.2.2.g Pod colour

Another character analyzed was pod colour. Figure 9A shows the percentages of plants with pods with spots on the total number of plants analyzed. The pod colour was always light brown (Fig. 9B) with spot pale brown (Fig. 9C), with some different nuances. The pod colour was always light brown (Fig. 9B) with spot pale brown (Fig. 14C), with some different nuances. The population with the greatest percentage is Conca Casale, which has manifested the character pod spot for more than 90% of the plants, while Salamanca was the population with the lowest percentage of pod spot (40%; Fig.9A). The lentils Castelverrino have reached 60% of plants with pods with spots, slightly higher than the value reached by Precoz with 65%, while the lentils Capracotta has reached 68% of pod spot, just below the lentils of Castelluccio di Norcia who have reached 70%. The only two varieties that have not experienced pod spot were Pant-L-406 and Syrian (Fig.9A).

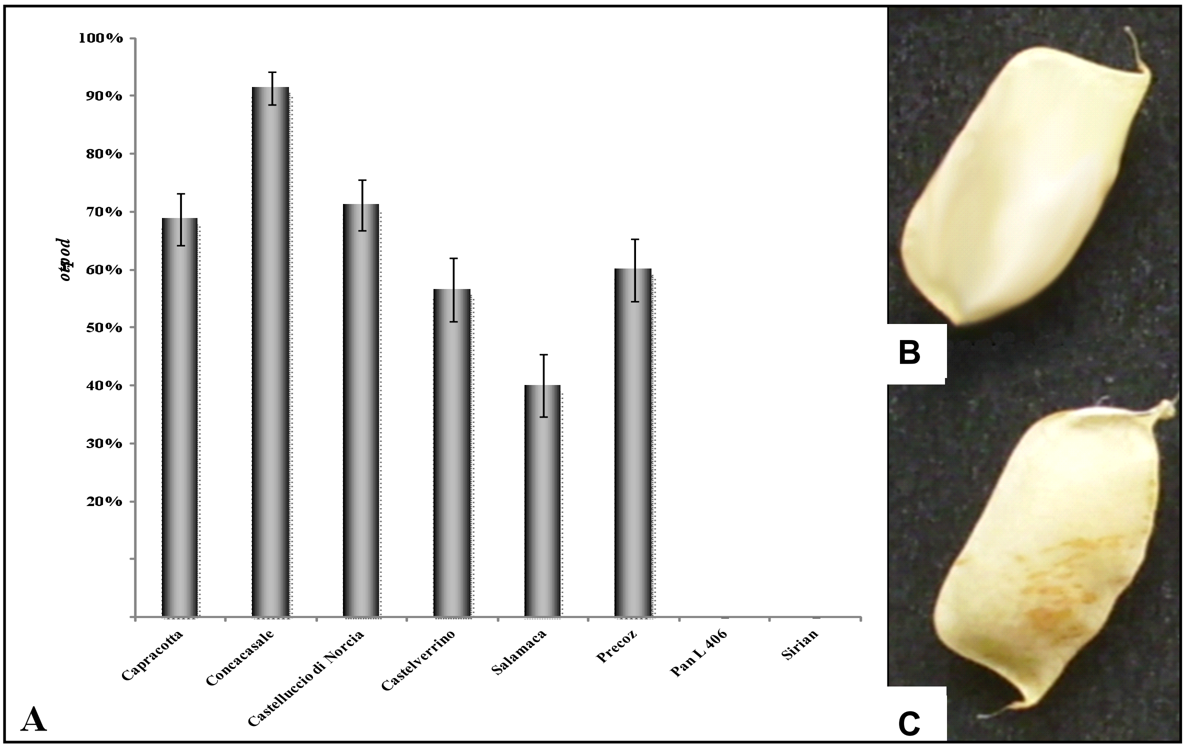

Figure 9. Percentage of numerous plants with spot-pod (A). The pod colour was always pale brown (B) with spot pale brown (C).

Only 5 plants belonging to the population of Capracotta, one plant from Conca Casale and one from Castelluccio di Norcia, exhibited patches of purple colour (Photo 14).

Photo 14. Pods with purple colour patches.

4.2.2.h Presence of a terminal tendril and the plant habit.

In the phase before flowering, some plants showed a well developed terminal tendril at the distal end of the leaf rachis, while in other plants was only slightly developed. After flowering, all plants exhibited well developed tendrils which were rolled up at the end. Therefore it was not considered as a character discriminating between varieties of lentils from Molise and controls, but it is still useful, for the morphological description of landraces, knowing that the character "tendril" is well developed. Also, plants have a tendency to attach to adjacent plants just before flowering, to be completely flattened at maturity. Therefore, even for the character "Habitus of the plant" there was no distinction between the controls (Castelluccio di Norcia, Salamaca, Pant-L-406, Syrian and Precoz) and the varieties of Molise.

4.3 Physiological traits

Immediately after sowing was recorded: “days to seed germination”. Moreover, the germination ability was also considered. It represented the percentage of number of seeds germinated on a total of 1022 places seeds in seedbeds.

4.3.1 Days to seed germination

In Figure 10 was reported the number of days that lentil seed, from different populations, need to germinate. Data showed that the lentils of Castelverrino and Castelluccio di Norcia have an early germination and homogeneous (7 days), while the landraces of Conca Casale and Capracotta are germinated in 11 days on average with maximum values of 15 days. The other controls such as Salamanca, Precoz, Pant-L-406 and Syrian have germinated in a uniform way in 11 days (Fig. 10).

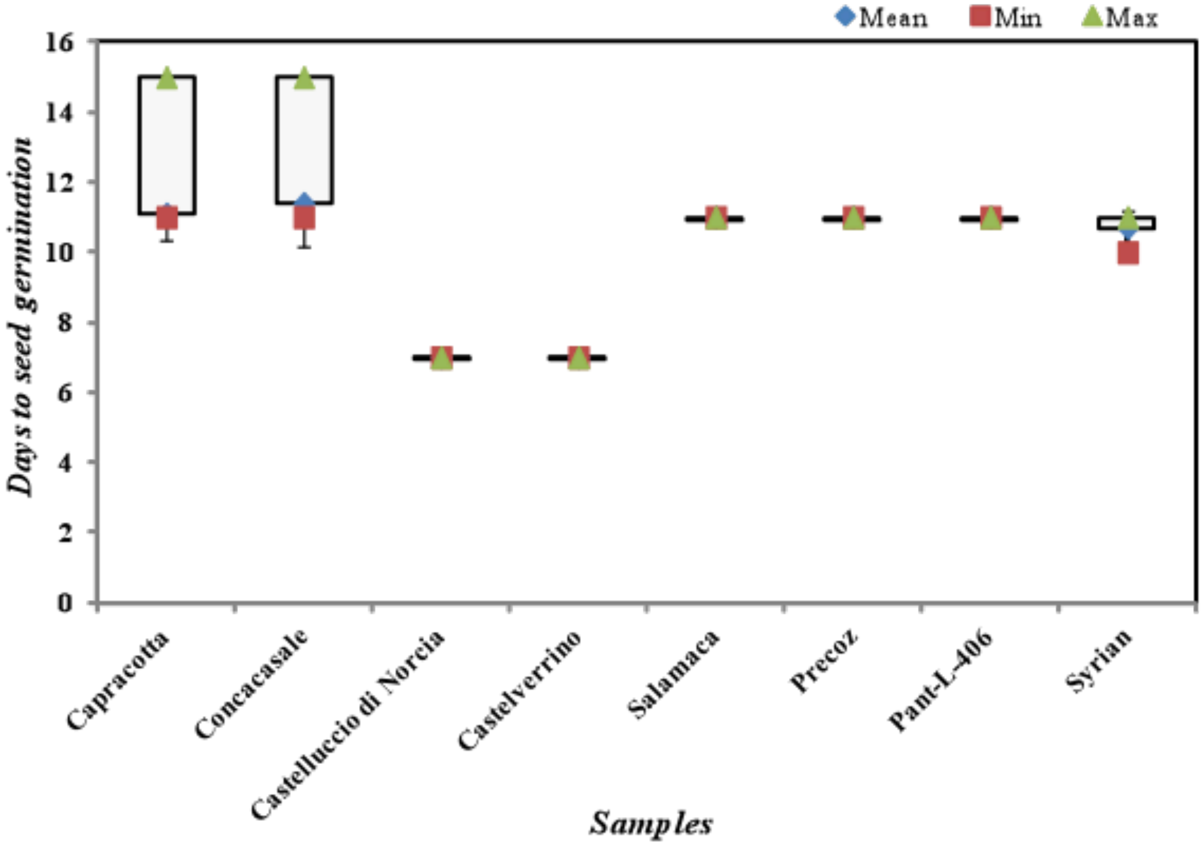

Figure 10. Days to seeds germination. Plot represents the lentil seeds germination trend for each lentil landraces from Capracotta, Conca Casale and Castelverrino; and cultivar Salamaca, Precoz, Pant-L-406 and Syrian. Minimum, Maximun and Mean of days to seeds germination (three replicates of 30 seeds for each sample) and standard deviation was reported. Reed rectangle was used to indicate the *minimum* of days to seed germination; green triangle was used to indicate the *maximum* of days to seed germination and blue rumble was used to indicate the *mean* of days to seed germination.

4.3.2 Germination ability

Another parameter which is generally measured, to characterize the seeds, is the germination ability (Fig. 11), which is one of the qualitative tests for evaluating a batch of seeds, but which is not sufficient to express other quality components of the seed (APAT 2006). The population with a lower percentage of germination was the variety

Syrian (40%), while the variety with higher percentage of germinated seeds was the variety of Castelluccio di Norcia (93%), slightly higher than the variety Salamanca (90%). Among the varieties of lentils, of the Molise appears that population with higher percentage of germinated seeds was to Capracotta (86%), followed by lentils from Castelverrino (83%) and finally the lentils of Conca Casale (70%), which had the same percentage of Precoz and Pant-L-406 (Fig. 11).

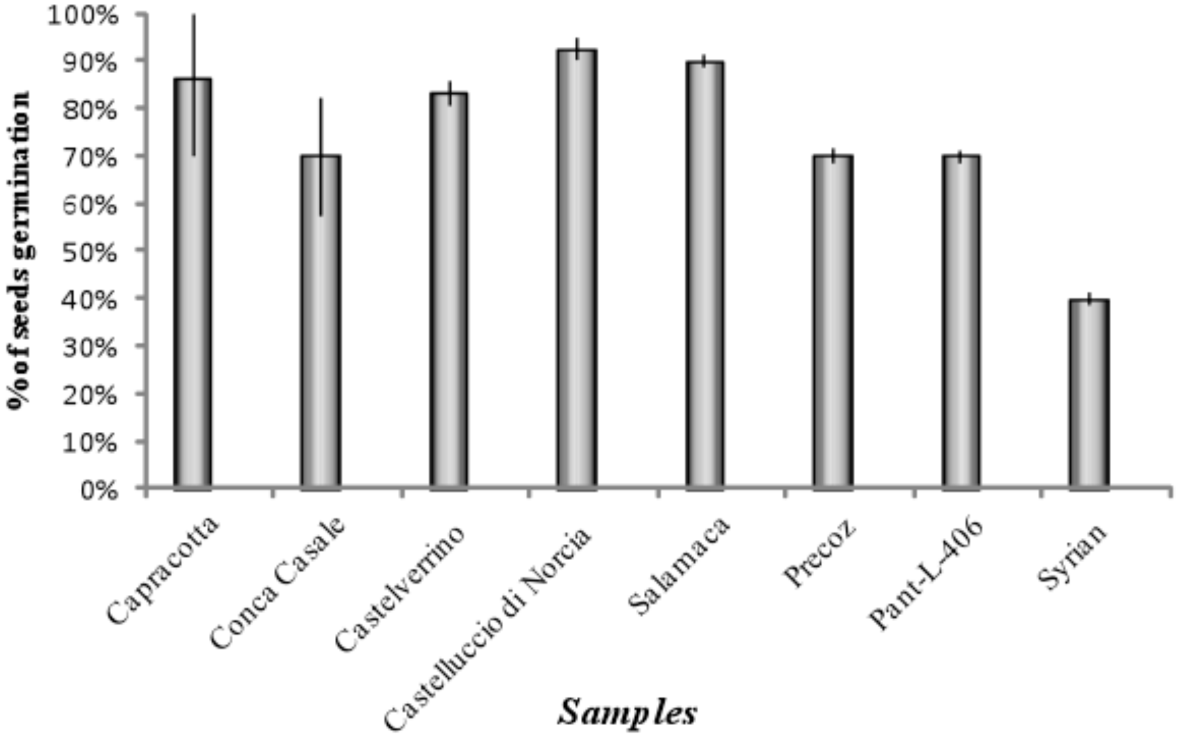

Figure 11. Percentage of germination ability. Lentil seeds germination percentage for each population (Capracotta, Conca Casale and Castelverrino) and cultivar (Salamaca, Precoz, Pant-L-406 and Syrian). Three replicates of 30 seeds for each sample were evaluated.

4.4 Plant agronomic traits

The agronomic traits measured in this test are: pods per plant, seeds per pod, days to flowering, days to pod and days to maturity (Table 15). The other agronomic traits such as resistance to disease and adaptation to certain stressful environments (environments with saline soils and / or dry, etc.) were not taken into account, because the test was conducted in a controlled environment condition.

4.4.1 Pod per plant

The variety that has reached the highest values for the character pods per plant was to Capracotta, with an average of 73.6 pods per plant, followed by the landrace of

Conca Casale with 66.4 pods per plant (Photo 15A; Table 15). Castelverrino showed an average of 41.8 pods per plant; Castelluccio di Norcia reached a data of 50 pods per plant. The cultivar Salamanca, Precoz, Pant-L-406 and Syrian have all expressed a low value compared to the Italian varieties, in fact, Salamanca has produced on average 27 pods per plant, Precoz 34.8, Pant L-406 31.2 and Syrian 29,2 (Table 15).

Photo 15. Lentil plant with pods (A) and pod with seeds (B) (Capracotta: Giardino della Flora Appenninica, P.to Dr. G. Pelino)

4.4.2 Seeds per pod

All populations have produced an average of 2 seeds per pod, except Salamanca and Syrian who have expressed a minimum value (1 seeds per pod) (Photo 15B; Table 15), while from Castelverrino has produced on average 1.5 seeds per pod, this result is due to the fact that some plants have product 2 seeds per pod while other one (Table 15).

4.4.3 Days from sowing to flowering

The landrace more late has been to from Castelverrino that employed an average of 80.3 days to flower, followed by the ecotype of Conca Casale, which took 75.1 days

(Table 15). The landrace of Capracotta flourished in 67.8, two days more of the variety of Castelluccio di Norcia. The variety that took fewer days to flower was Pant-L-406 with 56.8 days, while Salamanca, Precoz and Syrian have used 74, 64 and 70 days, respectively (Table 15).

4.4.4 Time to pod set

The variety was faster to attach Precoz with 5 days, while the later stages were Salamaca with 11 days and Pant-L-406 and Syrian with 10 days (Table 15). The Italian varieties expressed intermediate values to the above, particularly Castelluccio di Norcia took 6.4 days to attach, while Capracotta and Conca Casale took 7.6 and 7.8 respectively. In the end the landrace of Castelverrino was earlier attached to the varieties of Molise with 6.1 days (Table 15).

4.4.5 Time to maturity

Among all the varieties was the most precocious Pant-L-406 with 102 days to mature, while the later was Salamanca which took 134 days, one more than the Syrian (Table 15). Among the earliest Italian varieties was Castelluccio di Norcia, 115 days, followed by the landrace of Capracotta with 123 days. While the ecotype of Conca Casale and Castelverino have both spent 131 days (Table 15).

Table 15 Agronomic traits measurements of five different characters of the Capracotta, Conca Casale and Castelverrino lentil populations and of the commercial lentil cultivars (Castelluccio di Norcia, Precoz, Salamaca, Pant-L-406 and Syrian). For each character average of 60 plants and standard deviation was calculated.

Population	Pod per plant	Seeds per pod	Days to flowering	Days to pod	Days to maturity
Capracotta	73,6 ±27,47	1,9 ±0,37	67,8 ±5,12	7,6 ±3,74	123 ± 8,81
Conca Casale	66,4 ±19,71	2,0 ±0	75,1 ±2,8	7,8 ±2,18	131 ±2,58
Castelverrino	41,8 ±17,37	1,5 ±0,53	80,3 ±4,6	6,1 ±2,74	131 ±2,88
Castelluccio di Norcia	50,1 ±14,36	2,1 ±0,51	65,6 ±6,57	6,4 ±2,79	115 ±5,92
Salamaca	27,0 ±11,53	1,0 ±0	73,8 ±4,02	11,8 ±4,09	134 ±6,26
Precoz	34,8 ±6,69	1,8 ±0,45	64,0 ±2,74	5,0 ±0	121 ±7,52
Pant-L-406	31,2 ±8,01	2,0 ±0	56,8 ±5,17	10,2 ±5,17	102 ± 2,74
Syrian	29,2 ±4,78	1,0 ±0	69,2 ±5,5	10,0 ±1,15	133 ±6,65

4.5 Multivariate statistical analysis.

To summaryze the information, for the most important morphological and agronomic traits (F.J. Muehlbauer et al., 1989) were performed a uni- and multi-variate statistical analysis: ANOVA (Analysis of Variance), pairwise comparison, APC (Analysis of Principal Component) and Ascending Hierarchical Classification (AHC).

4.5.1 Pairwise comparison

The ANOVA showed in all populations of lentils that is statistically significant difference (p <0.05) for each character, but did not distinguish among populations were indeed different. For these appeals to the pairwise comparison which showed that the population of Molise lentil differed statistically significantly from controls.

From Table 16 shows that the lentils Capracotta differ statistically significantly for the first three characters with all the control populations, except with the lentils from Castelluccio di Norcia. For the character seed weight difference is only Precoz and Salamanca. For the character plant height differs from Castelluccio di Norcia and Precoz, differs significantly from the same plants for the character height of the first pod in addition to Syrian.

Table 16. P values of pairwise tests on *morphological traits.* The significant discriminant character was indicated in bold (P< 0,05).

Morphological traits	Population	Castelluccio di Norcia	Precoz	Salamaca	Pant-L-406	Syrian
Area of seeds	Capracotta	1,000	**0,0001**	**< 0.0001**	**0,003**	**< 0.0001**
	Conca Casale	**0,0001**	**< 0.0001**	**< 0.0001**	0,122	**< 0.0001**
	Castelverrino	**0,001**	0,957	**0,002**	**0,002**	**0,002**
Perimeter of seeds	Capracotta	1,000	**0,0001**	**< 0.0001**	**0,006**	**< 0.0001**
	Conca Cassale	**0,0001**	**< 0.0001**	**< 0.0001**	0,217	**< 0.0001**
	Castelverrino	**0,001**	0,540	**0,002**	**0,002**	**0,002**
Diameter of seeds	Capracotta	1,000	**0,001**	**< 0.0001**	**0,004**	**< 0.0001**
	Conca Casale	0,178	0,108	0,108	0,083	0,108
	Castelverrino	**0,001**	0,755	**0,002**	**0,002**	**0,002**
Weight of seeds	Capracotta	0,943	**0,020**	**0,020**	0,077	0,064
	Conca Casale	0,980	**0,030**	**0,030**	0,288	0,084
	Catelverrino	**< 0.0001**	**0,040**	1,000	**0,019**	0,293
Plant height	Capracotta	**< 0.0001**	**0,014**	1,000	0,720	0,118
	Conca Casale	**< 0.0001**	**0,007**	0,991	0,098	0,053
	Castelverrino	0,529	0,999	**0,006**	**0,006**	0,156
First pod eight	Capracotta	**< 0.0001**	**0,022**	0,999	0,060	**0,034**
	Conca Casale	0,117	0,097	0,161	**0,006**	0,093
	Castelverrino	**0,0001**	0,473	**0,006**	**0,006**	0,058

The ecotype of Conca Casale does not differ statistically only from the cultivar Pant-L-406 both for the character area of the seed both for the perimeter. For the character seed diameter is no different from any control variety. For seed weight, and is distinguished from Precoz Salmaca. For the character height of the plant can be distinguished from Castelluccio of Norcia and Precoz, while for the height of the first pod is distinguished only by Pant-L-406 (Table 16).

The landrace of Castelverrino showed statistically significant differences in the character area, perimeter and diameter of the seed from all control populations except by Precoz. From this variety of lentils Castelverrino differ only in the character of the seed weight, for the same character is distinguished from Castelluccio di Norcia and Pant-L-406. For the character height of the plants has significant difference with the cultivars Salamanca and Pant-L-406, from which it differs for the height of the first pod, as well as Castelluccio of Norcia (Table 16).

In Table 17 are compared statistically the varieties of Molise with control cultivars for agronomic traits, it is clear that the landrace of lentils of Capracotta has significant differences with all control cultivars for the character pod per plant, while for the character seeds per pod differs only from Castelluccio di Norcia, Salamanca and Syrian. For the character days to flowering is distinguished from the cultivar Pant-L-406. There are no significant differences with the control varieties for the character days to pod, while remaining distinct from Castelluccio di Norcia and Pant-L-406 for the character of days to maturity (Table 17).

Table 17. P values of pairwise tests on *agronomic traits.* The significant discriminant character was indicated in bold (P<0,05).

Agronomical traits	Population	Castelluccio di Norcia	Precoz	Salamaca	Pant-L-406	Syrian
Pod per plant	Capracotta	**< 0.0001**	**0,009**	**0,006**	**0,007**	**0,021**
	Conca Casale	**< 0.0001**	**0,010**	**0,005**	**0,006**	**0,018**
	Castelverrino	0,102	0,950	0,395	0,715	0,604
Seeds per pod	Capracotta	**0,012**	0,983	**< 0.0001**	1,000	**< 0.0001**
	Conca Casale	**0,002**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
	Castelverrino	**< 0.0001**	0,985	0,236	0,644	0,367
Days to flowering	Capracotta	0,557	0,592	0,193	**0,011**	0,998
	Conca Casale	**< 0.0001**	**0,003**	0,782	**0,003**	0,106
	Castelverrino	**< 0.0001**	**0,003**	0,124	**0,003**	**0,026**
Days to pod	Capracotta	0,198	0,619	0,184	0,916	0,486
	Conca Casale	**< 0.0001**	**0,014**	0,241	0,988	0,281
	Castelverrino	1,000	0,908	0,055	0,760	0,115
Days to maturity	Capracotta	**< 0.0001**	1,000	0,059	**0,005**	0,230
	Conca Casale	**< 0.0001**	0,082	0,260	**0,002**	0,799
	Castelverrino	**< 0.0001**	**0,038**	0,258	**0,002**	0,630

The landrace of lentils from Conca Casale is distinguished statistically by control cultivars for characters pods per plant and seeds per pod, while is not distinguished from Salamanca and Syrian for the character days to flowering. For the character days to pod landrace of Conca Casale has significant differences by Castelluccio di Norcia and Precoz. Finally the character days to maturity Conca Casale is distinguished only from Castelluccio and Pant-L-406 (Table 17).

The ecotype of lentils from Castelverrino is indistinguishable statistically significantly to the character 2pods per plant” and “days to pod” from the control cultivars, while the character “seeds per pod” is distinguished only by Castelluccio di Norcia. For the character of days to flowering cultivar is distinguished from all control except by Salamanca (Table 17).

Finally, for the character of days to maturity lentils Castelverrino differ statistically from Castelluccio di Norcia Precoz and Pant-L-406 (Table 17).

4.6 Principal Component Analysis (PCA) and Ascending Hierarchical Clustering (AHC)

To visualise even more clearly and easily interpretable similarities (or differences) both between landraces of Molise and controls, and between the same landraces, the data was analyzed using two statistical techniques that process easy to understand graphs: Principal Component Analysis (PCA) and Ascending Hierarchical Clustering (AHC).

To avoid an overload of data in the charts, which are difficult to organize in a two-dimensional space, these were made after selecting the data for those characters that were morphological and agronomic most important (Yadav et al., 2007). The means and median were then calculated and divided into morphological and agronomic traits.

4.6.1 Principal Component Analysis (PCA)

Statistical analysis was computed on the eleven quantitative agro-morphological characters (see ‘Materials and methods’) analyzed for the Conca Casale, Castelverrino and Capracotta lentil populations, one commercial lentil varieties (Castelluccio di Norcia) and four commercial cultivar (Salamaca, Syrian, Precoz and Pant-L-406). In detail, PCA was computed on a standardized matrix of 8 populations for a total (eleven characters) quantitative agro-morphological traits and for a quantitative agronomic (five characters) and morphological traits (six characters), separately.

In the scatter plot of PC1 and PC2 scores (Fig. 12a) of quantitative agro-morphological traits analysis, which accounted for 77.1 and 12.1% of the total variance respectively, the characters are all very influential in discriminating the populations represented. The character of "days to maturity" isn't considered influential because showed a position on scatter plot contrary to the rest of the group of characters.

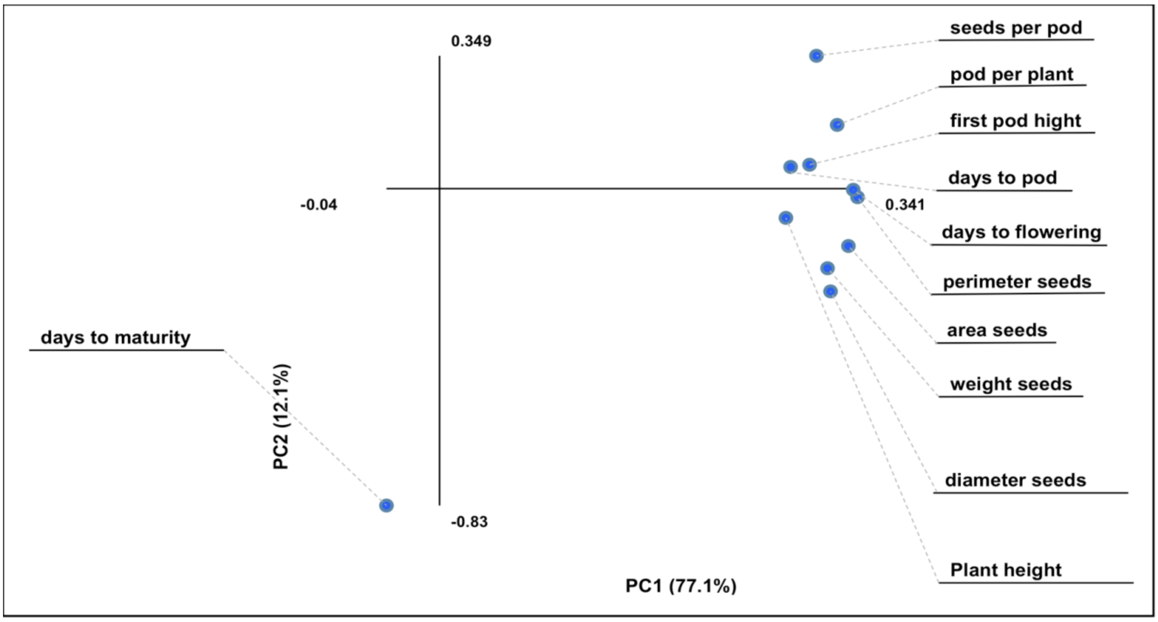

Figure 12a. Mean of eleven quantitative agronomical and morphological traits (variables) chosen. Each variable was represented by vectors.

In Figure 12b was reported the principal component analysis (PCA) results. It is highlighted the great variability of the populations of Capracotta (pink area) that the margin includes the population of Conca Casale, while Castelverrino is distant than the other two landraces of Molise.

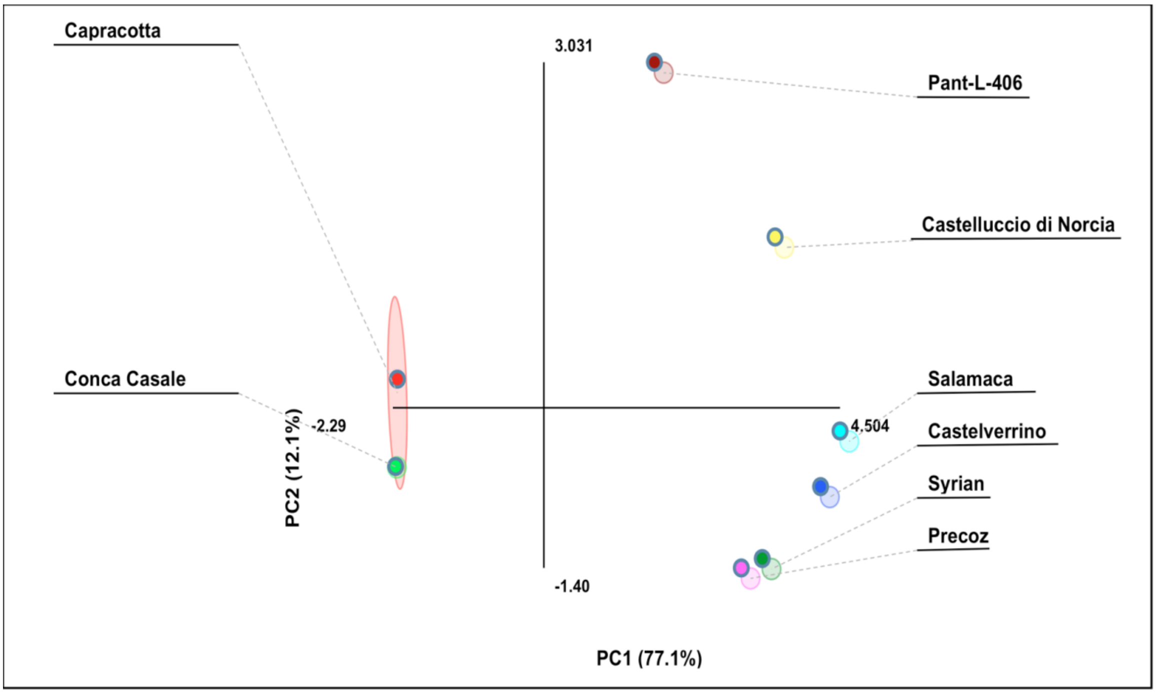

Figure 12b. Scatter plot of the first two principal components from the principal component analysis (PCA) computed among 8 populations of Lens culinaris Medik., using eleven quantitative agro-morphological features.

In the scatter plot of PC1 and PC2 scores (Figure 13a) of five quantitative agronomic traits analysis, which accounted for 73.7 and 20.1% of the total variance respectively, the characters are all very influential in discriminating the populations represented; days to maturity remains influential because of the position opposite to the rest of the traits considered.

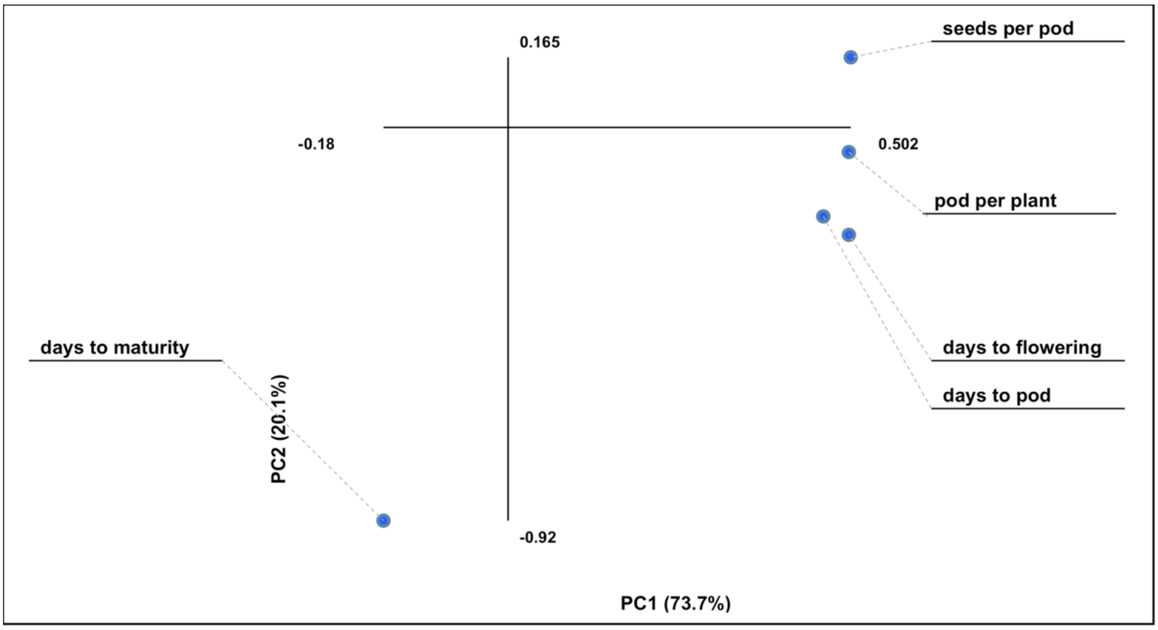

Figure 13a. Mean of five quantitative agronomical traits (variables) chosen. Each variable was represented by vectors.

Figure 13b Conca Casale remains in the "variability" of Capracotta; the cultivars of Salamanca is the only record that makes a significant shift compared to the previous graphic. For the rest the distribution of the population remains the same.

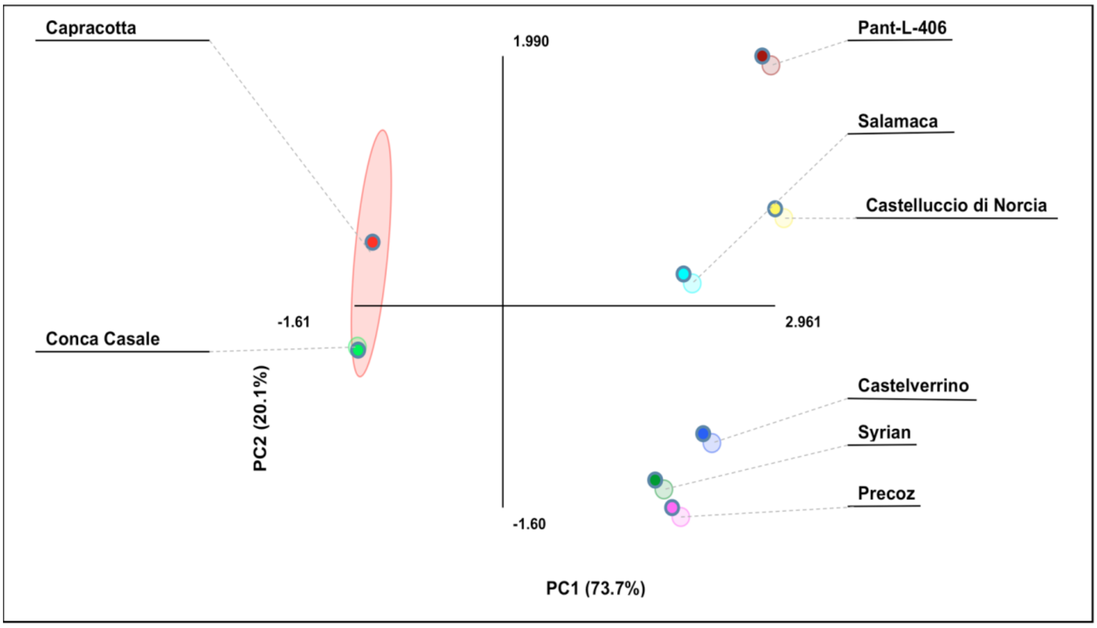

Figure 13b. Scatter plot of the first two principal components from the principal component analysis (PCA) computed among 8 populations of Lens culinaris Medik., using five quantitative agronomic features.

Also in the scatter plot of PC1 and PC2 scores (Figure 14a) of six quantitative morphological traits analysis, which accounted for 88 and 6% of the total variance respectively, the characters are all very influential in discriminating the populations represented. From the figure we see that the characters first pod and plant height are less influential in the multi-dimensional distribution showed in figure 14b.

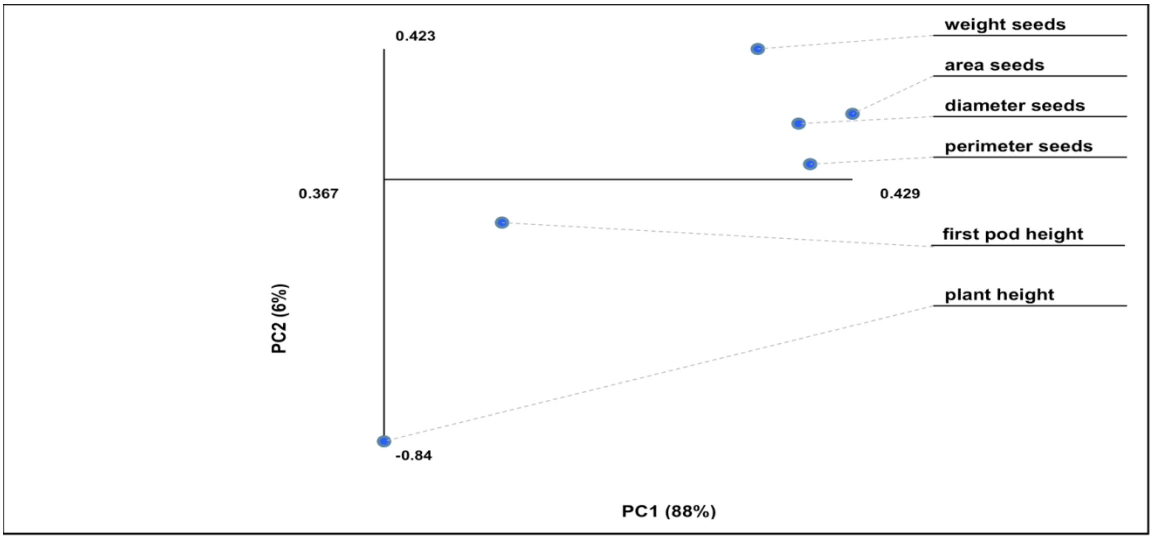

Figure 14A Mean of six quantitative morphological traits (variables) chosen. Each variable was represented by vectors.

In the graph of the Figure 14b, the PCA obtained by averaging the morphological characters we have that the populations of Conca Casale and Capracotta overlap almost coincide, while Castelverrino remains distant and close to the population of Castelluccio di Norcia.

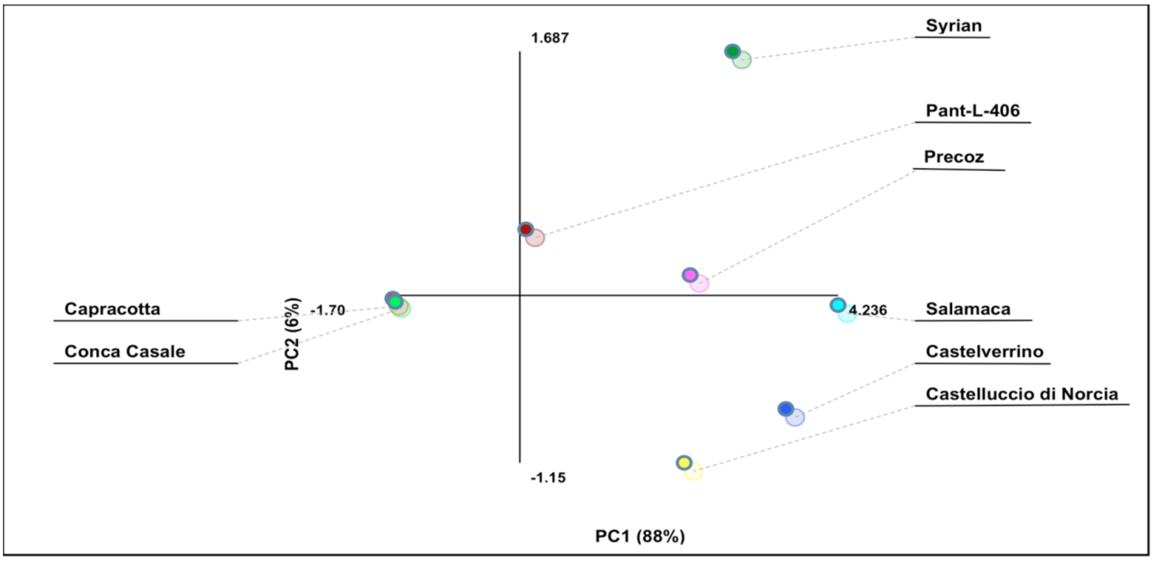

Figure 14b. Scatter plot of the first two principal components from the principal component analysis (PCA) computed among 8 populations of Lens culinaris Medik., using six quantitative morphological features.

4.6.2 Ascending Hierarchical Clustering (AHC)

The Figure 15 shows the AHC obtained by considering the mean of all the characters of the populations; it follows that the varieties of lentils from Conca Casale, Capracotta, Castelluccio di Norcia and Pant-L-406, form the first group, although the variety of Pant-L-406 remains detached; Castelverrino and Salamaca form the second group and Precoz and Syrian the third. Between the first and second group is note the closeness of the varieties of Castelluccio and Castelverrino.

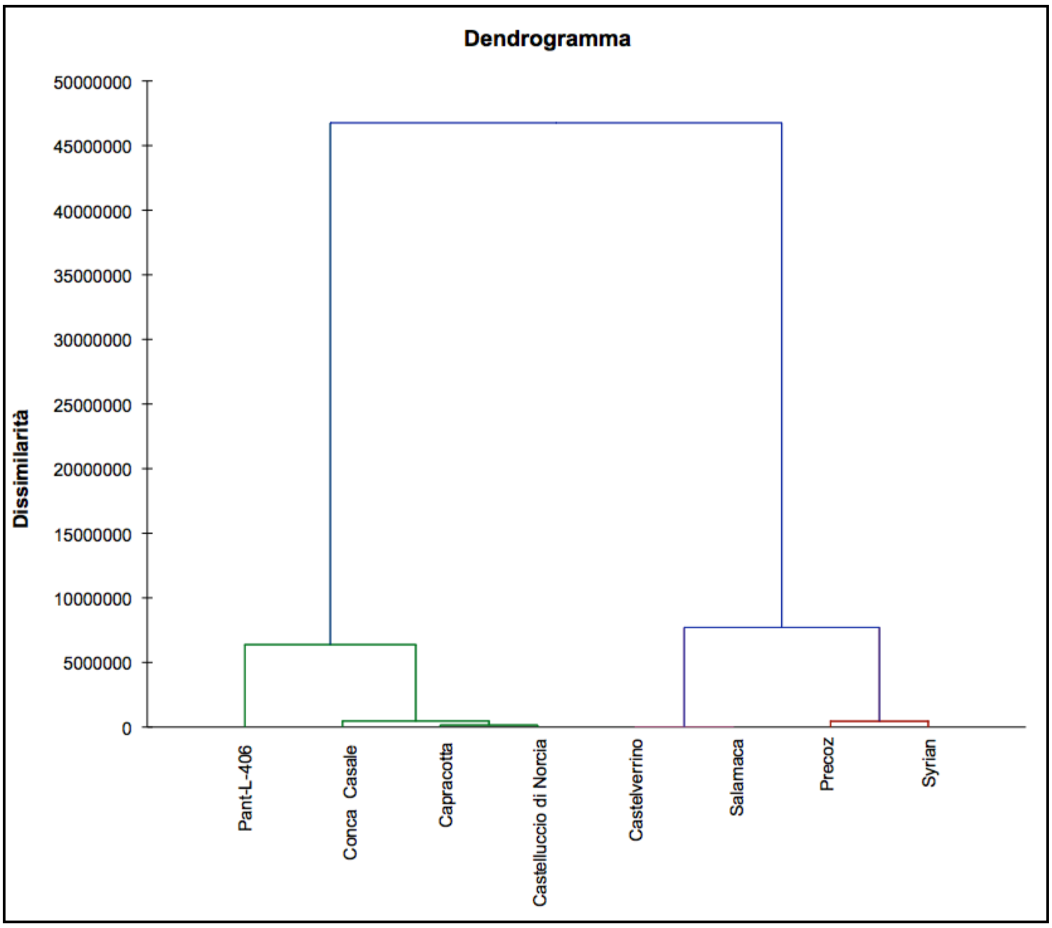

Figure 15 Dendrogram computed among 8 populations of the L. culinaris, using eleven agro-morphological features. Hierarchical clustering was performed using Euclidean distance, Ward method for agglomeration and mean method and average linkage criterion for linkage.

Figure 16 shows the results obtained from the AHC analysis of agronomic traits, making it possible to decrease the level of dissimilarity between populations. In this case the first group is formed by the ecotypes of Capracotta and Conca Casale, while the second group is formed by the landrace of Castelverrino, Precoz and Syrian. Finally, the last group is made up of Salamanca, Castelluccio di Norcia and Pant-L-406.

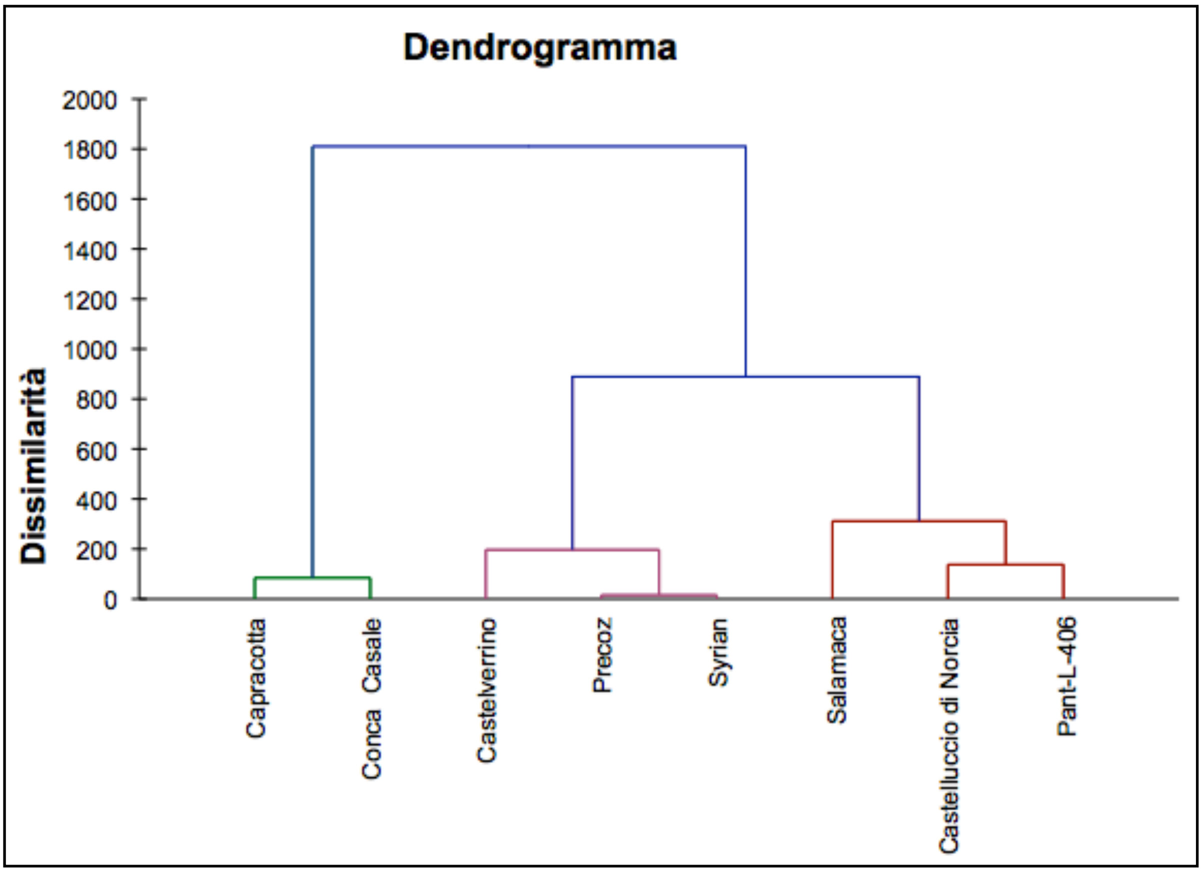

Figure 16. Dendrogram computed among 8 populations of the L. culinaris, using six agronomic features. Hierarchical clustering was performed using Euclidean distance, Ward method for agglomeration and mean method and average linkage criterion for linkage.

In the graph of Figure 17, obtained from the AHC morphological characters, there is again an increase in the level of dissimilarity between the varieties analyzed and the distribution of populations is identical to that of Figure 20 (obtained from AHC of all characters).

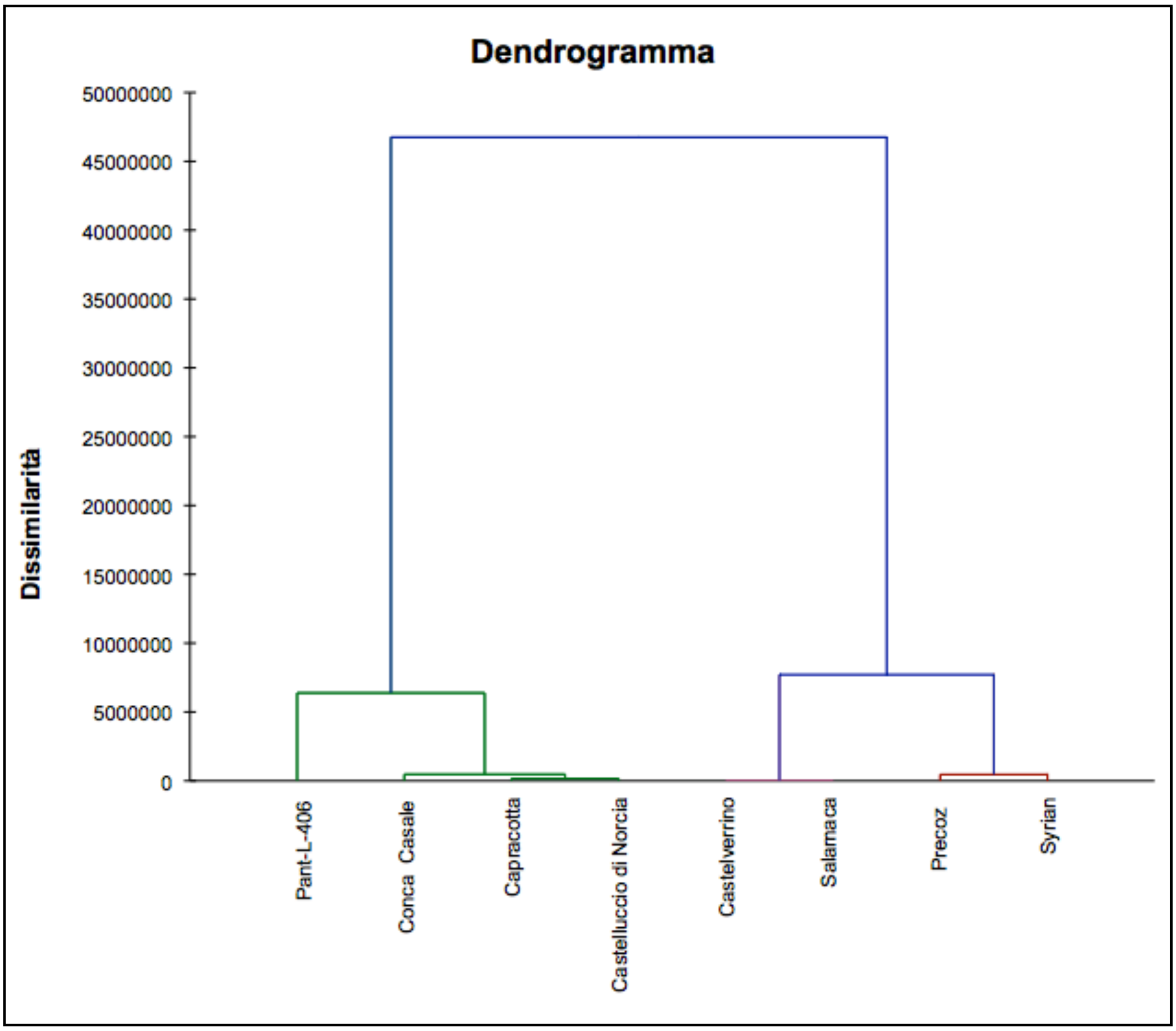

Figure 17. Dendrogram computed from the Euclidean distance matrix among 8 populations of the L. culinaris, using five morphological features. Hierarchical clustering was performed using Euclidean distance, Ward method for agglomeration and mean method and average linkage criterion for linkage.

4.7 Discussion

Genetic diversity created in the farmers' fields over millennia, complemented by the diversity present in wild relatives of crops, provides the raw material for improving crop productivity through plant breeding (FAO 1998).

The need to increase the knowledge about genetic variation and relationships between accessions or genotypes is important: (1) to understand the genetic variability available and its potential use in breeding programs, (2) to estimate any possible loss of genetic diversity, (3) to offer evidence of the evolutionary forces shaping the genotypic diversities, and (4) to choose genotypes to be given priority for conservation (Thormann et al., 1994).

Many horticultural varieties are grown for local consumption and farmers often include small amount of exchanged seeds to maintain the high variability in crop and safeguard the production. The varieties used for home consumption every year run the risk of genetic erosion and extinction, because they are only grown by elderly farmers who are disappearing gradually as the years go on (Torricelli, R., et al., 2011; Piergiovanni , AR, 2000). To avoid losing these genetic resources throughout Italy in recent years have initiated studies on the collection, conservation and genetic characterization, proteomics, and agro-morphological varieties of many locality (Torricelli, R., et al., 2011; Viscosi, V., et al., 2010; Scippa, G. S., et al., 2008; Piergiovanni, A. R., 2000) and the possibility of using this crop also in alternative ways, for example in lawns monophite or polyphite for the production of quality hay.

Various local landraces have evolved in several Italian regions thanks to an optimal combination of climate, soil and moisture, although modern agricultural practices are eroding the genetic richness of this plant species. Some of them are on the limit of extinction; others are grown only by elderly farmers, mainly in marginal lands, for their own consumption and, occasionally, for local markets (Piergiovanni, 2000). Unlike modern cultivars selected (with low, or no variability) for their performance in specific environmental conditions, landraces have a high genetic variability, suitable to growing in a range of environments.

In fact, they are highly adapted to different environmental conditions because have evolved adaptive gene complexes conserved by genetic linkage or natural or human selection.

Molise region as many other parts of Italy is very rich of local crop landraces, that are mainly cultivated by elder farmers. These landraces are often characterized by high environment adaptation traits and have a strong link with the local historical traditions. Within these cultures a particular interest was taken in that of lentil (*Lens culinaris* M.). This crop is particularly rustic and grown in all Italian regions including the islands (Piergiovanni 2000) for both home consumption and for local markets. Extensive differentiation of *L. culinaris* over millennia has resulted in a myriad of different landraces. However, in more recent times many landraces have disappeared consequent to environmental and socioeconomic changes. A wide variety of methods has been used to investigate genetic similarities and relations among *L. culinaris* Medik. Landraces (Gallo et al., 1997; Senatore et al., 1992; Sonnante and Pignone, 2001; Scippa et al., 2008) but only a few varieties have been able to be marketed throughout the country and even internationally.

Thus far, genetic variation of the lentil germplasm has been evaluated based on agro-morpho-physiological traits (Erskine et al., 1989; Lazaro et al., 2001; Tullu et al., 2001; Yan'kov et al., 2001, Bioversity International 2011), isozymes (De La Rosa and Jouve, 1992; Rodriguez et al., 1999), seed storage proteins (De La Rosa and Jouve, 1992, Echeverrigaray et al., 1998, Piergiovanni and Taranto, 2005) or such molecular markers as random amplified polymorphic DNA (Sharma et al., 1995), ISSR (inter-simple sequence repeat) (Alvarez et al., 1997; Sonnante and Pignone, 2001). More recently, proteomics, an innovative approach involving the comparison of several hundred to several thousand gene products revealed by two-dimensional gel electrophoresis of protein extracts, has been successfully applied to investigations of natural variations within these plant species populations (Scippa et al., 2008, 2010). From these studies, protein spots resolved by two-dimensional gel electrophoresis resulted in de-facto genetic and physiological markers that can be used to assess genetic variability and to establish genetic distances and phylogenetic relationships between lines, species and genus (Scippa et al., 2008).

However, despite the power of genetic and proteomic approach, with the increasing size of genetic resources collections, methods of analyzing and classifying their genetic variability together with the assessment of their genetic hereditability are becoming more required. In the case of large size collections, like those of lentils, their rationalization is very important to identify gaps and encourage their efficient and full evaluation. For these reason samples in such collections are generally described by a mixture of both quantitative and qualitative characters following different lists of descriptors recommended by the International Plant Genetic Resources Institute, national breeders, etc. Characterization by descriptors explains plant traits that are highly heritable, easily seen by the eye and equally expressed in all environments in order to distinguish phenotypes. In this way, curators can select among all this information a minimum set of quickly identified, easy scoring and highly discriminate traits as a practical solution towards the establishment of comprehensive germplasm collections.

In this context the aim of the presented thesis is to characterize at agronomical morphological and physiological level some lentil ecotypes (*Lens culinaris* M.) of Molise region, characteristic of municipalities of Isernia provence, namely Capracotta, Conca Casale and Castelverrino.

It was chose these three varieties of lentils of Molise because their cultivation was practiced on marginal land in the mountains and hills of Molise Apennines up to a maximum altitude of 1525 m (share of Giardino della Flora Appenninica of Molise in the village of Capracotta). During the ecotype germplams collection, interview of local farmers were carried out, and information about cultivation techniques and associated traditions were gathered. It emerged that cultivation techniques are not specialized, as the seeding is done by hand by a broadcaster and on the ground worked in autumn in the preceeding year, the seeds are not tanned and pesticide treatments are not performed on the plants during growth. For the particular hardiness of the landraces and their ability to fix atmospheric nitrogen, the crops are not fertilized and cultivated in the portion of garden less suited to other more profitable harvests. No irrigation intervention is undertaken, thanks to the plant's ability to withstand drought. In this way, however, the quantities produced each year are limited, a few tens of square meters per farmer, with an average yield of 35 grams/m^2 seed. In the cases studied the seed is used for family consumption; while only in one case was the crop sold as a local (village of Capracotta), reaching a price of 11 euros per kilogram, far above other domestic varieties marketed under European brand (PGI).

The characterization had two main goals: (1) fill the gaps in knowledge of landraces of Molise, which have already been analyzed from a genetic and proteomic point of view, and (2) implement a quickly discriminate protocol for characterization that can be used also for other crops that need to be safeguarded before they are extinguished.

For this study, 23 descriptors (13 quantitative and 10 qualitative characters) were used to characterized the three local lentil populations (Capracotta, Conca Casale, Castelverrino) compared to one Italian commercial landraces (Castelluccio di Norcia) and four International commercial pure line (Precoz, Salamaca, Syrian local large and Pant-L-406).

Results of the seed morphological characters analysis showed that colour combinations of both cotyledons (yellow, red, orange, etc.) and testa (gray, with or without spots, etc.) were all expressed in the landraces of Capracotta and Castelverrino while Conca Casale always presented red cotyledons and coloration of the tegument more or less extensive and no spots. The genetic combinations for the coloring of the seed teguments and cotyledons are very important for landrace characterization. However the control of inheritance has been very little investigates remaining still poorly understood. Indeed the only aspect that seems well assessed is that the spot character is dominant over its absence (Vandeberg, V., et al., 1990; Saingh, JP et al., 1993, Yadav, SS, et al., 2007). An aspect particularly appreciated by consumers is the color of the seeds, as the market often prefers pure lines which have the characteristic of having uniform seeds for the colour magnitude (Vandeberg, V., et al. 1990). However, as shown in the results of this study, although the seeds of landraces of Molise are very varied in colour, it does not preclude the possibility of obtaining pure lines, thanks to the ability of lentils to be self-pollinating.

The results of morphometric characters analysis showed that greater values of the area, perimeter and diameter were expressed by the Molise seed ecotypes according to the following order Castelverrino, followed by the Capracotta and finally Conca Casale. In fact it was found that Castelverrino reached an average area of 0.24 cm^2, a perimeter of 1.85 cm and an average diameter of 0.559 cm, at the limit between the microsperma (diameter <6 mm) and the macrosperma (diameter >6 mm). Capracotta lentils have reached an area of 0.159 cm^2, a perimeter of 1.44 cm and a diameter of 0.44 cm, showing intermediate values of lentils and returning between the varieties analyzed microsperme. From interviews with farmers it became known that in the past two varieties were grown in Capracotta: the "*miccula*", small lentil, and "*micculona*", larger lentil. Currently *micculona* has disappeared, no longer cultivated and remains only a small amount in the Germplasm Bank of Molise from the University of Molise.

Lentils of Conca Casale showed a smaller morphometric value of the seed (0.11 cm^2 area of the seed, perimeter of 1.25 cm and diameter 0.38 cm) and were more homogeneous than the other two varieties and were easily distinguishable by eye. Even the character of the seed weight influenced the result. In fact, the landace of Castelverrino reached a weight of 100 seeds of 5.61 g; Capracotta had reached a weight of 3.47 g and the landrace of Conca Casale reached a weight of 2.74 g. Interviews with local farmers and the literature examined report that the varieties with small seeds were the most grown in the central Apennines (Piergiovanni, A. R, 2000). In fact, this feature is found throughout the central-southern Apennines and was the engine that drove local producers of Castelluccio di Norcia to focus efforts to increase production and build a commercial variety with a European label. However, the focus on the production of small seeded lentil ecotypes makes the Castelverrino landrace (larger varieties) recognized as a rarity even in other towns of the hinterland of Isernia province.

The morphometric analysis of plant characters highlighted that among the Molise ecotypes Castelverrino is the largest even in terms of plant height and height of the first pod (69.3 cm and 51.8 cm respectively), while the leaves reached an "average" value, equal to the most varieties analyzed (including controls).

The differences between the three landraces of Molise were also found in the number of pods produced per plant, days to flowering and rate of maturation. In general, the ecotypes of Conca Casale showed homogeneity in all these characters, while Capracotta exhibited a very wide variability. These data are in accordance with a previous work were these two local landraces were characterized at morphological and molecular level, showing that while Conca Casale population were very homogeneous, Capracotta presented a wide variability between populations (Scippa et al., 2008, 2010). However, in this last case can’t be excluded a genetic intercross with varieties usually sold in local markets occurred. A technique often used by local farmers to replace the failed areas resulting from the late frosts which kill off the young plants.

Interesting features from an agronomic point of view were shown by Molise lentils such as the number of pods per plant, seeds per pod, time to flowering, time to maturity and complete the production cycle. All these features, in fact, give important indication on crop yield.

In particular, Conca Casale and Capracotta ecotypes have produced many more pods than Castelverrino and compared to all the commercial varieties. Additionally, an interesting result to be highlighted is that Capracotta ecotype produced an average of 171 pods per plant, while the average of all populations varied between 27 and 73 pods per plant. All populations analyzed have expressed an average of almost equal seeds per pod (1.9 and 2 respectively), except the landrace of Castelverrino which produced an average of 1.5. However, despite the lower number of seed produced as discussed previously, Castelverrino seed is characterized by higher morphometric values being classified between microsperma and macrosperma.

Furthermore, the literature reports a positive correlation between seed size and the number of pods per plant (Latief, A., et al. 2011; Yadav, S., S., 2007). This correlation does not apply in the case of Molise ecotypes. In fact, the populations of lentils from Conca Casale and Capracotta have the seed smaller than that of Castelverrino, but have produced an average of many more pods. A single plant of the landrace of Castelverrino produced only 4 pods. This data, however, may have been influenced by environmental conditions and also suggests the possibility of some factor associated with infertility, because some plants continued to flourish and grow without setting consistently (data not shown).

Time is the other important parameter to be taken into consideration in agriculture since it impacts crop yield and it is strongly influenced by environmental adaptation (Review H. Ulukan, 2008). In particular three steps must be analyzed in ecotypes agronomic characterization: 1. time to germinate, 2. time to flower and 3. time to mature. Knowing the length of these three steps allows farmers to plan the work in the field, from sowing, through tilling to harvesting. Although the data obtained in the trial of this thesis are only indicative, because the parameters were measured in a controlled environment, they might be still useful for phenotypic and genotypic characterization. It is known from literature that the biological cycle (which in this case coincides with the crop cycle) of the lentil is strongly influenced by climate, soil exposure, soil, cultivation techniques and all other environmental parameters (Yadav, S. S., et al, 2007; Review H. Ulukan, 2008).

The physiological character “days to germinate” distinguished Castelluccio di Norcia and Castelverrino populations from all others. In fact seeds in these populations germinated on average of 7 days only, while the other varieties tested germinated on average over 11 days. The differences in germination rates, and percentage among seeds of different lentils population, may be interpreted as a result of the adaptation of lentil seeds to different environmental conditions (Covell et al., 1985).

Although the similarity between Castelverrino and Castelluccio di Norcia landraces may be attributed to a common geographical origin, as these landraces are cultivated in close central Italian areas. Nevertheless differences observed in relation to the other two local landraces may results from a diverse ‘‘survival kit’’ allowing the seeds (i) to face various abiotic/biotic stresses, (ii) to provide various nutritional protein and non-protein compounds for the growing plantlet, and (iii) to enhance germination by ready-protein machineries present in the seed (Scippa et al., 2010).

The earliest flowering line was Pant-L-406 which took only 56 days and the more delayed was Castelverrino (80.3). In between there were Conca Casale with 75.1, Salamanca with 73.8, and finally Capracotta landrace which took approximately 68 days.

The appropriate timing of flowering is a pivotal adaptive trait controlling the propagation and survival of a plant species and a wide variability exist in lentils. Plants have evolved sophisticated mechanisms capable of responding to environmental cues such as day length and exposure to low temperature (Wang et al., 2010). In fact timing of flowering, is a critical stage of development in the life cycle of most plants when seed number is determined, and it is important for adaptation to the abiotic stresses such as temperature and water deficit, and to biotic (pest and disease) constraints (Curtis, 1968). In this context the differences reported in the time of flowering of the populations analyzed, may be linked to the specific environmental conditions adaptation.

Time to maturity is also the result of the adaptation to environmental condition and it is used to classify crops as early and late, with all the intermediate values that varieties may have, differentiating the different cultivars in modules of seven days. Thus considering the parameters are expressed by the variety analyzed that has Pant-L-406 was the most precocious (102 days), while the later has been Salamanca (134 days). Between these two extremes are cataloged all the other varieties: Precoz early, Castelluccio di Norcia could be considered medium-early, Capracotta medium late and all other late. This classification is not applied directly to natural field, where the factors influencing the useful days to maturity are manifold. However, in the case of lentil landraces of Molise it can be assumed that the shorter time needed for flowering and maturation of Capracotta populations, compared to the other two local ecotypes, may be explained with the short period of good environmental conditions occurring in the area of cultivation, characterized by short summers with average temperatures lower than the other two locations. Indeed Capracotta land is made available for cultivation after several days of melting snow, while in Conca Casale and Castelverrino average temperatures are milder and is the ideal time to grow larger. This has allowed the culture to adapt even after the selection made by the environment and humans (Yadav, S. S., et al, 2007).

The character "time to pod", that the plant uses to fruit set, is not generally used for agricultural purposes but it was here measured since it is important for the characterization of varieties. Results show that commercial varieties take from few days to fruit set as Precoz that took only 5 days, to more than a week as for Pant-L-406, Syrian, and Salamaca that took up to almost 12 days. Capracotta, Conca Casale, Castelverrino together with Castelluccio di Norcia took around 6-8 days to fruit set. Also in this case the different timing observed can be attributed to the need of setting fruits before environmental conditions become unfavorable. As for example, the length of time for entry into production in mountain areas, typical of the Apennines, where there is a risk of late frosts, is quite short, so that ensure its reproduction local landraces must complete their own cycle in a less extended time compared with varieties grown in better environmental conditions.

The second important point remaining concerns the two characters, one morphological, the other morpho-agronomic, namely the “length of the internode” (internode close to the first flower from the base of the plant) and the number of pods per pedicel. These characters were found to always express the same values (ie. Invariate and therefore uniform) except in very rare cases where some plants showed some exceptions. Therefore it was considered not appropriate to include these data.

To distinguish which of the morphometric and agronomic traits have higher weight in discriminating among and within landraces a uni- and multivariate statistical analysis was performed.

The differences observed between landraces of Molise (Conca Casale, Capracotta, Castelverrino) and the commercial varieties (Castelluccio di Norcia, Precoz, Syrian, Salamaca and Pant-L-406) are confirmed by the pairwise test, which showed a statistically highly significant variance among landraces and controls. The coefficients resulting from pairwise test reported in Tables 16 and 17, show that all the morphometric and agronomic traits considered, although with a different significance, well discriminate landraces of Molise from the other. In particular morphometric traits were more important in discriminating of Molise landraces from Precoz and Salamanca, whereas the agronomic traits in differing of Molise landraces from Castelluccio Norcia.

Principal component analysis (PCA) confirmed that all the traits, except the "days to maturity", are highly influencing in the populations discrimination (Fig. 12a). In particular, PCA shows that the landraces of Capracotta and Conca Casale are very similar between them although different from Castelverrino and from all the commercial varieties. Castelverrino landrace distributed together with Salamanca, Precoz and Syrian. This result is also confirmed by the Ascending Hierarchical Cluster, AHC, (Fig. 15), where two main clusters were obtained, the first grouping Conca Casale, Capracotta Castelluccio di Norcia, in the same branch, and Pant-L-406; the second grouping Castelverrino together with the other commercial varieties. The results of PCA performed taking into consideration the agronomic and morphometric traits separately (Fig. 13b and 14b), confirm the similarity between Conca Casale and Capracotta while Castelverrino remains distant from them and very close to Castelluccio di Norcia and all the other commercial varieties. The distribution obtained by the AHC for agronomic (Fig. 16) and morphometric (Fig.17) traits resulted slightly different since this graph was obtained from the normalized averages of the population and not by the normalized values of individual plants. However, in both cases Conca Casale and Capracotta grouped in the same cluster despite Castelverrino which was clustered with the commercial varieties.

While genetic analysis of Conca Casale and Capracotta populations showed that these two landraces are well differentiated (Scippa et al., 2008), not data are available on Castelverrino genetic characterization. Furthermore, previous studies showed that Conca Casale and Capracotta were well distinguished when the morphological traits of seed were analyzed, with Capracotta landrace very close to Castelluccio di Norcia (Scippa et al., 2008). In the present thesis although grouping in the same branch, Conca Casale and Capracotta remained distinguished and Capracotta appeared close to Castelluccio di Norcia, confirming the previous results. However, when the agronomic traits were taken into consideration the level of dissimilarity decreased. A possible explanation of this different distribution can be related to the confined small area in which the two local landraces are cultivated. Moreover similar agronomic practices probably used by farmers may have influences the agronomic traits of these two local landraces. Castelverrino landrace shows traits very similar to the commercial varieties and the lack of information at genetic level, makes difficult to evaluate the authenticity of these ecotypes. Further analyses are required to verify the origin of this ecotype and plant appropriate germplams conservation plans.

CHAPTER V

5.1 Conclusions and prospective

Autochthonous plant germplasm, characterized by a wide genetic variability and high adaptation to different but localized environmental conditions, are often more subjected to genetic erosion risks. In Italy, several different lentil landraces evolved thanks to the combination of different geographical and socio-economic characteristics.

The literature reports a wide number of methods that have been used to investigate genetic similarities and relations among landraces of L. culinaris Medik. Different methods have different powers of resolution and provide different information: neutral DNA markers are useful tools to describe genetic relations in terms of time divergence (Thiellemnt et al., 1999; Scippa et al., 2008), whereas phenotypic markers can provide information about adaptive responses to macro-environmental conditions (David et al., 1997; Thiellement H., et al., 1999).

In light of this new knowledge, technologies and increasing attention to the environment and the importance of native crops, are trying to draw from the past to rediscover sustainable lifestyles that are less stressful and less limiting to biodiversity, but rather support and promote it in a more sustainable manner.

The pressure for an alternative economic development may support the recovery of local crop varieties, selected over the years by farmers who have continued to use native seed. These crops are usually associated with high genetic variability (typical of landraces), from which it is possible to extract and establish new varieties with special characteristics: resistance to adverse environmental, drought, low temperatures, etc., resistance to pests and diseases in general; frugality; quality nutrition and cooking, etc.

There are many varieties that can be retrieved, while others were lost irretrievably. Once germplams have been collected they may have different fates: stored in banks, partly used for scientific analysis (all types of characterization: agronomic, physiological, morphological, genetic and proteomics), to obtain a card identification to protect the genetic diversity of possible fraud (Torricelli, R., 2011); part reused to be distributed to “farmers guardian”, and the keepers will be directly used by farmers for commercial purposes.

In order to achieve a district rural production, which has the purpose to protect crops and revalue those typical of Molise and promote them on local and national markets, it is necessary to monitor and recover part of agricultural biodiversity in the region and to characterize in as detailed manner as possible and in accordance to international standards.

In this study we used a combination of different agro-morpho-physiological descriptor to characterize three Molise autochthonous lentil landraces of different provenances and compared them to commercial cultivar or landraces.

Results showed that the differences between the local landraces were principally related to their sites of origin, where climate conditions and human activity over many successive generations has resulted in local accessions characterized by specific agro-morpho-physiological traits of seeds and plants. It is almost unknown the relation between these phenotypic markers and the environmental characteristics of the landrace provenance areas which fell outside the scope of this study.

The characteristics of the three varieties can be used as such to create a niche market, or wider, under a single brand that enables the commercialization of local varieties, with all the positive consequences that follow both the environmental and socio-economic level. Taking as an example from what has been developed to Castelluccio di Norcia, in Umbria (Italy), where following the recognition of the European PGI mark with local lentils, there was an extension of cultivated land and increase production of lentil the production has gone from just 250 hectares before European recognition to about 600 hectares with a production increase from 100 to 400 tonnes of seeds (Torricelli. R., et al., 2011). It is possible to envisage a similar development in inland areas of Molise, relying not only on the crop of lentil but extended to a wide range of horticultural crops and in some cases even fruit trees discovered during this research.

Starting from the lentil crop that has been shown to be a plant frugal, easy to grow even with biological systems, both low energy inputs, ameliorate of soil fertility, easy to maintain and appreciated in the market for its culinary characteristics, it can continue to characterize and commercialize other vegetables that are also appreciated by the market.

Other possibilities, which can be developed using some of the characteristics expressed by the Molise lentils, are the establishment of inbred lines, which can have two specific markets: lentil seeds as food for humans with uniform characteristics (size and color of the seed, homogeneous cooking times, high-protein, high-quality protein, etc.) much appreciated by the consumer (Vandeberg, A., Slinkard, AE, 1990), or may be used for feeding livestock. In the latter case could be exploited by the population of

Castelverrino, which reported a greater height, and some plants showed rather large leaves which might be beneficial to animals.

The possibility to produce pure lines for the cultivation of lentils is simple thanks to the flower cleistogamous and self-pollinating. The main difficulty in the short term when considering the genetic improvement is the availability of staff with practical experience in the emasculation and artificial fertilization of the flower and in executing experimental designs to test for heritability and environmental interactions.

Whatever the fate of local crops, the original genetic variability in those collected has been preserved. In that remaining in cultivation it can often be compromised by intensive production systems (for example, specialized monoculture with the use of a single pure line), or replacement with other varieties that have little to do with local ecotypes and that can be marketed using the image of the original products, creating damage to the production of the local crops that is difficult to repair (Torricelli, R., 2010). Finally, a way to preserve the genetic variability of many local cultures and protect them from extinction is the establishment of an association of farmer guardians to interact with the banks of germplasm. The latter are the only ones that can preserve and protect even though some aspects remain to be elucidated. These points can be clarified only through the use of a multidisciplinary approach that includes the involvement of expertise in various fields: 1) agronomy, to improve agricultural techniques, which are more productive and sustainable; 2) biochemical, nutritional aspects to learn more. For example, the work by Scippa et al., 2010, showed that the Molise lentils are rich in storage proteins and were found to contain a protein anticancer, features that could represent an added value in marketing; 3) physiology, to use less demanding crops and use less fertile soils and in mixture with other species (grass mono and polyphite); 4) genetics, to conserve, evaluate and select the best varieties, without running the risk of genetic erosion or extinction of varieties; 5) economist, marketing plans, who can plan and promote the product on the market, sending to the consumer the goodness of the product both in terms of quality and environmental protection with a focus on biodiversity.

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