UNIVERSITÀ DEGLI STUDI DELLA TUSCIA DI VITERBO

DIPARTIMENTO DI ECOLOGIA E SVILUPPO ECONOMICO SOSTENIBILE

DECOS

DIVERSITY ANALYSIS IN SPACE AND TIME OF AN ANNUAL SELFING SPECIES (COMMON BEAN, *Phaseolus vulgaris*) AND A PERENNIAL OUTCROSSING SPECIES, (CARDOON, *Cynara cardunculus*)

CORSO DI DOTTORATO DI RICERCA

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Dedicated to my daughters, Shiana and Saesha, and future generations, in the hope that the world can always be a better place.

Contents

ABSTRACT

Evaluating changes in diversity over space and time are key in utilising and conserving biological resources. The aim of this study is to assess diversity in two different species with different breeding systems: in the cardoon a cross-fertilized, perennial and in the common bean a selfing, annual species. The study was designed such that wild types and traditional landraces for each species were obtained from the CIAT, IPK and University of Tuscia seed banks.

A total of 62 bean populations, totalling 498 individuals, were assessed in this study. There were eight wild populations from Latin America, 42 populations from Lazio, Italy, one Italian wild type and 10 Italian bean populations collected in 1950. DNA was extracted from all individuals in the study and amplified using 10 microsatellite markers. Results, based on 26 polymorphic loci, showed that the Lazio population were distinct from the cultivated varieties and from the wild types. The latter two groups of beans, however, were closer to each other than to the modern beans. Levels of polymorphism were high in all populations so that even if the populations from Lazio were different from the 1950 beans, no genetic erosion was found: AMOVA showed 37% diversity among populations and 63% within populations ($p<0.000$). Morphological data were also analysed through PCA for the Lazio beans. None of the 26 descriptors were significant in ascribing the diversity found, although seed and pod morphometrics were the relevant among the descriptors used.

There were 27 populations of cardoon in the study, of which nine were wild cardoon populations. The cultivated cardoon populations comprised eight European (non-Italian) populations (two of which collected in 1950), one Algerian population, and nine Italian populations. Seeds were obtained, germinated and DNA was extracted and amplified with four ISSR primers. The resulting 398 loci showed that two of the wild populations clustered together, but that there was no clear pattern as to diversity related to geographical origin or year of collection. Levels of polymorphism remained high in this species too, however, such that no genetic erosion was found. The morphological analysis involved data collection in the field and PCA performed. In contrast to the beans, diversity was defined by leaf length, plant height, total number of flowerheads and plant maturity.

Both species showed high levels of intra-varietal diversity, with the traditional varieties not clustering with the wild types. It is therefore possible that introgression has occurred from other sources of germplasm.

Arshiya Noorani: List of Publications/Posters 2008-2011

PUBLICATIONS

1. Noorani A., O. Temperini O, A. Temperini A., M.E. Dulloo M.E. and M.A. Pagnotta. (In Prep.) Genetic and morphological assessment of traditional varieties of common bean (*Phaseolus vulgaris*) from Lazio, Italy**.**

2. Noorani A., L. Mondini, and M. A. Pagnotta. (In Prep.) Conservation of Crop Genetic Diversity – An Overview

3. Mondini L., A. Noorani and M. A. Pagnotta. 2009. Assessing Plant Genetic Diversity by Molecular Tools. *Diversity 1*(1): 19-35

4. Pagnotta M.A., L. Mondini and A. Noorani. (In press). Plant Genetic Resources Conservation and Plant Breeding. In **Plant Breeding.** (N. Huttunen and Taavi Sinisalo, eds). Nova Science Publishers.

5. Noorani A., A. Temperini, N. Rey, F. Saccardo and M.A. Pagnotta. (In press). Assessment of Genetic Variation in Three Populations of Italian Wild Cardoon. *Acta Horticulturae*. (**ORAL PRESENTATION** at the 7th International Symposium of Artichoke, Cardoon and their Wild Relatives, France, 2009)

6. Noorani A., P. Crinò, N. Rey, E. Dulloo, F. Saccardo and M.A. Pagnotta. (In press.) Diversity Assessment of Seven Italian Globe Artichoke Varieties Using Agromorphological Parameters. *Acta Horticulturae*.

7. Rey, N. A. Noorani, P. Crinò, E. Dulloo and M. A. Pagnotta. (In Prep). Genetic Variability in Italian globe artichoke of the Romanesco typology.

8. Pagnotta M.A. and A. Noorani. European genetic resources of *Cynara* spp - the CYNARES Project. *Bioversity International Newsletter for Europe*. 36: 11

POSTERS

1. Noorani A., P. Crinò, N. Rey, E. Dulloo, F. Saccardo and M.A. Pagnotta. (2009) Diversity Assessment of Seven Italian Globe Artichoke Varieties Using Agromorphological Parameters. 7th International Symposium of Artichoke, Cardoon and their Wild Relatives, Saint Pol de Léon, France, 2009

2. Rey, N. A. Noorani, P. Crinò, E. Dulloo and M. A. Pagnotta. (In press). Genetic Variability in Italian globe artichoke of the Romanesco typology. Plant Conservation for the Next Decade: A Celebration of Kew's 250th Anniversary, 12-16 October, London, U.K.

3. Pagnotta M.A., A. Noorani, N. Rey, P. Crinò, R. Tavazza, A. Alercia and F. Saccardo (2009). Usability of Descriptors for Globe Artichoke. 19th EUCARPIA Conference – Genetic Resources Section, Slovenia, Ljubljana 26-29 May 2009.

4. Rey Muñoz N.A., A. Noorani, M.A. Pagnotta, P. Crinò, R. Tavazza, O. Temperini and F .Saccardo (2008). The first four clones selected from the traditional artichoke Romanesco populations. Proceedings of the 52nd Italian Society of Agricultural Genetics Annual Congress, 14-17 September, 2008, Padova, Italy.

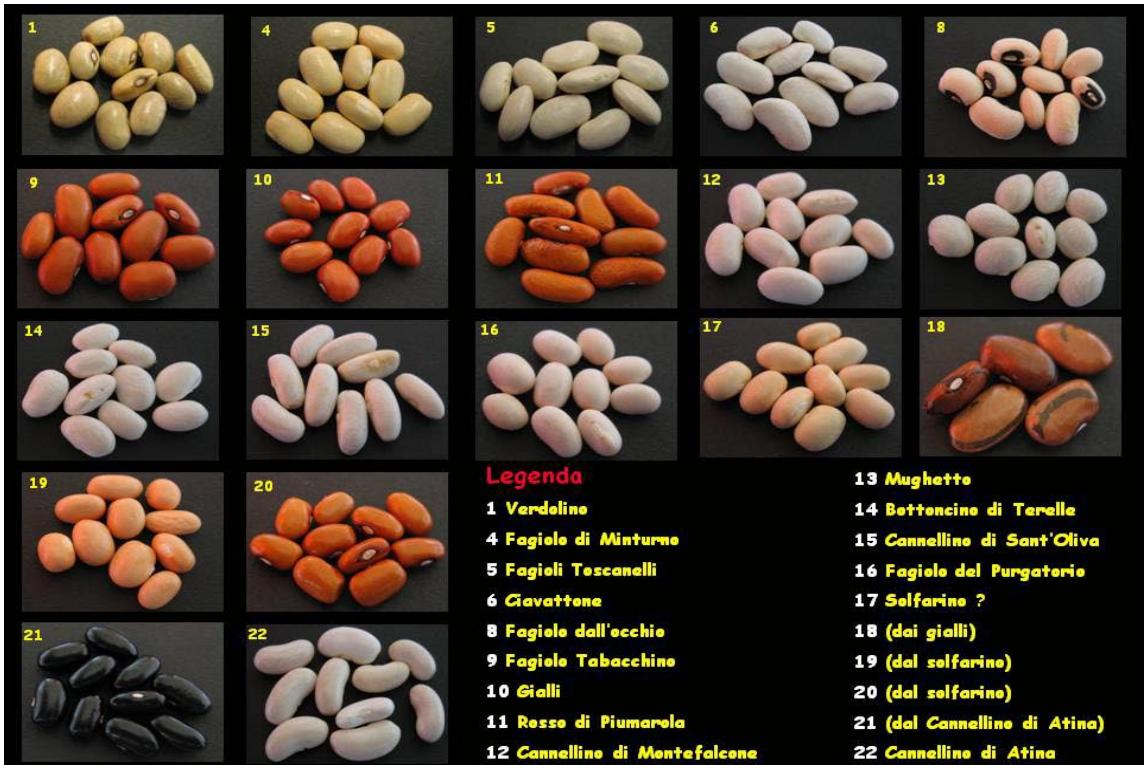
5. Rey N., M.A.Pagnotta, A. Noorani, P.Crinò, R.Tavazza, A. Alercia and F.Saccardo. (2009). Globe artichoke descriptors and their improvement. 53rd Annual Congress Societa' Italiana Di Genetica Agraria,16 -19 September 2009, Torino, Italy.

6. Rey N., A. Noorani, And M.A. Pagnotta. 2009. Assessment of genetic and morphological variation in Italian globe artichoke of the Romanesco typology. International Workshop: Plant Genetic Resources for Food and Agriculture - General Aspects and Research Opportunities. Rome, 5 - 6 November 2009.

INTRODUCTION

1.1 Agricultural Biodiversity

Crop genetic resources not only represent the basis of agricultural development, they are also an enormous reservoir of useful genes and gene complexes that endow plants with coping mechanisms for evolution and habitat changes (Fig.1.1). Fig.1.2 provides an overview of the plant diversity and its uses. The greater the diversity, the greater the chance that at least some of the individuals will possess an allelic variant suited to changing environments, and will produce offspring with that variant. The loss of genetic diversity can imply premature extinction due to a decrease in fitness through reduced genetic variation. Populations of endangered species are small and tend to lose genetic diversity as they have limited geneflows and are thus subject to genetic drift, founder effect and inbreeding (Jump and Peñuelas 2005). Diversity provides the basis for more productive and resilient production systems that are better able to cope with stresses such as drought or overgrazing.



(Photo: O. Temperini)

Fig.1.1. Bean diversity in Lazio, Italy.

The importance of crop genetic resources has been well recognized; however, the awareness of its importance and the danger of erosion and disappearance tended to be limited to scientists and farmers directly involved in plant breeding. Public opinion has tended to focus more readily on the extinction of endangered wild species, with less concern for the dramatic impact that the shrinking genetic variation in crop plants and their wild relatives may have on future agriculture. As well as providing resistance to biotic and abiotic stresses, the availability of a reserve of variation and different alleles allows the development of new products different in colour, shape, taste, etc. which is an additional factor in the use of socio-economically important species.

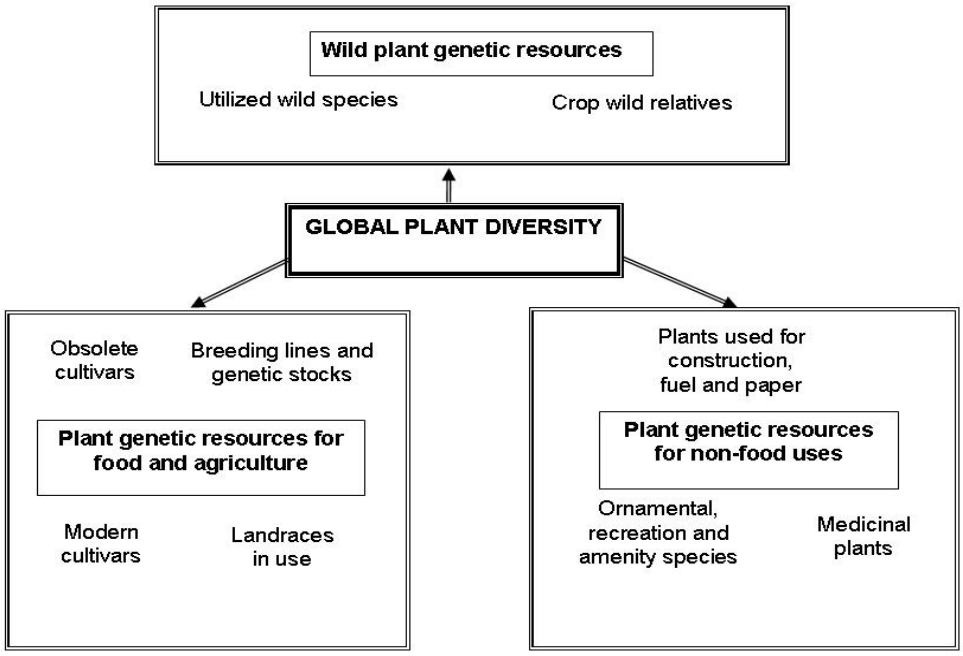


Fig.1.2. An overview of global plant genetic resources, diversity and uses (adapted from Maxted et al. 2008).

Unfortunately, the destruction of natural ecosystems has severely reduced the genetic variability in wild species. Exacerbating the situation is the replacement of local varieties with improved ones, thereby virtually eliminating traditional land races. As a result, most of the abundant genetic resources available even a few decades ago have been lost forever. Additionally, Brush (1995) showed that areas in marginal agronomic conditions, as those found in mountainous areas, and economically isolated areas, generally have a higher diversity. These areas are often most

vulnerable to climate change. Conversely, plains often have more homogenous environmental conditions, which could lead to stronger competition with commercial varieties.

Erosion of genetic resources in forest tree species is also a worrying and urgent issue. It is important to recognize that the variation in natural populations of forest species is key in devising the best strategies for their conservation and exploitation. The growth in molecular marker technologies, are particularly suitable for studying genetic variation and in assisting the breeder in forest planting.

Significant scientific and public concern for genetic resources began in the 1950s when scientists travelling on a global scale, and especially to developing countries, highlighted the cultivation of newly-acquired croplands and the spreading of modern, uniform varieties. A widespread movement from genetic resources to advanced biotechnology as possible source of variation and, to some extent, alleviated concerns of food production. The limitations of manipulations based on molecular and tissue culture techniques have been recognized during the last few years and it is becoming clear that only an integrated approach between traditional and advanced techniques will produce the best results.

In 1996, 186 nations' Heads of State met in Rome at the World Food Summit with the aim of devising methods to eradicate hunger; a target of halving the number of undernourished people by 2016 was set, but despite only being 5 years away the target is still far from being reached. It is estimated that currently over 800 million people do not have enough to eat, 73% of which live in poverty, often in marginal environments which is where diversity is crucial to agriculture (FAO 2006). The maintenance of diversity in plant populations, then, is key to their survival (Cooper et al. 2001). This survival is of even greater importance when the plants in question are crops on which human survival depends. It is estimated that 60,000 plant species, roughly 25% of the world's total, could be lost by the year 2025 (ICARDA 1996). Loss of diversity is a major issue in crops as most food crop cultivation today is based on single variety monocultures, thus making them vulnerable to man-made and environmental disasters. Over 50,000 species of edible plants are thought to exist but only 15 species provide 90% of the world's food energy intake. Rice, wheat, and corn are the staple foods for nearly two-thirds of the world's population (Hinrichsen 1997), grown as monocultures and thus highly vulnerable to environmental changes. These widely cultivated varieties display a very small amount of intraspecific diversity, leaving them susceptible to loss of genetic diversity through

small population effects. Increasing and maintaining diversity, especially in species of socio-economic importance, is therefore essential to human well-being and survival.

1.1.1 Conservation Strategies

In Situ *and* Ex Situ *Strategies of Plant Conservation*

In situ conservation focuses on conserving organisms in their natural environment while *ex situ* conservation entails the preservation of genetic resources in collections external to the natural environment in which the organisms are found. Table 3 gives an overview of the pros and cons of the two methods.

The conservation of plant genetic resources, and more specifically, crop genetic resources, historically focused mainly on *ex situ* collections as seed collection and seed storage was the main method of plant breeders to maintain their collections. The conservation of PGR *in situ* and *ex situ* are often seen as competing conservation strategies. The CBD's Article 9 stresses, however, that the two approaches must be complementary and applied in combination (CBD 1992). It is vital to maintain linkages between these two strategies. One of the key linkages is the use of *ex situ* material to improve *in situ* populations or to reintroduce extinct species or varieties into cultivation. Consequently, *ex situ* materials also perform a role of a safety net as species and varieties may be lost *in situ* due to extreme events or habitat destruction. Often, however, it is the breeding system of the species which dictates the strategy for preservation. In the case of apomictic species (which form asexually produced seed) or in vegetatively propagated species, it may be possible to choose between on-farm conservation, field genebanks as well as techniques such as cryopreservation (Engels et al. 2008). Once again, the use of both methodologies would be the most successful path to maximising chances of survival.

In Situ Conservation

Conservation *in situ* maintains the organisms in their original habitat, or agroecological environment, the plants are still undergoing evolution in the field, hence this method of conservation is dynamic. It tends to focus on protected areas such biospheres and nature reserves but also includes on-farm conservation which has now increased greatly in profile and importance (Brush 2000; Swaminathan 2002). This approach can target simply one species or can target the ecosystem as a whole. The latter is now preferred as the ecosystem functions can remain intact

while the former is used in cases where an extinct target species requires reintroduction or if there is a keystone/focal species whose presence is importance to the functioning of the ecosystem. Given below is an overview of the different forms of *in situ* conservation.

Genetic Reserves

These involve the establishment of protected areas, managed by conservationists with the aim of monitoring and maintaining the diversity present (Maxted et al. 2008). It is most likely that one area will not suffice to cover the diversity present and therefore a network of sites is required. A buffer zone and a transition zone for each reserve is also generally recommended (Dulloo et al. 2008).

On-Farm Conservation

The conservation of diversity within a farming system generally entails the preservation and use of agricultural varieties – landraces – which have been bred by farmers over time to allow for adaptation to local environments. In this form of conservation, not only does the agroecosystem maintain its integrity but the traditional knowledge required for the propagation and survival of those crops and varieties is also conserved. On-farm management, however, includes the introduction of modern cultivars in the agroecosystem, thus affecting landrace diversity originally present and is not generally included in the definition of on-farm conservation (Maxted et al. 2008).

Home Gardens

The resources conserved in home gardens are considered a form of *in situ* conservation as significant amounts of diversity are often found conserved in these gardens. The produce here is for local consumption, and more specifically, for consumption by the household within which they are grown (Eyzaguirre and Linares 2004). They are valuable reserves of local fruit, vegetable, medicinal and herb diversity.

***Ex Situ* Conservation**

Conservation *ex situ* maintains the organisms in a place different from where they originated to maintain the original gene and allele frequency, hence the conservation is static. Breeders and researchers most often obtain their resources from genebanks and botanical gardens. There are approximately 1500 genebanks around the world, containing 6.5 million samples; 83% of these are held in national government genebanks (Global Crop Diversity Trust 2007). Since most of the collections have a redundancy of material which slows up its utilization, it was proposed to build up

core collections (Frankel and Brown 1984) of material from within existing collections, but containing the maximum of diversity and the minimum of repetitions.

The major crops (wheat, rice, potato, banana/plantain etc.) are well represented in *ex situ* collections. Crop wild relatives and minor crops (such as yams, coconut and amaranth) are less well represented but these are now gaining in importance with genebanks now accepting regional responsibility for local minor crops (Scarascia-Mugnozza and Perrino 2002). *Ex situ* collections are, however, also maintained in facilities other genebanks, such as botanic gardens and field genebanks (Swaminathan 2002). Engels et al. (2008) provide a comprehensive overview with a list of useful references for each of the below *ex situ* conservation methods. Given below is a summary of the different types of *ex situ* conservation.

Botanic Gardens and Arboreta

Plants of major socio-economic importance such as medicinal, ornamental, aromatic are the kind of species most often found in these facilities. The plants are most often displayed in gardens with the focus of high species diversity but on not varietal diversity. These gardens often also maintain seed banks to maximise the amount of diversity conserved (Laliberté 1997). The first botanical gardens were set up in Italy during the second half of XIV century, in Pisa (1544), Padua and Florence (1545) followed by Holland (1593), Paris (1635) and Edinburgh (1690).

Field Genebanks

Field genebanks are the principal method for conserving species that do not “breed true”, or retain the production characteristics of the parent (i.e. fruit trees, forest plants), or those of which the seeds cannot tolerate the desiccation and cooling used for storage (i.e. recalcitrant seeds) (Fowler and Hodgkin 2004). These plants are kept either in fields or in greenhouses.

Seed and Pollen storage

This is the most commonly used form of *ex situ* conservation with 90% of the world’s 6 million accessions stored as seed (de Vicente 2006). The seeds are dried to between 3-7% seed moisture content (5% for pollen) and stored at sub-zero temperatures. The seeds need to be assessed at regular intervals for viability (as this tends to decrease with time) and regenerated when necessary (Gómez-Campo 2006). The regeneration process should be undertaken taking into account that the

same diversity and allele frequency should be maintained, hence avoiding actions that select changes in the original gene frequencies.

In vitro Storage

In vitro storage involves the storing of plants in a synthetic nutrient medium in a sterile environment. This approach is most commonly used for species with recalcitrant seeds, apomictic species and vegetatively propagated species. Endangered species are also being conserved using this method. A part of the plant (most often buds and embryos but also stems, leaves and flower buds) is maintained, often in a glass tube, with temperatures set according to the tolerance levels of the individual species. Pence et al. (2002) provide a detailed guide on *in vitro* collecting techniques.

Cryopreservation

Cryopreservation, very low temperature storage of seeds or embryos, is being developed as an alternative for a number of species. The germplasm is stored in liquid nitrogen (-196°C). A major disadvantage of this technique is that it is expensive as well as being specific to a particular species, sometime even variety-specific. The main advantage, however, the material may be stored, theoretically, for an unlimited period of time.

DNA Storage

In this method, DNA is extracted and stored at -20°C in an ethanol solution. This is not a conservation strategy per se but the information obtained is useful for monitoring genetic changes over time, assisting in the development of molecular markers and provide a greater understanding of taxonomy at the genetic level. Thus, alternative conservation measures are required to conserve the germplasm in its entirety. A detailed discussion of this subject is provided in de Vicente (2006).

Legal Frameworks for the Protection of (Plant) Biodiversity

In the 1960s genetic resources conservation started increasing in importance globally and its profile was further raised when the FAO organized two conferences in 1967 and 1972 to discuss the issue. In 1974, the International Board for Plant Genetic Resources (IBPGR) was established within FAO, which in 1992 later evolved into the International Plant Genetic Resources Institute (IPGRI), under the auspices of the Consultative Group on International Agricultural Research (CGIAR, also known as the CG), and is now known as Bioversity International.

During the 1970s, many countries and CG centres set up germplasm banks to collect and conserve plant genetic resources, possibly as a consequence of the 'Green Revolution' which so far has averted the pessimistic Malthusanian predictions. Thomas Robert Malthus (1809) noted that human population growth is geometric (exponential) while food was shown to have an arithmetic growth; the conclusion he therefore reached was that humanity was doomed to starvation. This was belied by the success of the Green Revolution, which occurred between 1960 and 1990, when there was a tremendous boom in agricultural productivity in the developing world. During these decades, in many regions of the world, especially in Asia and Latin America, the yield of the major cereal crops (rice, wheat and maize) more than doubled. India adopted IR8, a rice semi-dwarf variety developed by the International Rice Research Institute (IRRI) that produced more grains of rice per plant when grown properly with fertilizer and irrigation. These new varieties have been obtained through traditional plant breeding programmes (Swaminathan 2002).

Plant genetic resources developed by farmers over centuries were freely available and under international law, were considered as a "common heritage of mankind to be preserved and freely available for use, for the benefit of present and future generations" (FAO 1983 regarding the International Undertaking on Plant Genetic Resources for Food and Agriculture (IU); Le Buanec 2005). This free exchange ended during the last decade of the 20th century with the development of biotechnology and intellectual property rights. The IU in 1991 recognized the sovereignty of states of their own genetic resources.

With the **International Congress of Rio de Janeiro** and the signing of the **Convention on Biodiversity** in 1992, attention shifted from genetic resources to overall biodiversity conservation. The Convention on Biological Diversity (CBD) is a framework convention, with a broad mandate in relation to three goals:

- conservation of biodiversity
- sustainable use of the components of biodiversity
- the fair and equitable sharing of benefits arising from the utilization of such components

Article 2 of the CBD states that biological diversity is "…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 of ecosystems". The CBD includes the whole range of methodologies of conservation from *ex situ* to *in situ* conservation (Article. 8). The CBD entered into force in 1993 and confirmed the

sovereignty of the geo-political states over the resources within their national borders. It was recognized, however, that this impeded agricultural development, the global common heritage and the international interdependency of countries for food security (Laliberté 1997) as concerns over patenting and biopiracy decreased the access of PGR across borders.

The latest Conference of Parties of the CBD (COP 10) of the CBD was held in Nagoya, Japan, in October 2010 (http://www.cbd.int/nagoya/outcomes). It was of special importance as it was held during the UN-designated International Year of Biodiversity. The outcomes include a revised Strategic Plan, a mission and 20 targets, organized under five strategic goals which address the three objectives of the CBD. Of particular relevance to plant diversity in the revised Strategic Plan, and more specifically to crop diversity and the work undertaken here, is Strategic Goal C, Target 13:

"By 2020, the genetic diversity of cultivated plants and farmed and domesticated animals and of wild relatives, including other socio-economically as well as culturally valuable species, is maintained, and strategies have been developed and implemented for minimizing genetic erosion and safeguarding their genetic diversity."

It was due to this recognition that the International Treaty for Plant Genetic Resources (ITPGR; referred to as the Treaty henceforth) was signed in Rome in 2001 and ratified by 180 nations and entered into vigour on 29th June 2004. The objectives of the Treaty are "the conservation and sustainable use of plant genetic resources for food and agriculture and the fair and equitable sharing of the benefits arising out of their use, in harmony with the Convention on Biological Diversity, for sustainable agriculture and food security" (Article 1.1). In particular, Article 5 addresses the conservation, exploration, collection, characterization, evaluation and documentation of PGRFA, (the full text of the Treaty is available at http://www.fao.org/AG/cgrfa/itpgr.htm#text) and calls on contracting parties to:

- survey and inventory PGRFA;
- promote the collection of PGRFA that are under threat or of potential use, along with relevant associated information;
- promote or support farmers' and local communities' efforts to manage and conserve on-farm their PGRFA;
- promote *in situ* conservation of wild relatives of crops and wild plants for food production, including

in protected areas;

- cooperate to promote the development of an efficient and sustainable system of *ex situ* conservation;
- monitor the maintenance of the viability, degree of variation and genetic integrity of collections of PGRFA and take steps to minimize or eliminate threats to PGRFA.

The Treaty also provides a platform for facilitated global exchange of plant genetic resources, the Multilateral System (MLS) of Access and Benefit Sharing (ABS).The Multilateral System applies to all PGRFA and is implemented under a Standard Material Transfer Agreement (SMTA), which was agreed in 2006. The MLS aims to conserve and use PGRFA and the fair and equitable sharing of benefits arising out of their use include all plant genetic resources for food and agriculture listed in Annex I that are under the management and control of the Contracting Parties and in the public domain and in particular "The Contracting Parties agree that benefits arising from the use of plant genetic resources for food and agriculture that are shared under the Multilateral System should flow primarily, directly and indirectly, to farmers in all countries, especially in developing countries, and countries with economies in transition, who conserve and sustainable utilize plant genetic resources for food and agriculture" (Lewis-Lettington et al. 2006).

At present, Annex 1 of the Treaty comprises only 35 crops and a few forage species (Table 1), which were selected based on food security and interdependence issues and cover roughly 80% of human calorific intake from plants (on a global scale) (GFAR/IPGRI 2003).

Table 1. List of crops under the Annex 1 of the Multilateral System.

FOOD CROPS	**FORAGES**
Crop Genus Observations	LEGUME FORAGES
Breadfruit *Artocarpus*	*Astragalus chinensis, cicer, arenarius*
Asparagus *Asparagus*	*Canavalia ensiformis*
Oat *Avena*	*Coronilla varia*
Beet *Beta*	*Hedysarum coronarium*
Brassica complex *Brassica.* Genera included are:	*Lathyrus cicera, ciliolatus, hirsutus, ochrus, odoratus,*
Brassica, Armoracia, Barbarea, Camelina, Crambe,	*sativus*
Diplotaxis, Eruca, Isatis, Lepidium, Raphanobrassica,	*Lespedeza cuneata, striata, stipulacea*
Raphanus, Rorippa, and *Sinapis*. This comprises oilseed	*Lotus corniculatus, subbiflorus, uliginosus*
and vegetable crops such as cabbage, rapeseed, mustard,	*Lupinus albus, angustifolius, luteus*
cress, rocket, radish, and turnip. The species *Lepidium*	*Medicago arborea, falcata, sativa, scutellata, rigidula,*

FOOD CROPS	FORAGES
meyenii (maca) is excluded.	*truncatula*
Pigeon Pea *Cajanus*	*Melilotus albus, officinalis*
Chickpea *Cicer*	*Onobrychis viciifolia*
Citrus *Citrus* Genera *Poncirus* and *Fortunella* are	*Ornithopus sativus*
included as root stock.	*Prosopis affinis, alba, chilensis, nigra, pallida*
Coconut *Cocos*	*Pueraria phaseoloides*
Major aroids *Colocasia, Xanthosoma;* includes taro,	*Trifolium alexandrinum, alpestre, ambiguum,*
cocoyam, dasheen and tannia.	*angustifolium, arvense, agrocicerum,*
Carrot *Daucus*	*hybridum, incarnatum, pratense, repens, resupinatum,*
Yams *Dioscorea*	*rueppellianum,*
Finger Millet *Eleusine*	*semipilosum, subterraneum, vesiculosum*
Strawberry *Fragaria*	GRASS FORAGES
Sunflower *Helianthus*	*Andropogon gayanus*
Barley *Hordeum*	*Agropyron cristatum, desertorum*
Sweet Potato *Ipomoea*	*Agrostis stolonifera, tenuis*
Grass pea *Lathyrus*	*Alopecurus pratensis*
Lentil *Lens*	*Arrhenatherum elatius*
Apple *Malus*	*Dactylis glomerata*
Cassava *Manihot Manihot esculenta* only.	*Festuca arundinacea, gigantea, heterophylla, ovina,*
Banana / Plantain *Musa*; except *Musa textilis*.	*pratensis, rubra*
Rice *Oryza*	*Lolium hybridum, multiflorum, perenne, rigidum,*
Pearl Millet *Pennisetum*	*temulentum*
Beans *Phaseolus*; except *Phaseolus polyanthus*.	*Phalaris aquatica, arundinacea*
Pea *Pisum*	*Phleum pratense*
Rye *Secale*	*Poa alpina, annua, pratensis*
Potato *Solanum* Section *tuberosa* included, except	*Tripsacum laxum*
Solanum phureja.	OTHER FORAGES
Eggplant *Solanum*; section *melongena* included.	*Atriplex halimus, nummularia*
Sorghum *Sorghum*	*Salsola vermiculata*
Triticale *Triticosecale*	
Triticum; includes *Agropyron, Elymus, Secale*.	
Faba Bean / Vetch *Vicia*	
Cowpea *Vigna*	
Maize *Zea*; excludes *Zea perennis, Zea diploperennis,*	
and *Zea luxurians*.	

The Millennium Development Goals (MDGs) are time-bound, quantifiable targets set by world leaders in 2000 at the Millennium Summit, with the aim of reducing poverty at a global scale.

(Source: http://www.unmillenniumproject.org/goals/index.htm)

Fig.1.3. The Millennium Development Goals.

Goal 7 is the most directly relevant to the assessment and sustainable management and use of biological (including crop) diversity. The initial target set in 2000 was to achieve a significant reduction in the rate of loss by 2010. This target has not been achieved, despite increases in investment in conservation planning and action (UN 2010). High rates of consumption, habitat loss, invasive species, pollution and climate change currently threaten the poorest peoples, the most vulnerable as well as the most directly dependent on diverse plants and animals for their survival.

The European Environment Agency developed the Driver-Pressure-State-Impact-Response (**DPSIR**) framework (EEA 1998), which the CBD also adopted. The abbreviation DPSIR stands for a conceptual framework for the description of the environmental problems and of their relationships with the socio-economic domain, and relevant to policy-makers. This framework is frequently used in decision making, especially in understanding pressures on the conservation of biodiversity.

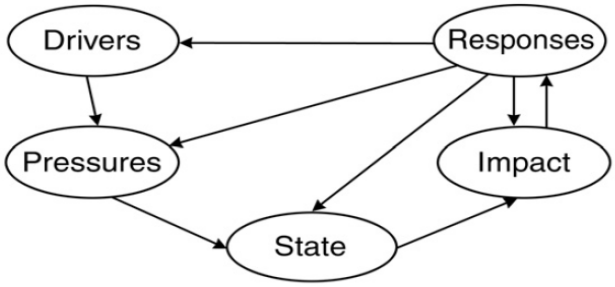


Fig.1.4. The DPSIR framework

Fig.1.4 shows the relationships between the framework components: social and economic developments (Driving Forces, D) exert Pressures (P) on the environment and, as a consequence, the State (S) of the environment changes. This leads to Impacts (I) on ecosystems, human health, and society, which may elicit a societal Response (R) that feeds back on Driving Forces, on State or on Impacts via various mitigation, adaptation or curative actions (Gabrielsen and Bosch 2003; Smeets and Weterings 1999). Thus, the DPSIR is described as a "causal framework for describing the interactions between society and the environment" (EEA 2006; Delbaere 2002).

1.1.2 Diversity Assessment

Adequate knowledge of existing genetic diversity, where it exists and how to best utilize it, is therefore vital for continued successful management of agricultural resources (see Table 1). Information regarding the threat and rate of genetic erosion is paramount, yet there is very little work in quantifying the magnitude of any trends. Standard methods for the collection and analysis of data over time for crops, forage and wild relatives have yet to be developed, although there is an increased awareness of the need. What work exists using time series data tends to focus only on specific crops based on small subsets stored in gene banks and using molecular techniques.

Table 1.2. Measures of genetic diversity

What diversity is measured and the methods employed
Diversity in single genes
Biochemical analysis
Mendellian analysis
Polygenic diversity
Multivariate analysis of morphological variation in traits whose expression is determined by more than one gene
Latent diversity of genome
Genealogical analysis
Analysis of cytoplasm donors
Molecular (DNA) analysis and probes
Pedigree complexity
Genealogical characteristics
Performance-based complexity
Analysis of genotypic variance and genotype by environment interactions
Analysis of yield variance at farm, district, national, or regional level
***Ex situ* diversity**
Analysis of numbers of accessions within and among species
Morphological analysis of accessions
Spatial diversity
Number of cultivars by percentage of area
Percentage distribution of area by cultivar
Temporal diversity
Average age of cultivars
Rate of cultivar replacement

(after Brookfield and Padoch 2007; Gaines et al.1999)

Genetic diversity may be assessed at scales other than at the genetic level *per se* (see Table 2.). At the molecular level diversity within and between populations is typically measured using various laboratory based techniques such as allozyme or DNA analysis, directly measuring levels of variation. A close correlate to genetic variation is that of morphological variation where appropriate physical traits can be used as a measure of genetic variation. At a species level various species diversity indices, taxonomic counts, population estimates, etc. can be used as an indicator of levels of diversity. Even at the landscape level, there are attributes that can be indicative of genetic diversity and erosion. For example field sizes, both in the present day and in historic reference can be related to numbers of different crops grown in an area in the past and compared to the current situation. This paper gives an overview of the main methodologies at each level and their potential for use in monitoring genetic erosion.

Table 1.3. Overview of levels at which genetic diversity may be assessed.

Measures/Assessments of Diversity and the Tools/Methods employed
Genetic Level
Diversity within and between populations
Allozyme analysis
DNA analysis
Assessment of morphological variation
Species Level
Impacts
Species diversity indices
Functional diversity
Functional group analyses
Changes in number of species/varieties
Taxonomic counts
Abundance indices
Population estimates
Landscape Level
Changes in landscape diversity
Indices of landscape patterns
Historic references
Remote sensing/GIS
Changes in habitat distribution
Indices of landscape patterns
Historic references
Remote sensing/GIS
Changes in landscape elements (e.g. field sizes, usage)
Indices of landscape patterns
Historic references
Remote sensing/GIS

Genetic Diversity

There are several different ways of measuring diversity, and especially the diversity or 'distance' between two or more populations (Mohammadi and Prasanna 2003). In general the various methods will give the same rank order of diversity, but they may give very different numerical values.

Given any two measurements of the same trait, subtracting one from the other gives a raw difference or interval. These intervals are themselves quantities in the same dimension as the trait itself, and can be added, multiplied, averaged, etc, to obtain such measures of diversity as the standard deviation, the Gini coefficient, the inter quartile interval, or the mean absolute-value deviation. The Unweighted Pair Group Method (UPGM) uses arithmetic averages to generate dendrograms that illustrate the genetic distances between different varieties (e.g. Garcia-Mas et al. 2000)

Genetic assessment is more complex given a non-quantitative trait, such as genetic material. Given any two stretches of DNA, one can identify and list the differences between them. It is in the weighting of the differences that the difficulties arise. For example, whether non-coding regions or synonymous codons should be included can be a major issue. This depends in part on the underlying motive for measuring diversity. Measures of diversity, such as Wright's FST, were devised mainly to reconstruct phylogenies and population history and all differences are therefore treated as equal (Nei 1973; Charlesworth 1998).

Genetic diversity may be spatially structured at different scales (geographic, population, subpopulation, etc.), due to environmental influence, life history, and demographic traits of the species (Loveless and Hamrick 1984; Slatkin 1985; Oostermeijer et al. 2003). Consequently, spatial genetic structure provides a valuable tool for inferring causal factors and underlying operating evolutionary forces such as selection, gene flow, and drift (Nevo et al. 1982; Nevo et al. 1986; Barbujani 1987; Epperson 1990; Wilson 2004). Levin (1974), Loveless and Hamrick (1984), Wade and McCauley (1988), Oostermeijer et al. (1994) and Grassi et al. (2004) have highlighted the influence of environmental factors (including human activities and various interactions) and life history traits of plant species on population viability and genetic variability.

An increasing number of studies have also integrated data from ecology, population biology, genetics, and reproductive biology in order to formulate reliable conservation and management

strategies of populations (Schaal and Levin 1976; Guerrant 1992; Widén 1993; Alvarez-Buylla et al. 1996; Oostermeijer et al. 2003). In these investigations, spatial analysis methods are of a special interest (Sokal and Oden 1978a; Sokal and Oden 1978b; Legendre and Fortin 1989; Escudero et al. 2003). Indeed, this technique may be helpful, for example, for the improvement of sampling strategies in collecting seeds for ex situ conservation, the selection of populations that should be protected in situ, the determination of the area size necessary for the conservation of a particular population, the selection of a specific site for the establishment of a corridor population, etc.

Molecular markers are divided into (see Fig.1.5 for an illustration of the types of methods; see Table 1.4 for an overview of the methods):

Non-Polymerase Chain Reaction (PCR) based techniques

These include Restriction length Polymorphisms (RFLPs) and minisatellites analysis (also known as Variable Number of Tandem Repeats (VNTR) analysis).

PCR based techniques

These include DNA sequencing and Sequence-Tagged Sites (STS). The latter method has undergone technological development and has spawned a variety of methods; the most well known are microsatellite analysis (also known as Simple Sequence Repeats (SSR)), Random Amplified Polymorphic DNA (RAPD) analysis and Amplified Fragment Length Polymorphism (AFLP).

Non-PCR based techniques

Restriction fragment length polymorphism (RFLP) analysis was the first technology developed which enabled the detection of polymorphisms at the sequence level. This approach involves: (i) digesting DNA with restriction enzymes; (ii) separating the resultant DNA fragments by gel electrophoresis; (iii) blotting the fragments to a filter, and; hybridizing probes to the separated fragments. A probe is a short sequence of oligonucleotides that share homology and are thus able to hybridize, with a corresponding sequence or sequences in the genomic DNA. RFLP analysis is used extensively in the construction of genetic maps and has been successfully applied to genetic diversity assessments, particularly in cultivated plants (e.g. Castagna et al. 1994; Deu et al. 1994; Jack et al. 1995) but also in populations and wild accessions (e.g. Besse et al. 1994, Laurent et al. 1994, Bark and Harvey 1995).

Interspersed among the genomes of higher organisms are highly variable regions that are comprised of repeats of short simple sequences. These are known as "microsatellites", where the basic repeat

unit is around two to eight base pairs in length and "**minisatellites**" for longer repeat units of 16 to 100 base pairs. Because of the very high levels of polymorphisms detected, minisatellites are recognized as powerful tools, particularly for fingerprinting and cultivar identification in plants (e.g. Beyermann et al. 1992; Vosman et al. 1992). They have also been used for studying within and between population variation, for ecological studies (e.g. Alberte et al. 1994; Wolff et al. 1994) and for estimating genetic distances (e.g. Lynch 1990; Antonius and Nyborn 1994).

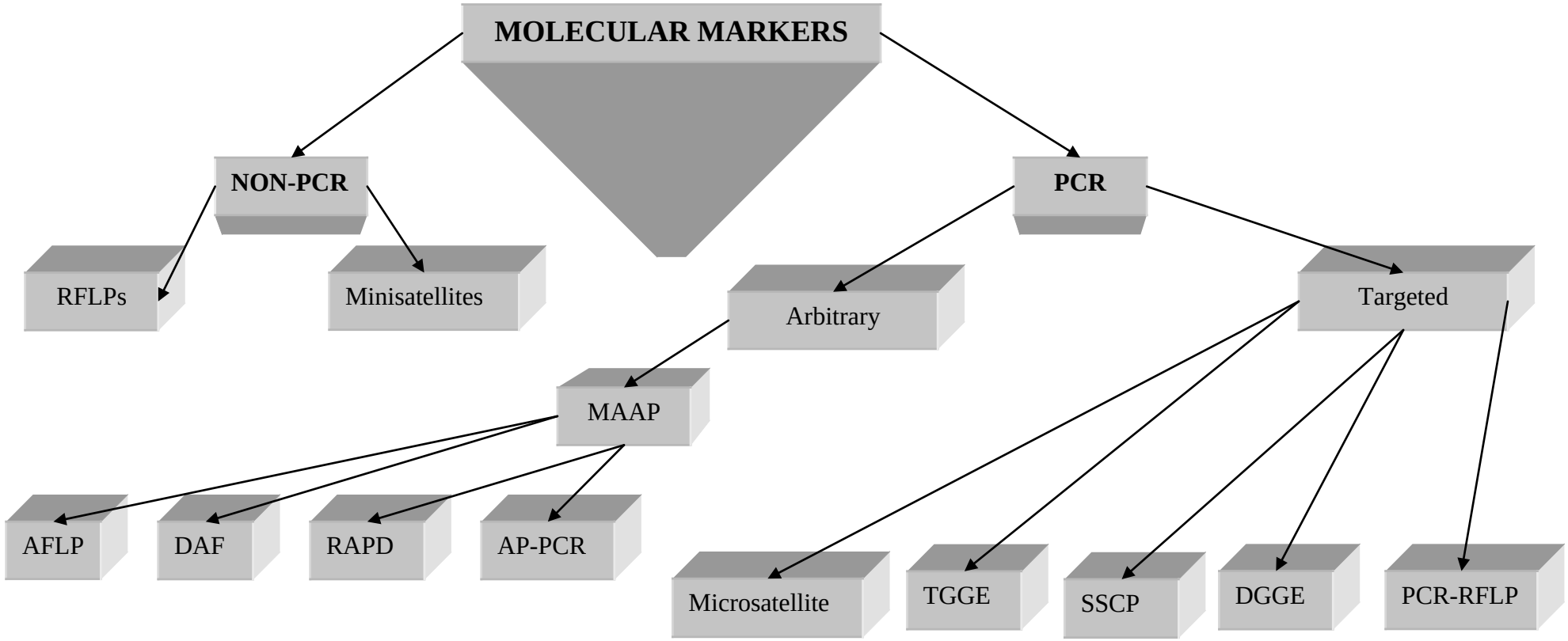
The success of multilocus fingerprinting is dependent on the probe/enzyme combination used, which has to be tested out each time a new species is studied. However, due to the complexity of the patterns obtained, in combination with the presence of an infinite number of alleles at each locus, means that alternative statistical procedures are required for the use of minisatellite data in classical population genetic models. This makes the accuracy of analyzing relationships using minisatellite data very problematic (Lynch 1990; Scribner et al. 1994). The problem can be reduced by selecting single locus minisatellites (or VNTRs) but this increases the pre-screening and selection process considerably. Taking all aspects of non-PCR based screening approaches into consideration, it is difficult to envisage that this would be the preferred choice today, given that alternative strategies are now available.

PCR based Techniques

The development of Polymerase Chain Reaction (PCR) sequencing allowed the amplification of any genomic region based on annealing a primer to a particular genomic site. Studying the distances between *microsatellite loci*, degrees of relatedness between organisms can be accurately measured, and there are various resources such as MoDAD (Measurement of Domestic Animal Diversity) which provides lists of such loci in various farm animals and recommendations of how to go about sampling individuals for genetic diversity studies (for examples of PCR based techniques see: Gonzalez et al. 2005, Fu et al. 2004, Budak et al. 2003, Guzman et al. 2003, Vergara and Bughara 2003, Negash et al. 2002, Potokina et al. 2002, Levi et al. 2001, Mengistu et al. 2000). The training required, laboratory work and the expense involved, may make this technique largely untenable in most field circumstances, although the results obtained from any such work carried out are invaluable for use within the analysis of data obtained by other methods such as species counts. A further result of the relative expense and difficulty involved is that it is the more commercially important varieties that are most studied and well known, underutilized and rare varieties less so.

Restriction-site analysis of mitochondrial DNA has been used, most recently leading to developments towards creating a ‘genetic barcode’ which can uniquely identify a species and how genetically distant it may be from its relatives. The barcode method holds great promise, but as mitochondria are organelles of animal cells is for now limited in its application to crops.

In a study by Bekessy et al. (2002), neutral DNA markers (RAPDs) and quantitative genetic techniques were used to characterize genetic heterogeneity within and among populations. Both the level and pattern of genetic variation estimated using the different techniques were essentially uncorrelated. An important discrepancy was found with the neutral markers failing to detect an important quantitative genetic divergence across the Andean Range relating to drought tolerance. This study clearly demonstrates the potential problems associated with making recommendations for conserving the genetic resource of threatened species based solely on neutral marker studies.



PCR	Polymerase Chain Reaction	DGGE	Denaturing Gel Gradient Electrophoresis
MAAP	Multiple Arbitrary Amplicon Profiling	RFLP	Restriction Fragment Length Polymorphism
DAF	DNA Amplification Fingerprinting	AFLP	Amplified Fragment Length Polymorphism
RAPD	Random Amplified Polymorphic DNA	AP-PCR	Arbitrary Primed PCR
SSCP	Single Strand Conformational Polymorphism	TGGE	Thermal Gel Gradient Electrophoresis

Fig.1.5. Diagram of the isozymes and molecular markers commonly used

Table 1.4. Description of the common molecular markers

Basic Description	Uses	Advantages	Disadvantages
Restriction Fragment Length Polymorphisms (RFLP)			
RFLPs are bands corresponding to DNA fragments (2-10kb), the result of digestion of genomic DNA with restriction enzymes. These are detected by agarose gel electrophoresis	Phylogenetic studies conducted at the intraspecific level and for closely related species Widely used in mapping studies due to high abundance of restriction enzymes Studies in hybridization and introgression	Highly reproducible between laboratories and the diversity profiles generated can be reliably transferred RFLPs are codominant markers, enabling heterozygotes to be distinguished from homozygotes Sequence-specific information is not required and, provided suitable probes are available, the approach can be applied immediately for diversity screening in any system	A good supply of probes is needed that can reliably detect variation at the below species level RFLPs are time-consuming and they are not amenable to automation without considerable capital investment RFLP analysis requires relatively large quantities of good quality DNA (e.g. 10µg per digestion) Insufficient polymorphisms are detectable at the below species level by RFLP analysis
Minisatellites (also known as Variable Number of Tandem Repeats (VNTR))			
Minisatellites are repeated sequences of around 10-50bp. They can be multilocus or single locus. Relatedness can be estimated from percentage band-sharing	Studies involving identification and parentage Identification of varieties and cultivars	High level of polymorphism High level of reproducibility	Random distribution of minisatellites across genome Similar sized fragments may not be homologous Hypervariability reduces value in taxonomic studies
Random Amplified Polymorphic DNA (RAPDs)			
RAPDs are random DNA fragments, amplified through PCR using arbitrary oligonucleotides primers. It is scored using the presence or absence of bands. Arbitrarily Primed PCR (AP-PCR) and DNA Amplification Fingerprinting are variants of this technique; they are collectively known as Multiple Arbitrary Amplicon Profiling (MAAP)	Studies at the individual level to related species Gene mapping studies to fill gaps not covered by other markers	Quick and easy to assay Low quantities of DNA required Random primers are available commercially High genomic abundance and randomly distributed through the genome	Low reproducibility, making comparisons between researchers problematic Purified, high molecular weight of DNA required – risk of contamination high RAPDs are not locus specific so band profiles cannot be interpreted in term of loci/alleles

Basic Description	Uses	Advantages	Disadvantages
for techniques using single arbitrary primers.			
Microsatellites (also known as Simple Sequence Repeats (SSR))			
Microsatellites are repeated sequences of around 1-6bp repeats, shorter than minisatellites. Microsatellites are amplified the by PCR through identifying flanking regions or by screening microsatellite sequence motifs and designing adjacent primers	**Due to high levels of polymorphism shown by microsatellites, they are extremely useful in population genetics studies and gene mapping studies.**	**High genomic abundance** **Low quantities and quality of DNA can be used** **Multiple microsatellites (non-overlapping size ranges) can be amplified and scored during PCR and gel electrophoresis, decreasing costs** **Use of automatic sequencing allows for greater efficiency in time management**	**High development costs of primers** **No amplification of the intended PCR may occur (occurrence of null alleles), leading to errors in screening** **"Stutter bands" may occur due to DNA slippage during PCR amplification, complicating the interpretation of band profiles** **High mutation rates of microsatellite loci make them unsuitable for higher taxonomic studies.**
Inter Simple Sequence Repeats (ISSR)			
ISSR analysis is a multilocus technique, consisting of DNA fragments made up of 100-3000bp, located between adjacent, oppositely-oriented microsatellite regions. Microsatellite core sequences are used as primers and amplified by PCR	**ISSR analysis is used in gene mapping studies as well as studies of genetic identity, parentage, clone and strain identification, and taxonomic studies of closely related species.**	**No sequence data are necessary** **Low quantities of DNA are required**	**Problems with reproducibility** **Possible non-homology of similar sized fragments**
Amplified Fragment Length Polymorphism (AFLP) **(also known as Selective Fragment Length Amplification (SFLA) or Selective Restriction Fragment Amplification (SRFA))**			
AFLPs are DNA fragments digested by restriction enzymes and then amplified by PCR. The AFLP banding profiles consist of variations in the restriction sites or in the intervening regions	**AFLP analysis is used in gene mapping studies as well as studies of genetic identity, parentage, clone and strain identification, and taxonomic studies of closely related species.**	**High genomic abundance and reproducibility** **Generation of many informative bands per reaction** **No sequence data for primer construction is required**	**Purified, high molecular weight of DNA is required** **Possible non-homology of co-migrating fragments belonging to different loci** **Subjectively determined criteria are needed for acceptance of bands in the analysis**

For the purposes of this study the following two types of markers were used:

Microsatellites (simple sequence repeats, SSRs) are short sequences of nucleotides (typically 1 to 5 bp, and no more than 6 bases long) which are tandemly repeated. Microsatellites alleles are characterized by different numbers of repeats, are abundant (they can occur every 30 kb region of the higher plant genomes) and exhibit high levels of polymorphism (see Brown et al. 1996 for a review). The majority of microsatellites occur in gene introns or other non-coding regions of the genome; thus variation in the number of repeats has no consequence on gene function, hence these markers are known as “selectively neutral”.

As a marker, the specific number of repeats in a given microsatellite is not important, but rather the difference in the number of repeats between alleles. The degree of polymorphism in microsatellites is proportional to the underlying rate of mutation. The variation in number of repeats affects the overall length of the microsatellite (Ellegren 2004).

In the past, SSRs have been expensive to develop and thus often limited to applications to the major commercial crops (Scott et al. 2000). The first report (Condit and Hubbell 1991) on the isolation and cloning of plant microsatellites was for tropical tree species

The use of microsatellites involves the isolation of microsatellite-containing DNA clones from enriched genomic DNA libraries, synthesizing primer sets to amplify the microsatellite-containing region, and mapping SSR loci that are polymorphic (Holton 2001). These single locus markers are characterized by their very useful in germplasm characterization and hypervariability, abundance, reproducibility, Mendelian mode of inheritance, and codominant nature (Scott et al. 2000).

Inter simple sequence repeat (ISSR) markers are generated from single primer polymerase chain reaction (PCR) amplifications in which the primers are based on dinucleotide or trinucleotide repeat motifs. The number of dinucleotide or trinucleotide repeats varies but is generally sufficiently long to make a primer sequence of at least 14 nucleotides. The microsatellite primer sequence may be anchored with one or two nucleotides on either the 5’ or the 3’ end of the oligonucleotide. An anchoring sequence on the 3’ end of the primer will eliminate the detection of fragment length differences resulting from simple sequence repeat variation, but this would be detectable only with the finest sieving gels and is not an issue for standard agarose gel techniques.

ISSR markers were introduced in 1994 (Gupta et al. 1994; Zietkiewicz et al. 1994) for studies of cultivated plants but have been used for studies of hybridization and hybrid speciation (Archibald et al. 2004; Wolfe et al. 1998a,b), population and conservation genetics (Culley and Wolfe 2001; Esselman et al. 1999), and systematic investigations in natural populations (Crawford et al. 2001; Mort et al. 2003; Wolfe and Randle, 2001). ISSRs are also being used in population studies of fungi (Kerrigan et al. 2003; Sawyer et al. 2003) and animals (Chatterjee and Mohandas 2003; Haig et al. 2003; Nagy et al. 2002). The hypervariable nature of ISSRs combined with minimal equipment requirements and ease of use has made them extremely useful and cost-effective molecular markers for many ecological and systematic investigations (Wolfe et al. 1998b; Yang et al. 1996).

A number of primers work on a wide range of angiosperm groups, including Acanthaceae, Asteraceae (Mort et al. 2003), Ericaceae, Lactoridaceae (Crawford et al. 2001), Orchidaceae, Orobanchaceae (Wolfe and Randle 2001), Poaceae (Esselman et al. 1999), Scrophulariaceae (Archibald et al. 2004; Wolfe et al. 1998a,b), and Violaceae (Culley and Wolfe 2001).

Studies of genetic diversity (e.g. population and conservation biology) require a quantitative approach in which the goal is to obtain as many ISSR bands as possible in as many individuals and populations as possible. A typical ISSR study will include 3–10 primers and several hundred individuals. Both qualitative and quantitative approaches require the assignment of ISSR bands to genetic loci. In practice, each ISSR band visualized on the gel is assigned as a locus identified by its molecular weight. After all molecular weights have been calculated and loci assigned, the data are converted to a matrix of 1s and 0s where 1 = band present and 0 = band absent for each locus individual combination.

Several studies using the ISSR technique have been described to properly assess genetic diversity in crops such as lentil (Sonnante and Pignone 2007a), mulberry (Kar et al. 2008), *Chondrus crispus* (Wang et al. 2008), *Eruca vesicaria* (Egea-Gilabert et al. 2009), and broccoli (Lu et al. 2009).

ISSR studies (Lanteri et al. 2004b; Crinò et al. 2008; Lo Bianco et al. 2010) have been performed in globe artichoke and cardoon (Sonnante et al. 2004; Raccuia et al. 2004; Itoiz et al. 2004; Portis et al. 2005a,b; Acquadro et al. 2006). Knowledge of genetic variation between different cultivars and within cultivars would provide great scope for crop improvement through judicious selection and

breeding to develop a desired genotype. Plant breeders can then use the knowledge of genetic relationships among cultivars as a parental line selection tool.

Analysis of Genetic Data

There are several different ways of measuring diversity, and especially the diversity or 'distance' between two or more populations (Mohammadi and Prasanna 2003). In general the various methods will give the same rank order of diversity, but they may give very different numerical values.

Given any two measurements of the same trait, subtracting one from the other gives a raw difference or interval. These intervals are themselves quantities in the same dimension as the trait itself, and can be added, multiplied, averaged, etc, to obtain such measures of diversity as the standard deviation, the Gini coefficient, the inter quartile interval, or the mean absolute-value deviation. The Unweighted Pair Group Method (UPGM) uses arithmetic averages to generate dendrograms that illustrate the genetic distances between different varieties (e.g. Garcia-Mas et al. 2000)

Genetic assessment is more complex given a non-quantitative trait, such as genetic material. Given any two stretches of DNA, one can identify and list the differences between them. It is in the weighting of the differences that the difficulties arise. For example, whether non-coding regions or synonymous codons should be included can be a major issue. This depends in part on the underlying motive for measuring diversity. Measures of diversity, such as Wright's FST, were devised mainly to reconstruct phylogenies and population history and all differences are therefore treated as equal (Nei 1973; Charlesworth 1998).

Statistical analyses are also used to identify similar accessions and cultivars and assess genetic and phenotypic relatedness; one such method, cluster analysis, is used to perform a classification on a large collection of individuals (Anderberg 1973). Multiple characters for each individual are used to group accessions into cluster classes. Individuals within a given cluster class are similar, while individuals from different classes are not. Similarity measurements among clusters were also determined so that relationships between groups can be established.

Differences at the intraspecific level are seen in the *frequency* (proportion) of different alleles than in their simple presence or absence. Most measures of genetic diversity at the intraspecific (and

therefore varietal) level, are based on some index of *heterozygosity*: the probability that two genes at a given locus, selected at random from the relevant population(s), will be different (Gitzendanner and Soltis 2000).

With sufficient data on the frequency of different alleles, it is possible to calculate the probability that two genes at a given locus, selected at random from the relevant population or populations, will be either identical (homozygous) or different (heterozygous). The result provides an average expected number of genetic differences between individuals. Diversity is said to be higher, other things being equal, when there are more alleles in the system, and when their frequencies are evenly spread rather than concentrated in one or a few alleles. Conversely, diversity is said to be low when most of the frequency is concentrated in one or a few alleles.

A simple equation that can be used to discover the probable genotype frequencies in a population, and to track their changes from one generation to another, is the Hardy-Weinberg equilibrium equation. In this equation ($p^2 + 2pq + q^2 = 1$), p is defined as the frequency of the dominant allele and q as the frequency of the recessive allele for a trait controlled by a pair of alleles (A and a). In other words, p equals all of the alleles in individuals who are homozygous dominant (AA) and half of the alleles in people who are heterozygous (Aa) for this trait in a population. The Hardy-Weinberg law is an essential theory in investigating genetic varieties in a population, and interpreted as following: the gene frequencies in a large population remain constant from generation to generation, if mating is at random and there is no selection, migration or mutation. If two alleles A and a are segregated at a locus, and each has a frequency of p and q respectively, then the frequencies of the genotypes AA, Aa and aa are P^2 2pq and q^2 respectively (Groombridge 1992; Mallet 1996). By comparing genotype frequencies from the next generation with those of the current generation in a population, one can also learn whether or not evolution has occurred and in what direction and rate for the selected trait. However, the Hardy-Weinberg equation cannot determine which of the various possible causes of evolution were responsible for the changes in gene pool frequencies.

The effective population size (N_e) based on the frequency of marker alleles measured over time is a well known method for estimating the rate of change due to genetic drift, which is especially salient in small populations (see Wang and Whitlock 2002, for a current overview) .

Once genetic material has been classified into a number of variant forms or 'alleles' (at whatever level), the level of diversity within a population can then be measured by the number of different alleles within that population, while the diversity between two populations can be measured by the number (and/or proportion) of alleles found in one population and not the other. This approach is valid at the inter specific level but at the intraspecific level, most alleles are common to most populations (except perhaps for mitochondrial or Y chromosome haplotypes).

1.1.3 Genetic Erosion

Genetic erosion generally refers to the loss of genetic diversity within a species, and ranges from the loss of an entire species to the gradual loss of alleles/allele combinations within a species. Once this intraspecific diversity is decreased, the potential for extinction is greater due the concurrent loss of elasticity in adaptability. Even if restoration efforts are carried out, there is no guarantee that the original levels of diversity will be restored, leading to either inbreeding depression or outbreeding depression (Williams and Davis 1996; Frankham et al. 2005).

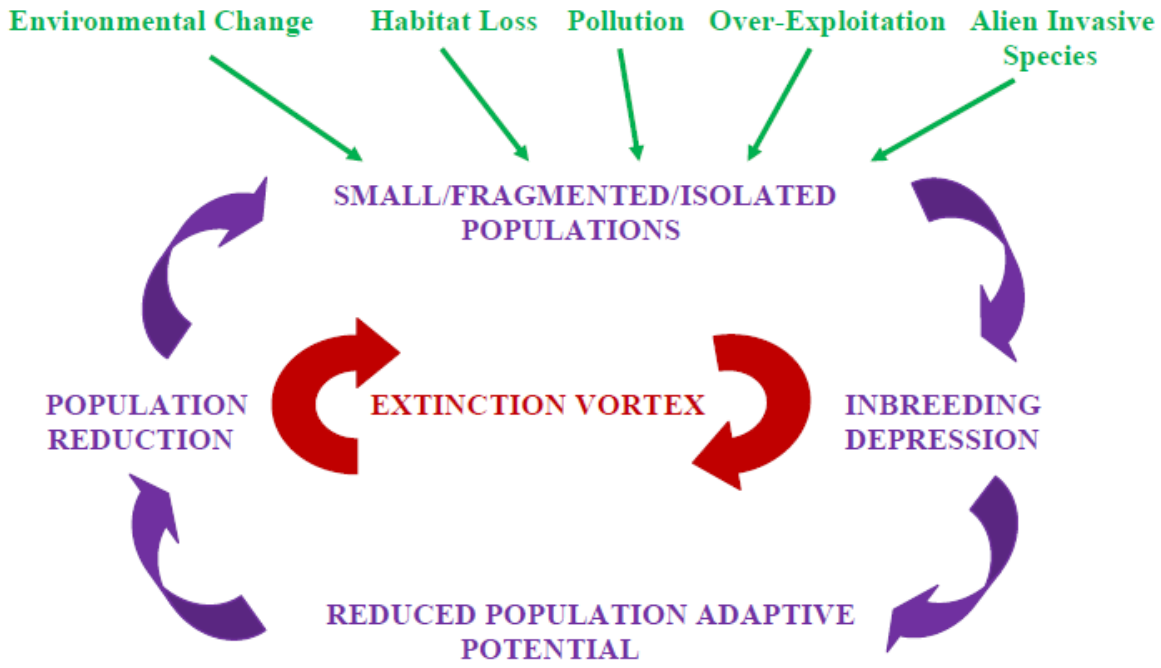


Fig.1.6. The extinction vortex, with the arrows indicating positive feedback mechanisms, leading to subsequent decreases in genetic variance, and therefore, a corresponding decrease in population fitness (Frankham et al. 2005)

Harlan (1970), one of the early contributors to the science of plant genetic resources, stated: ‘The varietal wealth of the plants that feed and clothe the world is slipping away before our eyes, and the human race simply cannot afford to lose it’.

In order to mitigate the effects of the extinction vortex (first coined by Gilpin and Soulè in 1986), studies have to take into account the demographic and environmental stochasticity (Fig.1.6). The elements involved in assessing the effects of include reproduction rates, number of offspring per generation, sex determination, and mortality rates (Fagan and Holmes 2006; Blomquist 2010). This is easier in animals as their reproductive biology, strategies and sexual reproduction is clearer those that of plants.

Estimating genetic erosion is not straightforward. Some studies have used species abundance over time as an estimate of genetic erosion (Hammer et al. 1996). In those cases, the sites selected for assessment need to be taken into account, where factors such as the geology and geography will be of major relevance.

“Genetic erosion may thus be defined as a permanent reduction in richness or evenness of common localized alleles or the loss of combination of alleles over time in a defined area” (IPGRI/Bioversity International). Drivers of genetic erosion in agricultural biodiversity include (Guarino 1995):

- Introduction of modern varieties and exotic crops
- Loss of seed-saving and vegetative and propagation skills
- Acculturation of traditional farmers (or their death)
- Change in economic base
- Conversion of land to industrial agriculture
- Destruction (urbanization) of habitat and farmland
- Impact of herbicides and pesticides
- Environmental contamination
- Introduction of exotic pests
- Loss of seeds to pests
- Inadvertent crossing of varieties.

Globally, this process has led to a decrease in crop diversity in many farming systems, frequently because traditional varieties are being replaced by modern varieties (FAO 1997). However, as landraces are often well adapted to specific environments, they do have a clear advantage in marginal areas. Besides their direct use, these genetic resources have an important potential value in future breeding programs as well (IPGRI 2004), and therefore need to be conserved. Observing this process of changing crop genetic diversity, Frankel (1970) already stated in 1970 that ‘‘it is now generally recognized that many of the ancient genetic reservoirs are rapidly disappearing’’.

Modern cultivars have undoubtedly replaced many traditional varieties. However, the adoption rates of modern cultivars vary considerably between countries, regions and crops. In the highly developed agricultural systems of North America and North-Western Europe, the replacement of traditional landraces of major field crops with cultivars had practically been completed when, in the 1970s, the Green Revolution in the developing world started. For example, in the United Kingdom, only a few historical landraces of cereals survived into the 20th century as most had already been replaced by cultivars before that time (Scholten et al. 2006), and also in the Netherlands most landraces of field crops had disappeared by mid 20th century (van der Meer and van den Ban 1956).

Fujisaka et al. (1998) reported the highest losses of wild plant diversity in the Brazilian Amazon in areas where conversion to pasture has taken place. Communities where a high number of farmers reported sourcing of planting material from markets also reported a loss of varieties. This could indicate that the planting material available at local markets does not possess a high diversity, which could lead to further erosion of the present diversity. This finding corresponds with other studies that stress the importance of local planting material in relationship to diversity trends (Brush et al. 2003; Coomes and Ban 2004). In Southern Africa, for crops with relatively minor breeding activities such as sorghum and millets, only 14 and 15% of the total cultivated area was planted with improved cultivars in 1995/1996, respectively (Maredia et al. 2000).

Until recently, genetic erosion of ancient crop varieties was not a problem in the mountain areas of Georgia, which until the 1990s constituted a depository of local crop varieties of wheat, barley, rye, oat, common millet, traditional legumes, vegetables, herbs, and spice plants with specific varieties adapted to mountain conditions. In Georgia the main reason for genetic erosion of ancient crop varieties is demographic decline in mountain regions due to harsh economic conditions and lack of modern infrastructure (Nakhutsrishvili et al. 2009).

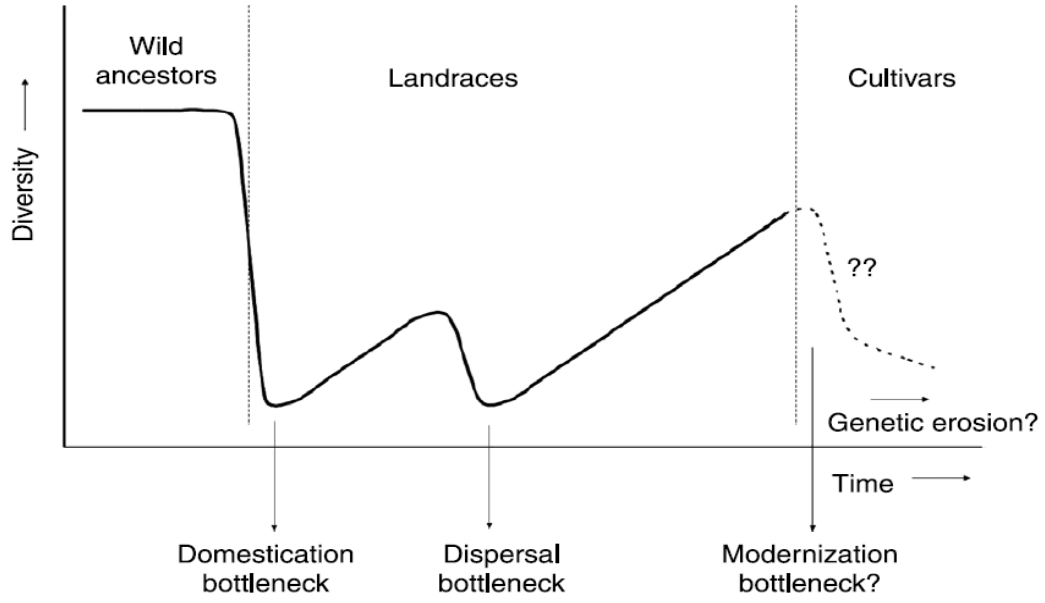


Fig.1.7. Model of trends in diversity of crops from wild ancestors to modern cultivars (van de Wouw et al. 2009).

Two phases in the modernization bottleneck can be distinguished, the initial replacement of landraces with modern cultivars, and the further trends in crop diversity as a result of new cultivar releases. Landraces and cultivars differ in their access to sources of new alleles (Fig.1.7). Landraces can gain new diversity through introgression of alleles from wild relatives, other landraces or cultivars.

For crops with a high naturally occurring gene flow between the domesticated plants and their wild relatives, diversity in the genes that are not selected for could increase gradually after the initial reduction in diversity as a result of the domestication and dispersal bottlenecks. Farmers do select and use introgressed types (Jarvis and Hodgkin 1999). As a consequence, the diversity found in landraces often increased after the initial bottleneck, sometimes to near similar levels as found in the wild species, as has been observed for eastern Mediterranean barley landraces (Jana and Pietrzak 1988) and in Mexican *Capsicum annuum* (Hernandez-Verdugo et al. 2001).

Currently over 900 cultivated plant species, of which the vast majority was never strongly domesticated, are thought to be endangered and 14 species are reported to have disappeared from agriculture (Hammer and Khoshbakht 2005). At the same time, new species are still being

domesticated: the highbush blueberry was domesticated as late as the 20th century (Boches et al. 2006).

The net effect of the disappearance and new domestication on the richness of the world's crop assemblage is not known. In several cases, crop species once thought to be threatened with extinction have found a new niche with the cultivation areas again expanding. Changing climates and changing consumer preferences have given new potential to species once thought to be redundant. Quinoa (*Chenopodium quinoa* Willd.) has now found a new niche due to its lack of gluten (Bonifacio 2003) and cultivation of maca (*Lepidium meyenii* Walp.), a once threatened tuber crop from the Andes, is rapidly expanding due to its alleged health benefits and medicinal properties (Brinckmann and Smith 2004).

Although modern agriculture has been blamed as one of the causes of genetic erosion, the increased yields of the staple crops as a result of modern agriculture might actually have freed land for other crops, as has happened in many countries in Asia, where the share of rice in the total harvested crop area has declined since the 1970s and crop diversity as expressed in levels of evenness has increased (Dawe 2003).

Genetic erosion as a loss of varieties (landraces and cultivars) is sometimes described as varietal erosion (Sperling 2001). Two approaches to quantify the loss of landraces have been used. The first approach is a comparison of the number of landraces or botanical varieties found in an area during collection missions at two different times (Ochoa 1975; Hammer et al. 1996; Buerkert et al. 2006). A possible problem with this approach is that a more intensive survey might yield more landraces, and it may be difficult to copy the approach of the original collection mission. A second approach is interviewing farmers about landraces formerly grown in the area (Peroni and Hanazaki 2002; Tsegaye and Berg 2007; Willemen et al. 2007).

There are several problems with using the number of varieties (landraces or cultivars) as a basis for studying genetic erosion. It is not always clear how distinct landraces or cultivars really are. Farmers often single out one character to identify a landrace, and through positive mass selection they ensure that this character is maintained, even in the presence of high gene flow between populations (e.g. ear type in Mexican maize, Louette and Smale 2000). The identification of landraces is not without pitfalls. Introduced cultivars adopted by farmers are often renamed with

local names (Jusu1999), the information about their origin might subsequently have been lost, and the varieties might be viewed by farmers as ‘traditional’. At the same time, landraces with the same name might in fact be different, as that name might only be a reflection of a limited number of characters, and landraces with different names might be identical, as was found for example with landraces of enset (Negash et al. 2002).

The addition of improved cultivars with a foreign origin to a group of closely related landraces could actually increase local diversity levels and also be a source of new, advantageous genes for these local landraces. Even without introgression from advanced cultivars, a current landrace will not remain genetically identical to that same landrace a decade ago, due to constant farmer selection and incorporation of new alleles.

Monitoring of genetic erosion should focus on those alleles that are locally common. Globally common alleles are highly unlikely to be in danger of disappearance, while alleles with a low frequency in a population can be lost and gained quite easily. The loss and gain of rare alleles are part of normal population genetic processes and although sometimes even the disappearance of rare alleles is considered as genetic erosion (Portis et al. 2004), the disappearance of rare alleles is too common an event to be a true reflection of genetic erosion. In contrast, an allele that is locally common is likely to be involved in adaptation to the local environment, agricultural practices or consumer preferences.

1.1.4 Crop Wild Relatives

In the 1920s Vavilov recognized the potential of crop wild relatives (CWR) for crop improvement and CWR have been routinely used since the 1940s for crop improvement (Table 1.5) (Plucknett et al. 1987; Hodgkin et al. 1992). They are broadly defined as any species in the same genus as a socio-economic crop. A crop wild relative is a wild plant taxon that has an indirect use derived from its relatively close genetic relationship to a crop; this relationship is defined in terms of the CWR belonging to gene pools 1 or 2, or taxon groups 1 to 4 of the crop (Maxted et al. 2006).

The gene pools concept, as proposed by Harlan and de Wet (1971), focuses neatly on the relationships between individuals and populations and is of particular relevance to plant breeders for the improvement of crop germplasm (Greene and Morris 2001). The concept is based on the division of the germplasm of plants into three gene pools:

- The primary gene pool (gene pool 1 or GP1) to which the crop species, crop wild relatives and related weedy species belong with crosses yielding fertile hybrids;
- The secondary gene pool (GP 2) comprises related taxa which are able to hybridize with the crop species but the gene transfer is poor and the progeny are often sterile and not viable;
- The tertiary gene pool (GP 3) includes distantly related taxa which do not cross readily in the wild and require anthropogenic assistance in gene transfer and hybridizing through sophisticated techniques, such as embryo culture, grafting, chromosome doubling, and the use of bridging species;

Current plant breeding now requires the addition of a quarternary gene pool (GP 4) where gene transfer could take place only through genetic engineering.

Table 1.5. Some examples of the contribution of crop wild relatives to the domesticated varieties.

Disease/Stress and Consequence	**Improved**
Rice *(Oryza sativa)*	
Grassy stunt virus, carried by the brown plant hopper, prevents flowering and grain production, affected millions of farmers in South and Southeast Asia	Resistance found in wild relative of rice in Uttar Pradesh, India (Raymond 2006)
Peanut *(Arachis hypogaea)*	
Root knot nematodes affected farmers globally	Three species of wild peanut found to have resistance (Raymond 2006)
Wheat *(Triticum spp.)*	
Low levels of micronutrients leading to malnutrition	Increased nutrients (zinc, iron and Vitamin A) found in wild relative in the Eastern Mediterranean (Raymond 2006)
Diseases: rust, septoria, leaf and spot blotch, fusarium scab and powdery mildew	Resistance found in wheat wild relatives in Armenia
Lettuce *(Lactuca sativa)*	
Downy mildew caused by the fungus *Bremia lactucae*	Resistance found in three wild *Lactuca* species (Nevo 2005)

Latest estimates report that there are between 50,000-60,000 CWR in the wild (Maxted and Kell 2009). Of these, 10,739 are important plant genetic resources for food and agriculture and 700**,** representing less than 0.26% of the world flora, are the most important in terms of global food security and the ones requiring urgent conservation measures.

The distribution of crop wild relatives is mostly correlated with the diversity of flora within a country. The greater the diversity of plant species, the greater the number of CWR occurring within a country. Many developing countries, located within centres of plant diversity and centres of crop diversity, contain large numbers of important crop relatives. The IUCN 2008 Red List Assessment classified 54% of the 1,155 Monocotyledons as endangered or facing a high risk of extinction in the wild, whereas 12% were listed as being critically endangered. This is all the more important when we consider that the Monocotyledon family includes economically important crops such as rice, wheat, maize, barley, sorghum/millet and sugarcane, which alone provide more than half of the dietary energy of the world's population (Crop Wild Relatives Global Portal http://www.cropwildrelatives.org/cwr/threats.html).

CWR used to mitigate climate change in crops, however, CWR are threatened by climate change itself. Jarvis et al. (2008) generated climatic envelopes for *Arachis* spp., *Solanum* spp. and *Vigna* spp. They compared current distribution with areas of current range in 2055 and found that for the three crop genera, 16–22% of CWR go extinct. Most species lost 50% of range and the range was highly fragmented. This varied depending on groups; in the worst case scenario, *Arachis* spp., with 24–31 (out of 50) species going extinct and the range decreasing by 85–94% for extant species.

Using bioclimatic modelling, possible scenarios of climatic change in Mexico were used to analyze the distribution patterns of eight wild *Cucurbitaceae* closely related to cultivated plants. Most of these taxa have restricted distributions, and many also have been proven to be resistant to various diseases. In these climate change scenarios, it was found that the eight taxa will be maintained in just 29 out of the 69 natural protected areas where they currently occur. The results of the bioclimatic analysis for the eight wild *Cucurbitaceae* taxa are similar to those in many other studies which aimed to predict the effect of climatic change in distribution patterns of other plant or animal groups in different areas of the world (Berry et al. 2002; Midgley et al. 2002; Téllez and

Dávila 2003; Thomas et al. 2004; Téllez et al. 2006, 2007; Levinsky et al. 2007; Midgley and Thuiller 2007). Consequently, major efforts are needed to fill these gaps for undertaking immediate *ex situ* conservation actions on these genera.

The seed of orthodox-seeded species can be routinely stored ex situ in seed banks using standard desiccation (5% moisture content) and freezing (-20°C) protocols (Hamilton et al. 2009). However, not all species are amenable to these procedures and require the development of alternative conservation technologies, particularly in vitro and cryopreservation approaches (i.e. storage at ultra-low temperatures), before long-term *ex situ* conservation can be achieved (Pritchard 2004; Ashmore et al. 2007). Conservation of these species is thus currently restricted to *in situ* approaches or field collections *ex situ*, making them particularly vulnerable to loss (Dulloo and Couturon 2009). Target 8 of the Global Strategy for Plant Conservation recognizes the importance of the development of new approaches to long-term *ex situ* conservation for recalcitrant (i.e. non-orthodox) seeded species, stating the need for 'additional resources, technology development and transfer, especially for species with recalcitrant seeds' (GSPC, 2002). Thus, there is an urgent need to develop conservation technologies (e.g. *in vitro* (Fig.1.8) and cryopreservation) to conserve the diversity of CWR.

Fig. 1.8. *In vitro* storage and propagation of *Citrus inodora* (Russell River lime) (vulnerable—protected under the Queensland Nature Conservation (Wildlife) Regulation 1994). (Hamilton et al. 2009)

There continues to be a strong emphasis on using pest and disease resistance genes (Ballhorn et al. 2010; Hajjar and Hodgkin 2007). CWR typically do not produce high yields (Hodgkin and Hajjar 2008; Pagnotta et al. 2005; 2009) but their agronomic and nutritive values, together with its use in health food products, made their cultivation economically viable in the marginal lands.

The conservation of crop wild relatives has received much attention due to high rates of genetic erosion occurring in commonly cultivated crop species (Smale et al 2004; Hou and Gao 1999). These wild species are at even greater risk of genetic erosion due to the destruction of natural habitats, and due to globalization, which causes a reduction in the diversity of species and varieties planted. Compounding this situation is an ever-increasing reliance on wild relatives for enhancing crop productivity. It is therefore essential to estimate diversity to effectively conserve and manage these resources.

1.2. The Common Bean, *Phaseolus vulgaris*

1.2.1 Origin and diffusion

Fig 1.9. The common bean, *Phaseolus vulgaris*.

The bean is the most widely cultivated legume globally and is often known as the "meat of the poor" due to its high nutritional values. It is highly diverse with over 40,000 recognized varieties (Voysest and Dessert 1991). The origin of the bean is Latin America, where domestication took place between 5000-3000 AD. Characterization studies of beans have shown the existence of two distinct genepools: (i) Mesoamerican, from Central America and Mexico, and (ii) Andean, from the highlands of South America (Blair et al. 2007).

Taxonomic Hierarchy

(Various taxonomic databases available from the National Centre for Biotechnology Information, at http://www.ncbi.nlm.nih.gov/)

Kingdom *Plantae* -- Plants
Subkingdom *Tracheobionta* -- vascular plants
Division *Magnoliophyta* -- angiosperms (flowering plants)
Class *Magnoliopsida* -- dicotyledons
Subclass *Rosidae*
Order *Fabales*
Family *Fabaceae* (=*Leguminosae*)
Genus *Phaseolus* L. -- bean, wild bean
Species *Phaseolus vulgaris* L. – common bean, wild bean

There are five recognized domestic *Phaseolus* species originally cultivated in Mexico and the central Andes (Delgado-Salinas et al. 2006; Ramìrez-Villegas et al. 2010; Debouck 1991).

Domestication of wild common beans occurred independently in Mesoamerica and Andean South America (Kaplan and Lynch 1999), thus giving rise to two major gene pools of the cultivated forms (Gepts et al. 1988). Studies supporting this taxonomy of *P. vulgaris* are based on a variety of methods, namely morphological, agronomical, and adaptation traits [Singh 1989; Singh et al. 1991a; Singh et al. 1991b), phaseolin type (Gepts et al. 1986; Gepts et al. 1988), isozymes (Debouck et al. 1993) and molecular markers (Blair et al. 2010; Blair et al. 2007). Cultivars from Mesoamerica usually are small- or medium-seeded (<25 g or 25–40 g/100 seed weight, respectively) and have S phaseolin type. The South American counterparts have larger seeds (>40g/100 seed weight) with T, C, H, and A phaseolin patterns.

The Bean in Italy

The common bean was introduced into Europe at least 500 years when numerous written records begin to appear (Zeven 1997; Dodonaeus 1521; Roesslin 1550; Fuchs 1543). The actual domestication of the bean, however, dates back over 7000 years (Piergiovanni and Lioi 2010). It is likely that sailors and traders brought the nicely coloured and easily transportable bean seeds already from the first journeys. Fuchs (1543) reported that the common bean had climbing habit, white or red flowers and red (red flowers are indicative of *Phaseolus coccineus* L)., white, yellow, skin-coloured or liver-coloured seeds with or without spots.

Picotral representations of the common bean are found in the festoons adorning the myth of Psyche decorating Villa Farnesina in Rome, painted by Giovanni di Udine in 1515 (Albala 2007). Mention of the plant in historical documents fixed 1532 as the year of common bean introduction in Italy.

The crop did well in Italy due to its similarity to the cowpea (*Vigna ungulata* (L.) Walp.), known as‘fagiolo dell’ occhio’ in Italian (Fig.1.10).

Fig.1.10. Illustration of ‘fagiolo dell’ occhio’ and its similarity to the common bean.
(Photo O. Temperini)

The common bean is a major protein source in Latin America and eastern Africa, and is increasing in production in developed countries due to concerns with healthier diets (Acosta-Gallegos et al. 2008). Over the last few decades, an inverse trend has been observed in Italy, where the crop covered barely 11,000 ha by the turn of the century (Ranalli et al. 2001). Despite this decrease, a number of landraces are presently grown in Italy, predominantly due to their nutritive, culinary and organoleptic attributes (Piergiovanni et al. 2000a; Piergiovanni et al. 2000b).

Adaptation to local soils and climatic conditions of the new environments, the geographical isolation of several growing areas, specific agricultural techniques (e.g. intercropping with maize), aesthetical and organoleptic preferences have led to the radiation of a large number of Italian landraces (Hammer et al. 1999).

The Italian varieties have diversified into the well known types, such as *cannellino, borlotto, ciabattone* and *tabacchino,* among others. Although the Lazio region of Italy does not have an intensive common bean production, there are varieties grown in the more remote and hilly areas of Lazio; these varieties are regarded as distinct landraces and are diverse in their morphology (Piergiovanni et al. 2000, 2006; Sicard et al. 2005; Foschiani et al. 2009). In these areas where traditional or low input agricultural systems are still present, Italian farmers still grow autochthonous varieties not only for personal consumption, but also for sale as niche products or specialties in farmer markets. Consequently, despite any formal efforts, local farmers have in effect undertaken on-farm conservation of traditional bean varieties. The landraces have been maintained by small-scale farmers over generations and command a higher market value.

Characterizing and evaluating the diversity of landraces is essential for understanding, managing conserving and utilizing the diversity present, both *in situ* and *ex situ* (Negri and Tosti 2002). This knowledge is fundamental in safeguarding and sustaining the on-farm survival of the elite landraces. This has implications for broadening the genetic base of improved cultivars or producing novel varieties new ones by using indigenous landraces, of particular importance in the face of climate change. Although the cultivation of beans in Italy has been decreasing (Lioi et al. 2005, 2007), the attention they are receiving as a functional food has been increasing (Perazzini et al. 2008). Its consumption has been linked to reduced risk of cardiovascular disease, diabetes mellitus, obesity, cancer and diseases of digestive tract (Cardador-Martínez et al. 2002).

Fig.1.11. Flowering bean in the field.

1.2.2. Botanical characteristics

The FAO describes the bean as a slightly pubescent, erect, herbaceous bush or as a twinning vine (Fig.1.9), with a well developed tap-root system. The stem can be angular or nearly cylindrical, the leaves trifoliate while the flowers are white, pink, lilac or purple. Bush cultivars (determinate growth form) are dwarf plants, 20-60 cm tall with lateral and terminal inflorescences. Climbing cultivars (indeterminate growth form) may reach a length of 2-5 m (Fig.1.12).

Fig.1.12. Beans in the fields, showing determinate and indeterminate growth forms

The pods range from 10 cm to 20 cm long, and can contain 4-6 seeds or more. The bean pods are often sold in processed forms, usually frozen, canned, or dehydrated. The seeds can be processed into dry flour or eaten boiled, baked or fried. They are good source of protein, vitamin B, and iron. Immature pods are eaten as vegetables (Uebersax and Occeña1991).

The leaves can be used as a potherb, while the plant as whole is also used as forage. Leaves and fruits have medicinal properties. The annual, growing season ranges from 50 to 90 days for fresh beans, and from 90 to 270 days for dry beans depending on variety and altitude. Most bean varieties are not affected by daylength.

The plant grows well in areas with medium rainfall but is not suited to the humid, wet tropics; excessive rain and hot weather cause flower and pod drop and increase the incidence of diseases (Laing et al. 1984). Optimum mean daily temperatures range between 15 and 20°C. The minimum mean daily temperature for growth is l0°C, the maximum 27°C. High temperatures increase the fibre content in the pod. Flowers are damaged at 5°C or below and the plant is not frost-tolerant. Germination requires a soil temperature of 15°C or more, and at 18°C germination takes about 12 days, and at 25°C about 7 days.

Water requirements for maximum production of a 60 to 120-day crop vary between 300 and 500 mm depending on climate (Laing et al. 1984). The water requirements during the ripening period depend very much on whether the pod is harvested wet or dry. When grown for its fresh product, the total growing period of the crop is relatively short and during the ripening, which is about 10 days long, the crop evapotranspiration is relatively small because of the drying of the leaves. When the crop is grown for seed the ripening period is longer and the decrease in crop evapotranspiration is relatively greater.

The growing period depends on the number of pickings, and when 3 or 4 pickings are taken the harvest period is 20 to 30 days. Crop coefficient (kc) relating reference evapotranspiration (ETo) to water requirements (ETm) for different development stages is, for common bean, green: during the initial stage 0.3-0.4 (15 to 20 days); the development stage 0.65-0.75 (15 to 20 days); the mid-season stage 0.95-1.05 (20 to 30 days); the late-season stage 0.9-0.95 (5 to 20 days) and at harvest 0.85-0.9. For common bean, dry, the kc value is: during the initial stage 0.3-0.4 (15 to 20 days); the

development stage 0.7-0.8 (15 to 20 days); the mid-season stage 1.05-1.2 (35 to 45 days); the late-season stage 0.65-0.75 (20 to 25 days); and at harvest 0.25-0.3.

The crop does not have specific soil requirements but friable, deep soils with pH of 5.5 to 6.0 are preferred. Fertilizer requirements for high production are 20 to 40 kg/ha N, 40 to 60 kg/ha P and 50 to 120 kg/ha K. Bean is capable of fixing nitrogen which can meet its requirements for high yields. However, a starter dose of N is beneficial for good early growth. Common bean is sensitive to soil salinity. The yield decrease at different levels of ECe is:0% at ECe 1.0, 10% at 1.5, 25% at 2.3, 50% at 3.6 and 100% at ECe 6.5 mmhos/cm.

The crop is sensitive to soil-borne diseases and should be grown in a rotation; in the subtropics in the USA wheat, sorghum, onion and potato are common rotation crops, whereas in tropical Africa and Asia maize, sweet potato and cotton are common (Van Schoonhoven and Cardona 1986; Abate and Ampofo 1996).

Normal sowing depth is about 5 to 7 cm. Spacing depends on variety. Bush types (erect) normally have a plant and row spacing of 5 to 10 x 50 to 75 cm, while pole-type (climbing) are 10 to 15 x 90 to 150 cm. Pole beans are also often grown on hills spaced 90 to 120 cm apart. Other spacings are possible, and these depend on the method of harvest.

1.2.3. Economic importance

Good quality commercial yield in favourable environments under irrigation is 6 to 8 tonne/ha fresh and 1.5 to 2 tonne/ha dry seed (Table 1.6). The water utilization efficiency for harvested yield (Ey) for fresh bean containing 80 to 90 percent moisture is 1.5 to 2.0 kg/m^3 and for dry bean containing about 10 percent moisture, 0.3 to 0.6 kg/m^3.

Table 1.6. Global green bean and dry bean production.

Global Green Bean Production: Top 10 Producers (2008)	Production (Metric Tonnes)	Global Dry Bean Production: Top 10 Producers (2008)	Production (Metric Tonnes)
People's Republic of China	2,566,748	Brazil	3,461,194
Indonesia	837,892	India	3,010,000
Turkey	563,056	Myanmar	2,500,000
India	420,000	People's Republic of China	1,709,151
Egypt	247,336	United States	1,159,290
Spain	207,100	Mexico	1122720
Italy	195,974	United Republic of Tanzania	850,000
Morocco	182,180	Uganda	440,000
Belgium	105,000	Argentina	336,779
Mexico	101,110	Indonesia	325,000
Source: FAOSTAT (2010) http://faostat.fao.org/			

In Italy, the average yields/ha for dry beans and green beans were 1.75 t/ha and 8.51 respectively. The most important bean producing regions of Italy are given below (Table 1.7.).

Table 1.7. Regional bean production in Italy

Dried Bean Production	Green Bean Production
Piedmont (3.005 ha)	Campania (5.033 ha)
Calabria (2.339 ha)	Emilia Romagna (4.386 ha)
Campania (775 ha)	Marche (2.496 ha)
Lombardy (303 ha)	Piedmont (1.666 ha)
Veneto (275)	Veneto (1.591 ha)
Puglia (257 ha)	Calabria (1.578 ha)
Tuscany (240 ha)	Sicily (860 ha)
Sicily (195 ha)	Puglia (758 ha)
Abruzzo (174 ha)	Lazio (509 ha)
Lazio (167 ha).	Abruzzo (496 ha)

Table 1.8. Chemical composition and calorific content of different legumes (Laghetti and Piergiovanni 2006).

Parameters	Species					
	Bean	**Lentil**	**Chickpea**	**Faba beans**	**Pea**	**Soya**
Calories (Kcal)	311,00	325,00	334,00	342,00	306,00	398,00
Water (g)	10,70	11,60	13,00	13,30	13,00	8,50
Proteins (g)	23,60	25,00	21,80	27,20	21,70	36,90
Fats (g)	2,50	2,50	4,90	3,00	2,00	18,10
Fibre (g)	17,00	13,70	13,80	7,00	15,70	11,90
Starch (g)	43,20	46,50	46,00	45,40	45,70	11,10
Sodium (mg)	4,00	8,00	6,00	0,00	38,00	4,00
Potassium (mg)	1.445,00	980,00	800,00	0,00	990,00	1.740,00
Iron (mg)	6,70	5,10	6,10	5,00	4,50	6,90
Calcium (mg)	137,00	127,00	117,00	90,00	48,00	257,00
Phosphorus (mg)	437,00	347,00	299,00	420,00	320,00	591,00
Vitamin B1 (mg)	0,40	0,57	0,36	0,50	0,58	0,99
Vitamin B2 (mg)	0,17	0,20	0,14	0,28	0,15	0,52
Niacin (mg)	2,30	1,80	1,70	2,60	2,20	2,50
Vitamin A (mg)	3,00	10,00	30,00	10,00	10,00	0,00
Vitamin C (mg)	3,00	3,00	5,00	4,00	4,00	0,00

The nutritional content of the bean varies between green bean and dried bean, ranging from 6.5% in green beans to 35% in dried beans of protein content. The bioavailability of proteins in the dried bean, at about 60%, is the highest of the legumes, while the amino acid composition is roughly the same as in soya.

1.3 The Cultivated (Leafy) Cardoon *Cynara cardunculus* var *Altilis*

1.3.1 Origin and diffusion

Fig.1.13. Immature flowerhead of the wild cardoon.

The wild cardoon (*Cynara cardunculus* var. *Sylvestris* (Lamk) Fiori) is the ancestor of the two cultivated subspecies: the artichoke (*Cynara cardunculus* var. *Scolymus* (L.) Fiori) and the cultivated cardoon (*Cynara cardunculus* var. *Altilis* DC) (Sonnante et al 2007; Basnizki and Zohary 1994; Rottenberg and Zohary 1996; Rottenberg and Zohary 2005). All three subspecies form fully fertile hybrids, although genetic studies of wild cardoon have shown them to be more closely related to the cultivated cardoon than to the artichoke (Sonnante et al. 2002, 2004, 2007). The wild cardoon belongs to the *Asteraceae* family, as do the Jerusalem artichoke and the sunflower. *C. cardunculus* is diploid (2*n*=34) and has its origins in the Mediterranean basin, ranging from Cyprus in the east to Portugal and the Canary Islands. The wild plant, a perennial, is found between 0-1100m asl, in arid, disturbed lands such as uncultivated areas, pastures and roadsides.

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Magnoliopsida* – Dicotyledons
Subclass	*Asteridae*
Order	*Asterales*
Family	*Asteraceae* – Aster family
Genus	*Cynara* L. – cynara
Species	*Cynara cardunculus* L. – cardoon

The cultivated cardoon, a perennial composite, is a native of South Europe. It grows up to 2m in height, and has large pinnate leaves, greyish-green above, almost white beneath. In some varieties there is a yellow or brown spine, often over ½ in. Long, in the angle of the leaf divisions. It is thought that Arabs probably played an important role in the diffusion of this crop, as in the case of other plants like eggplants and spinach; they had a particular interest in horticultural and garden crops (Idrisi 2005). The origin of the artichoke is therefore often associated with Arabs, who were prevalent in the southern Mediterranean during Medieval times.

A recent study used a cladistic method, based on morphological characters and on a large set of specimens, confirmed the inclusion of cultivated artichoke, leafy cardoon and wild cardoon, in a single species Wiklund (1992): *C. cardunculus* L. The study also indicated that *C. auranitica*, *C. syriaca* and *C. baetica* were close relatives of *C. cardunculus. C. algarbiensis* and *C. syriaca* showed only limited success in setting seeds and producing viable hybrids when crossed with *C. cardunculus*, while other wild Cynara show almost complete genetic isolation (Rottenberg and Zohary 1996).

Studies based on variation of isozymes and molecular markers such as RAPDs and AFLPs (Rottenberg et al. 1996; Sonnante et al. 2002, 2004; Lanteri et al. 2004; Raccuia et al. 2004b) have confirmed that both crops evolved from the wild cardoon gene pool, therefore confirming that it is the progenitor of both the cultivated types. AFLP marker studies have shown that demonstrated that

all *C. cardunculus* samples share a high genetic similarity compared with the other Cynara wild species. Despite this, artichoke germplasm is well separated from both wild and cultivated cardoon samples (Sonnante et al. 2004).

Studies based on rDNA spacer sequences, (Small et al. 2004; Robba et al. 2005; Sonnante et al. 2007), show close agreement with the phylogeny proposed by Wiklund (1992). The analyses showed that the leafy cardoon is genetically closer to wild germplasm of Spain rather than wild germplasm of Italy or Greece, thus supporting the view that the leafy cardoon was domesticated in the western part of the Mediterranean (Sonnante et al. 2006a, 2007). Results of these papers have revealed that the evolution of the whole genus is as recent as 20,000 years ago (Fig.1.14.). The domestication of artichoke and of cardoon are now believed to have been two distinctive events, separated in time and in space, which led to the two crops diverging for reproduction system and end use. The domestication of artichoke took place around the beginning of the first millennium (Foury 1989; Pignone and Sonnante 2004) while domestication of cardoon took place in the first half of the second millennium. Moreover, Pignone and Sonnante (2004) hypothesize that the artichoke was possibly domesticated in Sicily, while cardoon originated in the western range of the Mediterranean, probably within Spain and France (Sonnante et al. 2007).

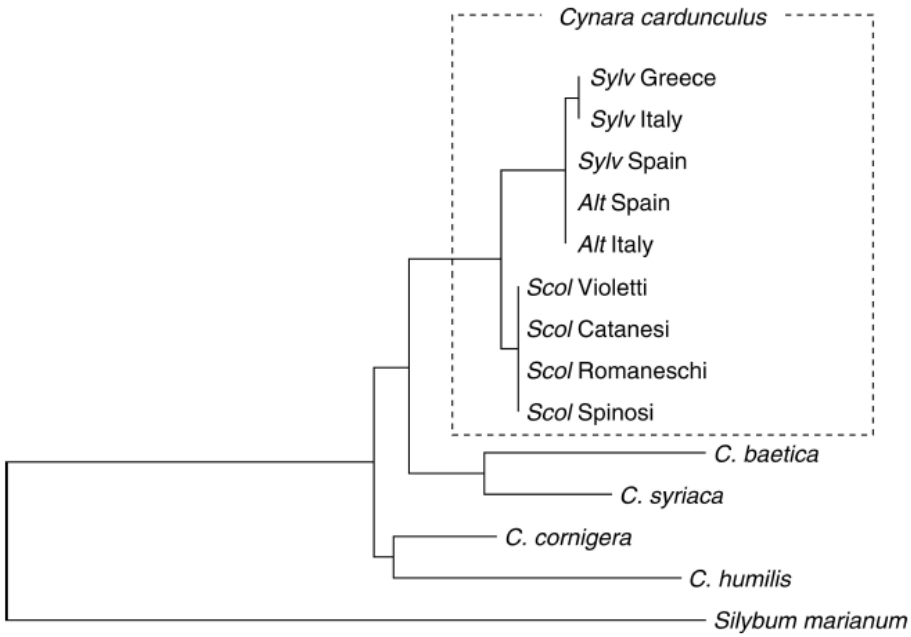


Fig. 1.14. Relationships among Cynara species and within *C. cardunculus* revealed by rDNA spacers parsimony analysis, redrawn from Sonnante et al. (2007). Abbreviations for the *Cynara*

cardunculus clade: Sylv = var. Sylvestris; Alt = var. Altilis; Scol = var. Scolymus, followed by geographic origin (Sylv and Alt) or varietal group (Scol).

1.3.2 Botanical characteristics

The cardoon is a crop of regional importance in Spain, Italy and the south of France, where it is used in traditional dishes. The plant has been collected for a variety of reasons, including as a rennet substitute in cheese-making, and as a vegetable (where the stalks are used). Fleshy stems and roots are generally collected in late autumn-early winter and before cooking are often blanched by tying together and wrapping with straw before being buried underground for about three weeks in order to accentuate the flavour. The fleshy leaf stalks when blanched, as well as the thick fleshy main roots, are the edible portions. While globe artichoke is usually vegetatively propagated by means of carducci (basal shoots) or ovoli (semi-dormant shoots with a limited root system), cultivated cardoon is raised from seed and cropped as an annual plant. It is believed that two crops are the result of human selection pressure for either large, non-spiny heads or non-spiny, large stalked tender leaves (Basnizki and Zohary1994). Sonnante et al. 2004 believed the two cultivated forms to be the result of concurrent directional selection for distinct traits, and not disruptive selection.

Cynara cardunculus is adapted to dry Mediterranean conditions where most precipitation occurs during the winter season. In its natural cycle it sprouts in autumn and passes the winter as a rosette. In the spring flowerheads are produced, which dry during summer and the whole crop can be harvested dry (10-15% water) in late summer (Fig.1.15.). It can withstand unfavourable summer conditions maintaining the root activity and by means of its latent basal buds, while the plant's aerial part dries in order to avoid transpiration. The root system is very powerful and can penetrate the soil by several metres (up to 5 metres has been proved experimentally) in order to obtain water from the subsoil.

Fig.1.15. Cardoon in the wild at the end of summer.

(Photo O. Temperini)

1.3.3. Economic importance

Due to these properties, total biomass production can rise to 20-30 tonnes dry matter per ha and year, with 2000-3000 kg of seeds, rich in oil (25%) and proteins (20%) (Fernandez 1998). The dry matter is potentially suitable for use as forage, in paper pulp or for energy production. The plant can also be used to yield a yellow dye and cardoon has become importance as a medicinal herb.

The leaves are also used medicinally much as those of the artichoke, with similar hepatoprotector and choleretic properties (Bruneton, 1995). The cultivated cardoon, the artichoke and the wild cardoon are a source of biopharmaceuticals (Adzet and Puigmacia 1985; Debenedetti et al. 1993; Slanina et al. 1993; Wagenbreth 1996; Sevcikova et al. 2002; Wang et al. 2003) as the roots contain inulin, improving human intestinal flora, while the leaves provide a source of antioxidant compounds.

Inulin molecules, with a chain length of up to 200, which is the highest degree of polymerization (DP) of inulin molecules known in plants, are present in *C. cardunculus*. Inulin belongs to a group of fructose-based polysaccharides called fructans, which are not digested in the small intestine because humans lack the enzymes required for hydrolysis of fructans. It is a complex polysaccharide and is effective in regulating the bacterial flora of the intestine through increasing the presence of Bifidobacteria and Lactobacilli while simultaneously reducing the presence of the more harmful gut flora. There are additional beneficial effects on mineral absorption, blood lipid composition, and prevention of colon cancer. Finally, inulin is a low-calorie fibre that has potential

for use in the production of fat-reduced foods (Frehner et al. 1984; Pollock 1986; Darwen and John 1989; Pontis 1990; Carpita et al. 1991; Rapaille et al. 1995; Hellwege et al. 1998; Roberfroid & Delzenne 1998; Van Loo et al. 1999; Hellwege et al. 2000).

Previous studies have demonstrated the species to be a promising source of seed oil, both with respect to quality and quantity, while the residual flour following oil extraction is usable as a component of animal feed. Seed yield is about 2.0 t/ha (at 5% w/v moisture), of which about 25% is oil of good quality {Foti et al. 1999), on the basis of a high and well-balanced content of oleic and linoleic acids, a low content of free acids, peroxides, saturated and linoleic acids, and favourable α-tocopherol content, which functions as an antioxidant agent (Maccarone et al. 1999).

Furthermore, recent studies (Quilho et al. 2004) have identified the potential of cultivated cardoon as a candidate for the production of paper pulp. Herbaceous plants provide 10-15% of the global production of cellulose paper pulp. The increase in demand is seen in the continual search of new species for paper production at ever lower costs and increased production. Gominho et al. (2000) evaluated the possibility of exploiting wild cardoon to this effect. Analyses were carried out on the plant fibre, which produced high yielding results of 47% of the total weight of the plant. Additionally, the extracted fibre was of high quality, traction resistant, reduced levels of lignifications and high biomass production (20-30 t/ha of dry material) at low costs.

2. MATERIALS AND METHODS

2.1 Materials

Materials for this study were sourced from various genebanks and university collections. These were as follows for the bean populations:

- 42 traditional varieties from Lazio, Italy
- eight varieties collected in 1950 by The Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany.
- one wild Italian variety collected by IPK,
- one black variety ‘bobby’ collected by IPK,
- eight wild beans from Latin America collected by CIAT.

The cardoon populations were sourced from:

- the wild populations of Sasso and Tarquinia were evaluated using 16 individuals per population as this was the first time they have been assessed
- four cultivated cardoon accessions collected by IPK
- six cultivated cardoon accessions collected by Vavilov Institute in Russia (VIR)
- 10 accesions (six wild, four cultivated) collected by ISAFOM
- eight accessions (five cultivated and three wild) collected by the University of Tuscia.

Given below is a description of the accessions and their origins mapped out where possible.

2.1.1. Bean Populations

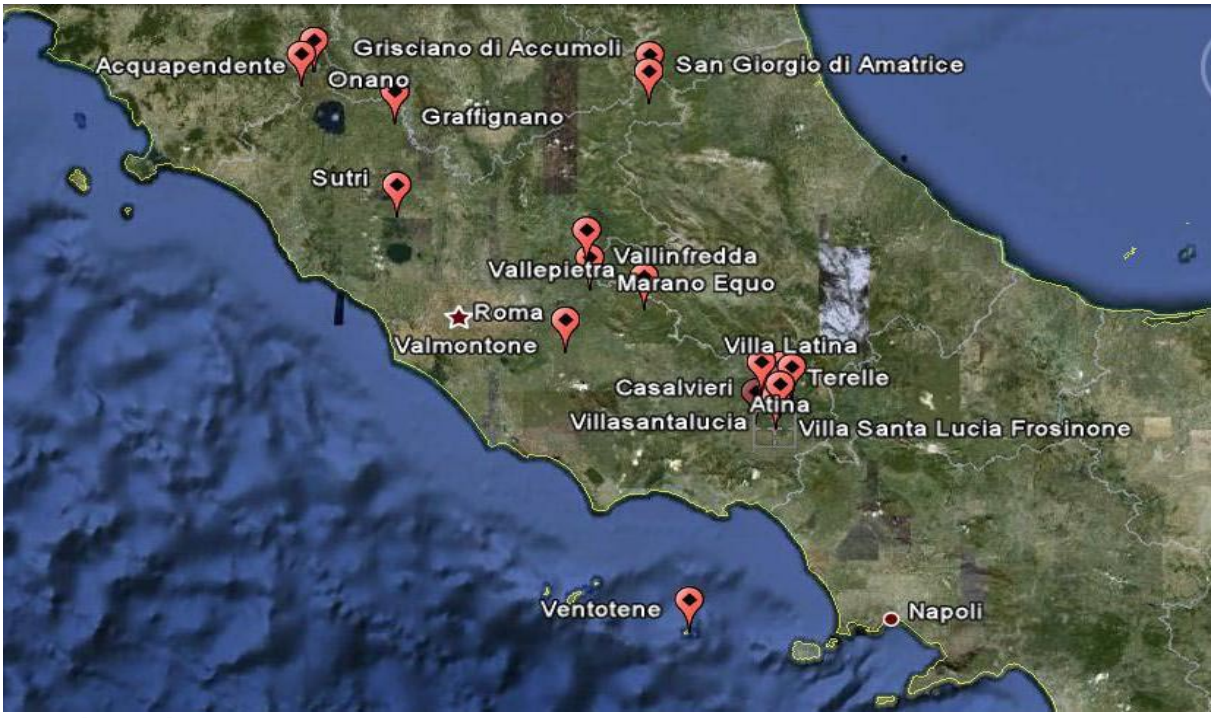
Populations from Lazio, Italy, collected in 2009

The 42 populations in this study originate from the Lazio region of Italy. They have been listed hereafter according to their province: Viterbo (VT), Rieti (RI), Frosinone (FR), Rome (RM) and Latina (LT). The study examines both genetic and morphological variation to assess whether varieties displaying similar morphology are also similar genetically.

Table 2.1. Common names and origin for the Lazio bean populations.

	Accession No. and Common Name	***Origin***
1.	2. Borlotto di Graffignano	*Graffignano (VT)*
2.	3. Cannellino di Atina	*Villatina (FR)*
3.	6. Carbonari	*San Giorgio di Amatrice (RI)*
4.	7. Confettino	*Casalvieri (FR)*
5.	10. Cannellino Moschetta	*Atina (FR)*
6.	13. Fagiolo Pisello	*San Giorgio di Amatrice (RI)*
7.	14. Mezzo cannellino di Ventotene	*Ventotene (LT)*
8.	17. Rosso di Piumarola	*Villasantalucia (FR)*
9.	18. Sanguinelli	*San Giorgio di Amatrice (RI)*
10.	21. Solfarino determinato	*Onano (VT)*
11.	26. Fagioletti di Vallepietra	*Vallepietra (RM)*
12.	27. Nero rosso di Piumarola	*Villasantalucia (FR)*
13.	28. Tabacchino	*Acquapendente (VT)*
14.	31. Verdolino (determinato)	*Onano (VT)*
15.	50. Borlotto Bingo Cileno	*Sutri (VT)*
16.	51. Borlotto di Grisciano	*Grisciano di Accumoli (RI)*
17.	53. Borlotto Lamon	*Valmontone (RM)*
18.	54. Borlotto Regina di Marano Equo	*Marano Equo (RM)*
19.	55. Borlotto Regina di Sutri	*Sutri (VT)*
20.	56. Bottoncino di Terelle	*Terrelle (FR)*
21.	57/58. Cannellino S.Oliva (semi-determinato)	*Castrocielo (FR)*
22.	59. Chiarinelli	*Grisciano di Accumoli (RI)*
23.	61. Ciabattone di Onano	*Onano (VT)*
24.	62. Cioncone di Vallinfreda	*Vallinfredda (RM)*
25.	64. Fagiolina Arsolana	*Vallinfredda (RM)*
26.	65. Fagiolo Burro	*Villalatina (FR)*
27.	69. Fagiolo di Fratta	*Villalatina (FR)*
28.	71. Gialli	*Onano (VT)*
29.	72. Mughetto	*Grisciano di Accumoli (RI)*
30.	74. Mughetto Grisciano	*Grisciano di Accumoli (RI)*
31.	75. Nocciolino	*San Giorgio di Amatrice (RI)*
32.	76. Romaneschi	*Vallepietra (RM)*
33.	77. Rugginosi	*San Giorgio di Amatrice (RI)*
34.	78. Toscanelli	*Acquapendente (VT)*
35.	79. Uova di Quaglia	*San Giorgio di Amatrice (RI)*

	Accession No. and Common Name	*Origin*
36.	81. Verdolino indeterminato	*Onano (VT)*
37.	83. Cappellette	*Vallepietra (RM)*
38.	84. Fagioletti di Vallepietra (indeterminato)	*Vallepietra (RM)*
39.	85. Fagiolone di Grisciano	*Grisciano di Accumoli (RI)*
40.	86. Fagiolone di Vallepietra	*Vallepietra (RM)*
41.	87. Pallini di Vallepietra	*Vallepietra (RM)*
42.	88. Cocco	*Onano (VT)*



(Google Earth 2010)

Figure 2.1. Origins of Lazio Populations

Bean populations collected in 1950

Beans were obtained from the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany. IPK possesses a unique collection of plant genetic resources in its central *ex situ* gene bank, with more than 3,000 botanic species of about 800 different genera. All accessions are from many plants, ie there are no single-plant or single-seed descent accessions. In the case of line splittings, the respective accession numbers are stated hereafter. Where nothing is written, the collected samples have not been split up.

Table 2.2. Descriptions of the beans collected in 1950.

Accession No.	Scientific Name	Country of Origin	Date of Collection
PHA 770	*Phaseolus vulgaris* L. subsp. *vulgaris* var. *Vulgaris*	Italy	1950
PHA 822	*Phaseolus vulgaris* L. subsp. *vulgaris* var. *Vulgaris*	Italy	1950
PHA 823	*Phaseolus vulgaris* L. subsp. *vulgaris* var. *Vulgaris*	Italy	1950
PHA 824	*Phaseolus vulgaris* L. subsp. *vulgaris* var. *Vulgaris/*	Italy	1950
PHA 825	*Phaseolus vulgaris* L. subsp. *vulgaris* var. *Vulgaris*	Italy	1950
PHA 828	*Phaseolus vulgaris* L. subsp. *vulgaris* var. *Vulgaris*	Italy	1950
PHA 865	*Phaseolus vulgaris* L. subsp. *vulgaris* var. *Vulgaris*	Italy	1950
PHA 939	*Phaseolus vulgaris* L. subsp. *vulgaris* var. *Vulgaris*	Italy	1950
PHA 942	*Phaseolus vulgaris* L. subsp. *vulgaris* var. *Vulgaris*	Italy	1950
PHA 951	*Phaseolus vulgaris* L. subsp. *vulgaris* var. *Vulgaris*	Italy	1950
OTHER			
PHA 5327	*Phaseolus vulgaris* L. subsp. *vulgaris* var. *Vulgaris* fagiolo selvatico, f. matto SaNr.11642c	Italy	1990
PHA 4317	Bobby SaNr. 8386b *Phaseolus vulgaris* L. subsp. *vulgaris* var. *nanus Asch.*	Italy	1988

SaNr.10859c = collection number 10859c (if there are several accessions with the same collection number, but different letters, it means the seed sample collected was already divided by the collector before the material was given to the genebank)

Fig. 2.2. Beans collected in 1950 by IPK. (From the accessions pictured above, accession no. 827 did not germinate).

Fig. 2.3. Beans collected in 1950 by IPK.

Fig. 2.4. Beans collected in 1950 by IPK.

Fig. 2.5. An Italian wild bean.

Fig. 2.6. A black variety, 'bobby'.

Fig. 2.7. Beans were germinated in petri dishes in the dark.

Wild type *Phaseolus vulgaris* from CIAT

The International Center for Tropical Agriculture (CIAT), based in Cali, Colombia, holds more than 27,000 samples of *Phaseolus* spp (dry bean) seeds. The CIAT genebank is one of 11 maintained worldwide by the CGIAR, where crop materials such as seeds, stems and tubers are held in trust with the United Nations Food and Agriculture Organization (FAO). All the wild varieties in this study have climbing (indeterminate) growth habit.

Table 2.3. Accession names and descriptions for wild beans from Latin America.

Accession	Seed Colour	100g Seed Weight (g)	Country of Origin	Date of Collection
G10017 – Mex1962a	Brown	4.8	Mexico	01/12/1962
G12858 – Peru 1959	Cream, Other, Brown	8	Peru	21/04/1959
G12875 – Mex 1966	Cream, Other, Black	6	Mexico	01/11/1966
G13034 – Mex 1962b	Cream	6.1	Mexico	01/12/1962
G24392 – Mex 1991	Yellow	5	Mexico	15/01/1991
G50386 – Guat 1995a	Pink	13.8	Guatemala	24/01/1995
G50388B - Guat 1995b	Pink, Purple, Other	17	Guatemala	24/01/1995
G50518B – Col 1979	Cream, Other, Black	15	Colombia	13/07/1979

Fig. 2.8. Origins of the wild bean populations collected by CIAT

(Google Earth 2010)

Fig. 2.9. Wild beans from Latin America.

2.1.2 Cardoon Populations

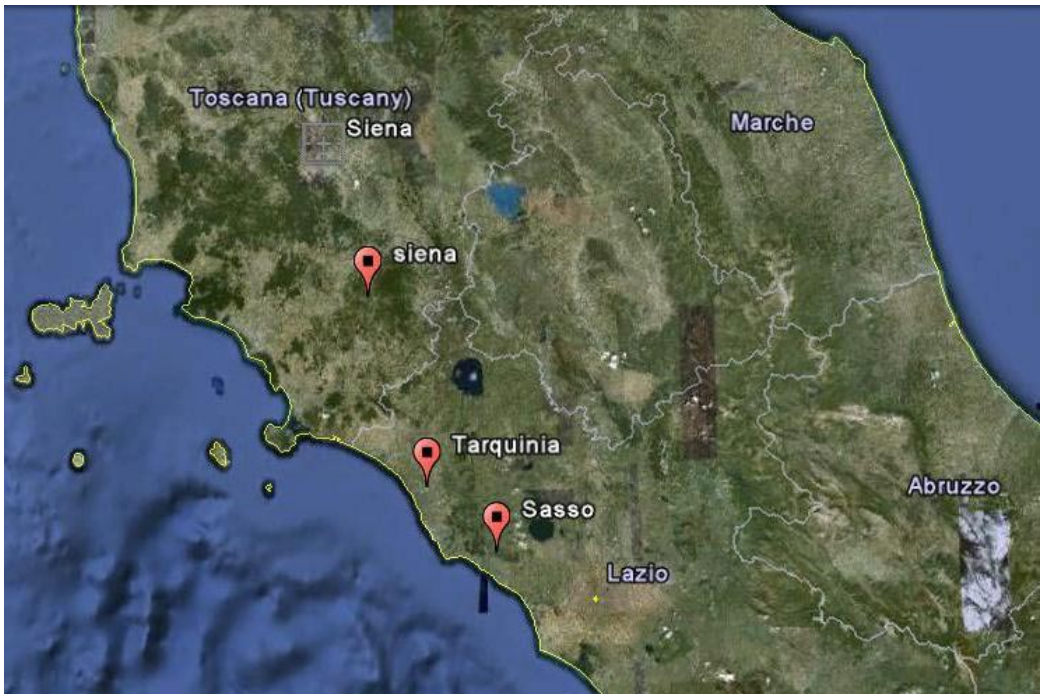
Wild Cardoon

The samples from Sasso and Tarquinia were collected in the region of Lazio, Italy. The samples from Siena, Tuscany, were collected from the area surrounding the city of Siena and planted in the University of Tuscia's (Viterbo, Italy) experimental field station. The seeds were collected from individual wild plants once flowering was complete.



Fig. 2.10., Fig. 2.11. and Fig. 2.12. Wild cardoon collected from Sasso and Tarquinia once the flowers were dry and germinated in the Unviersity of Tuscia greenhouses.

Seventeen plants from Sasso (Cerveteri, province of Rome) and 16 from Tarquinia (province of Viterbo) were selected for the study..



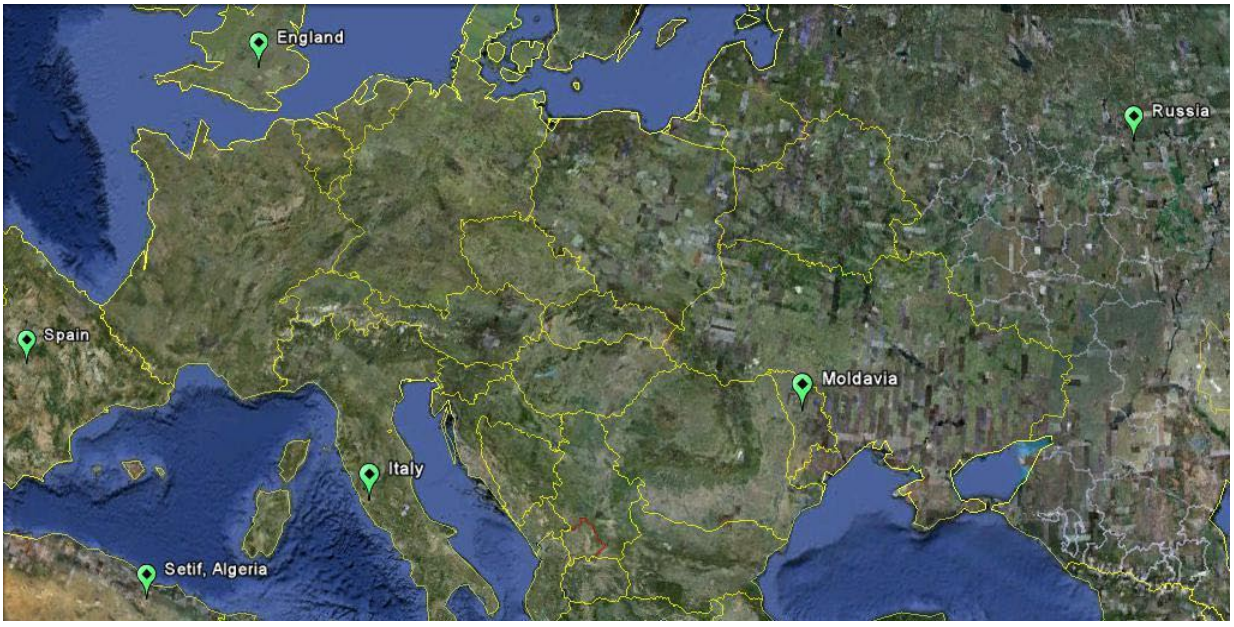
(Google Earth 2010)

Fig. 2.13. Map of Italy showing the areas of origin and collection of wild cardoons in the University of Tuscia collections.

Table 2.4. Description and origin of the cutlivated cardoon obtained from VIR.

Acc No.	VIR Catalogue Code	Acc Name	Country of Origin	Year
1	K3	Artishok	Moldavia	1975
2	K4	Maikopski 6 Zelenyi Krasnodar	Russia	1950
3	K5	Krupyi Zelenyi	England	1950
5	K7	Cardo Nostrano Bolognese	Italy	1974
6	K8	Cardo Blanco sin Espinas	Spain	1979
7	K9	Artishok	Setif-Algeria	1971

Fig. 2.14. Cultivated cardoon seeds from VIR.



(Google Earth 2010)

Fig. 2.15. Origins of cultivated cardoon populations collected by VIR.

Table 2.5. Cardoon Accessions from IPK, Germany.

Year	Accession Number	Scientific Name	Accession Name	Country of Origin
1985	CYN 9	*Cynara cardunculus* L.	Cardo Pieno Inerme	Italy
1985	CYN 10	*Cynara cardunculus* L.	Cardo Bianco Avorio	Italy
1989	CYN 12	*Cynara cardunculus* L.	Cardo gobbo	Italy
null	CYN 7	*Cynara cardunculus* L.	null	Unknown

Fig. 2.16. Cultivated cardoon accessions collected by IPK.

ISAFOM accessions

These collections were sent by CNR-ISAFOM (Italian National Research Council - Institute for Mediterranean Agriculture and Forest Systems) and the germplasm was evaluated from frozen leaves sent via courier from Sicily. The accessions are a modern collection; they are listed below:

‘Solco 1’: Cultivated Cardoon line selected for biomass production

‘Solco 2’: Cultivated Cardoon landrace Siciliana (Catania)

‘Solco 3’: Cultivated Cardoon landrace, Campana (Benevento)

‘Solco 4’: Cultivated Cardoon landrace, Romagnola (Bologna)

Fig. 2.17. Origins of the cultivated cardoon collected by ISAFOM.

(Google Earth 2010)

Wild Cardoon, Sicily

'Solco 1': Adrano (province of Catania)

'Solco 7': Francofonte (province of Siracusa)

'Solco 10': Centuripe 2 (province of Enna)

'Solco 12': Misterbianco (province of Catania)

'Solco 16': Paternò (province of Catania)

'Solco 20': Raccuja (province of Messina)

Fig. 2.18. Origins of the wild cardoon populations collected by ISAFOM.

(Google Earth 2010)

University of Tuscia Collections

The cultivated cardoon collected by the University of Tuscia are recent collections. They are listed below:

• Cardo gigante inerme

• Cardo scolimus madrid

• Cardo madrid

• Cardo nizza

• Cardo belgio

(Google Earth 2010)

Fig. 2.19. Origins of the cultivated cardoons collected by the University of Tuscia.

2.2. Methods

2.2.1. Agromorphological assessment

Morphological assessment of Lazio beans

Plants were evaluated for the following IPGRI descriptors (IPGRI 2001):

- germination time
- maturation time (when 90% of pods are mature)
- leaf length
- leaf width
- leaf form
- chlorophyll intensity
- persistency of the leaves (when 90% of the pods have fallen or are desiccated)
- number of flowers per inflorescence
- flower size
- texture of pod
- pod width
- pod length
- number of pods
- degree of petal aperture
- flower stem colour
- petal colour
- days to flowering
- number of seeds
- primary seed colour
- secondary seed colour
- secondary seed colour distribution
- seed shape
- seed weight
- seed length
- seed width

Once the germplasm was acquired from local farmers, it was planted at the University of Tuscia experimental field site, “Nello Lupori”, at Viterbo, Italy. Fifteen individuals per accession were planted 0.5m apart: eight individuals per accession were then selected at random for study. Thirty nine accessions were evaluated in this study.

Cardoon morphological assessment

The following descriptors were used to evaluate the wild and cultivated cardoon populations in the field:

- Plant Height
- No of offshoots
- Distance Flowerhead-1st Leaf
- Stem Diameter
- Leaf length
- No of leaf lobes
- Intensity of green colour
- Hue of green colour
- Main flowerhead length
- Main flowerhead diameter
- Shape of main flowerhead
- Shape of flowerhead top
- Main flowerhead readiness
- Total no of flowerheads
- Greyness intensity

2.2.2. DNA extraction

Leaves from bean varieties, eight per accession, were collected in the field in late summer 2008 and extraction protocol performed according to the EUR$_x$ GeneMATRIX Plant & Fungi DNA Purification Kit Universal kit for isolation of total DNA from plants, algae and fungi (Version 5.1) (See Annex 1 for the complete extraction protocol). A total of 496 extractions of *P. vulgaris* individuals, i.e. eight individuals each from 62 different accessions, were completed.

Cardoon DNA, also eight per accession, were either collected in the field or from frozen leaves sent from the relevant institution. Extractions were undertaken as per the Invitrogen ™ PureLink™ Plant Total DNA Purification Kit For purification of DNA from plants (Version A) (See Annex 1 for the complete extraction protocol). A total of 238 extractions of *C.cardunculus* individuals, eight individuals each from 26 different accessions, were completed.

2.2.3. Microsatellites (SSR)

The average mutation rate of microsatellites ranges from 10^{-2} to 10^{-6} per locus per generation, among different species and different types of microsatellites (Selkoe and Toonen 2006; Vigouroux et al. 2002; Ellegren 2004). In this study, the microsatellite was flanked with fluorescent PCR

primers then amplified to produce a pair of fluorescent allelic products which vary in size according to their repeat length.

Example of microsatellites:

a) Repeat units

AAAAAAAAAAA = $(A)_{11}$ = mononucleotide (11 bp)

GTGTGTGTGTGT = $(GT)_6$ = dinucleotide (12 bp)

CTGCTGCTGCTG = $(CTG)_4$ = trinucleotide (12 bp)

ACTCACTCACTCACTC = $(ACTC)_4$ = tetranucleotide (16 bp)

b) Homozygous microsatellite

…CGTAGCCTTGCATCCTT**CTCTCTCTCTCTCT**ATCGGTACTACGTGG… (46 bp)

…CGTAGCCTTGCATCCTT**CTCTCTCTCTCTCT**ATCGGTACTACGTGG… (46 bp)

5’ flanking region microsatellite locus 3’ flanking region

c) Heterozygous microsatellite

…CGTAGCCTTGCATCCTT**CTCTCTCTCTCTCT** ATCGGTACTACGTGG… (46 bp)

…CGTAGCCTTGCATCCTT**CTCTCTCTCTCTCTCTCT**ATCGGTACTACGTGG... (50 bp)

5’ flanking region microsatellite locus 3’ flanking region

2.2.4. ISSR markers

Visible bands are defined as dominant markers and are assigned to genetic loci with two alleles: 1 = present and 0 = absent. This should not be confused with dominance as it relates to mendelian inheritance patterns. The dominant allele of dominant markers can result from a heterozygous or homozygous genotype for a particular priming location. Inheritance patterns of ISSR markers can be established through genetic studies (Tsumura et al., 1996). In plants it is unlikely that ISSR markers result from amplification of plastid DNA because the microsatellites found in this genome are predominantly mononucleotide repeats (Powell et al., 1995).

There are different approaches for data gathering and scoring, depending on the type of study to be conducted. For example, in studies in which one is examining patterns of gene flow (e.g., hybridization and parentage analysis) among populations or species, the type of data needed are qualitative as opposed to quantitative. For qualitative analyses, one needs to identify ISSR markers typical of individuals, populations, or species that can be used to assess the amount and directionality of gene flow (Wolfe et al., 1998a,b).

The descriptive statistics often reported include the total number of genetic loci scored, the percentage of polymorphic loci, average number of bands per primer, the percentage of polymorphic bands per population, the number of species-typical or population-typical markers, and the number of shared and unique genotypes. The comparative statistics used in studies of natural populations include genetic similarities calculated by a bandmatching algorithm (e.g., Jaccard or Dice).

2.3. Genetic Analyses

2.3.1. Genetic assessment of beans using microsatellites

Genomic DNA was extracted from young leaves of eight individuals per accession, for a total of 496 individuals, using GeneMATRIX Plant and Fungi DNA Purification Kit (Eur_x). DNA was amplified using 10 SSR primers, one per chromosome arm: PVBR5, BM200, BM211, BM185, BM183, BM159, BM138, BM156, BM149, BM184.

Table 2.6. A description of the microsatellite primers used.

Publication	Marker	Oligo Sequence (5' to 3')	Chrom -osome	Repeat Array	Allele Size	Annealing Temp
Buso et al 2006	**PVBR5 F**	CACGACGTTGTAAAACGACATTAG ACGCTGATGACAGAG	1	$(GA)_{22}$	195	56
	PVBR5 R	AGCAGAATCCTTTGAGTGTG				
Gaitàn-Solís et al 2002	**BM200 F**	CACGACGTTGTAAAACGACTGGTG GTTGTTATGGGAGAAG	2	$(AG)_{10}$	221	52
	BM200 R	ATTTGTCTCTGTCTATTCCTTCCAC				
Gaitàn-Solís et al 2002	**BM211 F**	CACGACGTTGTAAAACGACATACC CACATGCACAAGTTTGG	3	$(CT)_{16}$	186	52
	BM211 R	CCACCATGTGCTCATGAAGAT				
Gaitàn-Solís et al 2002	**BM185 F**	CACGACGTTGTAAAACGACAAGG AGGTTTCTACCTAATTCC	4	$(CT)_{12}$	105	52
	BM185 R	AAAGCAGGGATGTAGTTGC				
Gaitàn-Solís et al 2002	**BM183 F**	CACGACGTTGTAAAACGACCTCAA ATCTATTCACTGGTCAGC	4b	$(TC)_{14}$	149	52
	BM183 R	TCTTACAGCCTTGCAGACATC				

Publication	Marker	Oligo Sequence (5' to 3')	Chrom -osome	Repeat Array	Allele Size	Annealing Temp
Gaitàn-Solíset al 2002	**BM159 F**	CACGACGTTGTAAAACGACGGTGC TGTTGCTGCTGTTAT	5	$(CT)_9$($CA)_8$	198	52
	BM159 R	GGGAGATGTGGTAAGATAATGAA A				
Gaitàn-Solís et al 2002	**BM138 F**	CACGACGTTGTAAAACGACTGTCC CTAAGAACGAATATGGAATC	7	$(GT)_{13}$	203	50
	BM138 R	GAATCAAGCAACCTTGGATCATAA C				
Gaitàn-Solís et al 2002	**BM156 F**	CACGACGTTGTAAAACGACCTTGT TCCACCTCCCATCATAGC	9	$(CT)_{32}$	267	52
	BM156 R	TGCTTGCATCTCAGCCAGAATC				
Gaitàn-Solís et al 2002	**BM149 F**	CACGACGTTGTAAAACGACCGATG GATGGATGGTTGCAG	10	$(TGC)_6$ $(TAG)_3$	273	50
	BM149 R	GGGCCGACAAGTTACATCAAATTC				
Gaitàn-Solíset al 2002	**BM184 F**	CACGACGTTGTAAAACGACAGTGC TCTATCAAGATGTGTG	11	$(AC)_{11}$	160	52
	BM184 R	ACATAATCAATGGGTCACTG				

PCR reactions were performed in a volume of 10 µl in Eppendorf and Biometra thermocyclers. The reaction mixture contained 0.2 µM of each primer, 4 µM of each deoxinucleotide, 0.05 units of Taq polymerase (Eppendorf), 10x Taq buffer containing 1.5 mM $MgCl_2$ and 10 ng of template DNA. Amplification regime consisted of 94°C/2min, followed by 30 cycles of 94°C/40 sec, 52-65°C (specific for each primer)/40 sec and 72°C/40 sec, and ending with an extension step of 72°C/10min.

The PCR products were separated on ABI 3130xl sequencer and the throughput was analyzed using GeneMapper Software version 4.0. An internal size standard (Gene Scan 500-Liz, Applied Biosystems) ensured accurate sizing. Each allele was named according to its molecular size. Figs 2.20 and 2.21) illustrate the allele calling once peaks are visualized.

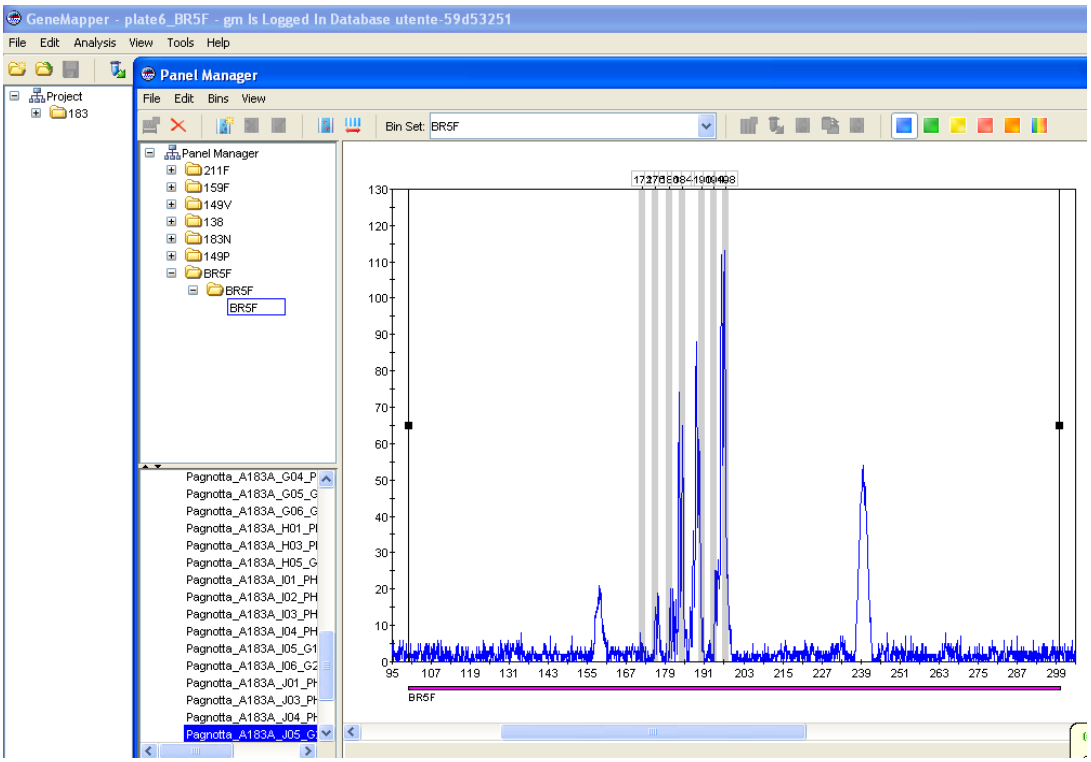


Figure 2.20. Visualization showing a single microsatellite marker .

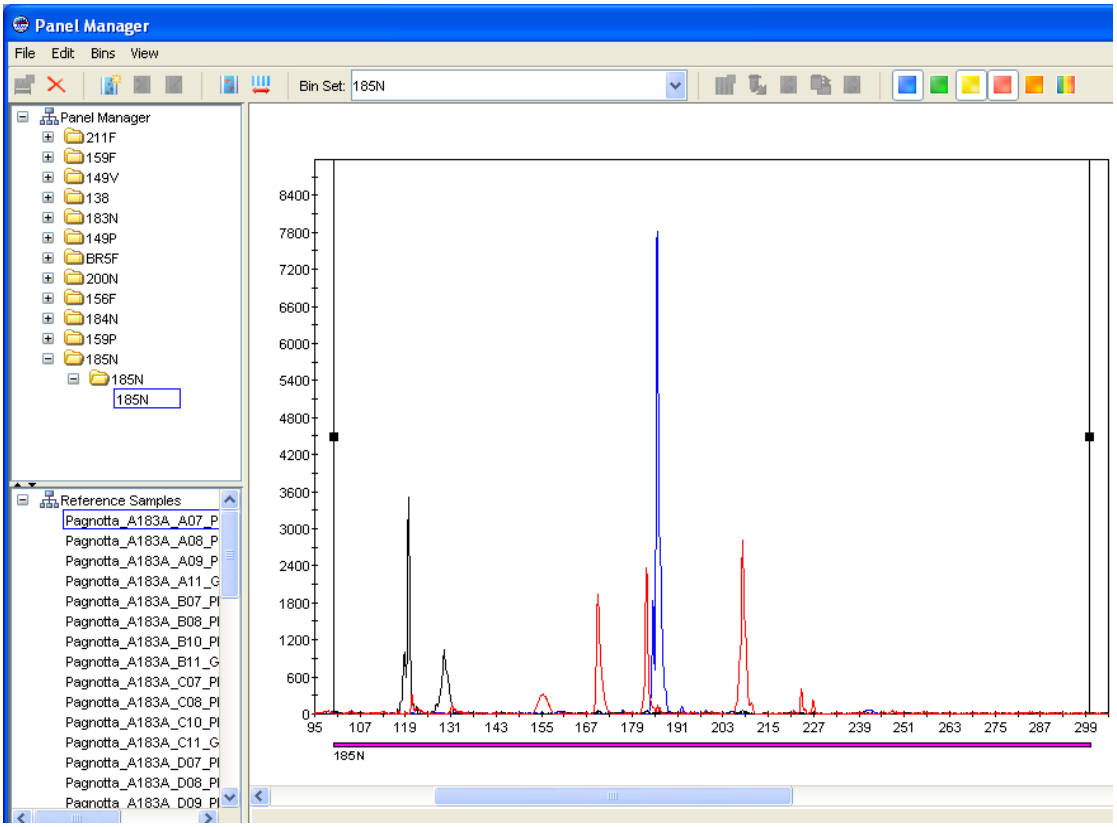


Figure 2.21. Multiple alleles, polymorphic loci, with three microsatellite markers.

2.3.2. Genetic assessment of cardoons using ISSR, a dominant marker

Nine ISSR primers were screened and four selected: 810, 811, 841, 857 (University of British Columbia, Canada).

Table 2.7. ISSR Primers and their annealing temperatures (T_a).

Primer	Sequence 3' → 5'	T_a
810	$(GA)_8T$	43
811	$(GA)_8C$	43
841	$(GA)_8CTC$	45
857c	$(AC)_8CTGC$	59
857g	$(AC)_8CTG$	58

The assessment required 10µl reactions containing 10ng DNA, 0.3µM primer, 100µM dNTP, 10mM Tris-HCl (pH 9.0) and 1U Taq polymerase. The amplification regime was 94°C/5min, followed by 35 cycles of 94°C/1min, 60°C/1min and 72°C/2min, and ending with an extension step of 72°C/10 min.

The PCR products were separated on ABI 3130xl sequencer and the throughput was analyzed using GeneMapper Software version 4.0. The number of possible bands for each ISSR primer (between 100 to 500 bp) was recorded; each significant peak was assumed to represent a single locus with two possible alleles (presence and absence) (Fig. 2.23).

Figure 2.23. Results visible through the use of the ABI sequencer and GeneMapper Software to assess allele frequencies.

2.4. Statistical Analyses

The data matrix of field surveys were analyzed using multivariate statistical analysis performed with PAST 1.89 (Hammer et al. 2001) useful for revealing patterns within large, multidimensional data sets. The PCA routine finds the eigenvalues and eigenvectors of the variance-covariance matrix or the correlation matrix. Correlations (normalized var-covar) were used as the variable assessed were measured in different units; this normalized all variables using division by their standard deviations. The eigenvalues, giving a measure of the variance accounted for by the corresponding eigenvectors (components) are given for all components. The percentages of variance accounted for by these components are also given.

For statistical analyses, the ISSR frequency data were converted in a 0 – 1 matrix (0 = absence and 1 = presence of the band). Descriptive statistics of the molecular data were computed for each accession, among these the percentage of polymorphism and the expected heterozygosity (H_e) were calculated using GDA (Lewis and Zaykin, 2001). The 1/0 marker matrix was used to compute genetic distances (Nei, 1972) between populations, and then used to conduct a cluster analysis and

to draw a UPGMA diagram (based on the total number of alleles per genotype). A similar procedure was followed for microsatellite analysis, whereby the allele sizes were used to construct a matrix for importing into the various software listed below. The numbers of common versus rare alleles were also analyzed to obtain an assessment of the richness within each population. Rare alleles were assessed as those that were present in ≤ 5% of each population.

Genetic data were analyzed using POPGEN 1.31 (Yeh et al. 1999), GenAlex 6 (Peakall and Smouse 2006), Arelquin 3.5 (Excoffier and Lischer 2010) and Genetic Data Analysis 1.1 (Lewis and Zaykin, 1996). The molecular data are used to evaluate the percentage of polymorphism (*P*), number of alleles per locus (*A*), number of polymorphic alleles per locus (*Ap*), expected (H_e) and observed (H_o) heterozygosity using GDA software. The genetic distance (Nei, 1972) matrix was utilized to construct, using GDA and TreeView 1.6.6. (Roderic 1996), cluster dendrograms. STRUCTURE 2.3.1 Pritchard et al. 2000) was also used for data analyses. Here Bayesian models (aim to infer the structure of a data set by assuming that observations from each sample are random draws from unknown gene frequency distributions, in which the marker loci are unlinked and at Hardy-Weinberg (HWE) and linkage equilibrium (LE).

Genetic measures of diversity used in this study were:

Polymorphism:

The number of polymorphic loci or the fraction of polymorphic loci among several loci studied in a population

Heterozygosity:

The fraction of heterozygous individuals in a population maybe be estimated as:

Expected heterozygosity (H_E, also referred a gene diversity): This is the mean genetic diversity or the mean theoretical heterozygosity. Its calculation is based on allele frequencies. It equals 1 minus the frequencies of homozygote:

$$H_E = \sum_{1}^{n} \frac{h}{n}$$ where n = number of loci

$h = 1 - \Sigma q^2$ and q^1 is the i^{th} allele

Observed heterozygosity (H_O): Mean observed heterozygosity and can be estimated for all loci:

$$H_O = \frac{\text{Number of heterozygous individuals}}{\text{Total number of individuals sampled}}$$

Wright's F-Statistics:

F-statistics (F_{IT}, F_{IS}, and F_{ST}) estimated from genetic markers provide information on the genetic structuring within and among populations (Weir and Cockerham, 1984). Of these indices, the value of F_{ST} indicates how much of the genetic variation is partitioned among populations and then, can be used as a measure of the genetic differentiation that can be expected as a consequence of low level of gene flow among populations or steady differential selection (Slatkin, 1985; 1987). Gene flow decreases F_{ST}, thus making allele frequencies and composition more similar between subpopulations (when $F_{ST} = 0$, it indicates complete geneflow, while when F_{ST} is 1, there is complete isolation between subpopulations:

$$F_{ST} = \frac{H_T - H_E}{H_T}$$

F_{IS} is the the reduction in heterozygosity due to inbreeding in subpopulations:

$$F_{IS} = \frac{H_E - H_I}{H_E}$$

F_{IT} is the reduction of heterozygosity due to inbreeding in the total population:

$F_{IT} = (1-F_{IS})(1-F_{ST}) = (1-F_{IT})$

where S = subpopulation, T = total population, I = Inbreeding, H_E = expected heterozygocity in subpopulations, H_T = heterozygosity expected without subdivision.

Geneflow

This is based on the introduction of genetic material (by interbreeding) from one population to another (usually of the same species), thereby affecting the composition of the genepool of the receiving population. The introduction of new characteristics through geneflow increases variability within the population and makes possible new combinations of traits.

Geneflow N_m= Fst = 0.25(1 - Fst)/Fst)

where N_m is the product of the effective size of individual populations (N) and the rate of migration among them (m).

AMOVA

The Analysis of Molecular Variance tests the differences among population and/or groups of populations in a way similar to ANOVA and the results interpreted via the Φ-statistic (Phi Statistic).

$$\Phi_{SG} = \frac{\sigma_b^2}{\sigma_b^2 + \sigma_c^2}$$

where σ_b^2 = variance among populations and σ_c^2 = variance within populations.

3. BEAN DIVERSITY IN SPACE AND TIME

3.1 Populations from Lazio, Italy

3.1.1. Morphological data analyses for Lazio Populations

The morphological data for the Lazio bean populations were collected by colleagues and provided for analysis for this study (Bruni 2008); De Santis 2007). In addition to these analyses, seeds from the Latin American populations and seeds from the 1950s populations were also planted in the field. The aim was to provide a complete morphological analysis of all the populations in this study. However, seeds from Latin American and 1950s populations failed to produce enough seedlings/population for a viable analysis (at least six plants/population were required, with eight plants/population set as an ideal target to match the eight plants/population assessed molecularly).

Fig. 3.1 Latin American bean populations as well as 1950s cultivated beans were planted in the field but failed to germinate and/or grow, possibly due to the heat.

There were 26 traits assessed for 39 populations from Lazio, Italy. Three further populations (totalling 42 in all) were assessed genetically as these were populations selected by the University of Tuscia from other populations also in the study. They are Mughetto (derived from Mughetto di Grisciano), Nero Rosso di Piumarola (derived from Rosso di Piumarola) and Verdolino indeterminate (derived from Verdolino determinate). This was undertaken as all the Lazio bean populations here are eco-types and have not previously been assessed.

Results of Principal Component Analysis showed that the main component of diversity was due to number of flowers per inflorescence, pod width, pod length as well as seed colour, weight, length and width (Fig 3.2 and 3.3). This is to be expected as selection pressure on beans is based primarily on the seed and its pod. Detailed descriptions of these bean populations as well as images of the seeds, flowers and leaves, are provided in De Santis (2007) and Bruni (2008); however, the work undertaken by De Santis (2007) and Bruni (2008) focused on morphological characterization and production descriptors, while the present work focuses on the analysis of the variable measured in the field. The descriptions of the beans collated by Bruni (2008) is provided in Annex 3.

None of the descriptors, however, show a heavy weighting on attributing diversity of these bean accessions as Axis 1 is responsible for about 23% of total variation and Axis 2 is responsible for about 15% of total variation (Table 3.1 shows the Eigen values for all the descriptors assessed).

As expected, the seed morphometrics (length, weight and width) were correlated among themselves: r = 0.7, 0.8 and 0.9 respectively). Seed width and seed length were negatively correlated with seed number (r = -0.5 and -0.7 respectively) such that plants with the larger the number of seeds, the fewer on that plant. Seed width was highly correlated to pod width (r = 1), as one would expect. Leaf length and leaf width were also correlated (r = 0.8), and leaf length was also correlated with chlorophyll intensity (0.5). This was also the case with the flower sizes and numbers (r = 0.5), and pod length and width (r= 0.7). The number of viable pods/plant and the total number of pods/plant showed complete correlation (r = 1); therefore, the number and proportion of non-viable pods/plant was not significant among the populations.

Of little characterization value, instead, were germination times, geographical origin (the bean populations were coded according to the five provinces from which they were obtained), leaf length, leaf form and persistency.

The Borlotti beans all related to one another with seed number being the main grouping variable. The Carbonari, a black sub-variety of the Cannellino, is characterized by flower size and number, while the Chiarinelli, the Cannellino S. Oliva and the Mezzo Cannellino di Ventotene are characterized by pod number and petal aperture. The Solfarino bean was characterized chiefly by seed morphometrics but the Fagiolone di Grisciano (the bean with the heaviest seed, weighing 1.96g), the Fagiolone di Vallepietra, the Cannellino di Atina and the Sanguinelli are those with the

largest pods. The Confettino bean was also large (7.74 mm) along with Fagiolo Pisello, Rosso di Piumarola, and Tabacchino. This agrees with the results of De Santis (2007) who found that Sanguinelli had large, and few, pods. The bean with the largest number of seeds per plant was the Carbonari (20.60).

Table 3.1. Eigen values of morphological descriptors for the Lazio bean populations.

Morphological Descriptors	Comp 1	Comp 2
Germination	0.00	0.00
Origin	0.10	-0.08
Maturation	0.46	-0.02
No. of Pods	-0.22	-0.71
Total No. Pods	-0.22	-0.73
Leaf Length	0.01	-0.10
Leaf Width	0.23	-0.33
Leaf Form	-0.10	-0.04
Chlorophyll Intensity	0.14	-0.34
Persistency	0.24	0.06
No. Flowers/Inflorescence	0.61	-0.54
Flower Size	0.37	-0.63
Petal Aperture	-0.26	-0.47
Stem Colour	-0.03	0.21
Petal Colour	-0.02	0.24
Days To Flowering	0.03	0.45
Texture Of Pod	0.17	0.54
Pod Width	0.93	0.07
Pod Length	0.76	0.31
Seed Number	-0.60	0.41
Primary Seed Colour	0.47	0.57
Secondary Seed Colour	0.43	0.45
Secondary Colour Distribution	0.19	0.30
Seed Shape	0.15	-0.39
Seed Weight	0.94	-0.13
Seed Length	0.91	-0.21
Seed Width	0.95	-0.06

The smallest beans, the Bottoncino di Terrelle and the Mughetto di Grisciano, are found close together but on the dendrogram based on correlations place them in separate clusters, suggesting that size is not their defining feature. The Bottonocino also had the lightest seeds (0.35) along with the Fagiolina Arsolana (0.35). The cluster diagram (Fig. 3.4) shows the grouping of the Borlotti beans, with the Borlotto di Graffignano slightly more distant from the other Borlotti. At a similar distance from the Borlotti was the Fagiolo Burro.

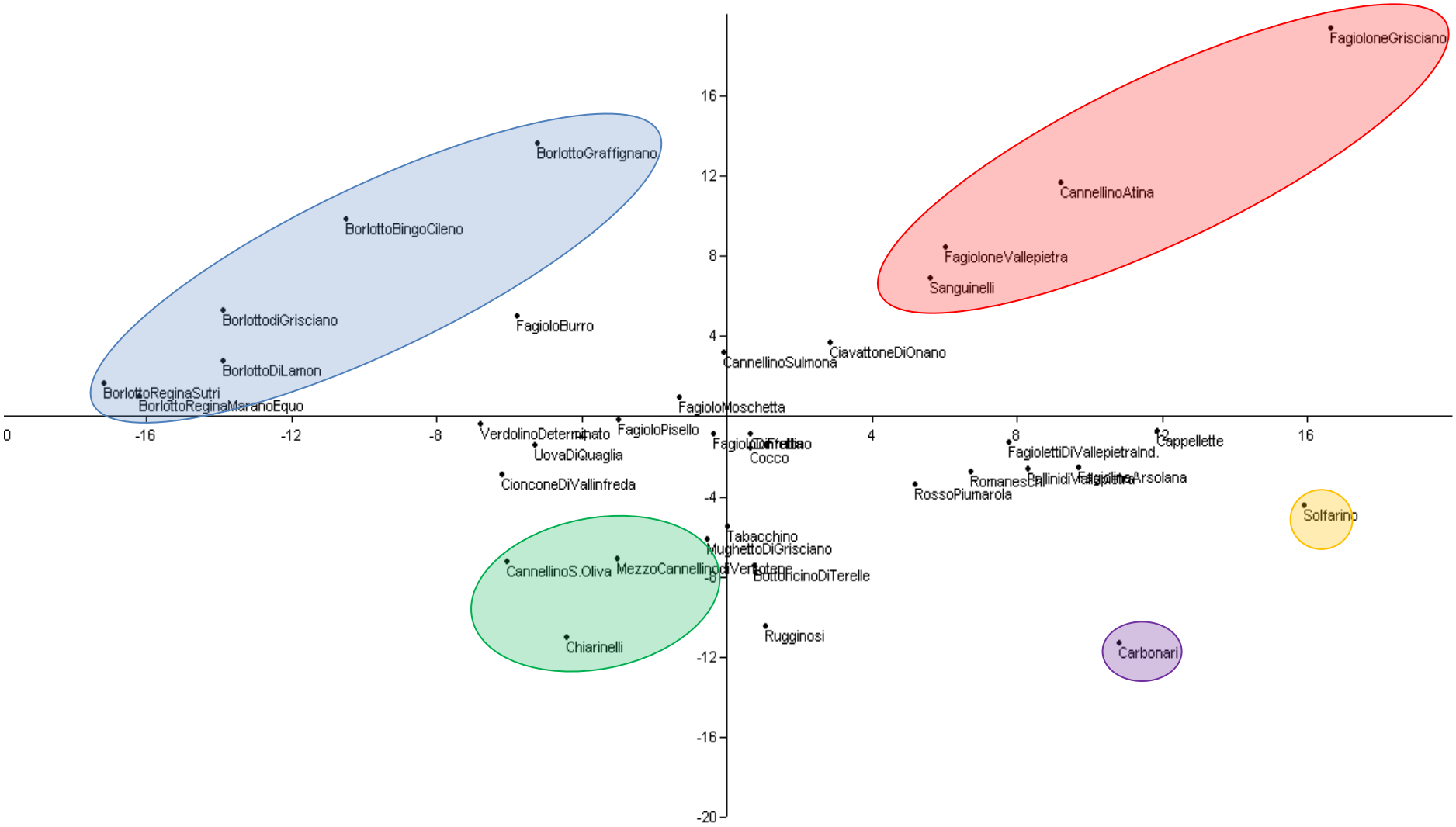


Fig.3.2. Results of Principal Components Analysis of the morphological traits for the Lazio bean populations, based on the varieties.

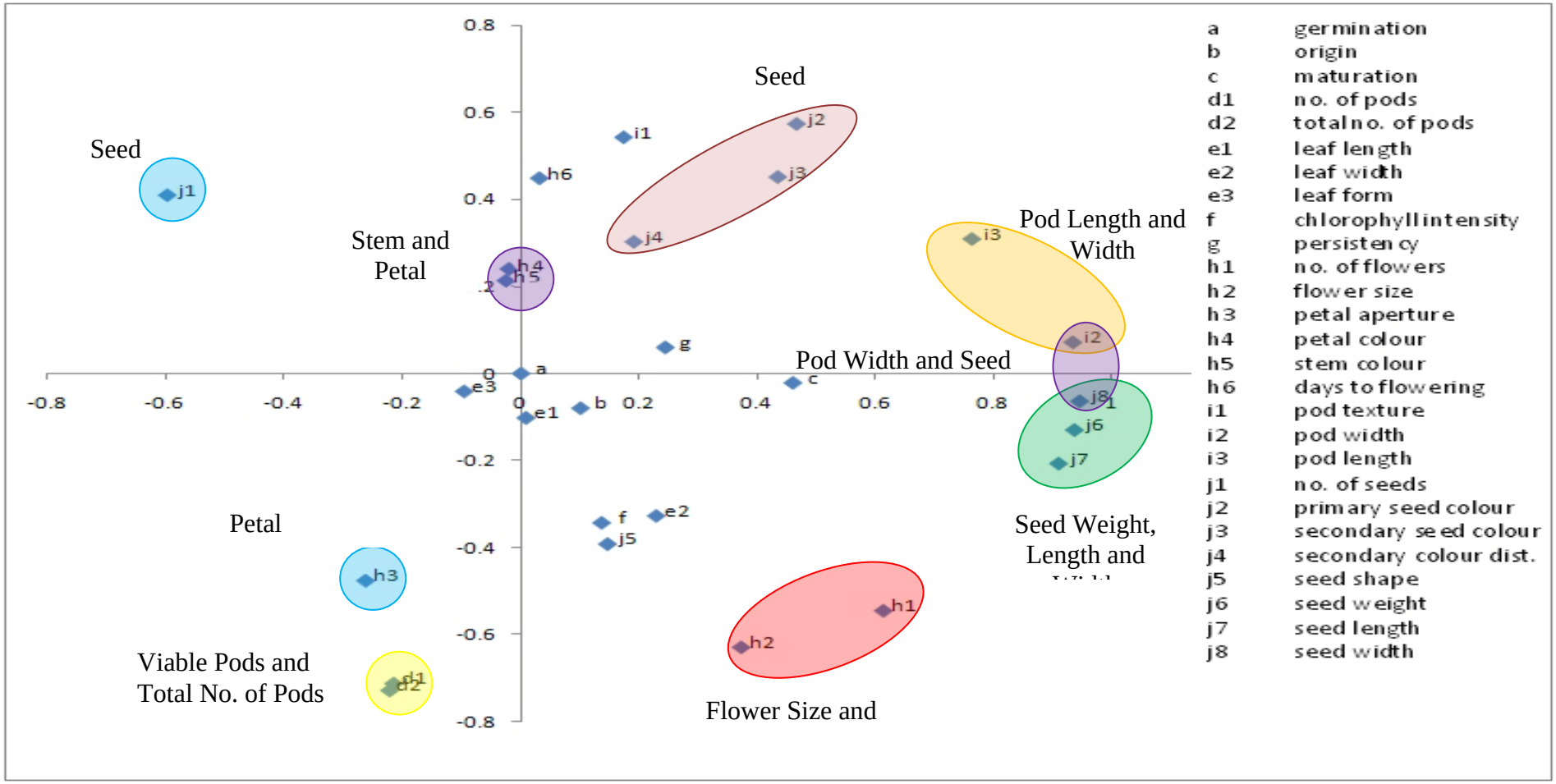


Fig.3.3. Results of Principal Components Analysis of the morphological traits for the Lazio bean populations, based on the agromorphological descriptors.

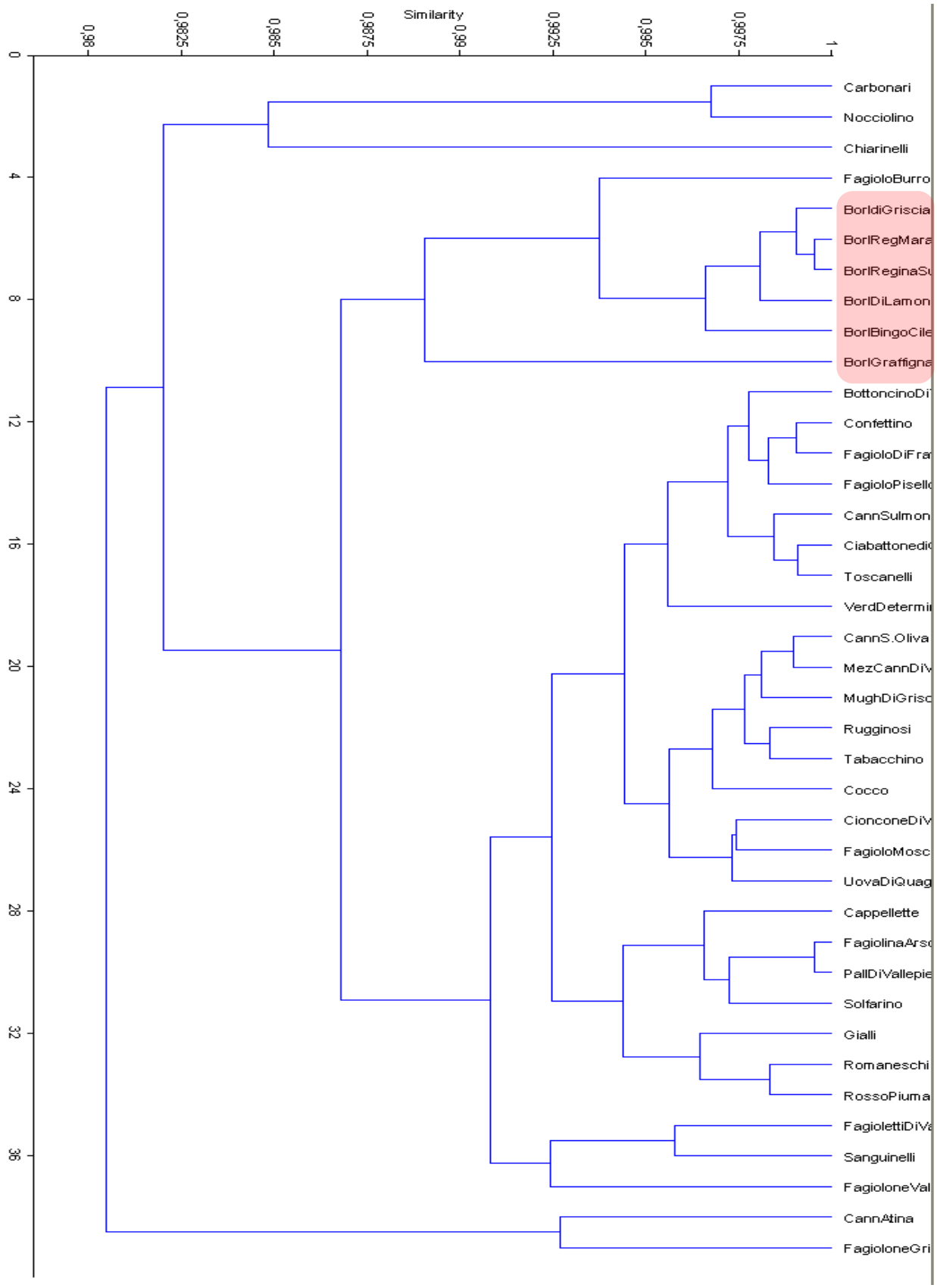


Fig. 3.4. Cluster analysis of the morphological data based on Pearson's correlation coefficient.

3.1.2. Molecular analyses of Lazio beans

Analyses were carried out on 42 Lazio bean populations (totalling 336 individuals) to assess diversity among and within them. The genotypic composition of the analysed populations showed deviation from the expected Hardy-Weinberg proportions. Analysis of Molecular Variance (AMOVA) was undertaken to assess diversity within and among populations, and highlighted the high levels of diversity once more: 38% among populations and 62% within populations ($p < 0.000$) (results obtained from both GenAlex and Arlequin) (Table 3.2).

Table 3.2. AMOVA and *F*-Statistics results for Lazio bean populations.

Source	d.f.	SS	MS	Est. Var.	%
Among Pops	41	4785.452	116.718	12.107	38%
Within Pops	294	5839.875	19.864	19.864	62%
Total	335	10625.327	136.582	31.970	

***F*-Statistics and Geneflow (N_m)**

F_{is} 0.4638

F_{it} 0.6660

F_{st} 0.3771

N_m 0.416

The genetic distance between populations (F_{ST} = 0.3771, i.e. 38% of the total variance of allele frequencies was due to the genetic differences among samples) did differ from the extent of inbreeding within groups (F_{it} = 0.6660). The degree of relatedness between markers within groups (F_{is} = 0.4638) was significantly low. The sizeable genetic differentiation among landrace populations was confirmed by the gene flow N_m that resulted <1, suggesting a local differentiation of Lazio populations. This value is not high enough to offset random genetic drift, which often leads to population divergence (Wright 1931; Slatkin 1985), also lending further weight to the population differentiation among populations.

The Cannellino di Atina population had the highest number of private alleles (P_A = 3), while Solfarino, Nero Rosso di Piumarola, Verdolino determinato, Borlotto di Grisciano, Bottoncino di Terrelle, Ciabbattone di Onano, Fagiolo di Fratta, Mughetto di Grisciano, Uova di Quaglia all had

P_A = 2 private alleles each. With P_A = 1 were Cannellino di Moschetta, Carbonari, Gialli, Borlotto Bingo Cileno, Borlotto di Sutri, Mughetto, Toscanelli and Verdolino indeterminate. It is interesting that the rare alleles in the Verdolino types were not the same. This was also the case between Mughetto and Mughetto di Grisciano, as well as within the Borlotti. The only allele which was found in two populations was allele 170, found in Cannellino di Atina and Nero Rosso di Piumarola. While the Nero Rosso has been derived from the Rosso di Piumarola, the latter does not possess the same rare allele. It is possible that there has been some introgression from the Cannellino di Atina as both beans originate from the province of Frosinone.

A dendrogram was constructed, based on Nei's Genetic Distance (UPGMA method) (Nei 1978) (Fig 3.5. and Table 3.3) using GDA and drawn up using TreeView. It shows that the beans separate into three distinct clusters, as opposed to the cluster diagram based on morphological data where the clusters were not as distinct.

Diversity was also measured using the percentage of polymorphism (P), number of alleles per locus (A), number of polymorphic alleles per locus (A_p), expected (H_e) and observed (H_o) heterozygosity. Fagiolo Burro, Gialli, Fagiolone di Grisciano, Cocco and Toscanelli (P = 100%). The Nero Rosso di Piumarola had the lowest levels of polymorphism (P = 57%), while still possessing two rare alleles. It therefore might be possible to use rare alleles use as a discriminant marker for variety/ecotype identification and fingerprinting.

The Nero Rosso di Piumarola, showing no difference between He and Ho, demonstrates that this population is in Hardy-Weinberg Equilibrium (Table 3.4).

The high levels of diversity are consistent with those found by Negri and Tosti (2002) when they assessed diversity in Lazio, as well as in the neighbouring regions of Abruzzo and Umbria. They also found a correlation between the geographical origin of the populations and levels of diversity observed. This was not the case for the 42 populations in this study which did not cluster according to their origin, possibly because the geographical area covered is small and levels of inter-breeding high.

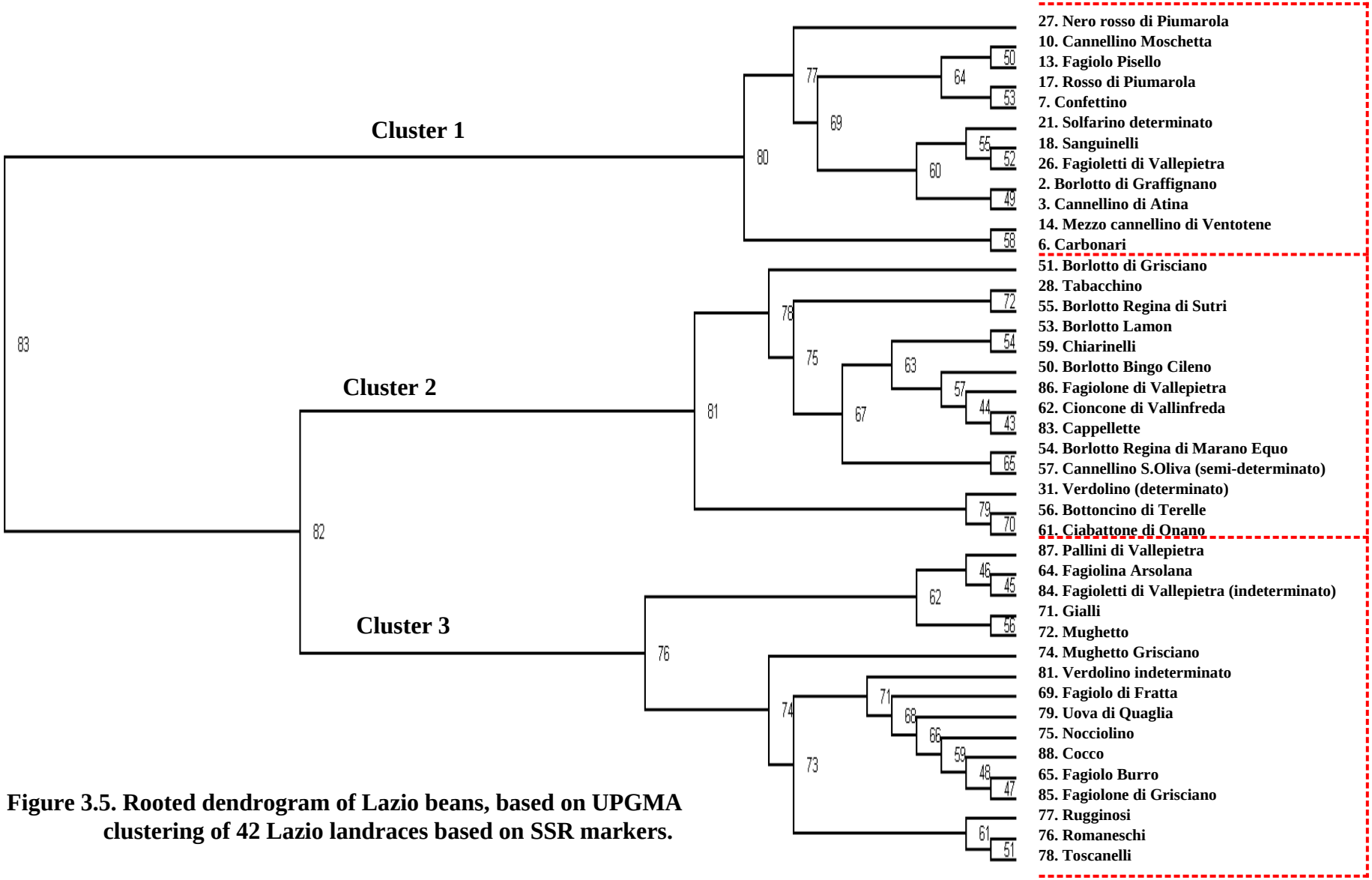


Figure 3.5. Rooted dendrogram of Lazio beans, based on UPGMA clustering of 42 Lazio landraces based on SSR markers.

Table 3.3. Lazio beans clustered according to the results generated by GDA.

	ACCESSION NO. AND COMMON NAME	ORIGIN
	Cluster 1	
1.	2. Borlotto di Graffignano	*Graffignano (VT)*
2.	3. Cannellino di Atina	*Villatina (FR)*
3.	6. Carbonari	*San Giorgio di Amatrice (RI)*
4.	7. Confettino	*Casalvieri (FR)*
5.	10. Cannellino Moschetta	*Atina (FR)*
6.	13. Fagiolo Pisello	*San Giorgio di Amatrice (RI)*
7.	14. Mezzo Cannellino di Ventotene	*Ventotene (LT)*
8.	17. Rosso di Piumarola	*Villasantalucia (FR)*
9.	18. Sanguinelli	*San Giorgio di Amatrice (RI)*
10.	21. Solfarino determinato	*Onano (VT)*
11.	26. Fagioletti di Vallepietra	*Vallepietra (RM)*
12.	27. Nero rosso di Piumarola	*Villasantalucia (FR)*
	Cluster 2	
13.	28. Tabacchino	*Acquapendente (VT)*
14.	31. Verdolino (determinato)	*Onano (VT)*
15.	50. Borlotto Bingo Cileno	*Sutri (VT)*
16.	51. Borlotto di Grisciano	*Grisciano di Accumoli (RI)*
17.	53. Borlotto Lamon	*Valmontone (RM)*
18.	54. Borlotto Regina di Marano Equo	*Marano Equo (RM)*
19.	55. Borlotto Regina di Sutri	*Sutri (VT)*
20.	56. Bottoncino di Terelle	*Terrelle (FR)*
21.	57/58. Cannellino S. Oliva (semi-determinato)	*Castrocielo (FR)*
22.	59. Chiarinelli	*Grisciano di Accumoli (RI)*
23.	61. Ciabattone di Onano	*Onano (VT)*
24.	62. Cioncone di Vallinfreda	*Vallinfredda (RM)*
37.	83. Cappellette	*Vallepietra (RM)*
40.	86. Fagiolone di Vallepietra	*Vallepietra (RM)*
	Cluster 3	
25.	64. Fagiolina Arsolana	*Vallinfredda (RM)*
26.	65. Fagiolo Burro	*Villalatina (FR)*
27.	69. Fagiolo di Fratta	*Villalatina (FR)*
28.	71. Gialli	*Onano (VT)*
29.	72. Mughetto	*Grisciano di Accumoli (RI)*

	ACCESSION NO. AND COMMON NAME	ORIGIN
30.	74. Mughetto Grisciano	*Grisciano di Accumoli (RI)*
31.	75. Nocciolino	*San Giorgio di Amatrice (RI)*
32.	76. Romaneschi	*Vallepietra (RM)*
33.	77. Rugginosi	*San Giorgio di Amatrice (RI)*
34.	78. Toscanelli	*Acquapendente (VT)*
35.	79. Uova di Quaglia	*San Giorgio di Amatrice (RI)*
36.	81. Verdolino indeterminato	*Onano (VT)*
38.	84. Fagioletti di Vallepietra (indeterminato)	*Vallepietra (RM)*
39.	85. Fagiolone di Grisciano	*Grisciano di Accumoli (RI)*
41.	87. Pallini di Vallepietra	*Vallepietra (RM)*
42.	88. Cocco	*Onano (VT)*

Table 3.4. Diversity parameters for Lazio bean populations: the percentage of polymorphism (*P*), number of alleles per locus (*A*), number of polymorphic alleles per locus (*Ap*), expected (H_e) and observed (H_o) heterozygosity.

LAZIO BEAN POPULATIONS	P	A	A_p	H_e	H_o
2. Borlotto di Graffignano	0.88	2.46	2.65	0.43	0.24
3. Cannellino di Atina	0.96	2.92	3.00	0.52	0.30
6. Carbonari	0.92	2.50	2.62	0.47	0.28
7. Confettino	0.92	2.54	2.67	0.45	0.24
10. Cannellino Moschetta	0.85	2.27	2.50	0.41	0.25
13. Fagiolo Pisello	0.92	2.5	2.62	0.42	0.19
14. Mezzo Cannellino Di Ventotene	0.88	2.42	2.61	0.43	0.26
17. Rosso di Piumarola	0.77	2.15	2.50	0.33	0.23
18. Sanguinelli	0.92	2.58	2.71	0.43	0.19
21. Solfarino Determinato	0.92	2.50	2.62	0.44	0.15
26. Fagioletti di Vallepietra	0.85	2.42	2.68	0.44	0.29
27. Nero Rosso Di Piumarola	0.58	1.73	2.27	0.27	0.27
28. Tabacchino	0.81	2.38	2.71	0.33	0.22
31. Verdolino (determinato)	0.96	2.92	3.00	0.53	0.27
50. Borlotto Bingo Cileno	0.88	2.58	2.78	0.45	0.26
51. Borlotto di Grisciano	0.81	2.65	3.05	0.46	0.26
53. Borlotto Lamon	0.92	2.69	2.83	0.48	0.27
54. Borlotto Regina di Marano Equo	0.88	2.46	2.65	0.44	0.19

LAZIO BEAN POPULATIONS	P	A	A_p	H_e	H_o
55. Borlotto Regina di Sutri	0.88	2.69	2.91	0.47	0.3
56. Bottoncino di Terelle	0.92	2.77	2.92	0.46	0.28
57/58. Cannellino S. Oliva	0.92	2.62	2.75	0.48	0.3
59. Chiarinelli	0.92	2.92	3.08	0.48	0.28
61. Ciabattone di Onano	0.92	2.92	3.08	0.5	0.27
62. Cioncone di Vallinfreda	0.88	2.81	3.04	0.48	0.23
64. Fagiolina Arsolana	0.92	3.00	3.17	0.54	0.27
65. Fagiolo Burro	1.00	3.27	3.27	0.6	0.23
69. Fagiolo di Fratta	0.92	3.38	3.58	0.6	0.24
71. Gialli	1.00	3.54	3.54	0.61	0.28
72. Mughetto	0.96	2.96	3.04	0.53	0.21
74. Mughetto Grisciano	0.92	2.88	3.04	0.49	0.16
75. Nocciolino	0.92	2.85	3.00	0.50	0.21
76. Romaneschi	0.92	2.69	2.83	0.51	0.23
77. Rugginosi	0.96	2.88	2.96	0.54	0.25
78. Toscanelli	1.00	3.38	3.38	0.57	0.23
79. Uova di Quaglia	0.92	2.81	2.96	0.5	0.22
81. Verdolino indeterminato	0.96	3.23	3.32	0.58	0.23
83. Cappellette	0.88	2.81	3.04	0.48	0.23
84. Fagioletti di Vallepietra (indeterminato)	0.92	3.00	3.17	0.54	0.27
85. Fagiolone di Grisciano	1.00	3.27	3.27	0.6	0.23
86. Fagiolone di Vallepietra	0.88	2.81	3.04	0.48	0.23
87. Pallini di Vallepietra	0.92	3.00	3.17	0.54	0.27
88. Cocco	1.00	3.27	3.27	0.6	0.23
MEAN	**0.91**	**2.77**	**2.94**	**0.49**	**0.24**

3.2. IPK Italian bean accessions from 1950, an Italian wild bean and a black variety ‘Bobby’

There were 10 bean populations from 1950, and for interest two further populations were included: Fagiolo Selvatico (translated as wild bean, originating in Italy) and a black variety, ‘Bobby’, also Italian. No passport data, apart from date of collection, were available from IPK for these populations, thus the habitat in which they were collected is not available for comparison.

The dendrogram of these populations shows two clear clusters (Fig 3.6): the Borlotti types (823, 824, 825, 828) cluster together while, Tabacchino types (822, 942), Cannellino type (939),

Confettino type (865) do not group together the Italian wild-type clusters close to Verdolino types; the beans are also similar morphologically.

Table 3.5. AMOVA and *F*-Statistics results for IPK bean populations.

Source	d.f.	SS	MS	Est. Var.	%
Among Pops	11	1299.514	118.138	12.442	40%
Within Pops	84	1562.633	18.603	18.603	60%
Total	95	2862.148	136.740	31.045	

***F*-Statistics and Geneflow (N_m)**

F_{is} 0.368

F_{it} 0.584

F_{st} 0.361

N_m 0.442

The above results are similar to those of the Lazio beans (Table 3.5), exposing a high level of diversity despite originating from *ex situ* collections and having been collected 60 years ago. There is no evidence of genetic erosion from maintaining these populations in the seed bank (Engelmann and Engels 2002). However, it is difficult, if not impossible, to assess whether genetic integrity has been maintained.

The highest level of polymorphism in these beans was seen in the Tabacchino-type 942 (P = 96%), while the Tabacchino-type 822, Cannellino-type 939 and Borlotto-type 828 all had levels of polymorphism P = 77%. There is no clear pattern to be found here as even the Borlotti types, which otherwise tend to show similar characteristics, range in levels of polymorphism.

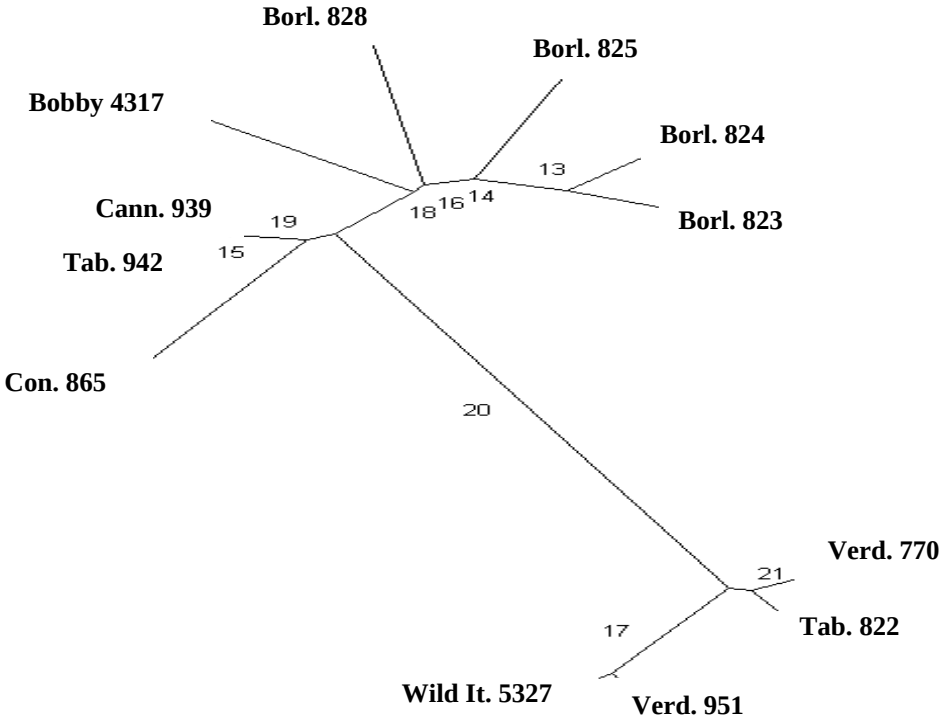


Figure 3.6. Unrooted dendrogram of IPK Italian bean accessions from 1950, the Italian wild bean and a black variety 'bobby'.

Table 3.6. Diversity parameters for IPK bean populations: the percentage of polymorphism (*P*), number of alleles per locus (*A*), number of polymorphic alleles per locus (*Ap*), expected (H_e) and observed (H_o) heterozygosity.

IPK Bean Populations	P	A	A_p	H_e	H_o
Verd. 770	0.88	2.62	2.83	0.49	0.21
Verd. 951	0.81	2.73	3.14	0.47	0.31
It. Wild 5327	0.92	3.19	3.38	0.57	0.28
Tab. 822	0.77	3.15	3.80	0.48	0.20
Tab. 942	0.96	3.19	3.28	0.58	0.33
Can. 939	0.77	2.58	3.05	0.47	0.35
Con. 865	0.81	2.73	3.14	0.47	0.35
Borl. 828	0.77	2.42	2.85	0.42	0.34
Borl. 825	0.81	3.08	3.57	0.52	0.34
Borl. 824	0.92	3.00	3.17	0.53	0.34
Borl. 823	0.85	2.85	3.18	0.51	0.40
Bobby 4317	0.85	2.88	3.23	0.50	0.33
Mean	0.84	2.87	3.22	0.50	0.32

3.3. Wild bean populations from Latin America

Eight populations of wild *P. vulgaris*, obtained from CIAT, were assessed using the same microsatellite markers as for the previous bean populations. Other populations were obtained for this study, 27 in total, but either did not germinate or did not produce enough seedlings.

There is also no clear pattern seen in the diversity parameters; the Peruvian population shows the highest number of alleles per locus (3.69) while the population from Mexico collected in 1991 shows the highest level of polymorphism (Table 3.7). There was no evidence, based on the markers used, of diversification between the Andean and Mesoamerican wild beans. Seed size is also not relevant here as both the Guatemalan beans are large but fall into separate clusters here (Fig 3.7). Origin with regard to distance between the origin of the populations does not seem to be a common factor as the four Mexican beans do not fall within the same cluster. The Italian wild bean was included in the dendrogram and it clustered close to G50386, from Guatemala and collected in 1995.

Table 3.7. Diversity parameters for IPK bean populations: the percentage of polymorphism (*P*), number of alleles per locus (*A*), number of polymorphic alleles per locus (*Ap*), expected (H_e) and observed (H_o) heterozygosity.

Population	P	A	Ap	He	Ho
G10017 – Mex1962a	0.96	2.88	2.96	0.56	0.26
G12858 – Peru 1959	0.92	3.69	3.92	0.59	0.33
G12875 – Mex 1966	0.81	2.54	2.90	0.46	0.26
G13034 – Mex 1962b	0.96	3.54	3.64	0.61	0.19
G50386 – Guat 1995a	0.81	2.85	3.29	0.47	0.25
G50388B - Guat 1995b	0.92	3.08	3.25	0.53	0.34
G50518B – Col 1979	0.92	3.12	3.29	0.55	0.29
G24392 – Mex 1991	1.00	2.88	2.88	0.54	0.29
Mean	**0.91**	**3.07**	**3.27**	**0.54**	**0.28**

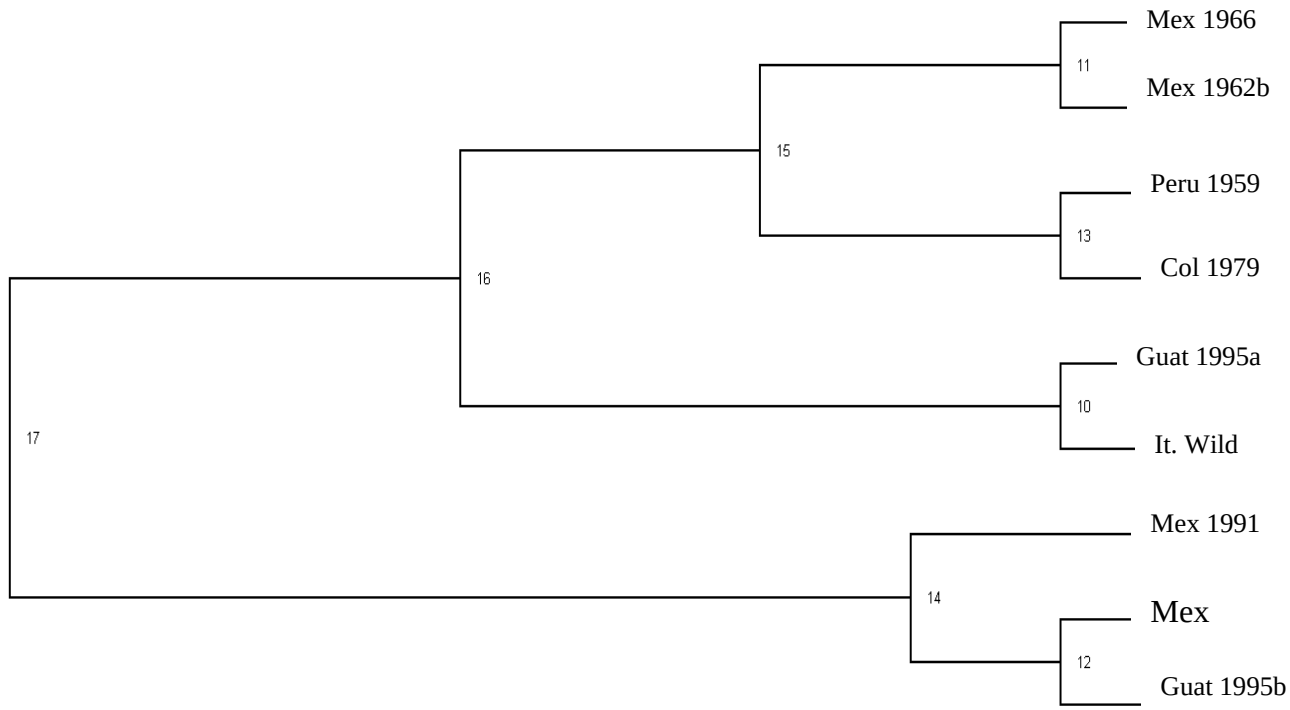


Figure 3.7. Rooted dendrogram of wild bean populations from Latin America and including the Italian wild bean from IPK.

3.4. Assessment of all bean populations in study

Genetic analyses were carried out using 10 SSR markers on a total of 62 populations (8 individuals per population, totalling 496 individuals) and 26 loci, all polymorphic, were identified. Analysis of Molecular Variance (AMOVA) was undertaken to assess diversity within and among populations, and highlighted the high levels of intra-varietal diversity once more: 37% among populations and 63% within populations ($p<0.000$) (results obtained from both GenAlex and Arlequin).

Source	d.f.	SS	Est. Var.	%
Among Pops	61	3484.511	3.22230	37%
Within Pops	930	5176.625	5.56626	63%
Total	991	8661.136	8.78857	

***F*-Statistics and Geneflow (N_m)**

F_{is} 0.437

F_{it} 0.663

F_{st} 0.401

N_m 0.373

All beans assessed in this study showed similar levels of diversity within and among the populations, ranging from 32% to 40% among populations, and from 60% to 68% between populations. While this does present differentiation among the diverse beans assessed, there is no significant difference between the wild beans, the 1950s beans and the more modern, traditional, varieties. The *F*-Statistics also confirm this uniformity of diversity levels among the populations, although, as the dendrogram shows clearly, the modern eco-types are different from the older varieties.

These results were in accord with those obtained from STRUCTURE (Fig. 3.6). Pritchard et al. (2000) described a Bayesian clustering method (implemented in the program STRUCTURE, http://www.stats.ox.ac.uk/~pritch/home.html), which uses multilocus genotypes to infer population structure and simultaneously assign individuals to populations. This model assume that there are K populations (where K may be unknown), each of which is characterized by a set of allele frequencies at each locus. Individuals in the sample are assigned probabilistically to populations, or jointly to two or more populations if their genotypes indicate that they are admixed. This method can be used to detect the presence of cryptic population structure and to perform assignment testing. Pritchard et al.'s model assumes Hardy-Weinberg Equilibrium (HWE) and Linkage Equilibrium (LE) among the unlinked loci. Departures from HWE and LE lead the population to be split into subpopulations, to which individuals are assigned. In this study the posterior probabilities of K (i.e., the likelihood of K as a proportion of the sum of the likelihoods for different values of K) are estimated assuming uniform prior values on K between 2 and 5 (option MAXPOPS = 2–5).

Population structure within a data set is detected by the presence of Hardy-Weinberg and linkage disequilibrium, and is modelled by assuming that the genotype of each individual is a mixture drawn at random from a number of different populations. The number of contributing populations can be estimated and, for a given number of populations, their gene frequencies and the admixture proportions for each individual are all jointly estimated. In this way the sampled population is subdivided into a number of different subpopulations that effectively cluster the individuals. Then, individuals of *a priori* known or unknown origin may be assigned probabilistically to the subpopulations.

No *a priori* information was available as to the likely origins of *P. vulgaris*. Thus, the number of genetically differentiated *P. vulgaris* populations, *K*, was estimated by employing a Bayesian

approach, implemented in the program STRUCTURE (Pritchard et al. 2000; Falush et al. 2003). STRUCTURE uses a clustering method to assign individuals with similar multilocus genotypes to probable common populations. Mean and variance of log likelihoods and posterior probabilities of the number of populations for K = (1, 2, … 11) were inferred from multilocus genotypes by running structure five times with 10^5 repetitions each (burn in = 10 000 iterations, or generations). The mean membership (q) for each bean variety describes the likelihood of that genet belonging to the respective cluster. Each variety can be assigned partial membership in multiple clusters, with membership coefficients summing to one across clusters. The 'no admixture ancestry model' (because the populations originated from diverse geographical regions) was run under the assumption of 'independent allele frequencies' rather than 'correlated allele frequencies' to improve clustering of closely related populations (Falush et al. 2003).

Table 3.8. Bayesian clustering analyses for the pooled bean samples (496 individuals; 26 loci) performed using STRUCTURE.

K	**Ln Prob of Data**
2	- 48301.3
3	- 34318.8
4	- 37454.7
5	- 42282.6

The Ln probability of the data, using 10,000 Burn-in period and 1000 Reps, was minimum with K = 2 populations (Ln = -48301.3) and maximum with K= 3 populations (Ln = - 34318.8), thus suggesting that the pooled bean populations were heterogeneous and contained three genetically distinct groups (Table 3.8).

Samples were placed into the respective subpopulation based upon the highest percentage of membership (q) which was $\geq$ 95% for all populations (Fig 3.8.). The exceptions to this still possessed a high q: Borlotto Regina di Marano Equo (population no. 18 in the bar plot) where q=93%, G13034 – Mex 1962b (population no. 45 in the bar plot) q=92% and Can. 939 (population 56 in the bar plot) q=90%.

The bar chart from STRUCTURE shows the Lazio populations (1 - 42; see Table 3.3 for the population order) closer to each other than to other bean populations (Fig. 3.8).

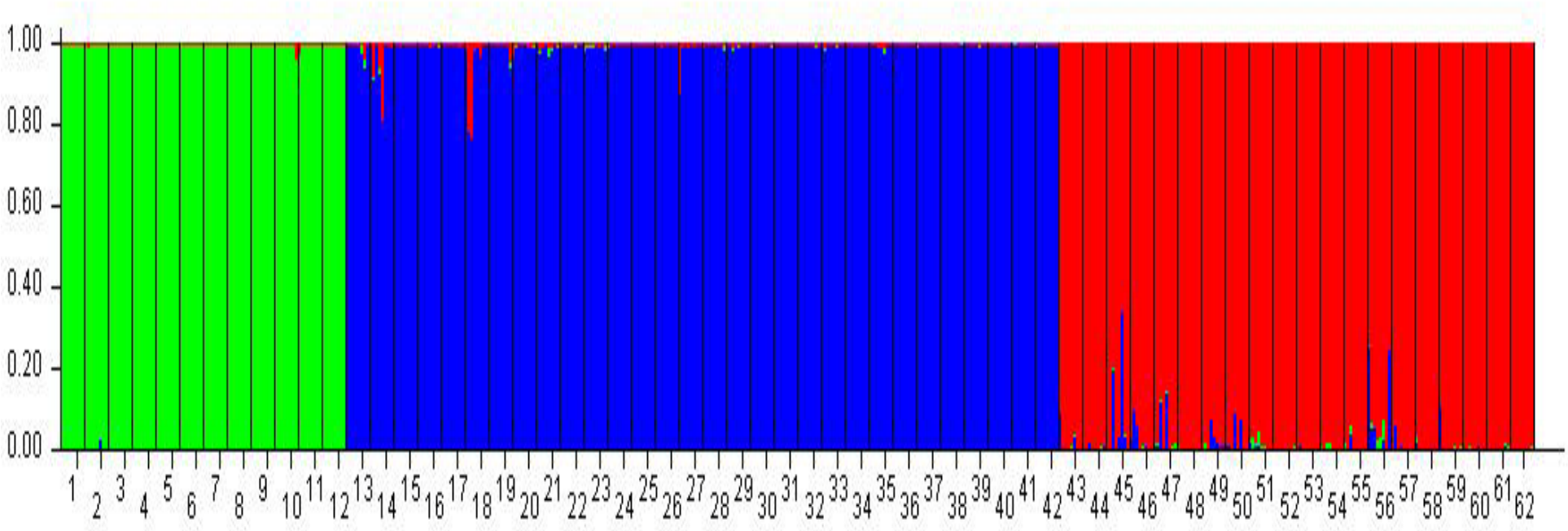


Fig 3.8. Bar plot of all beans in the study using STRUCTURE, showing the allocation of populations into three clusters, in agreement with the clusters designated by GDA

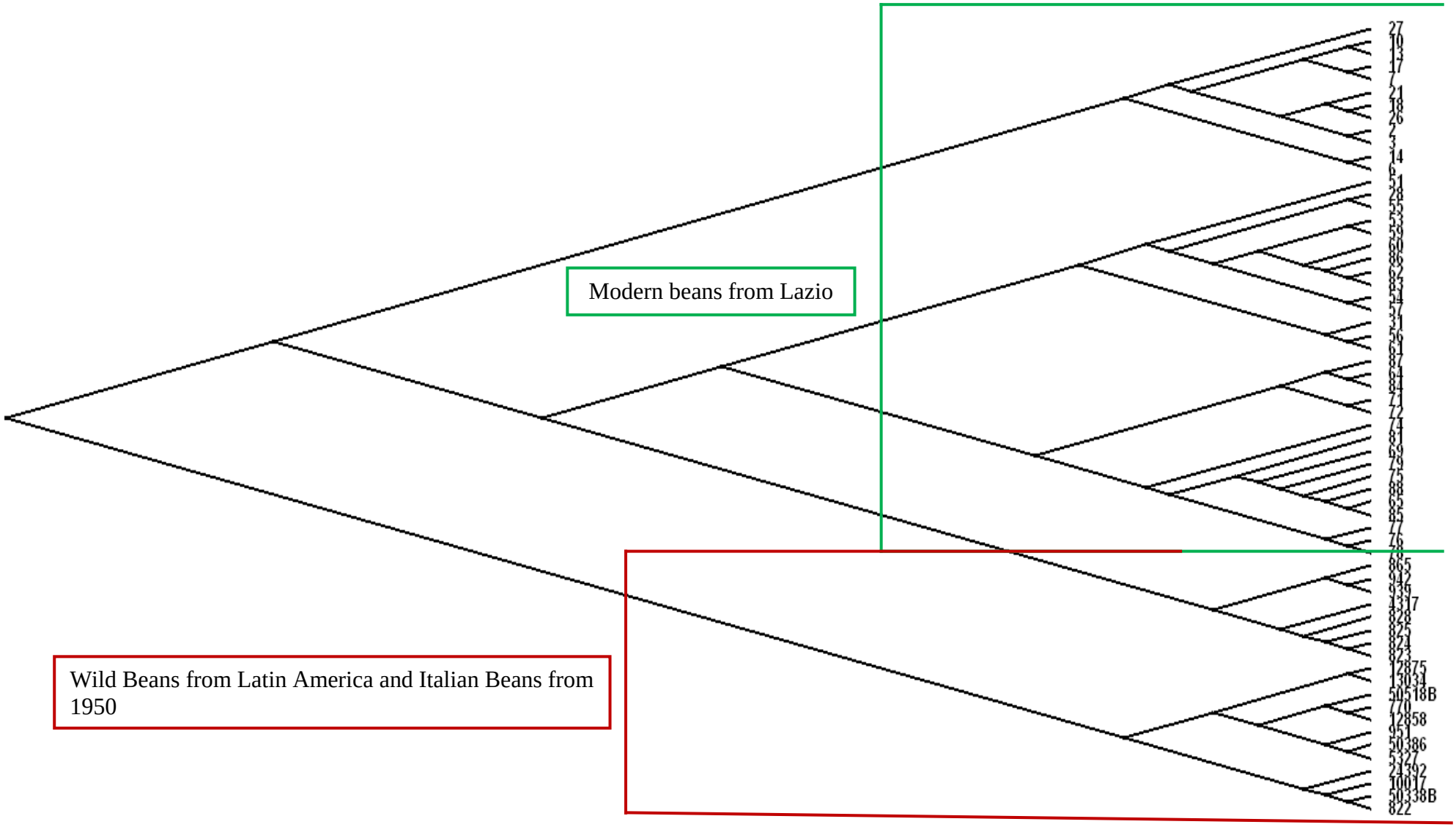


Fig 3.9. Dendrogram of all beans in the study using Nei's Genetic Distance based on the UPGMA method using data from GDA.

From the above dendrogram (Fig 3.9), it is clear that the modern Lazio varieties, despite being eco-types, do not cluster with the 1950s cultivated types. It is interesting that the 1950s Borlotti (823, 824, 828) do not cluster with the modern, Lazio Borlotti but do still cluster together.

One of the aims of analysing the diverse populations was to assess whether the populations would cluster according to whether they were of Mesoamerican or Andean origin. The evolution of the common bean (*Phaseolus vulgaris* L.) progenitor occurred before domestication, and so wild forms diverged into two major gene pools, each with a specific geographic distribution: the Mesoamerican (M) and the Andean (A) ones (Debouck and Tohme 1989). However, the wild types all fell into the same cluster so the markers in this study did not differentiate for origin.

It is possible to assess diversity through assessing names of traditional varieties (Negri and Tosti 2002). Local names mostly relate to seed colour and shape either directly (i.e. solfarino = yellow, tabacchino = tobacco brown, bianco = white seed coloured, respectively, tondo = round, pisello = pea-shaped) or indirectly referring to well-known types (i.e. cannellino = white, cuboid-seeded type) or to local folk language (ciabattone = old shoe shaped, fagiolone = large bean). The caution here is that farmers often use the same name for genetically diverse varieties, and vice versa, often use different names for the same variety. Therefore, assessing diversity through farmer-named varieties would always require genetic analyses to verify farmer-name differentiation.

With regard to this study, De Santis (2007) hypothesized that the varieties Cocco and Fagiolo Burro might be the same, especially as they both originate in the same area (Frosinone). Molecularly, they not only fall within the same cluster, they cluster together very closely. So it is possible they are the same variety. However, the Fagiolo Burro and the Fagiolone di Grisciano cluster even more closely together despite the latter originating from the Rieti area. Morphologically, though, the Fagiolone di Grisciano is in cluster of its own, along with Cannellino di Atina.

Hybridization might depend on the cultivation of several landraces neighbouring fields. This is might well be compounded by the exchange of seeds among neighbouring farms, resulting in occasional outcrossing and pollen flow.

While the accessions in this study showed high levels of diversity, other studies have shown that varieties from Sardinia and Basilicata regions of Italy show lower levels of diversity, possibly due to a founder effect based on the relative geographic isolation of these areas (Angioi et al. 2009). It is unfortunate that many of these varieties are now threatened with extinction due to a preponderance of *Cannellini* and *Borlotti*, at the expense of the other varieties, as these are less labour-intensive. The high levels of variation as well as the cultural values attached to these varieties highlight the need for on-farm and *ex situ* conservation and rural market incentives.

A case in point is the 18th century, the garden pea cultivation was completely replaced by the common bean in the neighborhood fields of Lamon village, Belluno province (Piergiovanni and Lioi 2010). Currently, common bean cultivation represents a lucrative activity for Lamon farmers with four recognized landraces (generically named ‘common bean from Lamon) cultivated in the area. These borlotto types (striped red seeds and striped pods) are recognized by the European Union which has designated this bean as ‘Protected Geographical Indication’ (PGI) (GUCE 163/96 2 July 1996).

4. CARDOON DIVERSITY IN SPACE AND TIME

4.1. Morphological Data Analysis

Agromorphological assessment of 12 populations of cardoon was undertaken over the summer of 2010, of at least 6 individuals per population, and averages then taken for each population. These populations are described in Table 4.1.

Table 4.1. Description of the cardoon populations used for the morphological data analysis

Accession Name	Acquired From	Common Name, Geographical Origin and Date of Germplasm Collection
CYN 7	IPK	Unknown
CYN 9	IPK	Cardo Pieno Inerme, Italy, 1985
CYN 10	IPK	Cardo Bianco Avorio, Italy, 1985
CYN 12	IPK	Cardo Gobbo, Italy, 1989
ACC1 K3	VIR	Artishok,Moldavia, 1975
ACC2 K4	VIR	Maikopski 6 Zelenyi Krasnodar, Russia, 1950
ACC3 K5	VIR	Krupyi Zelenyi, England,1950
ACC5 K7	VIR	Cardo Nostrano Bolognese, Italy, 1974
ACC6 K8	VIR	Cardo Blanco sin Espinas, Spain, 1979
ACC7 K9	VIR	Artishok, Setif, Algeria, 1971
CSASSO	Univ. Tuscia	Cardo selvatico, Cerveteri, Italy, 2008
CTARQ	Univ. Tuscia	Cardo selvatico, Tarquinia, Italy, 2008

Assessment consisted of the following descriptors (see Annex 4 for a description of how the data was measured):

- Plant Height
- No. offshoots
- Distance Flowerhead-1st Leaf
- Stem Diameter
- Leaf Length
- No. of lobes
- Intensity of green colour
- Hue of green colour
- Greyness intensity
- Main flowerhead length
- Main flowerhead diameter
- Shape of main flowerhead
- Shape of flowerhead top
- Main flowerhead readiness
- Total no. of flowerheads

Amount of hairiness and presences/absence of spines were removed as viable descriptor of diversity as they were not informative. All wild cardoons had long spines along the leaves and the flowerhead, while none of the cultivated varieties were spiny, except for the English population,

where half the cardoon had a few spines on the leaves. None of the populations, cultivated or wild, were hairy.

Given below is a description of the populations analysed in the field with the averages obtained for each descriptor.

CYN 7: Unknown Origin

Plant height	147.2
No. offshoots	3.7
Distance Flowerhead-1st leaf	26.4
Stem diameter	0.9
Leaf length	31.2
No. of lobes	5.8
Intensity of green colour	5.0
Hue of green colour	1.0
Greyness intensity	5.0
Main flowerhead length	4.1
Main flowerhead diameter	5.3
Shape of main flowerhead	5.0
Shape of flowerhead top	1.0
Main flowerhead readiness	179.0
Total no of flowerheads	4.4

CYN 9: Cardo Pieno Inerme, Italy, 1985

Plant height	149.3
No. offshoots	4.2
Distance Flowerhead-1st leaf	27.3
Stem diameter	0.8
Leaf length	29.2
No. of lobes	6.4
Intensity of green colour	5.0
Hue of green colour	1.3
Greyness intensity	4.6
Main flowerhead length	4.4
Main flowerhead diameter	5.7
Shape of main flowerhead	5.0
Shape of flowerhead top	1.0
Main flowerhead readiness	177.8
Total no of flowerheads	8.8

CYN 10: Cardo Bianco Avorio, Italy, 1985

Plant height	154.3
No. offshoots	4.2
Distance Flowerhead-1st leaf	30.8
Stem diameter	0.9
Leaf length	32.0
No. of lobes	6.9
Intensity of green colour	5.0
Hue of green colour	1.6
Greyness intensity	4.3
Main flowerhead length	4.5
Main flowerhead diameter	5.6
Shape of main flowerhead	4.7
Shape of flowerhead top	1.0
Main flowerhead readiness	173.9
Total no of flowerheads	10.0

CYN 12: Cardo Gobbo, Italy, 1989

Plant height	153.3
No. offshoots	4.2
Distance Flowerhead-1st leaf	30.0
Stem diameter	0.9
Leaf length	31.8
No. of lobes	6.9
Intensity of green colour	4.7
Hue of green colour	1.8
Greyness intensity	4.4
Main flowerhead length	4.6
Main flowerhead diameter	5.7
Shape of main flowerhead	4.8
Shape of flowerhead top	1.2
Main flowerhead readiness	172.1
Total no of flowerheads	10.3

ACC1 K3: Artishok, Moldavia, 1975

Plant height	148.5
No. offshoots	3.9
Distance Flowerhead-1st leaf	28.7
Stem diameter	0.9
Leaf length	31.5
No. of lobes	6.8
Intensity of green colour	4.6
Hue of green colour	2.0
Greyness intensity	4.3
Main flowerhead length	4.6
Main flowerhead diameter	5.7
Shape of main flowerhead	4.8
Shape of flowerhead top	1.1
Main flowerhead readiness	171.4
Total no of flowerheads	9.3

ACC2 K4: Maikopski 6 Zelenyi Krasnodar, Russia, 1950

Plant height	144.8
No. offshoots	3.8
Distance Flowerhead-1st leaf	28.4
Stem diameter	0.9
Leaf length	31.0
No. of lobes	6.6
Intensity of green colour	4.5
Hue of green colour	2.1
Greyness intensity	4.3
Main flowerhead length	4.7
Main flowerhead diameter	5.8
Shape of main flowerhead	4.8
Shape of flowerhead top	1.1
Main flowerhead readiness	170.8
Total no of flowerheads	9.0

ACC3 K5: Krupyi Zelenyi, England,1950

Plant height	147.0
No. offshoots	3.9
Distance Flowerhead-1st leaf	30.1
Stem diameter	0.9
Leaf length	35.9
No. of lobes	7.0
Intensity of green colour	4.5
Hue of green colour	2.1
Greyness intensity	4.4
Main flowerhead length	4.7
Main flowerhead diameter	5.8
Shape of main flowerhead	4.7
Shape of flowerhead top	1.1
Main flowerhead readiness	169.1
Total no of flowerheads	9.7

ACC5 K7: Cardo Nostrano Bolognese, Italy, 1974

Plant height	149.6
No. offshoots	3.9
Distance Flowerhead-1st leaf	28.9
Stem diameter	1.0
Leaf length	36.6
No. of lobes	6.8
Intensity of green colour	4.4
Hue of green colour	2.0
Greyness intensity	4.4
Main flowerhead length	4.6
Main flowerhead diameter	5.8
Shape of main flowerhead	4.4
Shape of flowerhead top	1.3
Main flowerhead readiness	167.6
Total no of flowerheads	10.5

ACC6 K8: Cardo Blanco sin Espinas, Spain, 1979

Plant height	152.1
No. offshoots	3.9
Distance Flowerhead-1st leaf	29.8
Stem diameter	1.0
Leaf length	37.5
No. of lobes	6.8
Intensity of green colour	4.4
Hue of green colour	2.1
Greyness intensity	4.5
Main flowerhead length	4.7
Main flowerhead diameter	5.8
Shape of main flowerhead	4.4
Shape of flowerhead top	1.3
Main flowerhead readiness	167.0
Total no of flowerheads	10.8

ACC7 K9: Artishok, Setif, Algeria, 1971

Plant height	151.5
No. offshoots	3.9
Distance Flowerhead-1st leaf	29.5
Stem diameter	1.0
Leaf length	38.6
No. of lobes	7.0
Intensity of green colour	4.4
Hue of green colour	2.2
Greyness intensity	4.5
Main flowerhead length	4.7
Main flowerhead diameter	5.8
Shape of main flowerhead	4.4
Shape of flowerhead top	1.2
Main flowerhead readiness	168.0
Total no of flowerheads	10.6

Wild Cardoon, 'Cardo selvatico', from Sasso, Cerveteri, Italy, 2008

Plant height	144.8
No. offshoots	4.5
Distance Flowerhead-1st leaf	27.8
Stem diameter	1.0
Leaf length	39.1
No. of lobes	7.4
Intensity of green colour	4.5
Hue of green colour	2.1
Greyness intensity	4.5
Main flowerhead length	4.6
Main flowerhead diameter	5.8
Shape of main flowerhead	4.4
Shape of flowerhead top	1.2
Main flowerhead readiness	168.2
Total no of flowerheads	13.3

Wild Cardoon 'Cardo selvatico', Tarquinia, Italy, 2008

Plant height	141.4
No. offshoots	4.9
Distance Flowerhead-1st leaf	27.1
Stem diameter	1.0
Leaf length	39.6
No. of lobes	7.9
Intensity of green colour	4.6
Hue of green colour	2.1
Greyness intensity	4.6
Main flowerhead length	4.5
Main flowerhead diameter	5.7
Shape of main flowerhead	4.5
Shape of flowerhead top	1.2
Main flowerhead readiness	168.3
Total no of flowerheads	14.5

Some of the variables were found to be highly correlated with others. The number of leaf lobes was correlated to leaf length, so that the larger leaves possessed a greater number of lobes. Stem diameter was found to be closely correlated to total number of flowerheads (r = 0.79), leaf length (r

= 0.94 and number of leaf lobes (r = 0.82), with leaf lobes and leaf lengths also beaing correlated (r = 0.75). Readiness of the main flowerhead, conversely, (measured in number of days from 1st January of that year) was found to be negatively correlated to flowerhead length (r = -0.83), flowerhead diameter (r = -0.82), leaf length (r = -0.82), and number of leaf lobes (r = -0.75).Therefore, the later the plant matured, the smaller the main flowerhead and leaves and fewer lobes on the leaves.

Overall the colour descriptors were not directly useful as they are subjective (depending on elements, such as quality of sunlight, and it is necessary to carry the Royal Botanic Garden colour chart in the field to be able to objectively assess colours of the leaves. This was especially true when assessing the level of green and grey of the leaves. Generally, however, the leaves of the cultivated populations of cardoon were greyer than those of the wild types, which had a yellower tinge to them.

Although peast resistance was not one of the variables assessed, it was found that the cultivated varieties were more susceptible to pests with aphid and ant attacks seen more frequently and in greater density on the cultivated varieties. In fact, it is recommended that future studies assess the level of pest damage as it is probable that the wild types are far more resistant to arthropod pests. All populations in this study will be assessed for resistance to *verticilium*, an ascomycete fungus; the flowers were wrapped in muslin prior to flowering to prevent any cross-pollination and the seeds saved for resistance studies.

Results of Principal Component Analysis, utilizing the 15 morphological traits, showed that the first two components characterised 89% of the diversity seen (Table 4.2). The first component (responsible for 61% of the variation observed) had high contributing factor loadings from flowerhead readiness, flowerhead shape and the intensity of green colour. Component 2 (responsible for 27.99% of the variation observed) was defined predominantly by plant height.

Table 4.2. Factor loadings of morphological descriptors for the cardoon populations.

Descriptor	Comp 1	Comp 2
Plant height	0.39	-0.92
No. of offshoots	-0.43	0.27
Distance Flowerhead-1st Leaf	-0.13	-0.86
Stem diameter	-0.96	-0.07
Leaf length	-0.94	-0.05
Leaf lobe no.	-0.86	0.01
Green intensity	0.83	0.20
Green hue	-0.86	-0.21
Greyness	0.27	0.50
Main flowerhead length	-0.62	-0.44
Main flowerhead diameter	-0.69	-0.29
Main flowerhead shape	0.90	0.35
Flowerhead-top shape	-0.80	-0.31
Main flowerhead readiness	0.93	0.27
Total_no of flowerheads	-0.83	-0.06

The plot of the PCA shows that the main useful descriptors were leaf length, total number of flowerheads, plant height, the distance from the main flowerhead to the first leaf and plant maturation (Fig. 4.1). The wild cardoons, Sasso and Tarquinia, were characterized by number of offshoots and total number of flowerheads. While the cultivated cardoon all had purple flowers, the wild populations' flowers were pale pink. The wild plants immature flowerheads, however, were a deeper purple while the cultivated varieties were all green. The wild types were also typified by a more stellate growth form, with narrower leaves and smaller in height that the cultivated varieties were (Fig.4.2).

Fig.4.2. Wild cardoon.

There was no clear pattern between the year of (cultivated) cardoon collection and levels of diversity measured (Fig 4.3 and Fig). Populations from the same decades did not cluster: K4 and K5 were collected in 1950 and are in separate clusters. The same applies to K7, K8, K9 and CYN 12, which were all collected in the 1970s. The remaining populations were collected in the 1980s.

Geographical origin, however, is more relevant as K3 (Moldavia) and K4 (Russia) cluster together, K8 (Spain) and K9 (Algeria) group together, CYN 10 and CYN 12 (both northern Italian varieties) also fall within the same cluster, and CYN 9 and CYN 7 are close (CYN 9 is also Italian but does not cluster with the other Italian varieties). While the physical distances between the countries might be similar (Spain to Italy versus Spain to Algeria), the countries linked in these clusters do tend to exhibit close cultural ties, e.g. Moldavia and Russia, even if Moldavia is geographically closer to Italy. It is also possible that pedo-climactic variables are a factor such that the soils and micro-climate in which the varities were found are similar. Therefore, it is possible that varieties from Spain and Algeria are similar due to their soil or micro-climate requirements.

Other studies have concluded that the level of variation observed in leafy cardoon is quite limited and only few landraces of this crop are known. These cardoon landraces were found to differ only slightly for minor characters relevant to domestication, such as the dimension of leaf stalk, which represents the edible part of this crop. These studies found that the process of domestication has increased the number of flowerheads when compared with wild cardoon (Dellacecca, 1990; Portis et al., 2005). The current study, however, found the opposite, where the wild cardoons possessed a greater number of flowerheads.

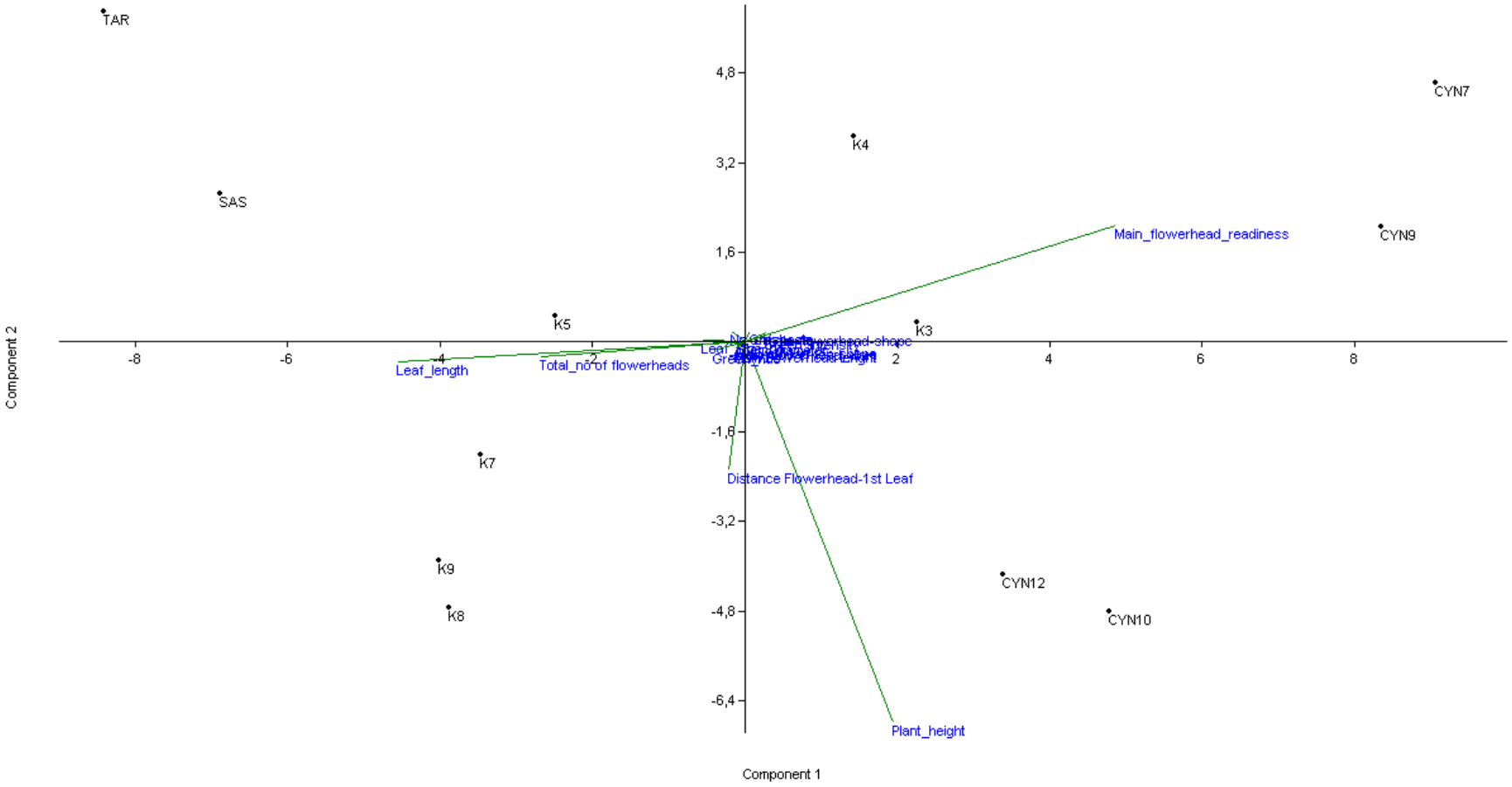


Fig. 4.3. Two dimensional PCA plot of the morphological data and the descriptors used to define the diversity found.

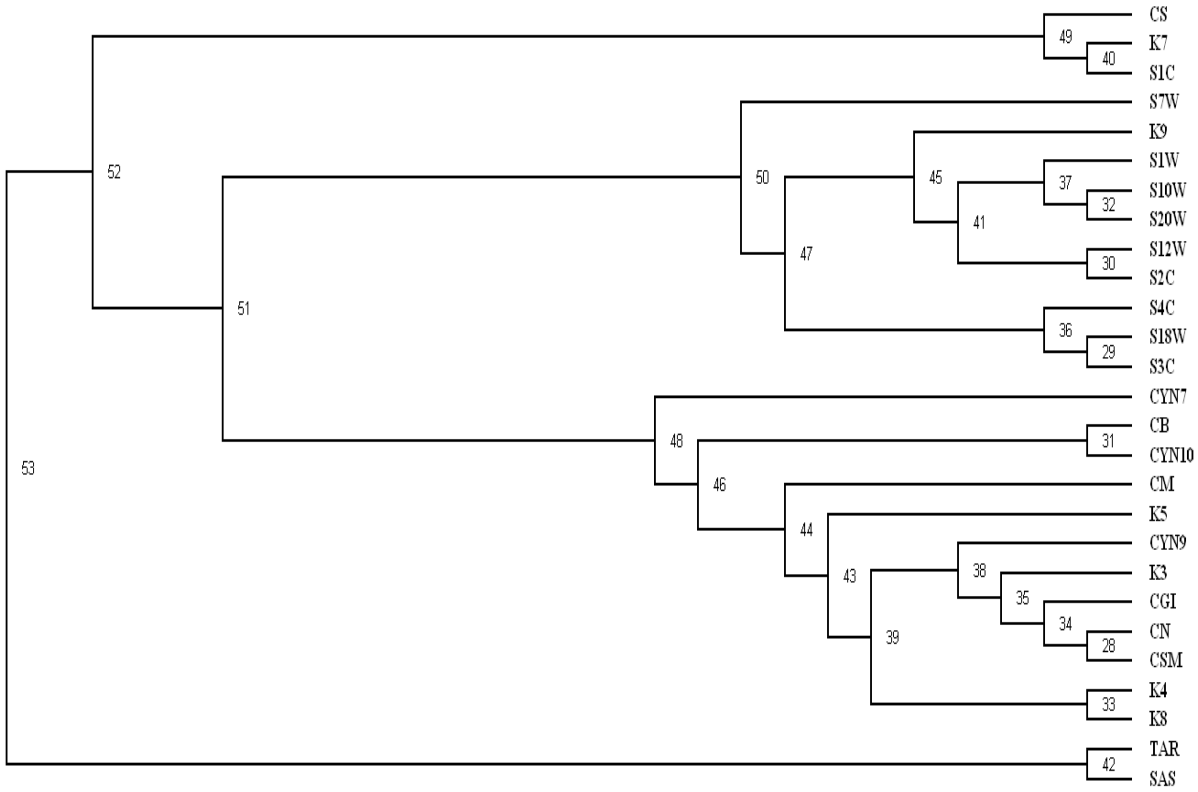


Fig. 4.4. Cluster analysis based on the morphological data.

4.2. MOLECULAR DATA ANALYSIS

This part of the study, in addition to the populations analysed above using agromorphological descriptors, included a further 10 populations (six wild Sicilian populations and four cultivated Italian populations) from ISAFOM, and five populations from the University of Tuscia (one wild and 4 cultivated). Therefore, a total of 27 cardoon populations were assessed, of which nine are wild types.

There were 398 loci found using 4 ISSR markers, and a total of 232 individuals assessed (Talbe 4.3). All populations consisted of eight individuals per populations analysed, except for two wild cardoon populations, Sasso and Tarquinia, which entailed 16 individuals per population as these populations have not been assessed prior to this study.

Table 4.3. A brief overview of the primers used and the alleles found.

ISSR Primer	Total No of Alleles	Rare Alleles	Common Alleles
811	71 alleles	21	50
857	51 alleles	21	28
841	112 alleles	45	67
810	174 alleles	58	116

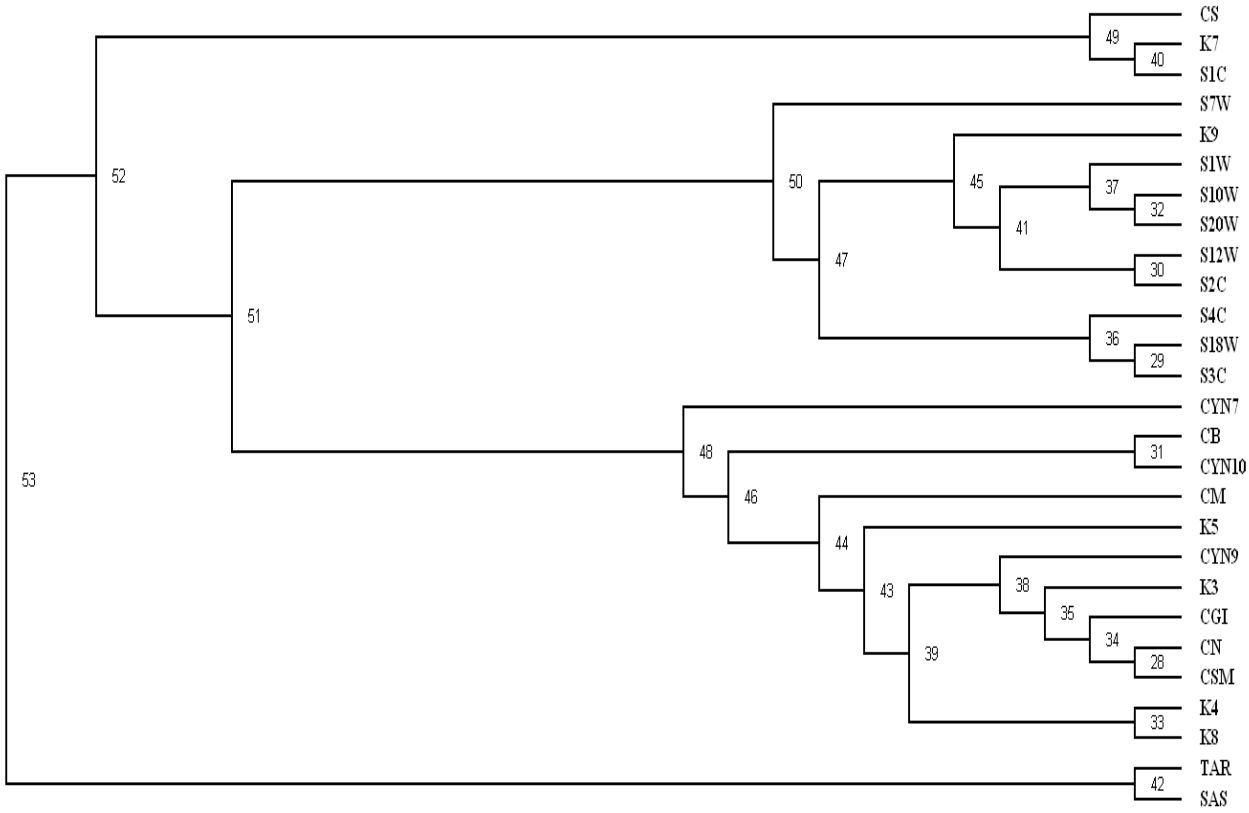


Fig. 4.5. Genetic similarity among 27 cardoon populations, based on Nei's genetic distance.

The cluster analysis based on genetic data shows that the Sicilian populations cluster together, both the wild and cultivated populations (Fig. 4.5). This is probably due to introgression between wild and cultivated island populations. The wild populations from Sasso and Tarquinia form a clear, separate grouping, while Siena, also a wild type, is found distant from Sasso and Tarquinia but close to the cultivar selected for biomass production, S1C, and K7, a cultivated cardoon from Bologna. K 4 and K5, both populations collected in 1950, do fall

within the same cluster but are not close together. There are three populations from Spain (where it is believed that the cardoon was domesticated) and all three do fall within the same cluster but again, not close to one another.

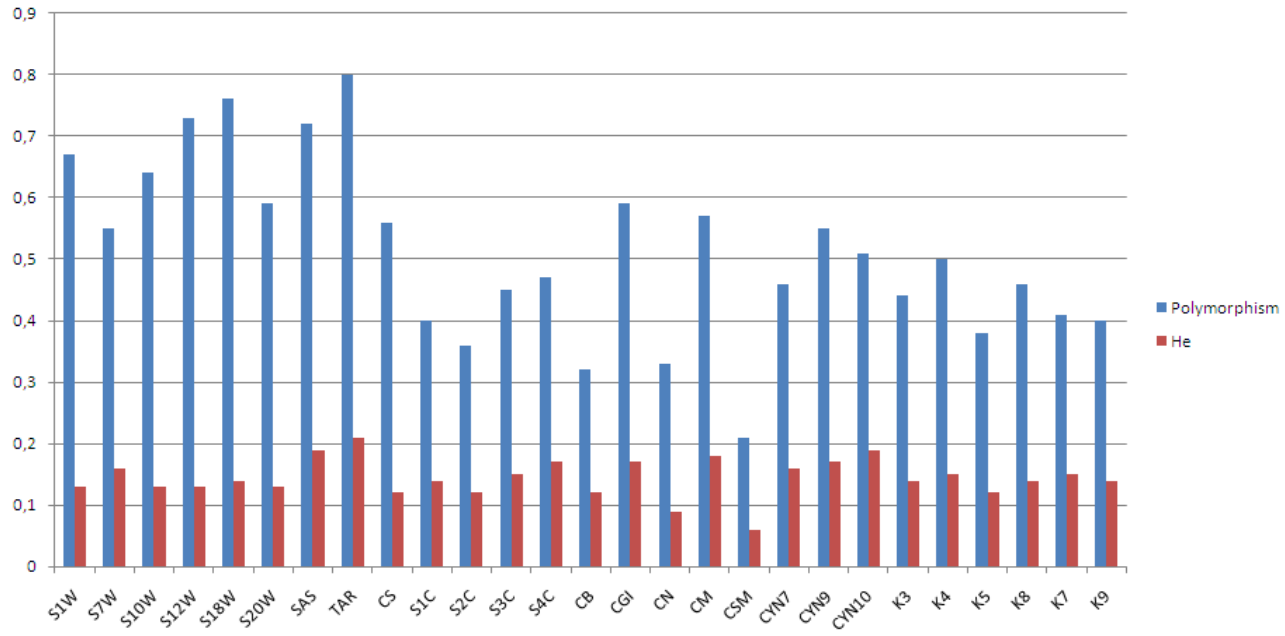


Fig. 4.6. Levels of H_e (expected heterozygosity) compared to observed polymorphism.

The above figure (Fig 4.6) shows the high levels of polymorphism found, when compared to the H_e. The wild populations of Sasso and Tarquinia show the highest levels of polymorphism, with Cardo Scolimus Madrid showing the lowest.

Table 4.4. Bayesian clustering analyses for the pooled cardoon samples (232 individuals; 398 loci) performed using STRUCTURE.

K	**Ln Prob of Data**
2	= -27003.9
3	= -25357.4
4	= -27475.6
5	= -28941.4

The inferred ancestry from a population of the 27 genotypes can also be divided into two major subpopulations using Pritchard's structure analysis (Pritchard et al. 2000) (Table 4.4

and Table 4.5) (an overview of this analysis is provided in Chapter 3).The natural log (ln) probability of the data, which is proportional to the posterior probability, is maximized at k=3 subpopulations (= -25357.4).

The barplot (Fig 4.8) shows intermediate levels of introgression with only Cardo Bologna (CB), Cardo Nizza (CN) and Cardo Scolimus Madrid (CSM) falling into the same cluster with $q > 90\%$. However, these are all population planted in the University of Tuscia field station and it is possible that there has been some crossing taking place, causing hybridization and leading to a skewed result (Fig.4.7).

Fig. 4.7. Cardoons at the University of Tuscia field station.

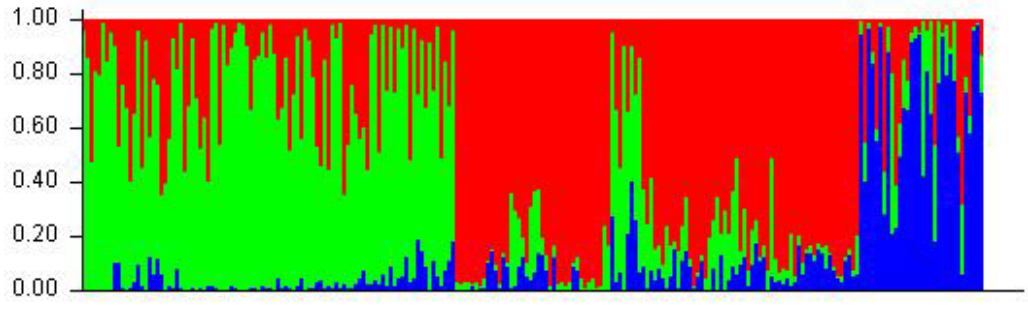


Fig. 4.8. Bar plot of the cardoon populations using STRUCTURE showing the probability of membership of a particular cluster.

Table 4.5. Cardoon populations showing the probability (*q*) of belonging to one of the three clusters. (the populations in purple are wild types)

Population Name	Population	Cluster 1	Cluster 2	Cluster 3
Cardo Nostrano Bolognese	K7	0.16	**0.83**	0.01
Artichok, Algeria	K9	0.33	**0.62**	0.05
Centuripe, Sicily	S10W	0.34	**0.61**	0.05
Francofonte	S7W	0.22	**0.70**	0.08
Misterbianco, Sicily	S12W	0.28	**0.70**	0.02
Paternò, Sicily	S16W	0.18	**0.81**	0.01
Adrano	S1W	0.22	**0.76**	0.02
Raccuja	S20W	0.31	**0.67**	0.02
Cultivated line for biomass	S1C	0.12	**0.88**	0.01
Sicily	S2C	0.28	**0.70**	0.02
Campana	S3C	0.23	**0.73**	0.05
Romagnola	S4C	0.17	**0.75**	0.08
Bologna	CB	**0.97**	0.02	0.01
Cardo Gigante Inerme	CGI	**0.84**	0.09	0.07
Cardo Madrid	CM	**0.80**	0.12	0.08
Cardo Nizza	CN	**0.93**	0.03	0.04
Cardo Scolimus Madrid	CSM	**0.93**	0.07	0.00
Unknown	CYN7	**0.77**	0.18	0.05
Cardo Pieno Inerme	CYN9	**0.84**	0.07	0.09
Moldavia	K3	**0.76**	0.20	0.04
Russian 1950	K4	**0.78**	0.12	0.10
England 1950	K5	**0.85**	0.11	0.03
Spain	K8	**0.84**	0.04	0.12
Cardo Bianco Avorio	CYN10	**0.89**	0.03	0.08
Siena	CS	0.23	0.17	**0.60**
Sasso	Sas	0.20	0.11	**0.68**
Tarquinia	Tar	0.16	0.17	**0.67**

Studies of wild cardoon populations collected in different areas of Sicily exhibited variation for abiotic stresses, such as salinity and water stress resistance during seed germination (Raccuia et al. 2004a). A correlation was also found between genetic variation and geographical origin among seven populations of wild cardoon from Sicily and Sardinia (Portis et al. 2005). Raccuia et al. (2004b) found similar correlation was observed for Sicilian wild cardoon populations.

Further analyses would require the use of more numerous, and diverse types of, molecular markers. Previous studies have shown that wild cardoon and cultivated cardoon are closer to each other than they are to the artichoke (also a *C. cardunculus*) (Sonnante et al., 2002, 2004; Lanteri et al., 2004). It would also have been useful to have included artichoke germplasm as a comparison in this study as well as other wild Cynara species.

6. CONCLUSION

There are many studies utilizing various indicators of diversity, which are useful when one needs to assess the *loss* of diversity. This was not the case with the beans in this study; it is clear that the modern beans are different from the older beans and from the wild beans which were closer to each other than they were to the modern landraces but that diversity loss is not significant. The same applies to the cardoon populations assessed here as the wild types did not possess significantly different levels of intra-varietal diversity from the cultivated varieties.

925 million people in the world today are hungry. It is estimated that that number will grow with an additional 600 million by 2080 due to climate change. In practice, sustainable agriculture can contribute to offsetting this issue in three ways: by reducing the use of fossil fuel through reducing fertilizer production and the use of fossil-fuel powered transport and machinery; by slowing the release of biotic carbon; and by increasing sequestration, particularly in soils. Resilience to climate change in agricultural systems requires the presence of overlapping elements: (i) agro-ecosystem resilience (the persistence and sustainability of yield from the land or sea in the face of a changing climate); (ii) livelihood resilience (achieved through livelihood strategy diversification, such as introducing fish into rice paddies or planting a wider variety of crop species, thus reducing dependence on external inputs). Assessing, maintaining and managing the genetic diversity of crops is essential to contributing to the resilience of a system.

Many traits found in indigenous agricultural varieties will become increasingly important as climate change alters the environment and the pattern of pathogen spread between and within countries. Their protection, along with the local knowledge that is critical to their management and breeding, is critical for the future.

Accurate methods for measuring and monitoring changes in genetic diversity over time are required in order to minimise the problem of genetic erosion. Current methods vary widely in terms of accuracy, ease of carrying out and expense. Obatining material collected over time is a challenge and highlights the need to collect germplasm and document the environmental and

agronomic variables surrounding the origin of those populations.

The molecular level of assessment is the most precise and gives a definitive result in terms of genetic variation within and between varieties. However, it is relatively expensive and time-consuming and requires specialist training and equipment. Necessarily, it can only deal with a limited number of varieties. The high level of technology involved and the subsequent accuracy may, ironically, also be a constraint. As the technology rapidly develops, incompatibilities between methods involved and the data gathered over time may limit the ability to make reliable comparisons over a number of years. Morphology is closely related to genetic makeup, so techniques differentiating varieties based on physical characteristics are useful, particularly in conjunction with the above methods. Local interpretations of characteristics are often incompatible, so the use of 'descriptors' to try and standardize what characters to assess provide a framework for comparison. Nevertheless, the ability of most plants to adapt to various environments over place and time while remaining genetically similar or, conversely, genetically distinct varieties to adopt a similar morphology under similar conditions may limit this approach.

The development of the use of indicators representing diversity within a community holds the greatest promise for useful and practical techniques for assessment over time. This study can contribute to the improvement of germplasm management and conservation through the genetic and morphological diversity assessments undertaken. Such studies are essential in order to evaluate the wild varieties in greater detail with regard to their resistance to biotic and abiotic stresses and thus to contribute to the continued health of the food/forage production systems.To this effect, environmental scientists and the agricultural sector need to work in greater synergy for the protection and management of crop wild relatives, both *in situ* and *ex situ*.

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ANNEX 1. Protocol Used for *Phaseolus vulgaris* DNA Extraction

GeneMATRIX Plant & Fungi DNA Purification Kit

Note 1: The kit is designed for isolation of DNA from different plant organs and tissues (leaves, seeds, fruits) as well as from fungi, algae and lichens. To obtain greatest yield from leaves it is recommended to use youngest leaves possible, as they contain less polysacharides and polyphenols.
Note 2: One minicolumn enables purification of DNA from up to 100 mg wet weight tissue or 20 mg dry weight tissue
(dried, lyophilized plant material).
Note 3: Once the kit is unpacked, store components at room temperature, with the exception of RNase A and Proteinase K. RNase A should be kept at 2÷ 8ºC and Proteinase K at -20ºC. In case of occasional buffer Lyse F ingredients precipitation, simply warm up in 37ºC water bath, until clarified.
Note 4: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.
Note 5: The kit does not contain 96% ethanol, the reagent required during the DNA isolation procedure. This reagent needs to be provided by the user.

1. Apply 40 µl of activation Buffer P onto the spin-column (do not spin) and keep it at room temperature till transfering lysate to the spin-column.

Note 1: Addition of Buffer P onto the center of the resin enables complete wetting of membranes and maximal binding of DNA.
Note 2: The membrane activation should be done before starting isolation procedure.

2. Homogenization of tissue.
Grind plant or fungal tissue under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place sample material (up to 100 mg wet weight tissue or 20 mg dry weight tissue) in 2 ml Eppendorf tube and centrifuge the powder to the bottom of the tube. Add
400 µl of buffer Lyse P (plants, algae, lichens) or buffer Lyse F (fungi). Suspend the precipitate thoroughly.

Note 1: To obtain high yield of DNA a tissue fragment should be thoroughly grinded to a fine powder.

3. Add 3 µl of RNase A and 10 µl of Proteinase K.
4. Mix by vortexing or several-fold inverting the tube and incubate the mixture for 30 min at 65°C (mix twice during incubation by inverting the tube).
5. Add 130 µl of buffer AC, mix thoroughly by inverting and incubate for 5 min on ice.
6. Centrifuge the lysate in a microcentrifuge for 10 min at 14000 rpm.
7. Carefully transfer 400 µl of the supernatant into a new tube.

Note 1: In some cases formed precipitates adhere loosely to the bottom of the tube. In such cases it is advised to transfer supernatant from only a few tubes simultaneously and continue centrifugation of remaining tubes.
Note 2: If it is impossible to transfer 400 µl of the supernatant into a new tube, reduce the starting weight of the sample or transfer as much liquid as possible and adjust the volume of buffer Sol P and 96 % ethanol proportionately in subsequent steps.

8. Add 350 µl of buffer Sol P.
9. Add 250 µl of 96 % ethanol. Mix thoroughly by several times inverting the tube.
10. Centrifuge for 1 min at 12000 rpm.
11. Transfer 600 µl of the supernatant to the spin-column placed in the collection tube.
12. Centrifuge for 1 min at 12000 rpm.

Note 1: Continue centrifugation at 14000 rpm if not all of the lysate passed through the column.

13. Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.
14. Transfer the remaining supernatant to the spin-column placed in the collection tube. Centrifuge again for 1 min at 12000 rpm to filtrate remains of the lysate through the resin.

Note 1: Continue centrifugation at 14000 rpm if not all of the lysate passed through the column.

15. Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.
16. Add 500 µl of buffer Wash PX to the spin-column and centrifuge for 1 min at 12000 rpm.
17. Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.

18. Add 500 µl of buffer Wash PX to the spin-column and centrifuge for 2 min at 12000 rpm.
19. Place the spin-column in a new collection tube (1.5-2 ml) and add 100-200 µl of Elution buffer
(10 mM Tris-HCl, pH 8.5) heated to 70°C to elute bound DNA.

Note 1: Addition of eluting buffer directly onto the center of the resin improves DNA yield. To avoid transfering traces of DNA between the spin-columns do not touch the spin-column walls with the micropippete.
Note 2: The following eluting solutions can be used:
1. 5-10 mM Tris-HCl buffer, pH 8.0-9.0
2. 0.5-1 x TE buffer, pH 8.0-9.0 (not recommended for DNA sequencing).
3. Other special application buffers can be used, provided that their pH and salt concentration is similar to that of
5-10 mM Tris-HCl,
pH 8.0-9.0.

20. Incubate the spin-column/collection tube assembly for 3 min at room temperature.
21. Centrifuge for 1 min at 12000 rpm.

Optional:

22. Repeat elution once again as described in steps 19-21.

Note 1: This step improves DNA recovery from the column. A new collection tube can be used to prevent dilution of the first eluate or collection tube from step 19 can be reused to combine the eluates.
Note 2: More than 200 µl should not be used to elute into a single 1.5 ml microcentrifuge tube, as the spin-column will come into contact with the eluate, causing DNA contamination.

23. Discard the spin-column, cap the collection tube. Genomic DNA is ready for analysis/manipulation. It can be stored either at 2÷8°C or at -20°C.

.

ANNEX 2: DNA extraction manual for cardoon

This quick reference sheet is included for experienced users of the PureLink™ Plant Total DNA Purification Kit.

Preparing Plant Lysate

1. For hard plant tissue, freeze the tissue in liquid nitrogen and grind the tissue to a powder. For soft, non-fibrous plant tissue, cut the tissue into small pieces. For lyophilized samples, proceed to Step 2. Add 250 µl Resuspension Buffer (R2) to 100 mg plant tissue.
2. Prepare lysate by homogenizing the soft tissue with a homogenizer/tissue grinder or vortexing the ground tissue/lyophilized sample until the sample is completely resuspended.
3. Add 15 µl 20% SDS and 15 µl RNase A (20 mg/ml) to lysate.
4. Incubate the lysate at 55°C for 15 minutes to complete lysis.
5. Centrifuge the lysate at high speed for 5 minutes to remove insoluble materials.
6. Transfer supernatant to a sterile microcentrifuge tube and add 100 µl Precipitation Buffer (N2) supplied with the kit to the clear lysate. Mix well and incubate **on ice** for 5 minutes.
7. Centrifuge at maximum speed in a microcentrifuge for 5 minutes at room temperature to produce a clear lysate.
8. Transfer 250 µl clear lysate to a new, sterile microcentrifuge tube and add 375 µl Binding Buffer (B4) with ethanol (page 5) to the lysate. Mix well.
9. Proceed to **Binding DNA**

Purification

ProcedureThe purification procedure is designed for use with a microcentrifuge capable of centrifuging >10,000 x g.

1. **Add** sample from Step 7, previous page to a PureLink™ Spin Cartridge in a collection tube.
2. 2. Centrifuge the cartridge at 10,000 × g for 30 seconds at room temperature. Discard the flow through and place the spin column into the Wash Tube supplied with the kit.
3. **Wash** the cartridge with 500 µl Wash Buffer (W4).
4. Centrifuge the cartridge at 10,000 × g for 30 seconds at room temperature. Discard the flow through and place the column into the tube.

5. **Wash** the cartridge with 500 µl Wash Buffer (W5) with ethanol (page 5).
6. Centrifuge the column at 10,000 x g for 30 seconds at room temperature. Discard the flow through and place the column into the tube.
7. **Repeat** Steps 5-6 one more time.
8. Centrifuge the column at maximum speed for 2 minutes at room temperature to remove any residual Wash Buffer (W5). Discard the collection tube.
9. Place the spin column in a sterile DNase-free 1.5 ml microcentrifuge tube.
10. **Elute** with 100 µl Elution Buffer (E1) or sterile, distilled water (pH >7.0).
11. Incubate at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute.

The elution tube contains the purified DNA.

12. To recover more DNA, perform a second elution step using 100 µl Elution Buffer. Centrifuge the column at maximum speed for 1 minute at room temperature. *The elution tube contains the purified DNA.* Remove and discard the column.
13. Store the purified DNA at -20°C or use DNA for the desired downstream application.

ANNEX 3. Description of Lazio Bean Populations (Bruni 2008)

4.1.1) ECOTIPI DETERMINATI:

BORLOTTO DI GRAFFIGNANO

Specie: *Phaseolus vulgaris* L.
Provenienza: Eusebio Temperini, Graffignano (VT)
Areale di coltivazione: parte orientale della Tuscia Viterbese
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 93 (U.C. 1086,79)

PIANTA
Accrescimento: determinato
Cirri: assenti Portamento:
eretto Altezza (cm): 44,48
± 2,29
Altezza 1°legume (cm): 25,70 ± 1,82
Numero legumi: 4,33 ± 0,69

FOGLIA
Lunghezza (cm): 10,95 ± 0,97
Larghezza (cm): 8,68 ± 0,9
Forma: 1 triangolare
Tonalità: 6
Persistenza: 4

FIORE
Numero fiori per infiorescenza: 3
Grandezza: 3 piccolo
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: viola
Colore petali: viola
N°giorni emergenza-inizio fioritura:
34 (U.C. 451,04)
N°giorni emergenza-piena fioritura:
41 (U.C. 555,41)

LEGUME
Grado di curvatura: 4
Grado di deiscenza: 6
Colore a maturazione fisiologica: giallo chiaro con striature viola chiaro
Presenza di strozzature: 1 assenti
Posizione mucrone: 2 non marginale
Orientamento mucrone: 5 dritto
Tessitura della superficie esterna: 1 liscia
Larghezza trasversale (mm): 12,31 ± 0,24
Lunghezza legume privato del mucrone (cm): 11,69 ± 0,34
Lunghezza mucrone (cm): 1,7 ± 0,1
Numero semi: 2,63 ± 0,26

SEME
Colore principale: marrone rosato scuro
Colore secondario: viola scuro
Distribuzione colore secondario: striato
Luminosità: 3 bassa
Forma: 3 a cubo
Peso (g): 0,65 ± 0,03
Lunghezza (mm): 17,23 ± 0,25
Altezza (mm): 8,9 ± 0,09

CANNELLINO DI ATINA

Specie: *Phaseolus vulgaris* L.
Provenienza: azienda Camilli Olimpio, Villalatina (FR)
Areale di coltivazione: Valle di Comino (FR)
N°giorni semina-emergenza: 7 (U.C.105,53)
N°giorni emergenza-maturazione dei legumi: 111 (U.C. 1185,65)

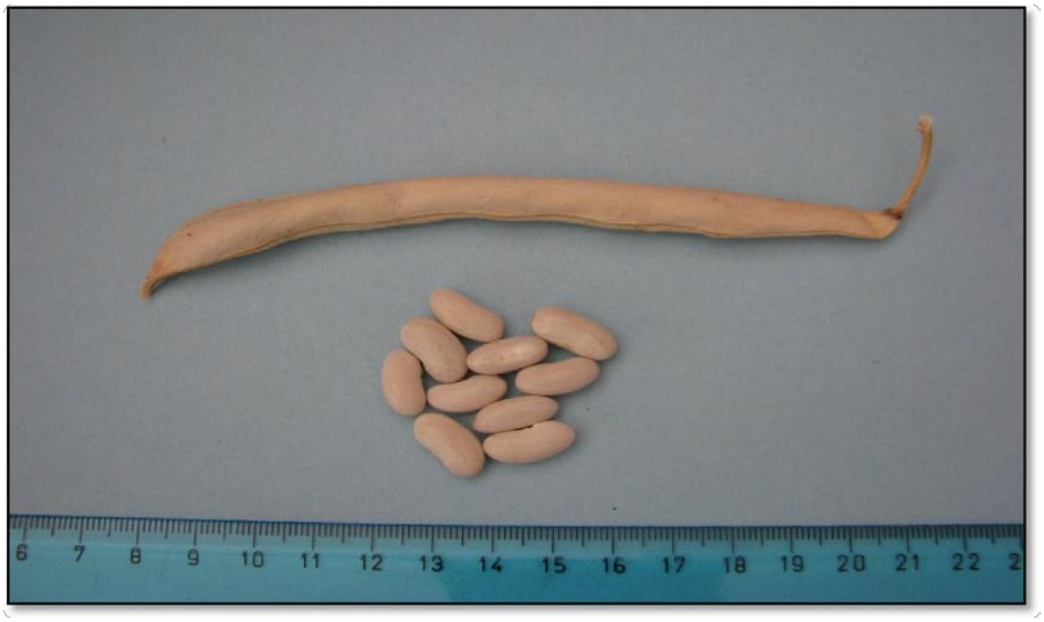
PIANTA
Accrescimento: determinato
Cirri: assenti
Portamento: semi-eretto
Altezza (cm): 59,9 ± 3,28
Altezza 1°legume (cm): 15 ± 2,4
Numero legumi: 11,9 ± 1,47

FOGLIA
Lunghezza (cm): 13,77 ± 0,88
Larghezza (cm): 9,82 ± 0,42
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 4-6
Grandezza: 5 medio
Grado di apertura dei petali: 2
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura:35 (U.C.464,81)
N°giorni emergenza-piena fioritura:41 (U.C. 555,41)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 5 leggermente curvo
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: 10-11 giallo chiaro tendente al bianco, giallo dorato o giallo forte
Presenza di strozzature: 2 leggere
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 1 liscia
Larghezza trasversale (mm): 9,65 ± 0,32
Lunghezza legume privato del mucrone (cm): 12,27 ± 0,37
Lunghezza mucrone (cm): 1,28 ± 0,09
Numero semi: 3,4 ± 0,35

SEME
Colore principale: 7 bianco puro Colore
secondario: 8 biancastro Distribuzione
colore secondario: 1 maculato Luminosità: 6
Forma: 4 reniforme
Grado di curvatura dei semi reniformi: 1 leggera
Peso (g): 0,51 ± 0,02
Lunghezza (mm): 14,99 ± 0,23
Altezza (mm): 7,37 ± 0,09



CANNELLINO DI SULMONA

Specie: *Phaseolus vulgaris* L.
Provenienza: azienda Camilli Olimpio, Villalatina (FR)
Areale di coltivazione: Frusinate
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 132 (U.C. 1287,4)

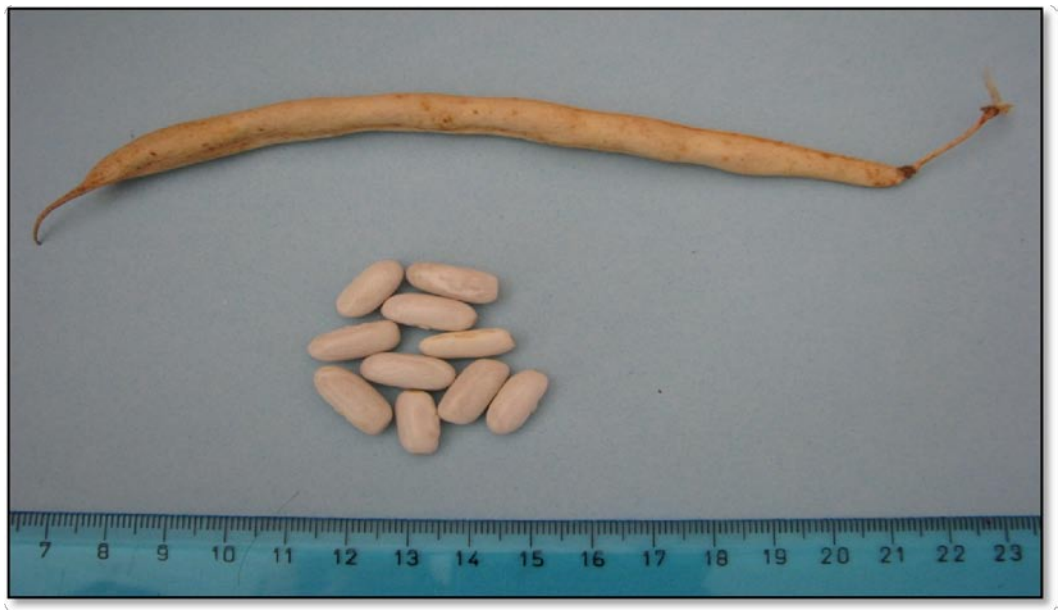
PIANTA
Accrescimento: determinato
Cirri: assenti
Portamento: semi-eretto
Altezza (cm): 51,71 ± 3,81
Altezza 1°legume (cm): 12,33 ± 2,76
Numero legumi: 8,83 ± 1,39

FOGLIA
Lunghezza (cm): 11,61 ± 0,75
Larghezza (cm): 9,16 ± 0,68
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 3 tutte le foglie cadono

FIORE
Numero fiori per infiorescenza: 2-3
Grandezza: 5 medio
Grado di apertura dei petali: 2
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 49 (U.C. 656,62)
N°giorni emergenza-piena fioritura: 58 (U.C. 777,72)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 2 piriforme
Grado di curvatura: 5 leggermente curvo
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: 10-11 giallo chiaro tendente al bianco, giallo dorato o giallo forte
Presenza di strozzature: 2 leggere
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 1 liscia
Larghezza trasversale (mm): 9,38 ± 0,22
Lunghezza legume privato del mucrone (cm): 12,47 ± 0,46
Lunghezza mucrone (cm): 1,28 ± 0,13
Numero semi: 3,28 ± 0,31

SEME
Colore principale: 7 bianco puro Colore secondario: 8 biancastro Distribuzione colore secondario: 1 maculato Luminosità: 6
Forma: 3 a cubo
Peso (g): 0,56 ± 0,02
Lunghezza (mm): 16,23 ± 0,28
Altezza (mm): 7,99 ± 0,15



CANNELLINO MOSCHETTA

Specie: *Phaseolus vulgaris* L.
Provenienza: Camilli Olimpio, Atina (FR)
Areale di coltivazione: Frusinate
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 132 (U.C. 1287,4)

PIANTA
Accrescimento: determinato
Cirri: assenti
Portamento: eretto
Altezza (cm): 35,5 ± 3,67
Altezza 1°legume (cm): 13,42 ± 1,88
Numero legumi: 6,65 ± 0,81

FOGLIA
Lunghezza (cm): 12,18 ± 0,85
Larghezza (cm): 7,68 ± 0,59
Forma: 1 triangolare
Tonalità: 4
Persistenza: 3 tutte le foglie cadono

FIORE
Numero fiori per infiorescenza: 2-6
Grandezza: 3 piccolo
Grado di apertura dei petali: 2
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 44 (U.C. 596,53)
N°giorni emergenza-piena fioritura: 58 (U.C. 777,72)

LEGUME
Grado di curvatura: 4
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: marrone tendente al giallo
Presenza di strozzature: 1 assenti
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 11,5 ± 0,29
Lunghezza legume privato del mucrone (cm): 10,68 ± 0,44
Lunghezza mucrone (cm): 1,1 ± 0,08
Numero semi: 3 ± 0,27

SEME
Colore principale: 8 biancastro
Colore secondario: marrone scuro
Distribuzione colore secondario: 6 campione di colore marginale
Luminosità: 4
Forma: 3 a cubo
Peso (g): 0,56 0,02
Lunghezza (mm): 14,59 ± 0,39
Altezza (mm): 8,74 ± 0,12

CARBONARI

Specie: *Phaseolus vulgaris* L.
Provenienza: azienda D'Apostolo, San Giorgio di Amatrice (RI)
Areale di coltivazione: Alto Reatino
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 118 (U.C. 1229,05)

PIANTA
Accrescimento: determinato
Cirri: assenti Portamento:
eretto Altezza (cm): 75,85
± 3,31
Altezza 1°legume (cm): 21,55 ± 2,55
Numero legumi: 16,6 ± 2,01

FOGLIA
Lunghezza (cm): 11,84 ± 0,78
Larghezza (cm): 7,99 ± 0,30
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 3 tutte le foglie cadono

FIORE
Numero fiori per infiorescenza: 2-4
Grandezza: 3 piccolo
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: fucsia
Colore petali: fucsia
N°giorni emergenza-inizio fioritura: 58 (U.C. 777,72)
N°giorni emergenza-piena fioritura: 63 (U.C. 845,42)

LEGUME
Colore a maturazione cerosa: verde normale con linea viola lungo la sutura
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 4
Grado di deiscenza: 6
Colore a maturazione fisiologica: 11 giallo chiaro tendente al bianco
Presenza di strozzature: 2 leggere
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 dalla parte del dorso
Tessitura della superficie esterna: 1 liscia
Larghezza trasversale (mm): 8,04 ± 0,19
Lunghezza legume privato del mucrone (cm): 8,83 ± 0,25
Lunghezza mucrone (cm): 0,63 ± 0,04
Numero semi: 5,20 ± 0,39

SEME
Colore principale: 1 nero
Luminosità: 3 bassa
Forma: 2 ovale
Peso (g): 0,23 ± 0,01
Lunghezza (mm): 8,83 ± 0,18
Altezza (mm): 5,14 ± 0,10

CONFETTINO

Specie: *Phaseolus vulgaris* L.
Provenienza: azienda Fanelli, Casalvieri (FR)
Areale di coltivazione: Frusinate
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 118 (U.C. 1229,05)

PIANTA
Accrescimento: determinato
Cirri: assenti
Portamento: semi-prostrato
Altezza (cm): 54,28 ± 2,88
Altezza 1°legume (cm): 13,62 ± 1,65
Numero legumi: 10,31 ± 1,39

FOGLIA
Lunghezza (cm): 12,92 ± 1,14
Larghezza (cm): 8,06 ± 0,74
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 3 tutte le foglie cadono

FIORE
Numero fiori per infiorescenza: 2-4
Grandezza: 3 piccolo
Grado di apertura dei petali: 2
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 44 (U.C. 596,53)
N°giorni emergenza-piena fioritura: 58 (U.C. 777,72)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 5 leggermente curvo
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: 11 giallo chiaro tendente al bianco
Presenza di strozzature: 4 pronunciate
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 dalla parte del dorso
Tessitura della superficie esterna: 3 rugosa
Larghezza trasversale (mm): 11,55 ± 0,28
Lunghezza legume privato del mucrone (cm): 9,18 ± 0,24
Lunghezza mucrone (cm): 0,60 ± 0,05
Numero semi: 4,25 ± 0,27

SEME
Colore principale: 7 bianco puro Colore secondario: 8 biancastro Distribuzione colore secondario: 1 maculato Luminosità: 5 media
Forma: 1 rotonda
Peso (g): 0,48 ± 0,03
Lunghezza (mm): 11,17 ± 0,21
Altezza (mm): 9,12 ± 0,16

FAGIOLO PISELLO

Specie: *Phaseolus vulgaris* L.
Provenienza: azienda D'Apostolo, San Giorgio di Amatrice (RI)
Areale di coltivazione: Alto Reatino
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 132 (U.C. 1287,4)

PIANTA
Accrescimento: determinato
Cirri: assenti
Portamento: semi-eretto
Altezza (cm): 54,68 ± 2,01
Altezza 1°legume (cm): 9,5 ± 1,88
Numero legumi: 9,18 ± 1,12

FOGLIA
Lunghezza (cm): 11,21 ± 0,99
Larghezza (cm): 7,37 ± 0,6
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 3 tutte le foglie cadono

FIORE
Numero fiori per infiorescenza: 2-3
Grandezza: 4
Grado di apertura dei petali: 2
Colore stendardo: bianco con striature rosa
Colore petali: rosa
N°giorni emergenza-inizio fioritura: 44 (U.C. 596,53)
N°giorni emergenza-piena fioritura: 63 (U.C. 845,42)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 3
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: 11 giallo chiaro tendente al bianco
Presenza di strozzature: 3 medie
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 dalla parte del dorso
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 11,57 ± 0,35
Lunghezza legume privato del mucrone (cm): 11,61 ± 0,41
Lunghezza mucrone (cm): 1,06 ± 0,06
Numero semi: 3,97 ± 0,37

SEME
Colore principale: 5 da giallo a giallo verdastro
Colore secondario: verde scuro
Distribuzione colore secondario: maculato
Luminosità: 5 media
Forma: 2 ovale
Peso (g): 0,61 ± 0,03
Lunghezza (mm): 12,51 ± 0,21
Altezza (mm): 8,99 ± 0,13

FAGIOLO TABACCHINO

Specie: *Phaseolus vulgaris* L.
Provenienza: azienda Donati Luciano, Acquapendente (VT)
Areale di coltivazione: Alta Tuscia
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 132 (U.C. 1287,4)

PIANTA
Accrescimento: determinato
Cirri: assenti
Portamento: semi-prostrato
Altezza (cm): 39,54 ± 2,83
Altezza 1°legume (cm): 9,27 ± 1,50
Numero legumi: 5,62 ± 1,59

FOGLIA
Lunghezza (cm): 11,08 ± 1,27
Larghezza (cm): 7,89 ± 0,74
Forma: 1 triangolare
Tonalità: 4
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 2-4
Grandezza: 4
Grado di apertura dei petali: 2
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 58 (U.C. 777,72)
N°giorni emergenza-piena fioritura: 63 (U.C. 845,42)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 5 leggermente curvo
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: 11 giallo chiaro tendente al bianco
Presenza di strozzature: 2 leggere
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 10,84 ± 0,36
Lunghezza legume privato del mucrone (cm): 9,46 ± 0,41
Lunghezza mucrone (cm): 1,09 ± 0,11
Numero semi: 3,03 ± 0,3

SEME
Colore principale: giallo arancione
Luminosità: 5 media
Forma: 5 allungata-troncata
Peso (g): 0,45 ± 0,02
Lunghezza (mm): 13,17 ± 0,29
Altezza (mm): 8,29 ± 0,18

ROSSO DI PIUMAROLA

Specie: *Phaseolus vulgaris* L.
Provenienza: azienda Coppola Nascenzo, Villasantalucia (FR)
Areale di coltivazione: Frusinate
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 114 (U.C. 1201,36)

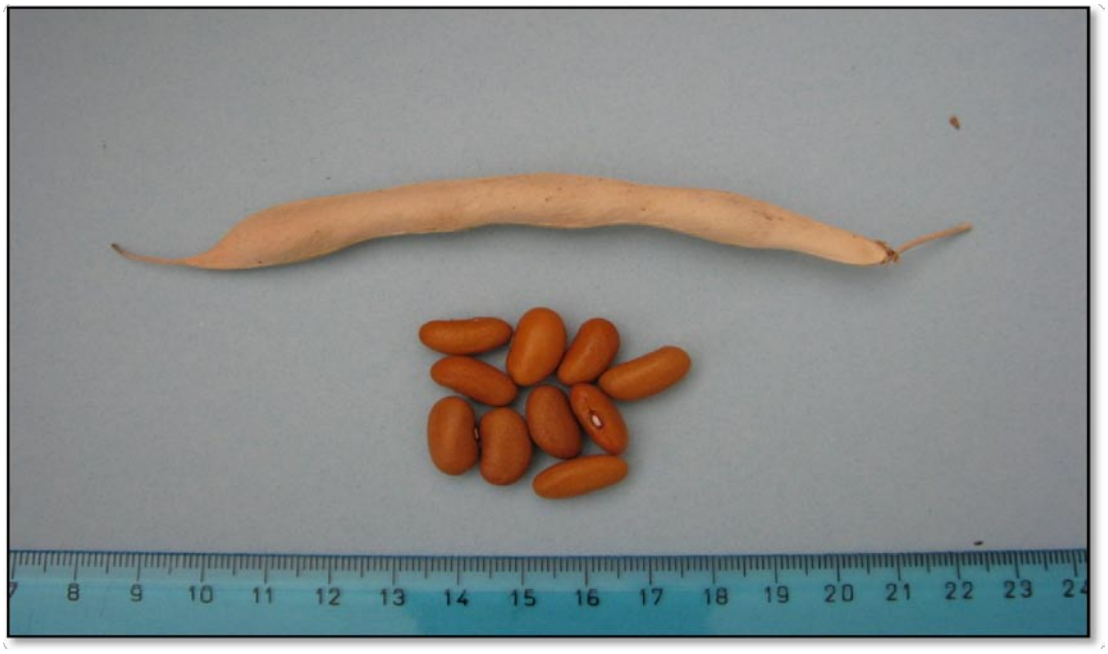
PIANTA
Accrescimento: determinato
Cirri: assenti
Portamento: eretto
Altezza (cm): 55,66 ± 2
Altezza 1°legume (cm): 9,83 ± 1,47
Numero legumi: 11,31 ± 1,2

FOGLIA
Lunghezza (cm): 10,82 ± 1,16
Larghezza (cm): 8,05 ± 0,76
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 3-5
Grandezza: 5 medio
Grado di apertura dei petali: 3
Colore stendardo: rosa
Colore petali: rosa
N°giorni emergenza-inizio fioritura: 41 (U.C. 555,41)
N°giorni emergenza-piena fioritura: 58 (U.C. 777,72)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 6
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: 11 giallo chiaro tendente al bianco
Presenza di strozzature: 2 leggere
Posizione mucrone: 1 marginale
Orientamento mucrone: 6
Tessitura della superficie esterna: 1 liscia
Larghezza trasversale (mm): 10,14 ± 0,29
Lunghezza legume privato del mucrone (cm): 12,15 ± 0,33
Lunghezza mucrone (cm): 1,32 ± 0,09
Numero semi: 3,43 ± 0,34

SEME
Colore principale: marrone chiaro Colore secondario: marrone scuro Distribuzione colore secondario: 1 maculato Luminosità: 5 media
Forma: 4 reniforme
Grado di curvatura dei semi reniformi: 1 leggero
Peso (g): 0,57 ± 0,02
Lunghezza (mm): 14,77 ± 0,31
Altezza (mm): 7,84 ± 0,16



SANGUINELLI

Specie: *Phaseolus vulgaris* L.
Provenienza: azienda D'Apostolo, San Giorgio di Amatrice (RI)
Areale di coltivazione: Alto Reatino
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 111 (U.C. 1185,65)

PIANTA
Accrescimento: determinato
Cirri: assenti Portamento:
eretto Altezza (cm): 45,62
± 2,45
Altezza 1°legume (cm): 10,21 ± 1,81
Numero legumi: 7,59 ± 1,21

FOGLIA
Lunghezza (cm): 11,71 ± 0,73
Larghezza (cm): 8,26 ± 0,64
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 3 tutte le foglie cadono

FIORE
Numero fiori per infiorescenza: 2-4
Grandezza: 5 medio
Grado di apertura dei petali: 4
Colore stendardo: bianco con striature rosa
Colore petali: bianco con striature rosa
N°giorni emergenza-inizio fioritura: 37 (U.C. 494,94)
N°giorni emergenza-piena fioritura: 44 (U.C. 596,53)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 2 piriforme
Grado di curvatura: 4
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: giallo chiaro tendente al dorato
Presenza di strozzature: 3 medie
Posizione mucrone: 1 marginale
Orientamento mucrone: 5 dritto
Tessitura della superficie esterna: 3 rugosa
Larghezza trasversale (mm): 10,81 ± 0,35
Lunghezza legume privato del mucrone (cm): 12,13 ± 0,45
Lunghezza mucrone (cm): 1,32 ± 0,08
Numero semi: 3,25 ± 0,31

SEME
Colore principale: rosso scuro
Luminosità: 7 brillante
Forma: 4 reniforme
Grado di curvatura dei semi reniformi: 1 leggero
Peso (g): 0,52 ± 0,02
Lunghezza (mm): 16,57 ± 0,35
Altezza (mm): 8,35 ± 0,15

SOLFARINO

Specie: *Phaseolus vulgaris* L.
Provenienza: Camilli Marco, Onano (VT)
Areale di coltivazione: Alta Tuscia
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 132 (U.C. 1287,4)

PIANTA
Accrescimento: determinato
Cirri: assenti
Portamento: semi-eretto
Altezza (cm): 42,19 ± 1,93
Altezza 1°legume (cm): 11,56 ± 1,19
Numero legumi: 19,04 ± 2,17

FOGLIA
Lunghezza (cm): 10,58 ± 1,01
Larghezza (cm): 7,39 ± 0,59
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 3 tutte le foglie cadono

FIORE
Numero fiori per infiorescenza: 3-4
Grandezza: 3 piccolo
Grado di apertura dei petali: 3 chiusi e paralleli
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 44 (U.C. 596,53)
N°giorni emergenza-piena fioritura: 58 (U.C. 777,72)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 4
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: marrone
Presenza di strozzature: 1 assenti
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 dalla parte del dorso
Tessitura della superficie esterna: 1 liscia
Larghezza trasversale (mm): 10,35 ± 0,25
Lunghezza legume privato del mucrone (cm): 8,52 ± 0,28
Lunghezza mucrone (cm): 1,09 ± 0,1
Numero semi: 3,6 ± 0,35

SEME
Colore principale: verde chiaro tendente al giallo
Luminosità: 4
Forma: 2 ovale
Peso (g): 0,34 ± 0,02
Lunghezza (mm): 9,81 ± 0,19
Altezza (mm): 7,37 ± 0,13

VERDOLINO

Specie: *Phaseolus vulgaris* L.
Provenienza: Camilli Marco, Onano (VT)
Areale di coltivazione: Alta Tuscia
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 111 (U.C. 1185,65)

PIANTA
Accrescimento: determinato
Cirri: assenti
Portamento: semi-eretto
Altezza (cm): 34,73 ± 1,87
Altezza 1°legume (cm): 10,62 ± 1,16
Numero legumi: 5,77 ± 0,68

FOGLIA
Lunghezza (cm): 10,09 ± 1,12
Larghezza (cm): 7,44 ± 0,78
Forma: 1 triangolare
Tonalità: 4
Persistenza: 4

FIORE
Numero fiori per infiorescenza: 2
Grandezza: 4
Grado di apertura dei petali:
5 petali moderatamente divergenti
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 44 (U.C. 596,53)
N°giorni emergenza-piena fioritura: 58 (U.C. 777,72)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 5 leggermente curvo
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: 11 giallo chiaro tendente al bianco
Presenza di strozzature: 3 medie
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 1 liscia
Larghezza trasversale (mm): 10,97 ± 0,31
Lunghezza legume privato del mucrone (cm): 9,23 ± 0,23
Lunghezza mucrone (cm): 1,04 ± 0,07
Numero semi: 3,38 ± 0,26

SEME
Colore principale: verdastro Colore secondario: 11 verde oliva Distribuzione colore secondario: 1 maculato Luminosità: 5 media
Forma: 2 ovale
Peso (g): 0,37 ± 0,02
Lunghezza (mm): 11,33 ± 0,22
Altezza (mm): 7,72 ± 0,17

4.1.2) ECOTIPI INDETERMINATI:

BORLOTTO BINGO CILENO

Specie: *Phaseolus vulgaris* L.
Provenienza: Zuchi Domenico, Sutri (VT)
Areale di coltivazione: Bassa Tuscia
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 132 (U.C. 1287,4)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 10,78 ± 0,97
Larghezza (cm): 7,71 ± 0,73
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 2
Grandezza: 3 piccolo
Grado di apertura dei petali: 1
Colore stendardo: bianco con striature rosa
Colore petali: bianco con striature rosa
N°giorni emergenza-inizio fioritura: 49 (U.C. 656,62)
N°giorni emergenza-piena fioritura: 58 (U.C. 777,72)

LEGUME
Grado di curvatura: 6
Grado di deiscenza: 5
Colore a maturazione fisiologica: marrone con striature violacee scure
Presenza di strozzature: 2 leggere
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 3 rugosa
Larghezza trasversale (mm): 15,49 ± 0,38
Lunghezza legume privato del mucrone (cm): 14,98 ± 0,46
Lunghezza mucrone (cm): 1,14 ± 0,10
Numero semi: 3,21 ± 0,31

SEME
Colore principale: marrone chiaro rosato
Colore secondario: viola scuro
Distribuzione colore secondario: 2 striato
Luminosità: 3 bassa
Forma: 3 a cubo
Peso (g): 1,04 ± 0,08
Lunghezza (mm): 17,96 ± 0,51
Altezza (mm): 10,52 ± 0,25

BORLOTTO DI GRISCIANO

Specie: *Phaseolus vulgaris* L.
Provenienza: cooperativa agricola zootecnica Grisciano, Grisciano di Accumoli (RI)
Areale di coltivazione: Alto Reatino
N°giorni semina-emergenza: 7 (U.C. 105,5)
N°giorni emergenza-maturazione dei legumi: 132 (U.C. 1287,4)

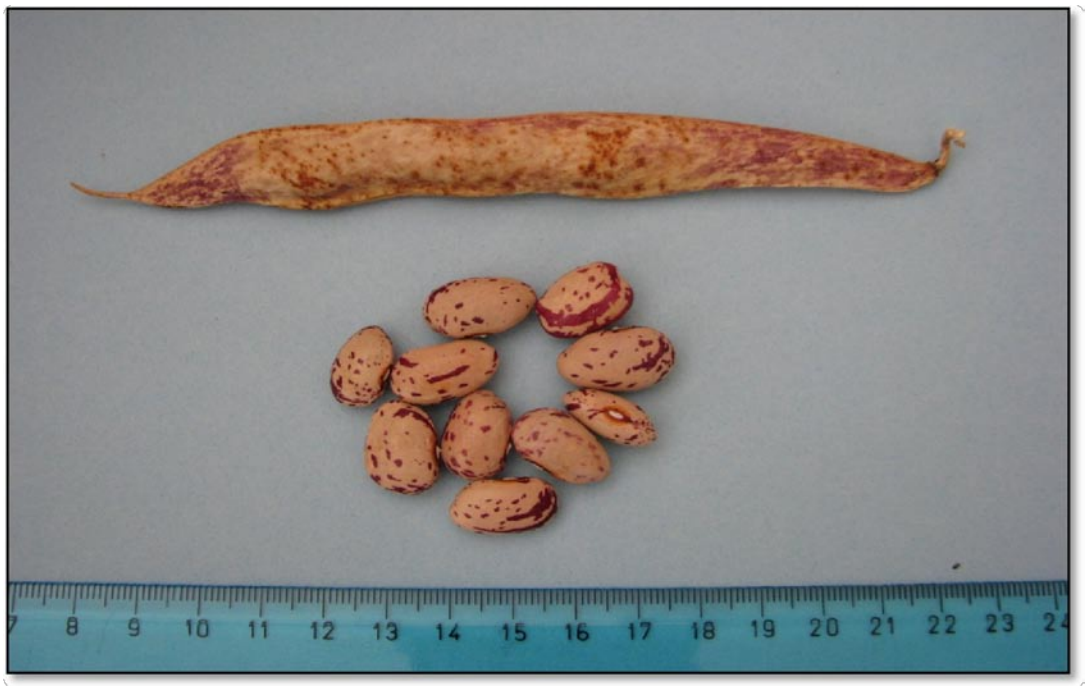
PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 10,2 ± 0,64
Larghezza (cm): 6,89 ± 0,48
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 2
Grandezza: 3 piccolo
Grado di apertura dei petali: 2
Colore stendardo: rosa
Colore petali: rosa
N°giorni emergenza-inizio fioritura: 49 (U.C. 656,62)
N°giorni emergenza-piena fioritura: 63 (U.C. 845,42)

LEGUME
Colore a maturazione cerosa: 4 verde strato di carmine
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 5 leggermente curvo
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: giallo chiaro con striature viola scuro
Presenza di strozzature: 3 medie
Posizione mucrone: 1 marginale
Orientamento mucrone: 7 dalla parte del ventre
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 15,3 ± 0,42
Lunghezza legume privato del mucrone (cm): 14,69 ± 0,48
Lunghezza mucrone (cm): 1,38 ± 0,2
Numero semi: 3,83 ± 0,28

SEME
Colore principale: marrone chiaro rosato
Colore secondario: rosso scuro o viola scuro
Distribuzione colore secondario: striato
Luminosità: 4
Forma: 3 a cubo
Peso (g): 0,81 ± 0,04
Lunghezza (mm): 16,05 ± 0,36
Altezza (mm): 10,52 ± 0,24



BORLOTTO DI LAMON

Specie: *Phaseolus vulgaris* L.
Provenienza: Cardinale Augusto, Valmontone (RM)
Areale di coltivazione: Valle del Sacco (RM)
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 132 (U.C. 1287,4)

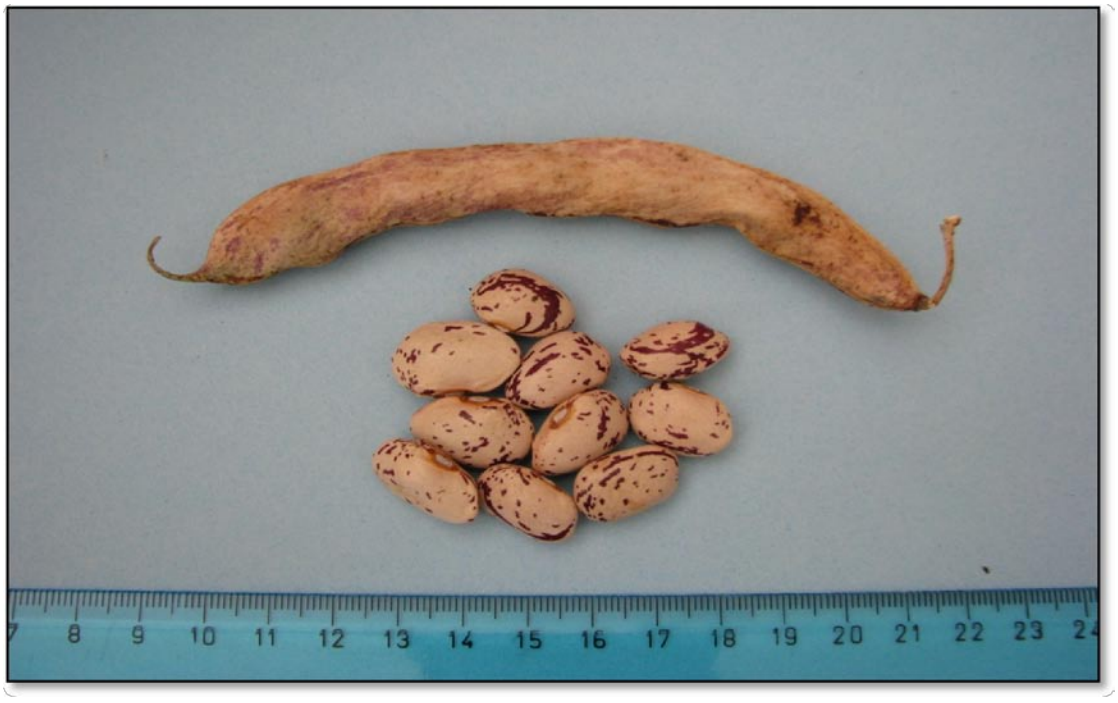
PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 10,21 ± 0,7
Larghezza (cm): 6,83 ± 0,73
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 3 tutte le foglie cadono

FIORE
Numero fiori per infiorescenza: 4-12
Grandezza: 3 piccolo
Grado di apertura dei petali: 2
Colore stendardo: rosa
Colore petali: rosa
N°giorni emergenza-inizio fioritura: 49 (U.C. 656,62)
N°giorni emergenza-piena fioritura: 69 (U.C. 928,3)

LEGUME
Grado di curvatura: 5 leggermente curvo
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: marrone con striature viola
Presenza di strozzature: 1 assenti
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 dalla parte del dorso
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 17,39 ± 0,34
Lunghezza legume privato del mucrone (cm): 12,75 ± 0,33
Lunghezza mucrone (cm): 1,34 ± 0,1
Numero semi: 3,03 ± 0,24

SEME
Colore principale: marrone chiaro Colore secondario: viola scuro Distribuzione colore secondario: 2 striato Luminosità: 3 bassa
Forma: 2 ovale
Peso (g): 1,09 ± 0,05
Lunghezza (mm): 17,92 ± 0,37
Altezza (mm): 12,49 ± 0,27



BORLOTTO REGINA

DI MARANO

EQUO

Specie: *Phaseolus vulgaris* L.
Provenienza: Rotili Giuseppe, Marano Equo (RM)
Areale di coltivazione: Valle dell'Aniene (RM)
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 132 (U.C. 1287,4)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 9,74 ± 0,45
Larghezza (cm): 6,53 ± 0,34
Forma: 1 triangolare
Tonalità: 4
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 2-3
Grandezza: 3 piccolo
Grado di apertura dei petali: 1
Colore stendardo: rosa
Colore petali: rosa
N°giorni emergenza-inizio fioritura: 58 (U.C. 777,72)
N°giorni emergenza-piena fioritura: 69 (U.C. 928,3)

LEGUME
Grado di curvatura: 6
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: marrone con striature viola chiare
Presenza di strozzature: 1 assenti
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 dalla parte del dorso
Tessitura della superficie esterna: 3 rugosa
Larghezza trasversale (mm): 16,20 ± 0,47
Lunghezza legume privato del mucrone (cm): 15,18 ± 0,39
Lunghezza mucrone (cm): 1,21 ± 0,11
Numero semi: 4,13 ± 0,21

SEME
Colore principale: marrone chiaro rosato
Colore secondario: viola chiaro
Distribuzione colore secondario: 2 striato
Luminosità: 3 bassa
Forma: 2 ovale
Peso (g): 0,92 ± 0,04
Lunghezza (mm): 15,64 ± 0,27
Altezza (mm): 10,45 ± 0,21

BORLOTTO REGINA DI SUTRI

Specie: *Phaseolus vulgaris* L.
Provenienza: Zuchi Domenico, Sutri (VT)
Areale di coltivazione: Bassa Tuscia
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 133 (U.C. 1288,53)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 10,47 ± 0,95
Larghezza (cm): 7,13 ± 0,71
Forma: 1 triangolare
Tonalità: 4
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 1-2
Grandezza: 3 piccolo
Grado di apertura dei petali: 1
Colore stendardo: rosa
Colore petali: rosa
N°giorni emergenza-inizio fioritura: 58 (U.C. 777,72)
N°giorni emergenza-piena fioritura: 69(U.C. 928,3)

LEGUME
Grado di curvatura: 5 leggermente curvo
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: marrone con striature viola
Presenza di strozzature: 1 assenti
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 3 rugosa
Larghezza trasversale (mm): 14,56 ± 0,40
Lunghezza legume privato del mucrone (cm): 16,01 ± 0,59
Lunghezza mucrone (cm): 1,07 ± 0,1
Numero semi: 4,29 ± 0,37

SEME
Colore principale: marrone chiaro rosato
Colore secondario: viola scuro
Distribuzione colore secondario: 2 striato
Luminosità: 3 bassa
Forma: 3 a cubo
Peso (g): 0,97 ± 0,07
Lunghezza (mm): 17,36 ± 0,43
Altezza (mm): 10,23 ± 0,22

BOTTONCINO DI TERELLE

Specie: *Phaseolus vulgaris* L.
Provenienza: azienda Tari, Terelle (FR)
Areale di coltivazione: Terelle
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 133 (U.C. 1288,53)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 9,94 ± 0,55
Larghezza (cm): 7,4 ± 0,47
Forma: 1 triangolare
Tonalità: 6
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 3-4
Grandezza: 4
Grado di apertura dei petali: 2
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 49 (U.C. 656,62)
N°giorni emergenza-piena fioritura: 69 (U.C 928,3)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 4
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: 11 giallo chiaro tendente al bianco
Presenza di strozzature: 2 leggere
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 dalla parte del dorso
Tessitura della superficie esterna: 1 liscia
Larghezza trasversale (mm): 10,29 ± 0,33
Lunghezza legume privato del mucrone (cm): 10,43 ± 0,46
Lunghezza mucrone (cm): 0,96 ± 0,08
Numero semi: 4,27 ± 0,41

SEME
Colore principale: 7 bianco puro Colore secondario: 8 biancastro Distribuzione colore secondario: 1 maculato Luminosità: 6
Forma: 2 ovale
Peso (g): 0,34 ± 0,03
Lunghezza (mm): 10,39 ± 0,27
Altezza (mm): 7,24 ± 0,20

CAPPELLETTE

Specie: *Phaseolus vulgaris* L.
Provenienza: Rodonti Iolanda, Vallepietra (RM)
Areale di coltivazione: Valle dell'Aniene (RM)
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 133 (U.C. 1288,53)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 11,04 ± 1,45
Larghezza (cm): 7,45 ± 1,24
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 3 tutte le foglie cadono

FIORE
Numero fiori per infiorescenza: 2-10
Grandezza: 3 piccolo
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: bianco con striature rosa
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 44 (U.C. 596,53)
N°giorni emergenza-piena fioritura: 58 (U.C. 777,72)

LEGUME
Grado di curvatura: 5 leggermente curvo
Grado di deiscenza: 3 legume fortemente contratto (tipo lomentaceo)
Colore a maturazione fisiologica: marrone tendente al giallo scuro
Presenza di strozzature: 1 assenti
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 3 rugosa
Larghezza trasversale (mm): 11,76 ± 0,21
Lunghezza legume privato del mucrone (cm): 10,63 ± 0,31
Lunghezza mucrone (cm): 0,52 ± 0,06
Numero semi: 3,72 ± 0,41

SEME
Colore principale: bianco
Colore secondario: rosso bordeaux
Distribuzione colore secondario: 9 chiazzato bicolore
Luminosità: 6
Forma: 2 ovale
Peso (g): 0,51 ± 0,03
Lunghezza (mm): 11,89 ± 0,26
Altezza (mm): 9,07 ± 0,16

CHIARINELLI

Specie: *Phaseolus vulgaris* L.
Provenienza: cooperativa agricola zootecnica Grisciano, Grisciano di Accumoli (RI)
Areale di coltivazione: Alto Reatino
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 102 (1126,94)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 11,23 ± 0,94
Larghezza (cm): 8,06 ± 0,99
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 4-6
Grandezza: 5
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: rosa
Colore petali: rosa
N°giorni emergenza-inizio fioritura: 58 (U.C. 777,72)
N°giorni emergenza-piena fioritura: 69 (U.C. 928,3)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 7 curvo
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: 10 giallo dorato o giallo chiaro
Presenza di strozzature: 2 leggere
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 dalla parte del dorso
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 14,35 ± 0,48
Lunghezza legume privato del mucrone (cm): 13,34 ± 0,71
Lunghezza mucrone (cm): 0,91 ± 0,1
Numero semi: 3,1 ± 0,42

SEME
Colore principale: marrone chiaro Colore secondario: marrone scuro Distribuzione colore secondario: 1 maculato Luminosità: 5 media
Forma: 3 a cubo
Peso (g): 0,87 ± 0,04
Lunghezza (mm): 15,12 ± 0,45
Altezza (mm): 10,41 ± 0,15

CIAVATTONE DI ONANO

Specie: *Phaseolus vulgaris* L.
Provenienza: azienda Camilli Marco, Onano (VT)
Areale di coltivazione: Alta Tuscia
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 132 (U.C. 1287,4)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 10,99 ± 0,67
Larghezza (cm): 8,9 ± 0,64
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 4
Grandezza: 4
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 49 (U.C. 656,62)
N°giorni emergenza-piena fioritura: 58 (U.C. 777,72)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 2 piriforme
Grado di curvatura: 7 curvo
Grado di deiscenza: 6
Colore a maturazione fisiologica: 11 giallo chiaro tendente al bianco
Presenza di strozzature: 3 medie
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 dalla parte del dorso
Tessitura della superficie esterna: 1 liscia
Larghezza trasversale (mm): 12,58 ± 0,3
Lunghezza legume privato del mucrone (cm): 12,41 ± 0,43
Lunghezza mucrone (cm): 1 ± 0,08
Numero semi: 3 ± 0,35

SEME
Colore principale: 7 bianco puro Colore secondario: 8 biancastro Distribuzione colore secondario: 1 maculato Luminosità: 5 media
Forma: 4 reniforme
Grado di curvatura dei semi reniformi: 1 leggero
Peso (g): 0,57 ± 0,03
Lunghezza (mm): 15,75 ± 0,42
Altezza (mm): 9,07 ± 0,23

CIONCONE DI VALLINFREDA

Specie: *Phaseolus vulgaris* L.
Provenienza: Proietti Teodora, Vallinfreda (RM)
Areale di coltivazione: Valle dell'Aniene (RM)
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 133 (U.C. 1288,53)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 11,34 ± 0,45
Larghezza (cm): 7,4 ± 0,41
Forma: 1 triangolare
Tonalità: 4
Persistenza: 6

FIORE
Numero fiori per infiorescenza: 2-7
Grandezza: 4
Grado di apertura dei petali: 2
Colore stendardo: rosa
Colore petali: rosa con striature rosa scuro
N°giorni emergenza-inizio fioritura: 49 (U.C. 656,52)
N°giorni emergenza-piena fioritura: 66 (U.C. 880,89)

LEGUME
Grado di curvatura: 5 leggermente curvo
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: marrone tendente al giallo
Presenza di strozzature: 1 assenti
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 14,82 ± 0,32
Lunghezza legume privato del mucrone (cm): 13,31 ± 0,35
Lunghezza mucrone (cm): 0,6 ± 0,06
Numero semi: 3,67 ± 0,37

SEME
Colore principale: verdastro
Colore secondario: marrone
Distribuzione colore secondario: 10 campione alla volta dell'ilo
Luminosità: 4
Forma: 2 ovale
Peso (g): 0,84 ± 0,03
Lunghezza (mm): 13,69 ± 0,24
Altezza (mm): 10,59 ± 0,2

COCCO

Specie: *Phaseolus vulgaris* L.
Provenienza: Camilli Marco, Onano (VT)
Areale di coltivazione: Alta Tuscia
N°giorni semina-emergenza: 7 (U.C. 105,76)
N°giorni emergenza-maturazione dei legumi: 92 (U.C. 1071,51)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 10,42 ± 1,1
Larghezza (cm): 7,42 ± 0,9
Forma: 1 triangolare
Tonalità: 4
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 2-4
Grandezza: 3 piccolo
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: rosa
Colore petali: rosa
N°giorni emergenza-inizio fioritura: 36 (U.C. 479,66)
N°giorni emergenza-piena fioritura: 48 (U.C. 641,34)

LEGUME
Grado di curvatura: 6
Grado di deiscenza: 5
Colore a maturazione fisiologica: 11 giallo chiaro tendente al bianco
Presenza di strozzature: 3 medie
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 9,71 ± 0,39
Lunghezza legume privato del mucrone (cm): 10,32 ± 0,31
Lunghezza mucrone (cm): 0,82 ± 0,08
Numero semi: 3,83 ± 0,45

SEME
Colore principale: marrone chiaro
Luminosità: 4
Forma: 3 a cubo
Peso (g): 0,49 ± 0,02
Lunghezza (mm): 12,32 ± 0,28
Altezza (mm): 6,52 ± 0,15

FAGIOLETTI DI VALLEPIETRA

Specie: *Phaseolus vulgaris* L.
Provenienza: Rodonti Iolanda, Vallepietra (RM)
Areale di coltivazione: Valle dell'Aniene (RM)
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 133 (U.C. 1287,9)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 11,49 ± 0,79
Larghezza (cm): 8,31 ± 0,72
Forma: 1 triangolare
Tonalità: 4
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 2-3
Grandezza: 4
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: viola
Colore petali: viola
N°giorni emergenza-inizio fioritura: 44 (U.C. 596,53)
N°giorni emergenza-piena fioritura: 59 (U.C. 790,6)

LEGUME
Grado di curvatura: 4
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: marrone tendente al giallo
Presenza di strozzature: 2 leggere
Posizione mucrone: 1 marginale
Orientamento mucrone: 6
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 12,92 ± 0,42
Lunghezza legume privato del mucrone (cm): 12,34 ± 0,38
Lunghezza mucrone (cm): 0,56 ± 0,05
Numero semi: 3,73 ± 0,34

SEME
Colore principale: 1 nero
Luminosità: 7 brillante
Forma: 3 a cubo
Peso (g): 0,65 ± 0,03
Lunghezza (mm): 14,37 ± 0,38
Altezza (mm): 9,44 ± 0,25

FAGIOLINA ARSOLANA

Specie: *Phaseolus vulgaris* L.
Provenienza: Proietti Teodora, Vallinfreda (RM)
Areale di coltivazione: Arsoli, Valle dell'Aniene (RM)
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 133 (U.C. 1288,53)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 11,71 0,81
Larghezza (cm): 8,93 0,51
Forma: 1 triangolare
Tonalità: 4
Persistenza: 3 tutte le foglie cadono

FIORE
Numero fiori per infiorescenza: 4-5
Grandezza: 4
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 49 (U.C 656,62)
N°giorni emergenza-piena fioritura: 58 (U.C. 777,72)

LEGUME
Grado di curvatura: 7 curvo
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: marrone chiaro tendente al giallo
Presenza di strozzature: 3 medie
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 10,14 ± 0,29
Lunghezza legume privato del mucrone (cm): 12,47 ± 0,4
Lunghezza mucrone (cm): 0,69 ± 0,05
Numero semi: 4,44 ± 0,4

SEME
Colore principale: bianco
Luminosità: 5 media
Forma: 3 a cubo
Peso (g): 0,36 ± 0,02
Lunghezza (mm): 12,72 ± 0,25
Altezza (mm): 7,71 ± 0,12

FAGIOLO BURRO

Specie: *Phaseolus vulgaris* L.
Provenienza: azienda Camilli Olimpio, Villalatina (FR)
Areale di coltivazione: Valle di Comino (FR)
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 133 (U.C. 1288,53)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 11,87 ± 0,67
Larghezza (cm): 8,26 ± 0,8
Forma: 1 triangolare
Tonalità: 4
Persistenza: 3 tutte le foglie cadono

FIORE
Numero fiori per infiorescenza: 3-4
Grandezza: 5 medio
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: rosa
Colore petali: rosa
N°giorni emergenza-inizio fioritura: 44 (U.C. 596,53)
N°giorni emergenza-piena fioritura: 58 (U.C.777,72

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 5 leggermente curvo
Grado di deiscenza: 4
Colore a maturazione fisiologica: 10 giallo dorato o giallo forte
Presenza di strozzature: 3 medie
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 dalla parte del dorso
Tessitura della superficie esterna: 3 rugosa
Larghezza trasversale (mm): 13,52 ± 0,36
Lunghezza legume privato del mucrone (cm): 11,99 ± 0,41
Lunghezza mucrone (cm): 0,85 ± 0,08
Numero semi: 3,42 ± 0,31

SEME
Colore principale: marrone chiaro tendente al verde
Colore secondario: 11 verde oliva
Distribuzione colore secondario: 1 maculato
Luminosità: 4
Forma: 2 ovale
Peso (g): 0,81 ± 0,03
Lunghezza (mm): 13,85 ± 0,25
Altezza (mm): 10,6 ± 0,15

FAGIOLO DI FRATTA

Specie: *Phaseolus vulgaris* L.
Provenienza: Camilli Olimpio, Villalatina (FR)
Areale di coltivazione: Valle di Comino (FR)
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 132 (U.C. 1287,4)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 10,54 ± 0,47
Larghezza (cm): 8,21 ± 0,41
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 3 tutte le foglie cadono

FIORE
Numero fiori per infiorescenza: 2-10
Grandezza: 3 piccolo
Grado di apertura dei petali: 2
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 58 (U.C. 777,72)
N°giorni emergenza-piena fioritura: 63 (U.C. 845,42)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 5 leggermente curvo
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: 11 giallo chiaro tendente al bianco
Presenza di strozzature: 2 leggere
Posizione mucrone: 1 marginale
Orientamento mucrone: 7 dalla parte del ventre
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 12,63 ± 0,43
Lunghezza legume privato del mucrone (cm): 10,30 ± 0,36
Lunghezza mucrone (cm): 0,57 ± 0,06
Numero semi: 3,48 ± 0,33

SEME
Colore principale: 7 bianco puro
Colore secondario: 8 biancastro
Distribuzione colore secondario: 1 maculato
Luminosità: 6
Forma: 2 ovale
Peso (g): 0,62 ± 0,03
Lunghezza (mm): 12,95 ± 0,05
Altezza (mm): 9,37 ± 0,13

FAGIOLONE DI GRISCIANO

Specie: *Phaseolus coccineus* L. (*multiflorum* Willd.)
Provenienza: cooperativa agricola zootecnica Grisciano, Grisciano di Accumoli (RI)
Areale di coltivazione: Alto Reatino
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 149 (U.C. 1272.78)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 11,09 ± 1,05
Larghezza (cm): 9,21 ± 0,96
Forma: 1 triangolare
Tonalità: 6
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 6-20
Grandezza: 6
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 37 (U.C. 494,94)
N°giorni emergenza-piena fioritura: 49 (U.C. 656,62)

LEGUME
Colore a maturazione cerosa: verde con riflessi viola
Sezione trasversale: 2 piriforme
Grado di curvatura: 6
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: 10 giallo dorato o giallo forte
Presenza di strozzature: 2 leggere
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 dalla parte del dorso
Tessitura della superficie esterna: 1 liscia
Larghezza trasversale (mm): 20,64 ± 0,85
Lunghezza legume privato del mucrone (cm): 13,27 ± 0,48
Lunghezza mucrone (cm): 1,39 ± 0,13
Numero semi: 2,37 ± 0,21

SEME
Colore principale: 7 bianco puro
Colore secondario: 8 biancastro
Distribuzione colore secondario: 1 maculato
Luminosità: 5
Forma: 4 reniforme
Grado di curvatura dei semi reniformi: 2 medio
Peso (g): 2,07 ± 0,14
Lunghezza (mm): 24,52 ± 0,53
Altezza (mm): 15,69 ± 0,49

FAGIOLONE DI VALLEPIETRA

Specie: *Phaseolus coccineus* L. (*multiflorum* Willd.)
Provenienza: Rodonti Iolanda, Vallepietra (RM)
Areale di coltivazione: Valle dell'Aniene (RM)
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 149 (U.C. 1272,78)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 10,56 ± 0,58
Larghezza (cm): 8,79 ± 0,58
Forma: 1 triangolare
Tonalità: 4
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 6-20
Grandezza: 6
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 41 (U.C. 555,41)
N°giorni emergenza-piena fioritura: 59 (U.C. 790,6)

LEGUME
Grado di curvatura: 6
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: marrone con sfumature verdi
Presenza di strozzature: 2 leggere
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 dalla parte del dorso
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 19,18 ± 0,41
Lunghezza legume privato del mucrone (cm): 14,08 ± 0,43
Lunghezza mucrone (cm): 1,33 ± 0,1
Numero semi: 2,59 ± 0,17

SEME
Colore principale: bianco
Luminosità: 6
Forma: 3 a cubo
Peso (g): 1,4 ± 0,08
Lunghezza (mm): 22,67 ± 0,59
Altezza (mm): 14,83 ± 0,32

MUGHETTO DI GRISCIANO

Specie: *Phaseolus vulgaris* L.
Provenienza: cooperativa agricola zootecnica Grisciano, Grisciano di Accumoli (RI)
Areale di coltivazione: Alto Reatino
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 132 (U.C. 1287,4)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 11,01 ±0,84
Larghezza (cm): 7,58 ± 0,48
Forma: 1 triangolare
Tonalità: 4
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 2-10
Grandezza: 3 piccolo
Grado di apertura dei petali: 2
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 59 (U.C. 790,6)
N°giorni emergenza-piena fioritura: 63 (U.C. 845,42)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 6
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: marrone tendente al giallo
Presenza di strozzature: 3 medie
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 11,34 ± 0,36
Lunghezza legume privato del mucrone (cm): 9,34 ± 0,3
Lunghezza mucrone (cm): 0,64 ± 0,07
Numero semi: 3,97 ± 0,32

SEME
Colore principale: 8 biancastro
Luminosità: 4
Forma: 1 rotonda
Peso (g): 0,5 ± 0,03
Lunghezza (mm): 10,52 ± 0,23
Altezza (mm): 8,7 ± 0,2

PALLINI DI VALLEPIETRA

Specie: *Phaseolus vulgaris* L.
Provenienza: Rodonti Iolanda, Vallepietra (RM)
Areale di coltivazione: Valle dell'Aniene (RM)
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 134 (U.C. 1288,46)

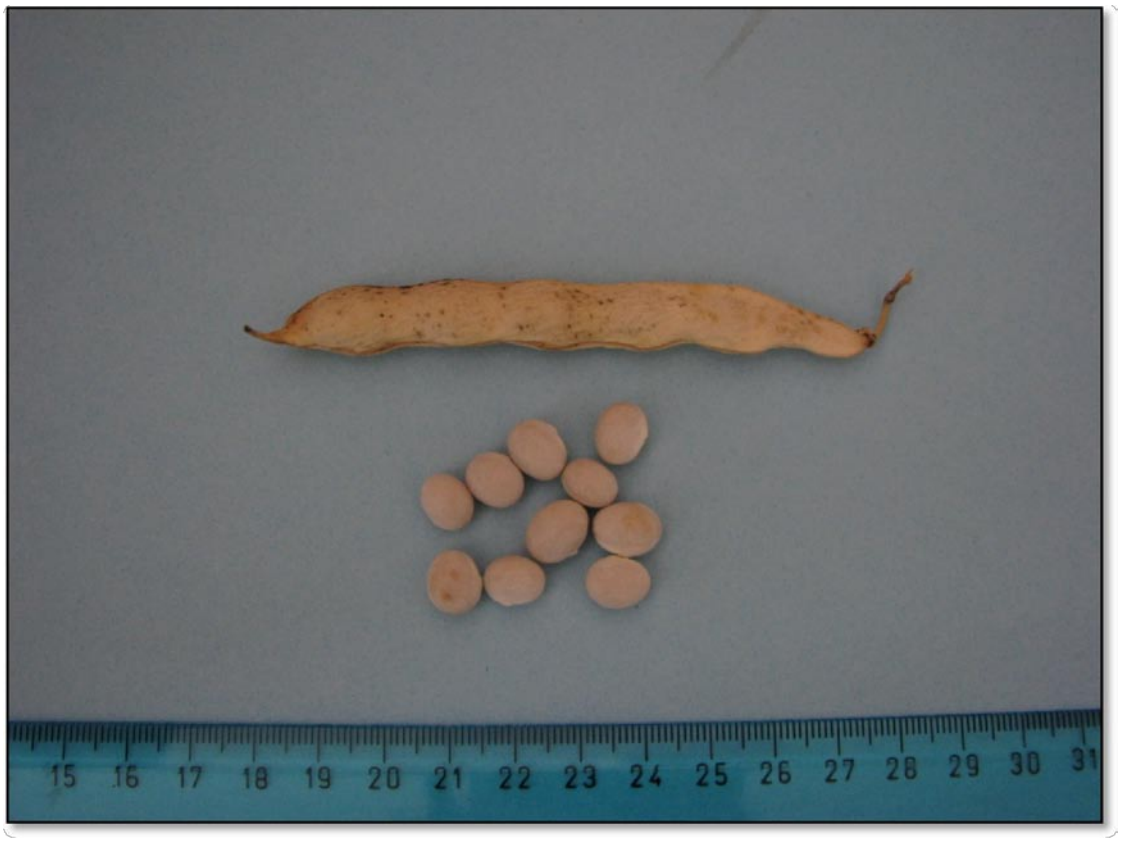
PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 12,03 ± 1,32
Larghezza (cm): 8,82 ± 0,76
Forma: 1 triangolare
Tonalità: 4
Persistenza: 5 intermedia

FIORE
Numero fiori per infiorescenza: 2-5
Grandezza: 3 piccolo
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 41 (U.C. 555,41)
N°giorni emergenza-piena fioritura: 59 (U.C. 790,6)

LEGUME
Grado di curvatura: 5 leggermente curvo
Grado di deiscenza: 3 legumi fortemente contratti (tipo lomentaceo)
Colore a maturazione fisiologica: marrone chiaro tendente al giallo
Presenza di strozzature: 1 assenti
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 3 rugosa
Larghezza trasversale (mm): 11,08 ± 0,27
Lunghezza legume privato del mucrone (cm): 11,4 ± 0,31
Lunghezza mucrone (cm): 0,85 ± 0,07
Numero semi: 4,29 ± 0,37

SEME
Colore principale: bianco
Luminosità: 5 media
Forma: 1 rotonda
Peso (g): 0,73 ± 0,05
Lunghezza (mm): 11,56 ± 0,42
Altezza (mm): 8,73 ± 0,3



ROMANESCHI

Specie: *Phaseolus vulgaris* L.
Provenienza: Rodonti Iolanda, Vallepietra (RM)
Areale di coltivazione: Valle dell'Aniene (RM)
N°giorni semina-emergenza: 7 (U.C 105,53)
N°giorni emergenza-maturazione dei legumi: 133 (U.C. 1288,53)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 11,93 ± 0,88
Larghezza (cm): 9,52 ± 0,61
Forma: 1 triangolare
Tonalità: 5 verde medio
Persistenza: 3 tutte le foglie cadono

FIORE
Numero fiori per infiorescenza: 2-6
Grandezza: 4
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 49 (U.C. 656,62)
N°giorni emergenza-piena fioritura: 63 (U.C. 845,42)

LEGUME
Grado di curvatura: 7 curvo
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: marrone tendente al giallo
Presenza di strozzature: 1 assenti
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 12,1 ± 0,26
Lunghezza legume privato del mucrone (cm): 11,76 ± 0,4
Lunghezza mucrone (cm): 1 ± 0,08
Numero semi: 3,66 ± 0,31

SEME
Colore principale: marrone chiaro
Colore secondario: giallo verde
Distribuzione colore secondario: 1 maculato
Luminosità: 4
Forma: 3 a cubo
Peso (g): 0,66 ± 0,03
Lunghezza (mm): 14,08 ± 0,36
Altezza (mm): 9,05 ± 0,17

UOVA DI QUAGLIA

Specie: *Phaseolus vulgaris* L.
Provenienza: azienda D'Apostolo, San Giorgio Amatrice (RI)
Areale di coltivazione: alto reatino
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 133 (U.C. 1288,53)

PIANTA
Accrescimento: indeterminato
Cirri: presenti

FOGLIA
Lunghezza (cm): 10,85 ± 0,69
Larghezza (cm): 7,79 ± 0,42
Forma: 1 triangolare
Tonalità: 4
Persistenza: 3 tutte le foglie cadono

FIORE
Numero fiori per infiorescenza: 2-4
Grandezza: 5 medio
Grado di apertura dei petali: 5 petali mediamente divergenti
Colore stendardo: rosa
Colore petali: rosa
N°giorni emergenza-inizio fioritura: 49 (U.C. 656,62)
N°giorni emergenza-piena fioritura: 63 (U.C. 845,42)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 3 ellettica-arrotondata
Grado di curvatura: 5 leggermente curvo
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: 11 giallo chiaro tendente al bianco
Presenza di strozzature: 3 medie
Posizione mucrone: 1 marginale
Orientamento mucrone: 6
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 13,92 ± 0,49
Lunghezza legume privato del mucrone (cm): 12,13 ± 0,43
Lunghezza mucrone (cm): 0,82 ± 0,1
Numero semi: 4,07 ± 0,42

SEME
Colore principale: marrone rosato
Colore secondario: verdastro
Distribuzione colore secondario: 2 striato
Luminosità: 6
Forma: 1 rotonda
Peso (g): 0,71 ± 0,04
Lunghezza (mm): 13,17 ± 0,27
Altezza (mm): 10,27 ± 0,22

4.1.3) ECOTIPI SEMI-DETERMINATI:

CANNELLINO S.OLIVA

Specie: *Phaseolus vulgaris* L.
Provenienza: Camilli Marco, Onano (VT)
Areale di coltivazione: Alta Tuscia
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 133 (U.C. 1288,53)

PIANTA
Accrescimento: semi-determinato
Cirri: assenti
Portamento: semi-prostrato

FOGLIA
Lunghezza (cm): 9,33 ± 0,92
Larghezza (cm): 6,67 ± 0,73
Forma: 1 triangolare
Tonalità: 4
Persistenza: 6

FIORE
Numero fiori per infiorescenza: 4-6
Grandezza: 5 medio
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 58 (U.C. 777,72)
N°giorni emergenza-piena fioritura: 69 (U.C. 928,3)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 2 piriforme
Grado di curvatura: 4
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: marrone tendente al giallo chiaro
Presenza di strozzature: 3 medie
Posizione mucrone: 1 marginale
Orientamento mucrone: 4
Tessitura della superficie esterna: 1 liscia
Larghezza trasversale (mm): 10,77 ± 0,30
Lunghezza legume privato del mucrone (cm): 12,29 ± 0,38
Lunghezza mucrone (cm): 1,17 ± 0,1
Numero semi: 3,58 ± 0,32

SEME
Colore principale: 8 biancastro
Luminosità: 6
Forma: 3 a cubo
Peso (g): 0,64 ± 0,04
Lunghezza (mm): 15,95 ± 0,19
Altezza (mm): 8,74 ± 0,1

MEZZO CANNELLINO

DI VENTOTENE

Specie: *Phaseolus vulgaris* L.
Provenienza: Gargiulo Guido, Ventotene (LT)
Areale di coltivazione: Ventotene
N°giorni semina-emergenza: 7 (U.C.105,53)
N°giorni emergenza-maturazione dei legumi: 132 (U.C. 1287,4)

PIANTA
Accrescimento: semi-determinato
Cirri: assenti
Portamento: prostrato

FOGLIA
Lunghezza (cm): 9,88 ± 0,61
Larghezza (cm): 8,1 ± 0,46
Forma: 3 rotonda
Tonalità: 5 verde medio
Persistenza: 5 intermedio

FIORE
Numero fiori per infiorescenza: 2-4
Grandezza: 4
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 58 (U.C. 777,72)
N°giorni emergenza-piena fioritura: 66 (U.C. 880,89)

LEGUME
Grado di curvatura: 6
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: marrone chiaro tendente al giallo
Presenza di strozzature: 5 molto evidenti
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 verso l'alto
Tessitura della superficie esterna: 1 liscia
Larghezza trasversale (mm): 11,20 ± 0,21
Lunghezza legume privato del mucrone (cm): 1,08 ± 0,06
Lunghezza mucrone (cm): 10,77 ± 0,63
Numero semi: 3,51 ± 0,32

SEME
Colore principale: bianco
Luminosità: 5 media
Forma: 3 a cubo
Peso (g): 0,33 ± 0,02
Lunghezza (mm): 12,8 ± 0,31
Altezza (mm): 7,79 ± 0,16

RUGGINOSI

Specie: *Phaseolus vulgaris* L.
Provenienza: azienda D'Apostolo, San Giorgio di Amatrice (RI)
Areale di coltivazione: Alto Reatino
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 133 (U.C. 1288,53)

PIANTA
Accrescimento: semi-determinato
Cirri: presenti

FOGLIA
Lunghezza (cm): 7,9 ± 0,64
Larghezza (cm): 5,63 ± 0,51
Forma: 1 triangolare
Tonalità: 3 verde chiaro
Persistenza: 6

FIORE
Numero fiori per infiorescenza: 2-4
Grandezza: 4
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 58 (U.C. 777,72)
N°giorni emergenza-piena fioritura: 66 (U.C. 880,89)

LEGUME
Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 3 ellittica-arrotondata
Grado di curvatura: 7 curvo
Grado di deiscenza: 6
Colore a maturazione fisiologica: 10-11 giallo dorato-giallo forte o giallo chiaro tendente al bianco
Presenza di strozzature: 2 leggere
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 dalla parte del dorso
Tessitura della superficie esterna: 2 mediamente rugosa
Larghezza trasversale (mm): 10,54 ± 0,25
Lunghezza legume privato del mucrone (cm): 10,12 ± 0,37
Lunghezza mucrone (cm): 1,04 ± 0,07
Numero semi: 4,12 ± 0,32

SEME
Colore principale: rosso scuro
Luminosità: 5 media
Forma: 5 allungata-troncata
Peso (g): 0,31 ± 0,01
Lunghezza (mm): 11,04 ± 0,19
Altezza (mm): 7,50 ± 0,14

TOSCANELLI

Specie: *Phaseolus vulgaris* L.
Provenienza: azienda Donati Luciano, Acquapendente (VT)
Areale di coltivazione: Alta Tuscia
N°giorni semina-emergenza: 7 (U.C. 105,53)
N°giorni emergenza-maturazione dei legumi: 133 (U.C. 1288,53)

PIANTA
Accrescimento: semi-determinato
Cirri: presenti

FOGLIA
Lunghezza (cm): 8,28 ± 0,55
Larghezza (cm): 6,53 ± 0,62
Forma: 1 triangolare
Tonalità: 4
Persistenza: 6

FIORE
Numero fiori per infiorescenza: 2-6
Grandezza: 3 piccolo
Grado di apertura dei petali: 3 petali chiusi e paralleli
Colore stendardo: 1 bianco
Colore petali: 1 bianco
N°giorni emergenza-inizio fioritura: 41 (U.C. 555,41)
N°giorni emergenza-piena fioritura: 59 (U.C. 790,6)

LEGUME

Colore a maturazione cerosa: 7 verde normale
Sezione trasversale: 2 piriforme
Grado di curvatura: 6
Grado di deiscenza: 5 legumi coriacei
Colore a maturazione fisiologica: marrone tendente al giallo
Presenza di strozzature: 2 leggere
Posizione mucrone: 1 marginale
Orientamento mucrone: 3 dalla parte del dorso
Tessitura della superficie esterna: mediamente rugosa Larghezza trasversale (mm): 10,84 ± 0,25
Lunghezza legume privato del mucrone (cm): 11,69 ± 0,35
Lunghezza mucrone (cm): 0,95 0,06
Numero semi: 4,43 ± 0,37

SEME

Colore principale: 7 bianco puro
~~Colore~~ secondario: 8 biancastro
Distribuzione colore secondario: 1 maculato Luminosità: 5 media
Forma: 4 reniforme
Grado di curvatura dei semi reniformi: 2 medio
Peso (g): 0,40 ± 0,01
Lunghezza (mm): 12,72 ± 0,22
Altezza (mm): 7,76 ± 0,11

ANNEX 4. Protocol for assessing morphological diversity in Cardoon (*Cynara cardunculus* var. *altilis DC)*

Morphological descriptors for the cardoon were based on those of the artichoke. Some of the descriptors relevant to the artichoke were not relevant to the cardoon, however, as where the quality of the inner bracts are major selective factors for artichoke, it is the leaves and stalks which are selected for in cardoon. Consequently, those variables were not taken into consideration for the purposes of this study.

PROTOCOL FOR DISTINCTNESS, UNIFORMITY AND STABILITY TESTS

Cynara scolymus
L.
(*Cynara cardunculus* var. *scolymus*

L.) GLOBE ARTICHOKE

Adopted on
25/03/2004

I SUBJECT OF THE PROTOCOL

The protocol describes the technical procedures to be followed in order to meet the Council Regulation 2100/94 on Community Plant Variety Rights. The technical procedures have been agreed by the Administrative Council and are based on general UPOV Document TG/1/3 and UPOV Guideline TG/184/3 dated 04/04/2001 for the conduct of tests for Distinctness, Uniformity and Stability. This protocol applies to varieties of ***Cynara scolymus*** **L. (*Cynara cardunculus* var. *scolymus* L.*)***

II SUBMISSION OF SEED AND OTHER PLANT MATERIAL

1. The Community Plant Variety Office (CPVO) is responsible for informing the applicant of

- the closing date for the receipt of plant material;
- the minimum amount and quality of plant material required;
- the examination office to which material is to be sent.

A sub-sample of the material submitted for test will be held in the variety collection as the definitive sample of the candidate variety.

The applicant is responsible for ensuring compliance with any customs and plant health requirements.

2. Final dates for receipt of documentation and material by the Examination Office

The final dates for receipt of requests, technical questionnaires and the final date or submission period for plant material will be decided by the CPVO and each Examination Office chosen.

The Examination Office is responsible for immediately acknowledging the receipt of requests for testing, and technical questionnaires. Immediately after the closing date for the receipt of plant material the Examination Office should inform the CPVO whether acceptable plant material has been received or not. However if unsatisfactory plant material is submitted the CPVO should be informed as soon as possible.

3. Plant material requirements

The final dates for request for technical examination and sending of Technical Questionnaire by the CPVO as well as submission date of plant material by the applicant can be found in the S2 supplement of the CPVO Official Gazette and the CPVO website.

Quality of seed: Should not be less than the standards laid down for certified seed in Annex 2 of Council Directive 2002/55/EC.

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Quality of plants: Should not be less than the standards laid down for plants in EC Directive 92/33 and implementing measures.

Seed Treatment: The plant material must not have undergone any treatment unless the CPVO and the examination office allow or request such treatment. If it has been treated, full details of the treatment must be given.

Special requirements: -

Labelling of sample:
- Species
- File number of the application allocated by the CPVO
- Breeder's reference
- Examination reference (if known)
- Name of applicant
- The phrase "On request of the CPVO"
- In the case of a split sample, the quantity of seed being submitted.

III CONDUCT OF TESTS

1. Variety collection

A variety collection will be maintained for the purpose of establishing distinctness of the candidate varieties in test. A variety collection may contain both living material and descriptive information. A variety will be included in a variety collection only if plant material is available to make a technical examination.

Pursuant to Article 7 of Council Regulation No. 2100/94, the basis for a collection should be the following:

- varieties listed or protected at the EU level or at least in one of the EEA Member States;

- varieties protected in other UPOV Member States;
- any other variety in common knowledge.

The composition of the variety collection in each Examination Office depends on the environmental conditions in which the Examination Office is located.

Variety collections will be held under conditions which ensure the long term maintenance of each accession. It is the responsibility of Examination Offices to replace reference material which has deteriorated or become depleted. Replacement

material can only be introduced if appropriate tests confirm conformity with the existing reference material. If any difficulties arise for the replacement of reference material Examination Offices must inform the CPVO. If authentic plant material of a variety cannot be supplied to an Examination Office the variety will be removed from the variety collection.

2. Material to be examined

Candidate varieties will be directly compared with other candidates for Community plant variety rights tested at the same Examination Office, and with appropriate varieties in the variety collection. When necessary an Examination Office may also include other candidates and varieties. Examination Offices should therefore make efforts to co-ordinate the work with other Offices involved in DUS testing of globe artichoke. There should be at least an exchange of technical questionnaires for each candidate variety, and during the test period, Examination Offices should notify each other and the CPVO of candidate varieties which are likely to present problems in establishing distinctness. In order to solve particular problems Examination Offices may exchange plant material.

3. Characteristics to be used

The characteristics to be used in DUS tests and preparation of descriptions shall be those referred to in the Annex 2. All the characteristics shall be used, providing that observation of a characteristic is not rendered impossible by the expression of any other characteristic, or the expression of a characteristic is prevented by the environmental conditions under which the test is conducted. In the latter case, the CPVO should be informed. In addition the existence of some other regulation e.g. plant health, may make the observation of the characteristic impossible.

The Administrative Council empowers the President, in accordance with Article 23 of Commission Regulation N° 1239/95, to insert additional characteristics and their expressions in respect of a variety.

4. Grouping of varieties

The varieties and candidates to be compared will be divided into groups to facilitate the assessment of distinctness. Characteristics which are suitable for grouping purposes are those which are known from experience not to vary, or to vary only slightly, within a variety and which in their various states of expression are fairly evenly distributed throughout the collection. In the case of continuous grouping characteristics overlapping states of expression between adjacent groups is required to reduce the risks of incorrect allocation of candidates to groups. The characteristics which may be used for grouping are the following:

(a) Leaf: incisions (10 to 12 leaf stage) (characteristic 9)
(b) Central flower head: shape in longitudinal section (characteristic 26) (c) Central flower head: time of appearance (characteristic 28)
(d) Outer bract: colour (external side) (characteristic 41)

5. Trial designs and growing conditions

The minimum duration of tests will normally be two independent growing cycles. For vegetatively propagated varieties, the duration of the testing may be reduced to one growing cycle if the results on distinctness and uniformity are conclusive. Tests will be carried out under conditions ensuring normal growth. The size of the plots will be such that plants or parts of plants may be removed for measuring and counting without prejudice to the observations which must be made up to the end of the growing period.

The test design is as follows

As a minimum, each test should include a total of 40 plants which should be divided between two or more replicates.

All observations determined by measurements or counting should be made on 10 plants or parts of 10 plants.

Unless otherwise indicated, all observations on the leaves should be made on fully developed leaves, on the 3rd or 4th leaf from the base (i.e. when the flower head is about 3 cm in diameter).

Unless otherwise indicated, all observations on the outer bract of the flower head should be made on the 5th whorl of bracts from the base of the central flower head.

Unless otherwise indicated, all observations on the inner bracts of the flower head must be made on the central flower head.

6. Special tests

In accordance with Article 83(3) of Council Regulation No. 2100/94 an applicant may claim either in the Technical Questionnaire or during the test that a candidate has a characteristic which would be helpful in establishing distinctness. If such a claim is made and is supported by reliable technical data, a special test may be undertaken providing that a technically acceptable test procedure can be devised.

Special tests will be undertaken, with the agreement of the President of CPVO, where distinctness is unlikely to be shown using the characters listed in the protocol.

7. Standards for decisions

a) **Distinctness**

A candidate variety will be considered to be distinct if it meets the requirements of
Article 7 of Council Regulation No. 2100/94.

b)
Uniformity

For the assessment of uniformity a population standard of 5% with an acceptance probability of at least 95% should be applied for seed propagated varieties, whilst for the assessment of uniformity of vegetatively propagated varieties a population standard of 1% with an acceptance probability of at least 95% should be applied.

Table of maximum numbers of off-types allowed for uniformity standards for seed propagated varieties.

Number of plants	off-types allowed
8-16	2
17-28	3
29-40	4
41-53	5

Table of maximum numbers of off-types allowed for uniformity standards for vegetatively propagated varieties.

Number of plants	off-types allowed
6-35	1
36-82	2

c) **Stability**

A candidate will be considered to be sufficiently stable when there is no evidence to indicate that it lacks uniformity.

IV REPORTING OF RESULTS

After each recording season the results will be summarised and reported to the CPVO in the form of a UPOV model interim report in which any problems will be indicated under the headings distinctness, uniformity and stability. Candidates may meet the DUS standards after two growing periods but in some cases three growing periods may be required. When tests are completed the results will be sent by the Examination Office to the CPVO in the form of a UPOV model final report.

If it is considered that the candidate complies with the DUS standards, the final report will be accompanied by a variety description in the format recommended by UPOV. If not the reasons for failure and a summary of the test results will be included with the final report.

The CPVO must receive interim reports and final reports by the date agreed between the CPVO and the examination office.

Interim reports and final examination reports shall be signed by the responsible member of the staff of the Examination Office and shall expressly acknowledge the exclusive rights of disposal of CPVO.

V LIAISON WITH THE APPLICANT

If problems arise during the course of the test the CPVO should be informed immediately so that the information can be passed on to the applicant. Subject to prior agreement, the applicant may be directly informed at the same time as the CPVO particularly if a visit to the trial is advisable.

The interim report as well as the final report shall be sent by the Examination Office to the CPVO.

*****

ANNEXES TO FOLLOW

I. Annex 1:

List of characteristics to be observed, explanations and methods.

ANNEX 1

TABLE OF CHARACTERISTICS TO BE USED IN DUS-TEST AND PREPARATION OF DESCRIPTIONS

CPVO N°	UPOV N°	Characteristics		Examples	Note
1. (+)	**1.**	**Plant: height (including central flower head)**	short	Violet de Provence, Tudela	3 [ ]
			medium	Blanc Hyerois, Camus de Bretagne, Vertu	5 [ ]
			tall	Caribou, Popvert, Salambo	7 [ ]
2.	**2.**	**Plant: number of lateral shoots on main stem**	few	Blanc Hyerois, Calico, Popvert	3 [ ]
			medium	Salambo	5 [ ]
			many	Chrysanthème, Vertu	7 [ ]
3. (+)	**3.**	**Main stem: height (excluding central flower head)**	short	Capitan	3 [ ]
			medium	Castel, Salambo	5 [ ]
			tall	Caribou	7 [ ]
4. (+)	**4.**	**Main stem: distance between central flower head and youngest well developed leaf**	short	Caribou, Violet de Provence	3 [ ]
			medium	Blanc Hyerois, Tudela	5 [ ]
			long	Castel	7 [ ]
5.	**5.**	**Main stem: diameter (at about 10 cm below central flower head)**	small	Violet de Provence	3[ ]
			medium	Castel, Vertu	5 [ ]
			large	Carène	7 [ ]
6.	**6.**	**Leaf : attitude (10 to 12 leaf stage)**	erect	Capitan, Pètre, Vert de Provence	1 [ ]
			semi-erect	Calico, Camus de Bretagne	3 [ ]
			horizontal	Blanc Hyerois, Popvert	5 [ ]

CPVO N°	UPOV N°	Characteristics		Examples	Note
7.	**7.**	**Leaf: long spines**	absent	Camus de Bretagne, Tudela	1 [ ]
			present	Spinoso sardo	9 [ ]
8.	**8.**	**Leaf: length**	short	Tudela, Violet de Provence	3 [ ]
			medium	Blanc Hyerois, Chrysanthème, Popvert	5 [ ]
			long	Camus de Bretagne, Caribou	7 [ ]
9.	**9.**	**Leaf: incisions (10 to 12 leaf stage)**	absent	Tudela, Violet de Provence	1 [ ]
			present	Camus de Bretagne, Vertu	9 [ ]
10.	**10.**	**Leaf: number of lobes**	few	Violet de Provence, Tudela	3 [ ]
			medium	Blanc Hyerois, Chrysanthème	5 [ ]
			many	Salanquet	7 [ ]
11.	**11.**	**Leaf: length of longest lobe**	short	Vertu	3 [ ]
			medium	Orlando, Popvert, Sybaris	5 [ ]
			long		7 [ ]
12.	**12.**	**Leaf: width of longest lobe**	narrow	Vertu	3 [ ]
			medium	Orlando, Popvert, Sybaris	5 [ ]
			broad		7 [ ]
13. (+)	**13.**	**Lobe: shape of tip (excluding terminal lobe)**	acute	Camus de Bretagne, Vertu	1 [ ]
			nearly right angle	Calico, Caribou, Salambo	2 [ ]
			obtuse		3 [ ]

CPVO N°	UPOV N°	Characteristics		Examples	Note
14. (+)	**14.**	**Lobe: number of secondary lobes**	none or very few	Violet de Provence	1 [ ]
			few	Camus de Bretagne	3 [ ]
			medium	Blanc Hyerois, Popvert	5 [ ]
			many	Orlando, Sybaris	7 [ ]
			very many		9 [ ]
15. (+)	**15.**	**Lobe: shape of tip of secondary lobes**	acuminate	Vert de Provence	1 [ ]
			acute	Blanc Hyerois, Tudela	2 [ ]
			rounded	Cric, Popvert	3 [ ]
16.	**16.**	**Leaf blade: shape in cross section**	flat	Salambo, Vertu	1 [ ]
			V shaped	Capitan, Castel	2 [ ]
17.	**17.**	**Leaf blade: intensity of green colour (upper side)**	light	Blanc Hyerois, Pètre	3 [ ]
			medium	Violet de Provence, Tudela, Vertu	5 [ ]
			dark	Camus de Bretagne, Cric	7 [ ]
18.	**18.**	**Leaf blade: hue of green colour**	absent	Salambo	1 [ ]
			yellowish	Blanc Hyerois	2 [ ]
			greyish	Camus de Bretagne	3 [ ]
19.	**19.**	**Leaf blade: intensity of grey hue**	weak		3 [ ]
			medium		5 [ ]
			strong		7 [ ]
20.	**20.**	**Leaf: hairiness on upper side**	absent or very weak	Camus de Bretagne, Castel, Vert Globe	1 [ ]
			weak	Vertu	3 [ ]
			medium	Carène, Popvert	5 [ ]
			strong	Violet de Provence	7 [ ]
			very strong		9 [ ]

CPVO N°	UPOV N°	Characteristics		Examples	Note
21.	**21.**	**Leaf blade: blistering**	absent or very weak		1 [ ]
			weak	Blanc Hyerois, Popvert	3 [ ]
			medium	Calico, Caribou	5 [ ]
			strong	Chrysanthème	7 [ ]
			very strong	Cric	9 [ ]
22.	**22.**	**Petiole: anthocyanin coloration at base**	absent or very weak	Capitan, Carène	1 [ ]
			weak	Castel	3 [ ]
			medium	Pètre	5 [ ]
			strong	Violet de Provence	7 [ ]
			very strong		9 [ ]
23.	**23.**	**Central flower head: length**	short	Pètre	3 [ ]
			medium		5 [ ]
			long	Vert de Provence	7 [ ]
24.	**24.**	**Central flower head: diameter**	small	Vert de Provence	3 [ ]
			medium		5 [ ]
			large	Camus de Bretagne, Salambo	7 [ ]
25.	**25.**	**Central flower head: size**	small	Vert de Provence, Violet de Provence	3 [ ]
			medium	Blanc Hyerois, Chrysanthème	5 [ ]
			large	Castel, Salambo	7 [ ]

CPVO N°	UPOV N°	Characteristics		Examples	Note
26. (+)	**26.**	**Central flower head: shape in longitudinal section**	circular	Castel, Green Globe	1 [ ]
			broad elliptic	Chrysanthème, Vert de Provence	2 [ ]
			ovate	Cric, Salambo	3 [ ]
			triangular	Tudela, Violet de Provence	4 [ ]
			transverse broad elliptic	Carène, Pètre	5 [ ]
27.	**27.**	**Central flower head: shape of tip**	acute	Violet de Provence	1 [ ]
			rounded	Camus de Bretagne	2 [ ]
			flat	Chrysanthème	3 [ ]
			depressed	Carène, Pètre	4 [ ]
28.	**28.**	**Central flower head: time of appearance**	early	Chrysanthème, Tudela	3 [ ]
			medium	Blanc Hyerois	5 [ ]
			late	Camus de Bretagne	7 [ ]
29.	**29.**	**Central flower head: time of beginning of opening**	early	Chrysanthème, Vert de Provence	3 [ ]
			medium	Camus de Bretagne	5 [ ]
			late	Popvert, Tudela	7 [ ]
30.	**30.**	**First flower head on lateral shoot: length**	short	Pètre, Popvert	3 [ ]
			medium		5 [ ]
			long	Vert de Pr(vence	7 [ ]
31.	**31.**	**First flower head on lateral shoot: diameter**	small	Vert de Pr(vence	3 [ ]
			medium	Blanc Hyerois	5 [ ]
			large	Salambo	7 [ ]

CPVO N°	UPOV N°	Characteristics		Examples	Note
32.	**32.**	**First flower head on lateral shoot: size**	small	Violet de Provence	3 [ ]
			medium	Chrysanthème	5 [ ]
			large	Blanc Hyerois, Castel	7 [ ]
33. (+)	**33.**	**First flower head on lateral shoot: shape in longitudinal section**	circular	Castel, Salambo	1 [ ]
			broad elliptic	Cric, Blanc Hyerois	2 [ ]
			ovate	Velours	3 [ ]
			triangular	Violet de Provence	4 [ ]
			transverse broad elliptic	Pètre, Popvert	5 [ ]
34.	**34.**	**First flower head on lateral shoot: degree of opening**	weak	Salambo	3 [ ]
			medium	Blanc Hyerois	5 [ ]
			strong	Chrysanthème	7 [ ]
35. (+)	**35.**	**Outer bract: length of base**	short		3 [ ]
			medium		5 [ ]
			long		7 [ ]
36. (+)	**36.**	**Outer bract: width of base**	narrow	Orlando	3 [ ]
			medium	Blanc Hyerois, Popvert, Vertu	5 [ ]
			broad	Pètre	7 [ ]
37. (+)	**37.**	**Outer bract: thickness at base**	thin		3 [ ]
			medium	Blanc Hyerois, Popvert, Vertu	5 [ ]
			thick	Pètre	7 [ ]

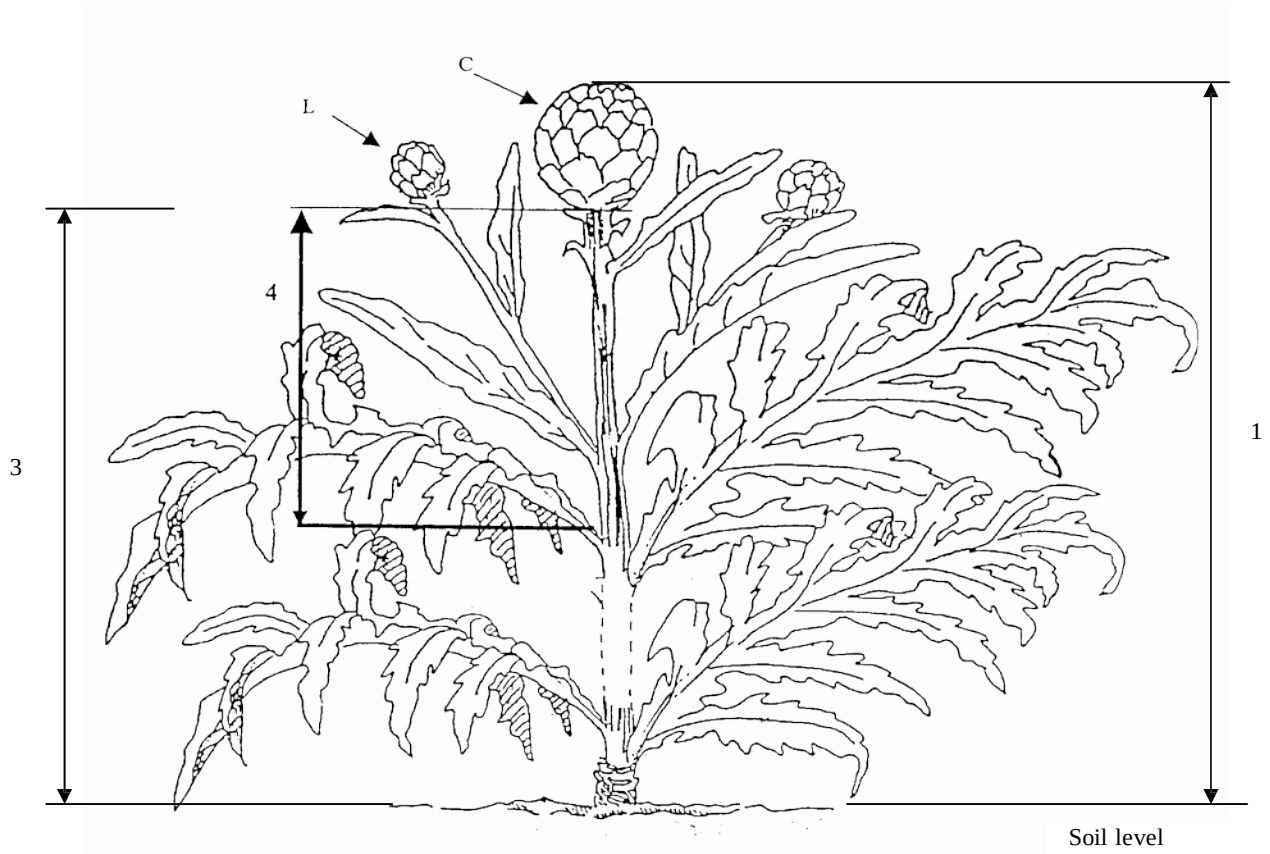
CPVO N°	UPOV N°	Characteristics		Examples	Note
38.	**38.**	**Outer bract: main shape**	broader than long	Calico, Cric, Pètre	1 [ ]
			as broad as long	Camus de Bretagne, Pètre	2 [ ]
			longer than broad	Vert de Provence, Vertu	3 [ ]
39.	**39.**	**Outer bract: shape of apex**	acute	Spinoso Sardo	1 [ ]
			flat	Talpiot	2 [ ]
			emarginate	Chrysanthème	3 [ ]
40.	**40.**	**Outer bract: depth of emargination**	shallow	Castel, Violet de Provence	3 [ ]
			medium	Blanc Hyerois	5 [ ]
			deep	Chrysanthème	7 [ ]
41.	**41.**	**Outer bract: colour (external side)**	green	Blanc Hyerois, Tudela, Vert de Prcvence	1 [ ]
			green striped with violet	Violet de Provence	2 [ ]
			violet striped with green	Chrysanthème	3 [ ]
			mainly violet	Cric, Salambo	4 [ ]
			entirely violet	Velours	5 [ ]
42.	**42.**	**Outer bract: hue of secondary colour (as 41)**	absent	Calico	1 [ ]
			bronze	Blanc Hyerois, Sakiz	2 [ ]
			grey	Camus de Bretagne, Popvert	3 [ ]
43. (+)	**43.**	**Outer bract: reflexing of tip**	absent	Castel, Salambo	1 [ ]
			present	Calico, Chrysanthème	9 [ ]

CPVO N°	UPOV N°	Characteristics		Examples	Note
44.	**44.**	**Outer bract: size of spine**	absent or very small	Calico	1 [ ]
			small	Chrysanthème, Vertu	3 [ ]
			medium	Violet de Provence	5 [ ]
			large		7 [ ]
			very large	Spinoso Sardo	9 [ ]
45. (+)	**45.**	**Outer bract: mucron**	absent	Chrysanthème, Pètre	1 [ ]
			present	Camus de Bretagne	9 [ ]
46.	**46.**	**Central flower head: anthocyanin coloration of inner bracts**	absent or very weak	Popvert	1 [ ]
			weak	Castel	3 [ ]
			medium	Blanc Hyerois	5 [ ]
			strong	Chrysanthème	7 [ ]
			very strong	Salambo	9 [ ]
47. (+)	**47.**	**Central flower head: density of inner bracts**	sparse	Camard, Calic0	3 [ ]
			medium	Camus de Bretagne	5 [ ]
			dense	Cacique, Compact	7 [ ]
48. (+)	**48.**	**Receptacle: diameter**	small	Violet de Provence	3 [ ]
			medium	Camus de Bretagne	5 [ ]
			large	Capitan, Salambo	7 [ ]
49. (+)	**49.**	**Receptacle: thickness**	thin	Blanc Hyerois, Tudela	3 [ ]
			medium	Pètre	5 [ ]
			thick	Camus de Bretagne, Castel	7 [ ]

CPVO N°	UPOV N°	Characteristics		Examples	Note
50. (+)	**50.**	**Receptacle: shape in longitudinal section**	flat	Carène	1 [ ]
			slightly depressed	Camus de Bretagne, Salambo	2 [ ]
			strongly depressed	Blanc Hyerois, Chrysanthème	3 [ ]
51.	**51.**	**Tendency to produce lateral shoots at base**	weak	Blanc Hyerois, Castel, Vertu	3 [ ]
			medium	Violet de Provence, Chrysanthème, Popvert	5 [ ]
			strong	Cacique, Calico	7 [ ]

EXPLANATIONS AND METHODS

Ad. 1, 3, 4: Plant: height (including central flower head) (1);
Main stem: height (excluding central flower head) (3);
Main stem: distance between flower head and last developed leaf (4)

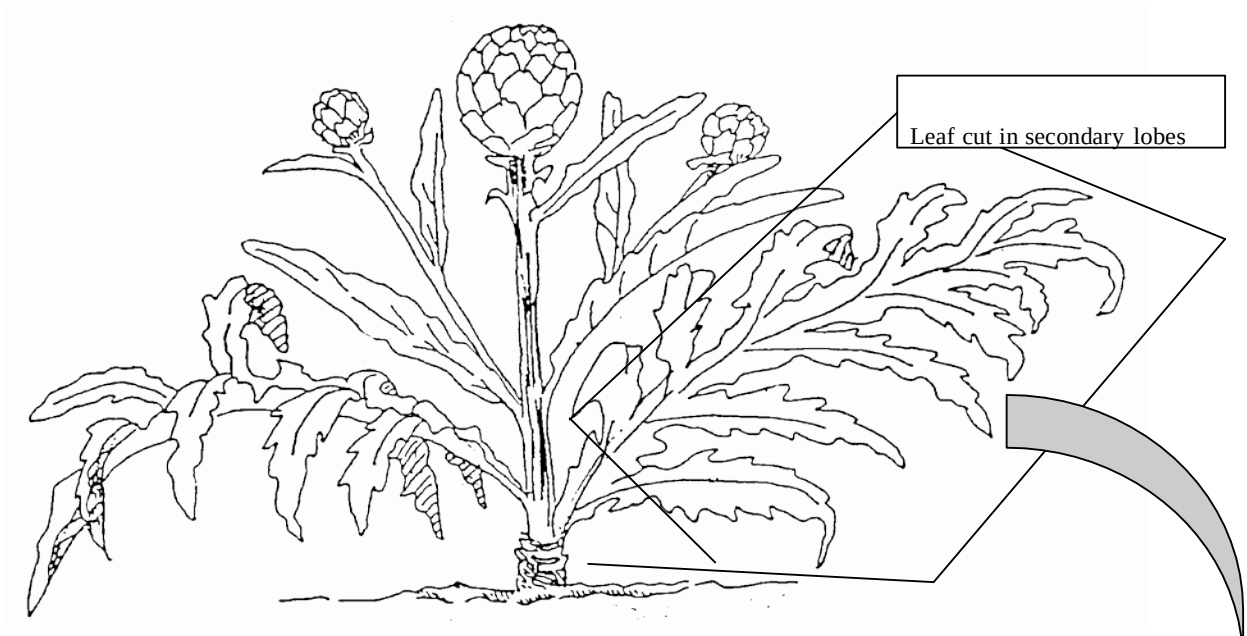


C: Central flower head
L: First flower head on lateral shoot

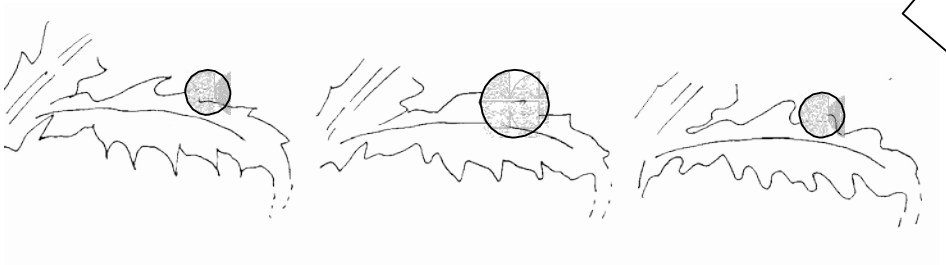
Ad. 13: Lobe: shape of tip (excluding terminal lobe)

1	2	3
acute	nearly right angle	obtuse

Ad. 14, 15: Lobe: number (14) and shape of tip (15) of secondary lobes (on the 3rd – 4th whorl of leaves)

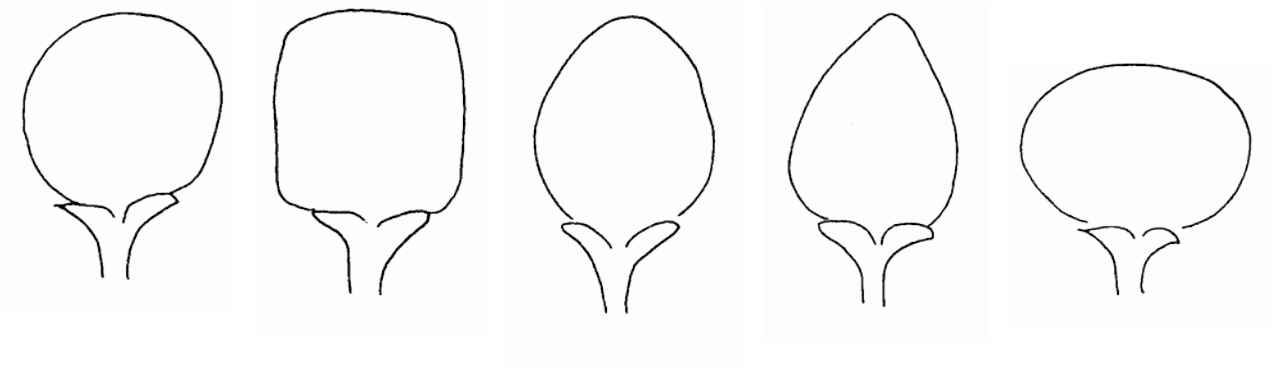


15



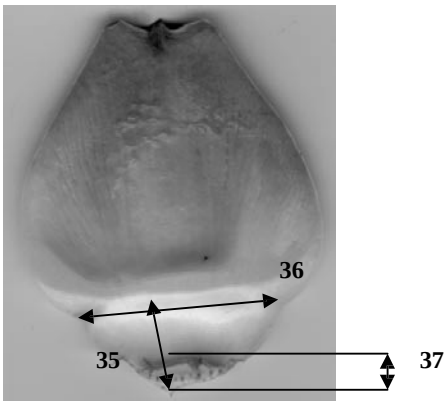
1 acuminate | 2 acute | 3 rounde

Ad. 26, 33: Central flower head (26) and First flower head on lateral shoot (33): shape in longitudinal section



1	2	3	4	5
circular	broad elliptic	ovate	triangular	transverse elliptic

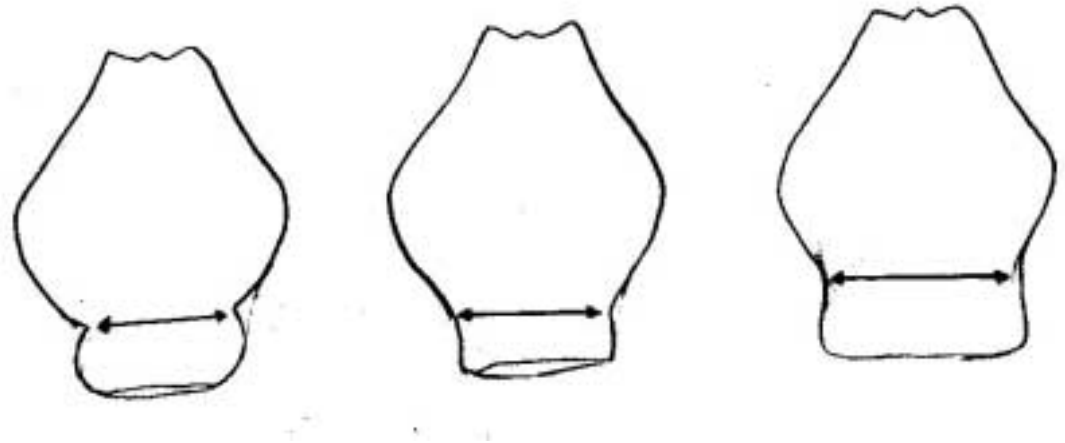
Ad. 35, 36, 37: Outer bracts: length of base (35), width of base (36), thickness at base (37)



Ad. 35: Outer bract : length of base

3	5	7
short	medium	long

Ad. 36: Outer bract : width of base



3
narrow

5
medium

7
broad

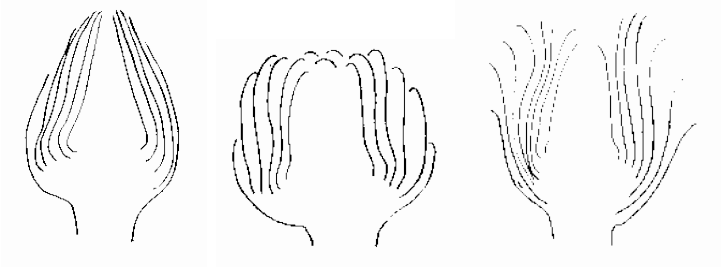
Ad. 37: Outer bract : thickness at base (*bract in profile*)

3
thin

5
medium

7
thick

Ad. 43: Outer bract: reflexing at tip



a b

1
absent

9
present

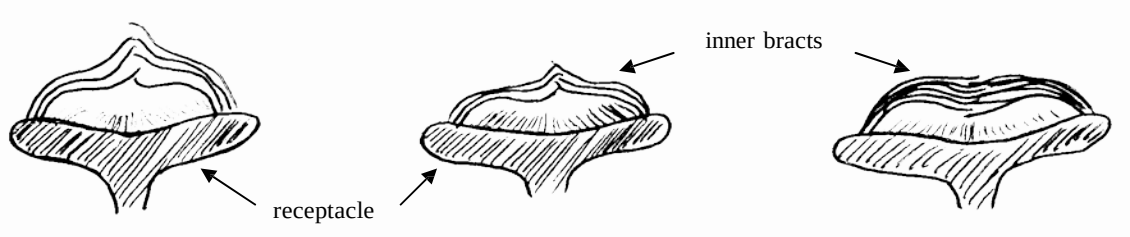
a: reflexing at tip (on Chrysanthème)
b: reflexing at tip (on Calico)

Ad. 45: Outer bract: mucron

1
absent

9
present

Ad. 47: Central flower head: density of inner bracts

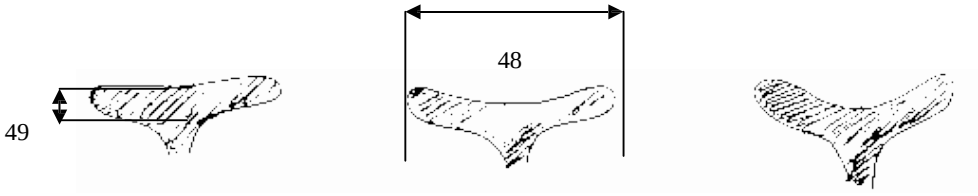


3
sparse

5
medium

7
dense

Ad. 48, 49, 50: Receptacle: diameter (48), thickness (49), shape in longitudinal section (50)



50

1
flat

2
slightly depressed

3
strongly

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European Union
Community Plant Variety Office

Application date ...../...../.....

File number ...../.....

(not to be filled in by the applicant)

TECHNICAL QUESTIONNAIRE

to be completed in connection with an application for Community Plant Variety Rights. Please answer all questions. A question without any answer will lead to a non-attribution of an application date. In cases where a field / question is not applicable, please state so.

1. Botanical taxon: Latin name of the genus, species or sub-species to which the variety belongs and common name:

Species ***Cynara scolymus* L. / *Cynara cardunculus* var. *scolymus* L.**

GLOBE ARTICHOKE

2. Applicant(s): Name(s) and address(es), phone and fax number(s), e-mail address, and where appropriate name and address of the procedural representative

3. Variety denomination

a) Where appropriate proposal for a variety denomination:

b) Provisional designation (breeder's reference):

4. Information on origin, maintenance and reproduction of the variety

4.1 Method of maintenance and reproduction

a) (i) hybrid [ ]
(ii) open-pollinated variety [ ]
(iii) parent line [ ]
(iv) other type (specify)

b) (i) seed propagated [ ]
(ii) vegetatively propagated [ ]

c) Other information on genetic origin and breeding method

4.2 Geographical origin of the variety: the region and the country in which the variety was bred or discovered and developed

4.3 Shall the information on data relating to components of hybrid varieties including data related to their cultivation be treated as confidential ?

[ ] YES [ ] NO

If yes, please give this information on the attached form for confidential information.

If no, please give information on data relating to components of hybrid varieties including data related to their cultivation:

Breeding scheme (indicate female component first)

Characteristics of the variety to be given (the number in brackets refers to the corresponding characteristic in the CPVO protocol; please mark the state of expression which best corresponds).

	Characteristics	Example Varieties	Note
5.1 (9)	**Leaf: incisions (10 to 12 leaf stage)**		
	absent	Tudela, Violet de Provence	1 [ ]
	present	Camus de Bretagne, Vertu	9 [ ]
5.2 (26)	**Central flower head: shape in longitudinal section**		
	circular	Castel, Green Globe	1 [ ]
	broad elliptic	Chrysanthème, Vert de Provence	2 [ ]
	ovate	Cric, Salambo	3 [ ]
	triangular	Tudela, Violet de Provence	4 [ ]
	transverse broad elliptic	Carène, Pètre	5 [ ]
5.3 (27)	**Central flower head: shape of tip**		
	acute	Violet de Provence	1 [ ]
	rounded	Camus de Bretagne	2 [ ]
	flat	Chrysanthème	3 [ ]
	depressed	Carène, Pètre	4 [ ]
5.4 (28)	**Central flower head: time of appearance**		
	early	Chrysanthème, Tudela	3 [ ]
	medium	Blanc Hyèrois	5 [ ]
	late	Camus de Bretagne	7 [ ]
5.5 (41)	**Outer bract: colour (external side)**		
	green	Blanc Hyèrois, Tudela, Vert de Provence	1 [ ]
	green striped with violet	Violet de Provence	2 [ ]
	violet striped with green	Chrysanthème	3 [ ]
	mainly violet	Cric, Salambo	4 [ ]
	entirely violet	Velours	5 [ ]

6. Similar varieties and differences from these varieties

Denomination of similar variety	Characteristic in which the similar variety is different [o)]	State of expression of similar variety	State of expression of candidate variety

[o)] In the case of identical states of expressions of both varieties, please indicate the size of the difference.

7 Additional information which may help to distinguish the variety

7.1 Resistance to pests and diseases

7.2 Special conditions for the examination of the variety

7.2.1 Main use:

a) Fresh market
   (i) large flower head [ ]
   (ii) small flower head [ ]

b) Canning
   (i) Receptacle [ ]
   (ii) Bottom [ ]
   (iii) pickling artichoke [ ]

c) Industrial use
   (i) leaf extraction [ ]
   (ii) biomass [ ]

d) Other (please specify)

7.2.2 Other conditions

[ ] YES, please specify

[ ] NO

7.3 Other information

8. GMO-information required

The variety represents a Genetically Modified Organism within the meaning of Article 2(2) of Council Directive EC/2001/18 of 12/03/2001.

[ ] yes [ ] no

If yes, please add a copy of the written attestation of the responsible authorities stating that a technical examination of the variety under Articles 55 and 56 of the Basic Regulation does not pose risks to the environment according to the norms of the above-mentioned Directive.

I/we hereby declare that to the best of my/our knowledge the information given in this form is complete and correct.

Date Signature Name

[End of document]