

## **Status of grain legume collections in Italy**

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In Italy grain legumes collections are conserved by different institutions (CRA, CNR, several Universities, *etc.*). Major collections are kept on common bean, pea and faba bean, but other minor species are well represented. In the different collections are kept landraces, local varieties but also breeding lines or wild accessions, according with the different missions of the diverse organizations.

A surely underestimated picture of the Italian germplasm could be summarized in the following numbers: *Phaseolus* spp. (5831), *Pisum sativum* (4657), *Vicia faba* (2302), *Vigna* spp. (948), *Cicer arietinum* (369), *Lens culinaris* (348), *Lathyrus* spp. (347), *Lupinus* spp. (188), but without doubt more accessions are conserved (and characterized) because the absence of a national Gene Bank.

This puzzled condition has positive and negative consequences: several collecting missions have been done also in the recent past at regional level and still other are planned in the future, with the opportunity to find other interesting landraces or local varieties, but the costs for their conservation and the risks of duplication of the material is quite significant. Regeneration of accessions is done routinely but according to different priorities; for example, breeding materials are not completely kept after the end of a project, and for this reason in the institutions devoted to grain legume improvement the dimension of germplasm is fluctuating.

In the last years a great impulse in the germplasm preservation was given by several projects carried out with the aim to preserve and promote traditional foods and markets, funded by the Italian Ministry of Agriculture or at different level by local institutions.

A significant picture of the “Byzantine” situation of the Italian germplasm could arise from the following contributions written by 6 diverse experts working in different institutions. This report has not the pretension to cover all the researches ongoing on grain legumes in Italy, because studies are surely more in numbers and results, since grain legumes are the second most characterized group of plants among the genetic Italian experts.

### **CRA-CIN Bologna**

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The CRA-CIN (formerly ISCI) grain legumes collection include diverse genetic materials belonging to 2 genera (1375 accessions). Since the mission of the Research Center is the study of genetic diversity, germplasm characterization and breeding improvement, the collection received a

great emphasis in the last years, according to diverse projects financed mainly by the Italian Ministry of Agriculture.

For this reason the collection is continuously increasing in number since the characterization activities for biotic and abiotic stresses improvement of common bean and pea requires the evaluation of large numbers of wild accessions and landraces. These accessions are collected from foreign gene banks as well from other national institutions or private companies. At the same time the collection contains traditional and new cultivars of Italian origin and a great numbers of breeder's lines.

**Table 1.** Description of the CRA-CIN collection.

Type:	<i>Phaseolus vulgaris</i>	<i>Pisum sativum</i>
Wild	784	-
Landraces	236	-
Breeding lines	more than 200	54
Cultivars	67	34
total	1287	88

The distribution of the accessions reflects the different goals of the Center (see table 1). Material of national origin accounts for only 20% of the collection (all the breeding lines, 23 landraces and 41 cultivars). Meso-America and South-America institutions (mostly CIAT) are the most important contributors of the collection.

Evaluation of common bean resources focused on yield, high temperature and drought tolerance, quality and morphological characters of pod and seed, *etc.* Resistance to some of the most important biotic stress such as virus, bacteria, nematodes, *etc.* is carried out in collaboration with researchers of different institution (CNR, Universities, regional institution for plant protection). These data are fundamental for the advancement of genetic materials (mapping populations, segregating progenies, improved lines, *etc.*), essential requirements for the development of molecular markers such as the new markers linked to root-knot nematode resistance. Together with the traditional activity of development of new molecular markers *Phaseolus* resistance genes are studied through innovative genomic strategies able to describe the dynamics of this gene family in common bean germplasm and to identify new resistance sources. Breeding is usually managed by molecular markers and MAS speeds up the development of new varieties, more than 20 in the last 10 years.

As far as pea concerns the characterization mainly is focused on cold tolerance.

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## CNR - The Bari Gene Bank

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The Bari Gene Bank unit (former Germplasm Institute) of the Plant Genetic Institute (IGV) belonging to the National Research Council (CNR) has made important progress to improve the collecting, conservation, evaluation and utilization of grain legumes genetic resources throughout partnerships in regional, national and international research projects.

The total number of legume accessions, including wild relatives collected since 1971, exceeds 10.514 (Table 1). and it is composed mostly of old cultivars and landraces collected in Italy. Numerous accessions have been acquired through the exchange with other Institutions. Between 1990-2007, collecting expeditions are organized each year jointly with the IPK of Gatersleben (Germany) in different regions of Italy to collect local forms and wild relative. Up to the moment, all legume entries conserved in the Bari Gene Bank have been multiplied in order to make them available for exchange. At the present the Bari Gene Bank database (Passport data) is upgrading and consequently the searchable Web-site service is temporarily out of order.

**Table 1.** Bari IGV food legume base collections (200?)

<b>Genus/Species</b>	<b>No. of accessions</b>
<i>Cicer</i>	357
<i>Lens</i>	348
<i>Lupinus</i>	188
<i>Phaseolus</i>	1.508
<i>Pisum</i>	4.558
<i>Vicia faba</i> L.	2.279
<i>Vigna</i>	944
<i>Lathyrus</i>	332
<b>TOTAL</b>	<b>10.514</b>

Stored entries with low germination rates, small amounts in active collections or which are insufficiently represented in the collection are subject to multiplication and regeneration programmes and are conducted on an *ad hoc* basis-in practice, almost every year. Our grain legume collections are regenerated in the open field, while outcrossing species such as faba beans are regenerated in bee-proof enclosures. Characterization and evaluation are continuously ongoing. Characterization according international standards for morphological and agronomical descriptors was carried out on accessions of pea, faba bean, lentil, chickpea and grass pea.

Financial support received from the Italian National Research Council (CNR) for genetic resources preservation is decreasing, which limits collecting activities, regeneration, characterization and evaluation. However, local institutions, farmer associations and private seed companies already

participate in regeneration and characterization research projects, since this activity offer an outstanding opportunity for them to handle new genetic variability with potential use for breeding and /or add value to local and typical products. The major qualitative characters studied in grain legumes are connected directly with current breeding objectives. They also take into account farmers' requirements regarding resistance to stress factors, productivity, resistance to the most commonly occurring diseases in Italy, and grain quality.

The following research activities and evaluation programmes are being carried out:

*Vicia faba* L.:

- phenotypic and genetic diversity studies of the faba bean collection;
- constitution of a "core collection" using biometric and biochemical (protein and enzymes) markers;
- agronomic evaluation including resistance to biotic (*Uromyces viciae-fabae*, *Orobanche crenata*) and abiotic stress;
- genotype x environmental interaction study to identify the more stable and productive biotypes;
- selection of more suitable biotypes for agroindustrial uses with particular attention to new products useful to animal and human nutrition.
- Improved populations of *V. faba* type *minor* included in national test by Annicchiarico, 2005 and characterized by high and stable yield with high protein content : MG 109307, MG 109263.
- Autochthonous improved populations of *V. faba* type *major* : Locale di Castellana (BA), Locale di Matera, Locale di Zollino (LE).

*Lathyrus sativus* L.:

- Phenotypic and genetic diversity studies of the grass pea collection;
- constitution of a "core collection" using biometric, biochemical (protein and enzymes), and molecular (RAPD, AFLP) markers;
- agronomic evaluation and selection of the most productive lines characterized by high protein content, low ODAP content( a neurotoxic compound), large and white seeds;
- genotype x environmental interaction study to identify the more stable lines.
- Lines of *Lathyrus sativus* characterized by , high and stable yield with medium ODAP content, high protein content, large and white seeds: MG 103203-1; MG 110492-4.

*Lens culinaris* Medik:

- Studies of variation within the collection for morpho-agronomic, biochemical (seed storage proteins, isozymes using by SDS-PAGE and Capillary Electrophoresis) and molecular markers (RAPD's, AFLP, Microsatellites)
- Correlation between antinutritional factor and storage pest resistance of seeds;
- Determination of parameters related to nutritional and commercial quality (seed composition, antinutritional factor levels, seed cooking time, etc.);
- Identification and valorization of some Italian landraces (Lenticchia di Altamura, Lenticchia di Villalba, Lenticchia di Mormanno, , Lenticchia di Ustica, Lenticchia di Pantelleria , Lenticchia di Castelluccio and others autochthonous populations from central regions of Italy).

*Phaseolus* spp.:

- Diversity and evolutionary studies in *P. vulgaris* and *P. lunatus* using biochemical (seed storage proteins, isozymes) and molecular markers (RAPD's, AFLP, Microsatellites)
- Correlation between antinutritional factor and storage pest resistance of seeds;

- Identification and characterization of some Italian agro-ecotypes by molecular markers (RAPD's, AFLP, Microsatellites): Fagioli di Sarconi (Verdolino, Ciuto, Riso, Tabacchino), Bianco di Bagnasco, Fagioli di Lamon (Spagnolet), Fagiolo di Saluggia, Castelluccisa di Rotonda (PZ), Poverello bianco, a Badde (Sicily), Fagiolo del Purgatorio and autochthonous populations from Basilicata and Abruzzo regions.

*Vigna* spp.:

- Diversity and evolutionary studies in *Vigna* spp. using morpho-agronomic and biochemical (seed storage proteins, isozymes) markers..
- Correlation between antinutritional factor and storage pest resistance of seeds;

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### ***Landraces of Phaseolus vulgaris collected in Basilicata Region.***

Basilicata Region for geographical position and for particular orography of area in the past was isolated so it keeps many genetic resources, in particular bean (*Phaseolus vulgaris* L.) landraces. These populations are not widespread in the area, but localized in different ecological niches

associated with traditional farming technique. They are still grown in small garden for family use and preserved. So genetic diversity of bean landraces was very high and is maintained and found in the inland of this region. Collecting expeditions and ‘*in situ / ex situ*’ conservation of bean landraces projects were carried out since 1998 at Basilicata University.

At the present bean collection consists of 571 accessions of *Phaseolus vulgaris*. 469 accessions of the collection were characterized for passport data and seed traits and grouped in 29 landraces (Masi et al. 1999, Masi 2001).

Besides, bean collection was characterized for 10 morpho-agronomic traits (5 quantitative: pod length, number of pods per plant, number of seeds per pod, seed weight per pod, 100 seed weight, and 5 qualitative: colour and shape of leaf, flower colour, colour and shape of pod) and for phaseolin pattern (Masi, 2001), table 1.

**Table 1.** Common bean (*Phaseolus vulgaris* L.) landraces identified in Basilicata: number of accessions collected for each landrace and its phaseolin pattern

<b>Landraces</b>	<b>n</b>	<b>Phaseolin pattern</b>
Borlotto type	85	T
Cannellino	35	T
Castelluccisa	7	C
Che si spacca	2	C
Ciuoto	5	T
Cocco bianco	5	T
Cornaiolo,	12	C
Fasul’ cu’ l’ucchieidd	3	C
Fasul’ bianco	30	C
Fasul’ di montagna	3	C
Gialletto	2	T
Lardaro	12	C
Lattino o Vaccarella	16	T-S
Mangiatutto	11	T
Marruchedd’	18	C
A’Marrozzo	44	C
Munacedd’	5	C
Nasiedd’	11	C
Panzaredd’	6	C
Poverella	23	C
Quarantano	9	C
Riso bianco	11	C
Riso giallo	7	T
Tabacchino	14	T
Tondino	60	C
Tuvagliedd’	21	C
Uocch’ di bue	3	C
Verdolino	22	T
Za’ Vicenza	9	C
Cultivars	24	T
Others	56	T-S
<b>Total</b>	<b>571</b>	

### ***Landraces of Phaseolus vulgaris collected in Campania Region (Cilento).***

In the Cilento area, at the boundary of Basilicata Region, in the Casalbuono (SA) district where bean is cultivated for a long time, 101 accessions were collected: 61 indeterminate and 40 determinate growth habit. In a preliminary analysis 16 landraces were identified by passport data and seed trait, table 2.

**Table 2.** Common bean (*Phaseolus vulgaris* L.) landraces identified in Campania (Cilento) and number of accessions collected for each landrace.

<b>Landraces</b>	<b>n</b>
<i>Determinate habit</i>	
Borlotto type	3
Cannellino	18
Risi	1
Rossi	1
Tabaccanti	16
Verdi	1
<b>Total</b>	<b>40</b>
<i>Indeterminate habit</i>	
Borlotto type	23
Ciutariedd	1
Lardari	1
Munaciedd	4
Panzariedd	7
Risi	13
Rossi	8
San Pasquale	2
Verdi	1
Fasul' nero	1
<b>Total</b>	<b>61</b>

All collections are kept at Centro Interdipartimentale per la Salvaguardia delle Risorse Genetiche Vegetali 'Pierino Iannelli' - Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Basilicata University, Potenza, Italy.

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#### ***Exploration and collection activities.***

Since the 1994 missions to explore and collect bean landraces (LRs) have been undertaken in different regions of Central Italy by DBVBAZ in farms and local markets. A few other accessions were donated to DBVBAZ by regional Institutions (Agencies for Agricultural Development of Tuscany and Abruzzo Region) or University collections (Turin University, prof. Lanteri and University of Basilicata, prof. Spagnoletti Zeuli).

Collecting germplasm, farmers were approached by the DBVBAZ staff in a friendly manner, explaining the reason of the visit. Only materials reproduced on their farm for generations was collected. Detailed informations were collected about adaptative, agronomic, qualitative and organoleptic traits, as well as seed exchange among farmers, use and local names, farm size,

farmer's age, reason for continuing to maintain landraces on-farm, traditions and social context. In addition notes about geographical characteristics (altitude, latitude, longitude) of collection site were recorded. LR(submitted) seeds were then stored under long term storage conditions (6-7% seed humidity, -18°C, vacuum sealed packets) in the DBVBAZ germplasm bank (Negri and Tosti 2002). Both *Phaseolus coccineus* L. and common bean (*P. vulgaris* L.) were found in 10 Italian Regions (Tiranti 2005). Many LRs were found in small farms or home gardens under traditional farming systems. LRs are mostly used by the farmers' families, only a little part is sold at local and wider markets. LRs have been maintained *on-farm* because of traditional reasons, organoleptic peculiarities (which make them highly valued on local and city markets), resistance/good productivity under difficult or harsh climatic conditions, or simply because they are appreciated by the families (Negri 2003)

#### ***Characterization of an Italian collection***

Attention was focused on 159 *P. vulgaris* LRs. These accessions were characterised for morphological and molecular traits. Growth habit, colour of flower, seed shape, seed colour, seed coat pattern (IBPGR 1982, IBPGRI 1984) and one hundred seed weight were recorded for morphological characterization.

A great phenotypic variation was found for growth habit (from bushy to aggressively climbing ability), colour of flower (commonly white, lilac, purple in the common bean), seed shape (mainly kidney, oval and cuboid), seed colour (mainly white, brown, pink and yellow, mixtures of different colours were also recorded), seed coat pattern (from absent to striped coats) and 100 seeds weight (ranged from 16.7 to 91.8 g). The genetic diversity of the collection was assessed using 10 SSR molecular markers. Our findings show that all Italian common bean LRs were distinguishable each other (Tiranti 2005, Tiranti et al, in press).

#### ***Appropriate strategy and promotion of in situ (on-farm) conservation***

Landraces should be preserved *in situ* (on-farm) in order to evolve with the environmental changes and maintain their potential utility for future generations.

In order to define an appropriate *in situ* conservation strategy, which can serve as a model for other populations, a LR (called 'A Pisello') case study was chosen. Five farmer populations of the threatened LR were examined with 28 SSR markers. Results showed that different farmers maintain distinct gene pools and diversity is maintained at the subpopulation level. As consequence the best strategy for preserving this LR would be to maintain the entire population on-farm (Tiranti and Negri, 2007).

A participatory breeding program for increasing disease resistance and dwarfing the size of this threatened LR was also carried out in order to promote its maintenance in cultivation (Tiranti, 2005).

#### ***In situ versus ex situ conservation***

Finally, genetic changes occurring *in situ* (on-farm) and *ex situ* were monitored in the above mentioned bean LR. Multiplication outside the original habitat changed the genetic structure in few years. The relevant genetic diversity of a landrace is much better maintained applying an *in situ* than an *ex situ* conservation strategy (Negri and Tiranti, submitted).

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## Università Politecnica delle Marche

Grain Legume genetic resources from the Plant Genetics group from the Università Politecnica delle Marche Italy (professor Roberto Papa, [r.papa@univpm.it](mailto:r.papa@univpm.it))

The major focus of our research group is the study of the evolution of the genetic diversity and its implication for plant breeding of the common bean and other legume species. For this purposes we have a large collection of germplasm accessions of *Phaseolus* and other legumes. In details we have

- 1) about 900 accessions of wild and domesticated *Phaseolus vulgaris* and other *Phaseolus* spp. from the centre of origin of the species obtained from gene banks and research institute (mainly USDA and CIAT).
- 2) about 1200 accessions of European landraces of *Phaseolus vulgaris* and *P. coccineus* obtained from the IPK gene bank and by others European research group. From this materials we have developed a core collection of European *P. vulgaris* (Logozzo et al., 2007).
- 3) Landraces of different grain legume species collected in the Marche region (*Phaseolus*, *Pisum*., *Lathyrus*, *Faba*, *Vicia*, *Cicer* ).
- 4) Two segregant populations (RILs) (BAT93XJaloEEP558 and MIDASXG12873) kindly provided by P. Gepts, Department of Agronomy and Range Science, UC Davis, USA).
- 5) Introgression lines developed from the cross between G12073 (a wild genotype from Morelos, Mexico) and MIDAS a domesticated Andean genotype
- 6) Two Cosmid libraries of G12073 and MIDAS developed in collaboration with E. Albertini from University of Perugia.

Our research activities on these materials have been carried out in a tight collaboration with several research groups from Italy (G. Attene University of Sassari, P. Spagnoletti Zeuli University of Basilicata, V. Negri University of Perugia, A. Carboni CRA, Bologna) and interanationally (P. Gepts UC Davis, USA, P. McClean North Dakota State University and H Knupffer IPK, Gatersleben, Germany).

### Published papers on European and Italian *Phaseolus* spp:

LOGOZZO G, DONNOLI R, MACALUSO L, PAPA R., KNUPFFER H, SPAGNOLETTI PZ. (2007). Analysis of the contribution of Mesoamerican and Andean gene pools to European common bean (*Phaseolus vulgaris* L.) germplasm and strategies to establish a core collection. *GENETIC RESOURCES AND CROP EVOLUTION* Published online April 2007: DOI 10.1007/s10722-006-9185-2

SICARD D., NANNI L., PORFIRI O., D. BULFON D., PAPA R., (2005). Genetic diversity of *Phaseolus vulgaris* L. and *P. coccineus* landraces in central Italy. *PLANT BREEDING*. vol. 124, pp. 464-472

### **Published paper on wild and domesticated *Phaseolus vulgaris*:**

PAPA R., BELLUCCI E, ROSSI M, LEONARDI S, RAU D, GEPTS P, NANNI L, ATTENE G. (2007). Tagging the Signatures of Domestication in Common Bean (*Phaseolus vulgaris* L.) by means of pooled DNA samples. ANNALS OF BOTANY

GONZALEZ A., WONG A., DELGADO-SALINAS A., PAPA R., GEPTS P., (2005). Assessment of Inter Simple Sequence Repeat Markers to Differentiate Sympatric Wild and Domesticated Populations of Common Bean (*Phaseolus vulgaris* L.). CROP SCIENCE . vol. 45(2), pp. 606-615

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PAPA R., GEPTS P., (2003). Asymmetry of gene flow and differential geographical structure of molecular diversity in wild and domesticated common bean (*Phaseolus vulgaris* L.) from Mesoamerica. THEORETICAL AND APPLIED GENETICS 106:239-250

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PAPA R., NANNI L., SICARD D., RAU D., ATTENE G., (2006). The evolution of genetic diversity in *Phaseolus vulgaris* L. In: T. J. Motley, N. Zerega, and H. Cross (Eds) Darwin 's Harvest: New Approaches to the Origins, Evolution, and Conservation of Crops. (pp.121-142) NY: Columbia University Press.

## **Università di Sassari**

Dipartimento di Scienze Agronomiche e Genetica Vegetale Agraria  
(professor Giovanna Attene, [attene@uniss.it](mailto:attene@uniss.it) )

The establishment of the Sardinian Grain Legume collection was carried out in two different periods (2003 and 2006) and interested all the regional territory. Each seeds sample was collected from a single farmer (and from a single field) and it was supposed as local variety only when this material was cultivated in the farm since 30 years (depending the farmer informations). All the sampled accessions are cultivated *in situ*. The collection comprehends the following species: *Cicer*, *Lathyrus*, *Phaseolus*, *Vicia faba* L., *Pisum* and *Vigna*, and common bean is the species most represented. In the year 2003 73 landraces were collected 73 but now the collection is made of 105 different local varieties. All the materials were characterized according with the IPGRI descriptors, and the initial 73 genotypes were also molecularly characterized (by seed proteins and by DNA molecular markers)

The phaseolin analysis was carried out at the University of Basilicata by Dr Giuseppina Logozzo. The molecular characterization was done using microsatellites (10 nuSSR e 14 cpSSR) on the local germplasm plus American controls and 15 commercial varieties chosen among the most cultivated in Sardinia. Other cultivars were provided by the CRA-ISCI of Bologna and they correspond to the most cultivated materials in Italy. Another part was collected in the local market.

Molecular markers were able to define the level and the structure of the genetic diversity of the Sardinian local varieties, emphasizing their originality, importance and necessity to preserve them as precious source of biodiversity. The collection is also completed by other species, precisely 65 landraces (12 chickpea, 15 grass pea, 23 faba bean, 11 peas and 4 vigna); all of them are characterized by passport data.

The collection is conserved *ex situ* at the “Centro per la Conservazione e Valorizzazione della Biodiversità” of the University of Sassari.

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