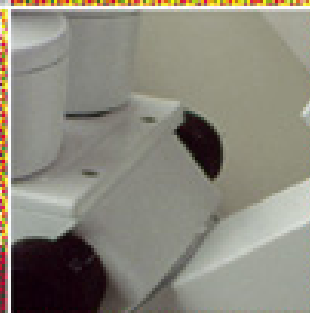




P H A S E L I E U

HANDBOOK
ON
EVALUATION
OF GERMLASM



**HANDBOOK ON EVALUATION
OF *Phaseolus* GERMPLASM**

HANDBOOK ON EVALUATION OF *Phaseolus* GERMPLASM

C. De La Cuadr a, A. M. De Ron and R. Schachl
(Editors)

A PHASELIEU Pub lication

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The PHASELIEU project

The PHASELIEU project (Improvement of sustainable *Phaseolus* production in Europe for human consumption, FAIR5-PL97-3463) developed under the FAIR Program of the European Union was born in 1998.

The overall aim of PHASELIEU was to coordinate the ongoing research on *Phaseolus* and to elaborate and integrated strategy model for the improvement of *Phaseolus* production in Europe for human consumption. Also, this project would like to avoid the duplication of current research and other RTD activities at national and transnational level on *Phaseolus*.

Therefore, the strategic aims of the project are the following ones, concerning to the organization and management of research and development in *Phaseolus*:

- * The **establishment of an EU wide network of experts** in order to exchange and disseminate the knowledge and expertise regarding the issues concerned. This includes also the exchange of genetic material within the participating groups and other outside the network.
- * The **organization of thematic workshops-group meetings**, as open as possible, in order to discuss specific subjects, to develop an integrative strategy model approach, and, on the basis of this model, to prepare follow-up research proposals to develop joint shared cost project in *Phaseolus* improvement.
- * The **publication of several scientific and technical documents** such as: **a)** progress and final reports, **b)** scientific and technical articles, **c)** handbooks and catalogues and **d)** contribution in international conferences. It is planned to publish all of them both as hardcopy version, electronic one in Internet and CD-ROM.
- * The **scientific exchange as training visits** are one of the aspects of the project. There will be two kinds of exchange visits among laboratories: 1) short visits, like targeted restricted meeting and 2) visits, for technology transfer and diffusion of information notably for younger scientist. First year all of them will be focused in genetic variability, cropping systems and diversification and quality analysis. During the following years (with the agreement required from the Commission) the subject of the visits will be focused in

transferring expertise on biotical and abiotic stresses, molecular markers, regeneration and transformation and breeding.

Eleven european countries (Austria, Belgium, France, Germany, Israel, Italy, Portugal, Spain, The Netherlands and the United Kingdom) and twelve partners with their research groups and fellowships participated in the PHASELIEU project (List of PHASELIEU participants is included). Also, well known scientific institutions such as International Plant Genetic Resources Institute (IPGRI), European Association for Grain Legume Research (AEP), and “Centro Internacional de Agricultura Tropical” (CIAT) supported PHASELIEU project as linked organizations.



PREFACE

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Bean genetic resources shall again contribute to the well-being of humans, to the quality and diversity of their food. This time, they undertake once more the journey back of the galleons through the Ocean Sea. In Europe, where cultural aspects so much reflect in every day food, *Phaseolus* beans, although all introduced crops, have integrated that human perspective, ending into many different dishes and recipes. Crop histories initiated on one side of the Atlantic Ocean enable the common bean, scarlet runner and Lima bean to continue their evolution beyond the other seashore. Bean uses were noted all across the Americas, and were tried again in the Old World on the beans that successfully made the journey. European peoples also tried with the newly adopted beans agronomic and culinary practices that were developed for centuries on peas, lentils, chickpeas, grasspeas, and haba beans, resulting thus in increased selection pressures. One should note that in comparison to these Old World pulses the newcomers react with astonishing variability in seed morphotypes exciting further the curiosity of European gardeners.

Maybe *Phaseolus* beans were domesticated first as snap bean for their young developing pods or for their young developing seeds, when the early Amerindians observed doves and parakeets feeding on them. And 8,000 years later snap bean is a key market in Europe and the Mediterranean region. It has become so popular that it has been named “Garden” bean, as there would be no other bean in your garden! It is also called “French” bean – a strong indication of its adoption by *gourmets* of both sides of North Atlantic. Although less favored by the European public now in comparison to snap bean, with fewer mutations, “String Bean” varieties have been grown by European gardeners from Brittany to Friesland. Perhaps the development of these cultivars would not have been possible without the “*exotl*” of the ancient Mexicas. Among the use of “greens” in beans, one should not forget the consumption of flowers and young leaves by the Tzotzils of Chiapas, and that of leaves by farming communities in the African Great Lakes region or in Java.

Green shelled beans, that once were so important for many Amerindians in cool and humid altitude regions of Mesoamerica and the Andes, because of obvious advantages in cooking time and digestibility, are making an important comeback in many regional dishes of southern Europe. The “pochas” of Navarra correspond to the plates of “petaco” of Antioquia or “ixich” of Huehuetenango. Popping beans were roasted before ceramics on hearthside in the Andes, and perhaps in other parts of ancient America as well. Dry beans once ceramics was discovered were part of the Amerindian plant trilogy and the food foundation of so many prestigious pre-Columbian civilizations. Dry beans under dozens of combinations of seed colors and sizes now form the daily food of millions of people in Latin America and Africa, but also enter into processed food for urban humans worldwide where time and health are a concern. Apart from snacks, salads and main courses, beans have been served as sweets for dessert from the Coast of Peru to Thailand. Dry beans are also the banner of many typical dishes in Europe: the *fabada asturiana* of Spain, the *cassoulet toulousain* of France, or the *uccelletti ai fagioli* of Italy. Sailors could not travel across all world seas without Navy beans. One can surely bet that astronauts will take some beans, perhaps nuñas, in their future odysseys!

Such a diversity of bean products and thus of opportunities for further crop development invites us to re-visit *Phaseolus* bean genetic resources and re-examine methods of evaluating them. This “Handbook on Evaluation of *Phaseolus* Germplasm” sums up recent advances in techniques for germplasm evaluation. Chapter 1 presents a practical methodology for the *ex situ* conservation of *Phaseolus* genetic resources in order to make them available for evaluation at any time now and in the future. Chapter 2 presents basic and additional passport descriptors as well as those needed for the sound management of *Phaseolus* collections. Chapter 3 complements the former one with descriptors for phenology and morphology of both vegetative and reproductive plant parts. The comparison of descriptor sources in both germplasm and seed industry (i.e. certification of new varieties) sectors makes these contributions particularly useful for genebank curators, agronomists, and bean breeders. Users will also appreciate a set of color pictures explaining some of the most used qualitative traits in pods and seeds. Chapter 4 presents a thorough compilation of heritability coefficients and correlation between different economically important traits, which shall help the breeder namely to focus on certain descriptors if not all can be evaluated at the same time. Chapter 5 introduces important nutritional characteristics and antinutritional factors of dry beans, and the necessary tasting protocol. Chapter 6 presents the use of bean germplasm in breeding with thorough information about sources of variability and useful genes, as well as breeding strategies. This practical handbook is a timely Phaselieu publication, for the further characterization of the rich heritage of bean landraces in Europe, and for the future breeding efforts, when the European peoples have a renewed and justified interest for these types of crops. It also serves beautifully as a link between researchers and disciplines, by providing contacts and a common technical language.

For sure, it deserves an enthusiastic welcome by all phaseologists!



Introduction

To improve the utilization of European *Phaseolus* germplasm collections is a current challenge. It is well known that a very low percentage of germplasm collection is used in breeding programs and this percentage is lowest if we are looking at accessions with the highest genetic variability as landraces are.

To make easy the use of PGR collections it is essential to offer to the users accessions well documented. It means that the general characterization and the evaluation specially interesting for the crop are made and the resulting data are correct presented in a data base easy to handle.

So, the aims of this handbook must be to serve as a good orientation to any research that need to manage a *Phaseolus* collection, to give them the descriptors more appropriated in each case and to offer the best methodology to obtain the more representative data for each descriptor.

All these objectives are developed of course under the light of the current state of art and ratified by us as the European specialists in *Phaseolus*.

Madrid, Pontevedra (SPAIN), Linz (AUSTRIA). March of 2001

The Editors,
C. DE LA CUADRA, A. M. DE RON, R. SCHACHL



Phaseolus GENETIC RESOURCES "EX SITU" CONSERVATION METHODOLOGY

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Introduction

Ex Situ Collection is the conservation of plant genetic resources outside of their natural environment by means of different technologies.

Phaseolus species are well conserved by seed because these species possess orthodox seeds. It means that the seed can be desiccated and cold stored to prolong dramatically its longevity.

Seed conservation must be performed as close as possible under the international rules of FAO/IPGRI (Genebank Standard, FAO/IPGRI 1994; International Code of Conduct for Plant Germplasm Collecting and Transfer, FAO 1994).

The most interesting species for breeding purposes are: crops, wild relatives and weedy types.

The "Ex Situ" conservation of plant genetic resources has the following steps:

1. Botanical prospecting and expedition to obtain the genetic material
2. Storage of the genetic material to look for viability preservation
3. Characterization of the genetic material
4. Documentation of the genetic material
5. Transfer of the genetic material

The different steps for *Phaseolus* accessions are detailed as follows:

1. Botanical prospecting and collecting

1.1 Prospecting

- Information analysis of the material already conserved
- Election of the area

- Ecogeographical, social and economic study of the area
- Meeting with the agronomic responsible of the area
- Collectors' permission if it is necessary
- Collecting strategy:
 - a) Best time to obtain seed from the plant (wild species), best time to obtain seed from the farmer (crop species).
 - b) Distance to be covered by day, depending upon road difficulties and number of stops.
 - c) Establish the best method to carry the seeds and to identify each accession, etc.

1.2 Expedition

- To collect all the interesting wild species that grow in the area, or making multicrop collection in the case of crop species
- To obtain and register in collection forms all the information about ecological, geographical, botanical and cultural aspects, and also about utilization.
- To assign an unique expedition number to each sample.

1.3 Return to the base

- All the seeds and data must be given to the Curator and Database Responsible respectively.
- Expedition report.

It is very important to follow up all the recommendations from the International Code of Conduct for Plant Germplasm Collecting and Transfer (FAO, 1994).

2. Storage of material and viability

2.1. Seed handlings

Cleaning

“Seeds for storage in germplasm collections should be as clean and free from weed seeds, pests and diseases as possible” (Genebank standards, FAO/IPGRI 1994).

The *Phaseolus* seeds must be shelled by hand. Then, they can be first cleaned with a winnowing machine to eliminate the majority of the vegetable and ground rests, but it is always necessary to finish with a hand made cleaning to eliminate broken seeds, deformed seeds and seeds with insects or any other detectable parasite.

A phytopathological study on accessions is also a desirable objective in seed storage of Plant Genetic Resources.



Viability control

“Genebank manager has the responsibility to provide conditions which will maintain the viability of each accession held within the genebank above a minimum value. Hence, accessions viability must be monitored” (Genebank standard, FAO/IPGRI, 1994).

Currently seed viability is regularly checked by germination tests. The problem in some species, such as *Phaseolus*, is that germination is a destructive method and it is difficult to obtain a large number of seeds by accession. An alternative is Tetrazolium tests, which need fewer seeds. But, if it is possible, it is better to carry out the germination tests.

Germination tests must be carried out under International rules (i.e. International Rules for Seed Testing, 1996, International Seed Testing Association). The International rules must be adapted to the specific problem of *Phaseolus* G.R.: the small quantity of seeds by accession. Some works organize the germination test with less seeds without any statistical problem.

The occurrence of soaking injury in dried seeds subjected to rapid imbibition has been reported for many legume species. Therefore a study of the suitable procedures of seed humidification prior the germination test is strongly recommended.

The Control of Viability Laboratory must have its own database, where methodology (including breaking dormancy), germination data (as percentage of germination, count times, fungus and bacteria development, etc) and origin and destination of the accession must be included.

If it is possible, control of viability must be developed on the original sample, after multiplication/regeneration and after ten years of conservation. All this data together offer us a good perspective about any deteriorative changes of stored seeds.

Drying

The objective of drying the seed is to reduce the moisture content to a level of 3-7% which prolongs longevity during storage and therefore increases the regeneration interval (Genebank standard, FAO/IPGRI, 1994).

As *Phaseolus* species have large size seeds, the use of a dehumidified drying chamber method seems to be more appropriated. Introduction of the material into the chamber must be made as soon as possible to avoid loss of viability.

It is very important to avoid any rehydration of desiccated accessions, so the containers where the accessions are stored must be hermetically closed. Hermeticity of containers is very important for two reasons:

- 1) It is very difficult and expensive to keep a very low humidity and temperature environment in the cold-room.

- 2) The relationship between the humidity inside the seed and the humidity in the environment of the seeds varies with the temperature.

2.2. Seed storage

The storage conditions change depending upon the focus on the collection. In Plant Genetic Resources there are two types of collections. In a **Base Collection**, the accessions are preserved for a long-term future and seeds will not be distributed from this collection. The **Active Collection** comprises accessions, which are immediately available for multiplication and distribution to satisfy requests, and they are maintained under medium-term storage conditions. A third type of collections can be distinguished: the Work Collections or Breeding Collections (for prebreeding or breeding purposes).

In general, sub-zero temperatures are very appropriate for a Base Collection and temperatures between 4 and -4 ° C for Active Collections. In all the cases seed moisture must be between 3-7%.

In the case of *Phaseolus* species, it seems advisable to avoid ultra-dry situations because their seeds are, as in other large seed size legumes species, fragile and problematic during management. Otherwise, cold temperatures have no detectable effects on dry seeds (anyway this aspect should be investigated). For this species, seed moisture between 6-7%, and temperature of -18 to -20° C in Base Collection and -4 to -5° C in Active Collection, it seems to be recommendable.

The PGR collections consist of a set of accessions distinct among them and FAO/IPGRI recommends as an acceptable standard 1000 viable seeds by accession and as a preferred standard 1500 to 2000 viable seeds by accession. According with our experience, *Phaseolus* species form a difficult material to multiply so, for a large number of seeds by accession, more than one multiplication is needed. The multiplications of material take erosion dangers, so the acceptable standard (1000 viable seeds/accession) is more appropriate in this particular case.

The viability of the seeds must be ensured, so in *Phaseolus* species the initial germination values should exceed 85%. Looking at the results of our control of viability after ten years of conservation, we can conclude that this Genus presents some conservation difficulties and the initial viability has a high influence on it. Therefore, initial multiplication for base collection must be done extremely carefully.

All the information coming from the seed storage routine (situation into the bank included) has to be recovered in the Management Database.

3. Characterization of the material

Multiplication

Multiplication is needed when the number of seeds by accession is small for distribution in Active Collections or when a duplicate of the accession is needed



for the Base Collection. This number can never be smaller than the seed needed for a correct multiplication that depends on the species.

In the case of *Phaseolus* species 60 to 100 seeds is the current number used.

Regeneration

Regeneration is needed to ensure that the seeds of the accession do not fall below acceptable levels of viability. The operation is the same as in the case of multiplication. The objective of multiplication is to obtain a new generation of seeds.

The control of seed viability after a period of storage is essential to know when regeneration is needed.

The frequency of regeneration depends on the species and the storage conditions. According to our experience the viability of *Phaseolus vulgaris* under long term conservation method (seed humidity below 7%, hermetically sealed tin containers and -18° C) and after ten years of conservation can be reduced around 12% of the initial value. Therefore, it is recommended to be very careful with the viability of a long-term conservation collection of *Phaseolus* species. Initial viability has a very high influence on the longevity of the stored seeds.

As regeneration requirements are determined by germination test it is necessary to assure that seed viability is not underestimated. As dry seeds of *Phaseolus* can be sensitive to imbibition, this aspect should be carefully treated.

Characterization

A general characterization is essential to cause the utilization of the collection. All the descriptors used for *Phaseolus* species and the selection of the more interesting ones are presented in the Chapter “Characterisation of *Phaseolus* accessions” of this Handbook.

Identification of duplicated accessions into the active collection and to get a core collection are two important tasks of the characterization.

4. Documentation of the material

It is essential to possess a well documented collection, with passport, management and characterization data.

As the subject is extensively presented in other Chapter of this Handbook it does not seem to be necessary to present it once more.

5. Transfer of material

The intention of Plan Genetic Resources (PGR) collections is not only to preserve genetic material from the erosion, but also its direct utilization by farmers or through breeding programs.

The collection needs to be presented to the potential users. In addition, to develop an “Index Seminum”, including the Institutions which are maintaining the PGR Collections, and its distribution by internet it would be very useful.

It is advisable to have a Database where all the information concerning material transfer can be recovered. As an example, the Material Transfer Database from CRF is presented.

Ideas from the International Code of Conduct for Plant Germplasm Collecting and Transfer must be straight and a Material Transfer Agreement must be written and signed.

Institution name
Institution Address
Telephone numberFax numberE-mail

REGISTRATION NUMBER:
PETITIONER:
DATE OF REQUEST:
DATE OF SENDING:
INSTITUTION ACRONYM:

NUMBER OF SAMPLES:

DOCUMENTACION ATTACHED: (P, Pasport; C, Characterization; O, Other)

PROJECT:

OBJECTIVE: (M, Multiplication; C, Characterization; U, Utilization; I, Investigation.; O, Other)

REMARKS:

LIST OF ACCESSIONS

| N REG. | GENUS | SPECIES | ACC. NUMBER | AMOUNT |
|--------|-------|---------|-------------|--------|
|--------|-------|---------|-------------|--------|

SIGN:

NOTE: Please, sign and send this paper to the above address as soon as possible



PASSPORT DATA AND MANAGEMENT DATA IN *Phaseolus* GERMPLASM COLLECTIONS

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Passport data

Effective documentation of plant genetic resources is essential to promote their utilisation and to ensure the coordination and rationalisation of conservation activities. In this context, the standardisation of descriptors is a fundamental aspect to make feasible the information exchange and the setting-up of global information systems at a national, regional or world level.

An important progress in passport data standardisation was made as for Europe, in the Workshop held in Budapest in October 1996 within the European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR) coordinated by IPGRI (Lipman *et al.* 1997). In this meeting a multi-crop descriptor list was set up for Central Crop Databases. These descriptors aim to be compatible with future IPGRI crop descriptor lists and with the descriptors used for the FAO World Information and Early Warning System (WIEWS) on PGR. The last version of this list contains two sections: the multicrop passport descriptors and a number of optional descriptor used in the FAO WIEWS.

Furthermore, in the 2nd meeting of the ECP/GR Working Group on Grain Legumes (Maggioni *et al.* 2000), the multicrop passport descriptor list was adopted for data exchange of grain legume collections. The Group also agreed to add to the Grain Legume Passport descriptor list a descriptor indicating whether the safety-duplicate was made or not and to include the attribute “genetic stock” under descriptor “status of sample”.

According with the spirit of the ECP/GR the inclusion of the Grain Legume passport descriptors (Table 1) or compatible ones in the passport information of *Phaseolus* collections is strongly recommended. FAO codes for the standardised designation of institutions are available in the FAO/WIEWS databases (<http://apps3.fao.org/wiews>).

Descriptors of Table 1 should be considered as a basic information at multicrop level and each seedbank should utilise additional descriptors in accordance with its particular requirements or characteristics. For *Phaseolus* collections additional descriptors of Table 2, including some grain/plant characteristics, are proposed. This information should not be taken up as genuine characterisation data but preliminary information to be obtained *de visu* or from the farmers during the collection of the material. In practise, seedbanks can not undertake the characterisation of all accessions in many cases and therefore, the inclusion in the passport data of some uncomplicated information about some relevant characteristics of *Phaseolus* has been considered of interest. Obviously, when data arising from characterisation work exist, this information will be prevailing.

Management data

Management data comprise the information generated through the preservation activities and are essential to organise the operation of a genebank. Management information is mainly destined to internal use and therefore it requires less standardisation among institutions than passport or characterisation data.

The information associated to a seedbank operation is usually divided in two main groups: seed storage and regeneration/multiplication data. General descriptors suggested for management data are outlined in the FAO/IPGRI Genebank Standards (FAO/IPGRI 1994) and more detailed information can be obtained from recent IPGRI crop descriptor lists (e.g. barley, black pepper, *Capsicum*, tomato) where these data are highly unified.

A minimum management descriptor list for *Phaseolus* is proposed in Table 3. Information involving destruction of genetic material has been highly limited considering the fact that, in large seeded species, the space availability frequently constitutes a limiting factor to the number of stored seeds per accession. Thus, if material saving is a priority, data corresponding to seed moisture content could be set aside provided that the seedbank desiccation procedure assures the required seed moisture. All additional available information about germination tests and regeneration/multiplication processes should be also structured and recorded.

Data related to the exchange of material constitute another information inherent to the seedbank management. An appropriate documentation of this information, including petitioner, type of material and aim (see an example in Chapter 2, section 5), will allow to analyse user priorities and can constitute a very useful tool for the future seedbank planning.

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Table 1. Grain Legume Passport Descriptors
(based on FAO/IPGRI Multi-Crop Passport Descriptors)

| GRAIN LEGUMES PASSPORT DESCRIPTORS | |
|--|--------------------|
| 1. Institute code | (INSTCODE) |
| Code of the institute where the accession is maintained. The codes consist of the 3-letter ISO 3166 country code of the country where the institute is located plus number or an acronym as specified in the Institute database that will be made available by FAO. Preliminary codes (i.e. codes not yet incorporated in the FAO Institute database) start with an asterisk followed by a 3-letter ISO 3166 country code and an acronym. | |
| 2. Accession number | (ACCENUMB) |
| This number serves as a unique identifier for accessions and is assigned when an accession is entered into the collection. Once assigned this number should never be reassigned to another accession in the collection. Even if an accession is lost, its assigned number should never be reused. Letters should be used before the number to identify the genebank or national system (e.g. IDG indicates an accession that comes from the genebank at Bari, Italy; CGN indicates an accession from the genebank at Wageningen, The Netherlands; PI indicates an accession within the USA system) | |
| 3. Collecting number | (COLLNUMB) |
| Original number assigned by the collector(s) of the sample, normally composed of the name or initials of the collector(s) followed by a number. This item is essential for identifying duplicates held in different collections. It should be unique and always accompany subsamples wherever they are sent | |
| 4 Genus | (GENUS) |
| Genus name for taxon. Initial Uppercase letter required. | |
| 5 Species | (SPECIES) |
| Specific epithet portion of the scientific name in lowercase letters with authority. Following abbreviation is allowed: "sp." | |
| 6. Subtaxa | (SUBTAXA) |
| Subtaxa can be used to store any additional taxonomic identifier and authority. Following abbreviations are allowed: "ssp." (for subspecies); "var." (for variety); "convar." (for convariety); "f." (for form). | |
| 7. Accession name | (ACCNAME) |
| Either a registered or other formal designation given to the accession. First letter uppercase. Multiple names separated with semicolon. | |
| 8. Country of origin | (ORIGCTY) |
| Name of the country in which the sample was originally collected or derived. Use the ISO 3166 extended codes, (i.e. current and old 3 letter ISO 3166 country codes) | |
| 9. Location of collecting site | (COLLSITE) |
| Location information below the country level that describes where the accession was collected starting with the most detailed information. Might include the distance in kilometers and direction from the nearest town, village or map grid reference point, (e.g. CURITIBA 7S, PARANA means 7 km south of Curitiba in the state of Parana) | |
| 10. Latitude of collecting site | (LATITUDE) |
| Degrees and minutes followed by N (North) or S (South) (e.g. 1030S). Missing data (minutes) should be indicated with hyphen (e.g. 10—S). | |
| 11. Longitude of collecting site | (LONGITUDE) |
| Degrees and minutes followed by E (East) or W (West) (e.g. 07625W). Missing data (minutes) should be indicated with hyphen (e.g. 25—W). | |

**12. Elevation of collecting site [m asl]****(ELEVATION)**

Elevation of collecting site expressed in meters above sea level. Negative values allowed.

13 Collecting date of original sample [YYYYMMDD]**(COLLDATE)**

Collecting date of the original sample where YYYY is the year, MM is the month and DD is the day.

14 Status of sample**(SAMPSTAT)**

- | | |
|---------------------------------|---------------------------------------|
| 1 Wild | 6 Genetic stock |
| 2 Weedy | |
| 3 Traditional cultivar/Landrace | 99 Other (Elaborate in REMARKS field) |
| 4 Breeders line | |
| 5 Advanced cultivar | |

15 Collecting source**(COLLSRC)**

The coding scheme proposed can be used at 2 different levels of detail; Either by using the global codes such as 1, 2, 3, 4 or by using the more detailed coding such as 1.1, 1.2, 1.3 etc.

| 1 Wild habitat | 2 Farm | 3 Market | 4 Institute/ Research organization |
|---------------------|-------------|--------------------|---------------------------------------|
| 1.1 Forest/woodland | 2.1 Field | 3.1 Town | |
| 1.2 Shrubland | 2.2 Orchard | 3.2 Village | |
| 1.3 Grassland | 2.3 Garden | 3.3 Urban | |
| 1.4 Desert/tundra | 2.4 Fallow | 3.4 Other exchange | |
| | 2.5 Pasture | system | 99 Other (Elaborate in REMARKS field) |
| | 2.6 Store | | |

16. Donor institute code**(DONORCODE)**

Code for the donor institute. The codes consist of the 3-letter ISO 3166 country code of the country where the institute is located plus number or an acronym as specified in the Institute database that will be made available by FAO. Preliminary codes (i.e. codes not yet incorporated in the FAO Institute database) start with an asterisk followed by a 3-letter ISO 3166 country code and an acronym.

17. Donor number**(DONORNUMB)**

Number assigned to an accession by the donor. Letters should be used before the number to identify the genebank or national system (e.g. IDG indicates an accession that comes from the genebank at Bari, Italy; CGN indicates an accession from the genebank at Wageningen, The Netherlands; PI indicates an accession within the USA system)

18. Other number(s) associated with the accession**(OTHERNUMB)**

Any other identification number known to exist in other collections for this accession. Letters should be used before the number to identify the genebank or national system (e.g. IDG indicates an accession that comes from the genebank at Bari, Italy; CGN indicates an accession from the genebank at Wageningen, The Netherlands; PI indicates an accession within the USA system). Multiple numbers can be added and should be separated with a semicolon

A. Safety duplication**(SAFEDUP)**

- 0 Sample is not safety-duplicated elsewhere
1 Sample is safety-duplicated elsewhere

19. Remarks**(REMARKS)**

The remarks field is used to add notes or to elaborate on descriptors with value "99"(=Other). Prefix remarks with the field name they refer to and a colon. Separate remarks referring to different fields by semicolons. (e.g. COLLSRC:roadside)

FAO WIEWS DESCRIPTORS ¹

1. Location of safety-duplicates (DUPLSITE)

Code of the institute where a safety-duplicate of the accession is maintained. The codes consist of 3-letter ISO 3166 country code of the country where the institute is located plus number or an acronym as specified in the Institute database that will be made available by FAO. Preliminary codes (i.e. codes not yet incorporated in the FAO Institute database) start with an asterisk followed by a 3-letter ISO 3166 country code and an acronym. Multiple numbers can be added and should be separated with a semicolon.

2. Availability of passport data (PASSAVAIL)

(i.e. in addition to what has been provided)

0 Not available

1 Available

3. Availability of characterization data (CHARAVAIL)

0 Not available

1 Available

4. Availability of evaluation data (EVALAVAIL)

0 Not available

1 Available

5. Acquisition type of the accession (ACQTYPE)

1 Collected/bred originally by the institute

2 Collected/bred originally by joint mission/institution

3 Received as a secondary repository

6. Type of storage (STORATYPE)

Maintenance type of germplasm. If germplasm is maintained under different types of storage, multiple choices are allowed, separated by a semicolon (e.g. 2;3). (Refer to FAO/IPGRI Genebank Standards 1994 for details on storage type)

1 Short-term

2 Medium-term

3 Long-term

4 *In vitro* collection

5 Field genebank collection

6 Cryopreserved

99 Other (elaborate in REMARKS field)

¹ FAO WIEWS Descriptors are used in the FAO World Inventory and Early Warning System. They are optional descriptors for the Grain legumes Central databases

**Table 2. Additional passport descriptors recommended for *Phaseolus* collections.****Use**

Multiple uses can be recorded and should be separated with a semicolon

- 1 Grain
- 2 Pod
- 99 Others (elaborate in REMARKS - Table 1 field).

Seed size (visually estimated)

- 1 Small (<1cm lenght, aprox.)
- 2 Medium (1-2 cm lenght, aprox.)
- 3 Large (>2 cm lenght, aprox.)

Seed colour

- 1 Light colour (white, pale yellow, pale grey, etc.)
- 2 Dark colour (brown, purple, black, etc)
- 3 Colour mixture (mottled, striped, bicolour, etc.)

Growth habit

- 1 Determinate
- 2 Indeterminate

Table 3. Minimum management descriptors for *Phaseolus* collections

1. Accession number
2. Location in storage (building, room, shelf numbers, etc)
3. Storage date [YYYYMMDD]
4. Germination at storage (initial) [%]
5. Date of last germination test [YYYYMMDD]
6. Germination at the last test [%]
7. Date of next germination test [YYYYMMDD]
8. Weight of seeds in storage [g]
9. Weight of 100 seeds [g]
10. Number of seeds in storage*
11. Date of regeneration/multiplication of the sample in storage [YYYYMMDD]
12. Regeneration/multiplication site
13. Number of times accession regenerated/multiplied

* Calculated from descriptors 8 and 9



CHARACTERISATION OF *Phaseolus* ACCESSIONS

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Introduction

There are several descriptor lists for *Phaseolus vulgaris* L., with a different number of variables each of them. In this chapter, information has been compiled proceeding from various lists which is showed in Table 1. The descriptor number, only for the morphological characterisation is variable and always excessive. The main descriptor lists for *Phaseolus vulgaris* are: COMECON (54), ECP/GR (41), IBPGR (56), MBG-CSIC (34), CRF-INIA (55), UPOV (45), and USDA (24) (in parenthesis is indicated the number of variables included in each list). These descriptors have been grouped in eight categories: phenology, plant, leaf, inflorescence, flower, pod immature, pod mature and seed (Table 2) which a total of 97 variables. A small number of variables was not included considering that they are not useful enough: leaf size (MBG-CSIC), emerging cotyledon colour (IBPGR), node number at harvest on main stem (IBPGR), pod break orientation (IBPGR), colour at immature seed (UPOV), pod: ratio transverse width/median width (UPOV), plant shape (COMECON) and fununculus stability (COMECON).

Among the different description systems, those developed by IPGRI and UPOV are the most common ones. The descriptors of IPGRI focus on wild and weedy material or landraces, and those of UPOV on improved cultivars. The application of both systems causes certain problems to genebanks as the accessions usually encompass both primitive and improved types. These descriptors are mainly designed to describe the plants during collection or at vegetative stage, indicating that description work can only be carried out on the field. For this reason is necessary to identify the accession and to follow it on all the stages of the plant development. Taking into account the large number of accessions (about 35.000) preserved in the European genebanks, descriptors following one of these systems can only be used for individual accessions.

Setting up the European *Phaseolus* Database, a synthesis of the most common evaluation and characterisation descriptors was made, mostly based on the IPGRI descriptor (Schachl, 2000).

The PHASELIEU consortium, after the study of several descriptor lists, mainly the elaborated by ECP/GR and those from the Spanish groups, the partners with a large experience in genetic resources into the consortium working in *Phaseolus*, has selected a minimum of 15 characters of plant, flower, pod and seed. They are the most interesting to take when an accession must be included in a collection.

Table 1. Definition of acronyms from descriptor sources.

| ACRONYM | DEFINITION |
|----------|---|
| COMECON | Council for Mutual Economic Assistance (1991) |
| ECP/GR | European Cooperative Program of Genetic Resources (1998) |
| IBPGR | International Board For Plant Genetic Resources (1982) |
| MBG-CSIC | Mision Biologica de Galicia. Consejo Superior de Investigaciones Científicas (1998) |
| CRF-INIA | Centro de Recursos Fitogenéticos-INIA (1998) |
| UPOV | International Union for the Protection of New Varieties of Plants (1994) |
| USDA | EEUU Departament of Agriculture (1998) |



Table 2. Descriptors compiled from the list mentioned in Table 1 grouped by phenology, plant, inflorescence, flower, pod and seed variables.

CATEGORY: PHENOLOGY

| Number | Descriptor | Source |
|--------|--|--|
| 1.01 | Days of first flower | MBG-CSIC, USDA |
| 1.02 | Days to flowering (50%) | COMECON, ECP/GR, IBPGR, MBG-CSIC, CRF-INIA, UPOV |
| 1.03 | Days to the end of flowering | MBG-CSIC, CRF-INIA |
| 1.04 | Days to beginning-end of flowering | ECP/GR, COMECON, IBPGR, MBG-CSIC, CRF-INIA |
| 1.05 | Days to sowing-beginning of ripen | COMECON, MBG-CSIC, CRF-INIA |
| 1.06 | Days from sowing-seeds maturity | COMECON, IBPGR |
| 1.07 | Days from sowing-50% pod non for green use | MBG-CSIC, CRF-INIA |

CATEGORY: PLANT

| Number | Descriptor | Source |
|--------|-------------------------------------|--|
| 2.01 | Type of germination | COMECON |
| 2.02 | Foliage | COMECON |
| 2.03 | Anthocyanin coloration of hypocotyl | COMECON, IBPGR, UPOV, USDA |
| 2.04 | Hypocotyl length | IBPGR |
| 2.05 | Type of growth | COMECON, ECP/GR, IBPGR, MBG-CSIC, CRF-INIA, UPOV, USDA |
| 2.06 | Initial growth rate | COMECON |
| 2.07 | Branches type | COMECON, ECP/GR, CRF-INIA |
| 2.08 | Height | COMECON, ECP/GR, IBPGR, UPOV |
| 2.09 | Height to first node | CRF-INIA |
| 2.10 | Stem diameter | IBPGR |
| 2.11 | Dwarf type (vining-no vining) | UPOV |
| 2.12 | Start of climbing | UPOV |
| 2.13 | Speed of climbing | UPOV |
| 2.14 | Pod per plant | ECP/GR, IBPGR, CRF-INIA |
| 2.15 | Lodging | COMECON, ECP/GR, IBPGR |

CATEGOR Y: LEAF

| Number | Descriptor | Source |
|--------|--|--|
| 3.01 | Shape of primordial leaves | COMECON |
| 3.02 | Shape of the base of primordial leaves | COMECON |
| 3.03 | Shape of the apex of primordial leaves | COMECON |
| 3.04 | Surface of primordial leaves | COMECON |
| 3.05 | Colour of leaves | COMECON, ECP/GR, IBPGR, UPOV |
| 3.06 | Shape of middle leaflet | COMECON, ECP/GR, IBPGR, MBG-CSIC, CRF-INIA, UPOV |
| 3.07 | Shape of middle leaflet apex | COMECON, UPOV |

CATEGOR Y: INFLORESCENCE

| Number | Descriptor | Source |
|--------|--|---------------------|
| 4.01 | Length | COMECON, IBPGR |
| 4.02 | Pedicle length | IBPGR |
| 4.03 | Nr of flowers | COMECON, IBPGR |
| 4.04 | Node Nr from base to first inflorescence | IBPGR |
| 4.05 | Location | COMECON, UPOV, USDA |
| 4.06 | Racemes per plant | IBPGR |

CATEGOR Y: FLOWER

| Number | Descriptor | Source |
|--------|------------------------------|--|
| 5.01 | Bud size | IBPGR |
| 5.02 | Size of bracts | COMECON, IBPGR, MBG-CSIC, UPOV |
| 5.03 | Shape of bracts | IBPGR, MBG-CSIC |
| 5.04 | Bracts/Calyx length relation | IBPGR |
| 5.05 | Calyx/Bracts colour | IBPGR |
| 5.06 | Style protrusion | IBPGR |
| 5.07 | Wings opening | IBPGR |
| 5.08 | Pattern of colour | COMECON, USDA |
| 5.09 | Vexillum colour | COMECON |
| 5.10 | Wings colour | COMECON, ECP/GR, IBPGR, MBG-CSIC, CRF-INIA, UPOV, USDA |
| 5.11 | Standart colour | ECP/GR, IBPGR, MBG-CSIC, CRF-INIA, UPOV |
| 5.12 | Standart veings | MBG-CSIC, USDA |
| 5.13 | Secondary colour of keel | COMECON |

**CATEGORY Y: POD IMMATURE**

| Number | Descriptor | Source |
|---------------|--------------------------------|--|
| 6.01.01 | Position | ECP/GR, USDA |
| 6.01.02 | Length | COMECON, ECP/GR, IBPGR, MBG-CSIC, CRF-INIA, UPOV, USDA |
| 6.01.03 | Width | COMECON, ECP/GR, IBPGR, MBG-CSIC, CRF-INIA, UPOV |
| 6.01.04 | Degree of curvature | COMECON, ECP/GR, IBPGR, CRF-INIA, UPOV |
| 6.01.05 | Shape of curvature | COMECON, UPOV |
| 6.01.06 | Length cord | MBG-CSIC, CRF-INIA |
| 6.01.07 | Parchment coating | COMECON, ECP/GR, IBPGR |
| 6.01.08 | Presence of fiber | COMECON, ECP/GR, IBPGR, MBG-CSIC, CRF-INIA, UPOV, USDA |
| 6.01.09 | Ground colour | COMECON, ECP/GR, IBPGR, MBG-CSIC, CRF-INIA, UPOV, USDA |
| 6.01.10 | Pigmentation | COMECON, ECP/GR, CRF-INIA, UPOV |
| 6.01.11 | Colour of pigmentation spots | COMECON, ECP/GR, CRF-INIA, UPOV |
| 6.01.12 | Location of pigmentation spots | COMECON, ECP/GR, UPOV, USDA |
| 6.01.13 | Shape of distal part | UPOV |
| 6.01.14 | Beak position | ECP/GR, IBPGR, MBG-CSIC, CRF-INIA |
| 6.01.15 | Beak shape | COMECON, MBG-CSIC, CRF-INIA, UPOV |
| 6.01.16 | Beak length | COMECON, ECP/GR, IBPGR, UPOV |
| 6.01.17 | Mass | MBG-CSIC, CRF-INIA |

CATEGOR Y: POD MATURE

| Number | Descriptor | Source |
|---------------|---------------------|-----------------------------------|
| 6.02.01 | Position | ECP/GR, IBPGR, USDA |
| 6.02.02 | Degree of curvature | COMECON |
| 6.02.03 | Locules per pod | IBPGR, UPOV, USDA |
| 6.02.04 | Seeds per pod | ECP/GR, IBPGR, MBG-CSIC, CRF-INIA |
| 6.02.05 | Colour | COMECON, ECP/GR, IBPGR |
| 6.02.06 | Surface | COMECON, UPOV |
| 6.02.07 | Mass | MBG-CSIC |

CATEGOR Y: SEED

| Number | Descriptor | Source |
|---------------|-------------------------|--|
| 7.01 | Cotiledon colour | IBPGR, USDA |
| 7.02 | Shape | COMECON, ECP/GR, IBPGR, CRF-INIA, UPOV, USDA |
| 7.03 | Shape cross | ECP/GR, CRF-INIA, UPOV |
| 7.04 | Ground colour | COMECON, ECP/GR, IBPGR, MBG-CSIC, CRF-INIA, UPOV, USDA |
| 7.05 | Secondary ground colour | COMECON, ECP/GR, IBPGR, CRF-INIA, UPOV, USDA |
| 7.06 | Character of pattern | COMECON, ECP/GR, IBPGR, UPOV, USDA |
| 7.07 | Veining | COMECON, ECP/GR, IBPGR, CRF-INIA, UPOV |
| 7.08 | Hilum ring colour | COMECON, UPOV, USDA |
| 7.09 | Weigth (100 seeds) | IBPGR, MBG-CSIC, CRF-INIA, UPOV, USDA |
| 7.10 | Length | ECP/GR, MBG-CSIC, IBPGR, CRF-INIA |
| 7.11 | Width | ECP/GR, IBPGR, MBG-CSIC, CRF-INIA, UPOV |
| 7.12 | Heigth | ECP/GR, IBPGR, MBG-CSIC, CRF-INIA, UPOV |
| 7.13 | Brillance | IBPGR, CRF-INIA, USDA |
| 7.14 | Water absortion | CRF-INIA |
| 7.15 | Nr of hard seeds | CRF-INIA |
| 7.16 | Volume (100 seeds) | IBPGR |

.../...



| | | |
|------|-------------|-------------------------------------|
| 7.17 | Proteins | COMECON, ECP/GR, MBG-CSIC, CRF-INIA |
| 7.18 | Fatty acids | MBG-CSIC, CRF-INIA |
| 7.19 | Starch | MBG-CSIC, CRF-INIA |
| 7.20 | Sugar | MBG-CSIC, CRF-INIA |

Field Trials Methodology

In this section some schematic guidelines are included which are proposed by the PHASELIEU group to characterize and evaluate bean accessions focussed on different purposes as germplasm characterisation, analysis of cropping systems or breeding.

- Germplasm characterisation. Usually the number of accessions to be characterised in a genebank is large enough to be done by means of a sophisticated design. So the simplest ones could be more appropriated. It could be recommended some designs as the following ones:
 - Hill-plot: 4-6-8 plants of one accession in each hill being 1 m apart each hill from other. This simple design gives the possibility of evaluate 10000 accessions by hectare.
 - By rows: 10-15 plants of each accession in each row. Recommended distances are 0.25 cm among plants and 0.80 among rows.
- Analysis of cropping systems. It is the case of the study of monoculture versus intercropping (frequently with maize) and other research regarding agronomic management. The split-plot design would be the most efficient one, in order to get information about the different components and sources of variation involved in the experiments.
- Breeding. For breeding purposes, morpho agronomical and adaptation evaluation of varieties is needed as well as comparative trials for yield:
 - Morpho agronomical and adaptation evaluation: the design must include two or three replications in each experiment –depending on the number of accessions– and in different locations and years to get information about varieties differences, environmental effects and interactions. Each individual plot must consist of one or two rows to include about 30-50 plants. Recommended distances are 0.25 cm among plants and 0.80 among rows
 - Yield trials: each variety tested must be represented by individual plots with 500-1000 plants. The experimental design could be the same that in the previous case

The Alphanumeric Code System Developed by PHASELIEU

The existing description systems mentioned above are appropriate for description of newly collected material rather than for already existing collections. In general,

the accessions of almost all genebanks are very well described by passport data. The description by characterisation and evaluation data, however, appears poor. There is an absolute need to standardise the characterisation and evaluation descriptors.

The description system presented here aims to describe a reasonable high number of accessions as quick as possible. It largely uses descriptive tables with photographs with an alphanumeric code, whilst also not going into a highly sophisticated identification system and neglecting fine nuances of colours. By that, the system is kept as simple as possible, but as mentioned above it offers the possibility for quick description of large collections. For more detailed information of individual accessions, if needed, one of the existing description and evaluation systems still might be used, and other methods like genetic ones could be included.

The system is based on four main-points with emphasis on the grain:

- 1) grow habit
- 2) seed, in accordance to colour, shape and size additional botanical characters
- 3) flower
- 4) pods again in accordance to colour, shape and size.

The basic characterisation, therefore, can be done immediately and directly in the store, and from information gained during collection. Thus rejuvenation in the field is required only in exceptional cases. The final idea is to include this basic information in the *Phaseolus* data base of ECP/GR.

In the section below are described the minimum descriptor proposed by the PHASELIEU group and the alphanumeric code developed for some of them.

PLANT

1. First flower days: days from sowing to 50% of plants are some flower.

2. Plant type

- 1 Determinate bush.
- 2 Indeterminate bush, with erect stems.
- 3 Indeterminate prostrate, with many lateral guides.
- 4 Indeterminate climber.

LEAF

3. Shape .

- 1 Triangular
- 2 Quadrangular
- 3 Round
- 4 Ovate
- 5 Ovate/lanceolate
- 6 Lanceolate
- 7 Hastate



FLOWER

4. Colour of standard

- 1 White
- 2 Greenish
- 3 Lilac
- 4 White with lilac edge
- 5 White with lilac stripes
- 6 Dark lilac with purple outer edge
- 7 Dark lilac with purplish spots
- 8 Carmine red
- 9 Purple
- 10 Others

5. Colour of wings

- 1 White
- 2 Greenish
- 3 Lilac
- 4 White with carmine stripes
- 5 Strongly veined in red to dark lilac
- 6 Plain red to dark lilac
- 7 Lilac with dark lilac veins
- 8 Purple
- 9 Others

6. Veins in the standard

- + Present
- 0 Absent

POD

7. Position in the plant

- 1 Base
- 2 Centre
- 3 Top
- 4 Combination of 1, 2 and 3
- 5 Others

8. Fibre hardness

- 1 Absent
- 5 Strongly present.

9. Colour (fresh pod)

- 1 Green
- 2 Yellow
- 3 Green with purple stripes
- 4 Yellow with purple stripes

10. Colour (mature pod)

White

WH



White yellowish /brownish

WY



Yellow - brownish

YB



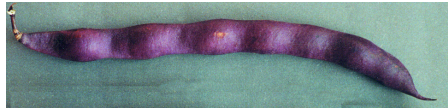
Green

GR



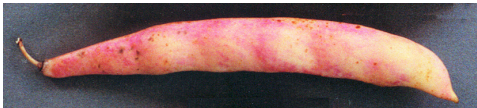
Violet

VI



Pink mottled

PM



Red mottled

RM



Red stripped

RS



Violet mottled

VM



Lilac mottled

LM



Violet stripped

VS





Seed

11. Size: average (mm) of 10 seeds

length, measured parallel to the hilum

width

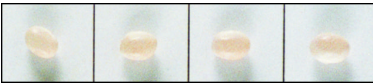
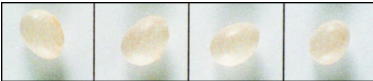


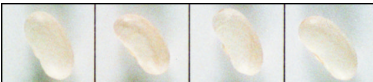

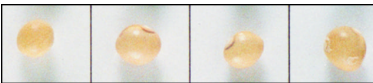


height, measured from the hilum to the opposite side

12. Shape

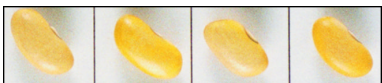
- 1 Round/circular
- 2 Oval/circular to elliptic
- 3 Cuboid/elliptic
- 4 Kidney shaped
- 5 Truncated

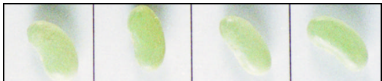
13. 100 seeds weight

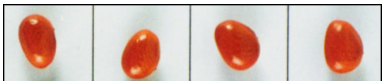
14. Colour (primary and secondary) and 15. Coat pattern n.

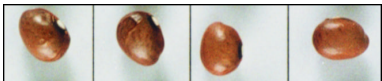
| COLOUR | SHAPE | SIZE | CODE | |
|--------|-------|--------|-------|--|
| WHITE | Round | Small | WH 11 |  |
| | | Medium | WH 12 |  |
| | | Large | WH 13 |  |
| | Long | Small | WH 21 |  |
| | | Medium | WH 22 |  |
| | | Large | WH 23 |  |
| YELLOW | Round | Small | YE 11 |  |
| | | Medium | YE 12 |  |
| | | Large | YE 13 |  |

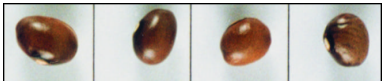
| COLOUR | SHAPE | SIZE | CODE | | |
|--------|-------|--------|-------|--------|------|
| YELLOW | Long | Small | YE 21 | | |
| | | Medium | YE 22 | | |
| | | Large | YE 23 | | |
| GREEN | Round | Small | GR 11 | | |
| | | Medium | GR 12 | | |
| | | Large | GR 13 | | |
| | Long | Small | GR 21 | | |
| | | Medium | GR 22 | | |
| | | Large | GR 23 | | |
| BROWN | Round | Small | B 11 | | |
| | | Medium | B 12 | | |
| | | Large | B 13 | | |
| | Long | Small | B 21 | | |
| | | Medium | B 22 | | |
| | | Large | B 23 | | |
| | | CREAM | Round | Small | C 11 |
| | | | | Medium | C 12 |
| | | | | Large | C 13 |
| Long | Small | C 21 | | | |

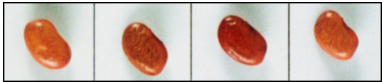





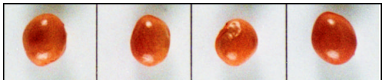


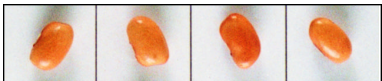




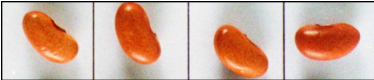
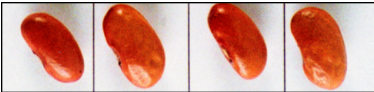

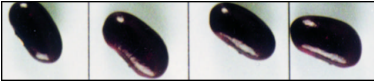







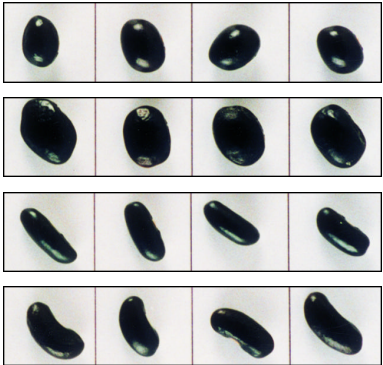






| COLOUR | SHAPE | SIZE | CODE | |
|-------------------|-------|--------|-------|--|
| CREAM | Long | Medium | C 22 |  |
| | | Large | C 23 |  |
| PURPLE | Round | Small | PP 11 | |
| | | Medium | PP 12 |  |
| | | Large | PP 13 | |
| | Long | Small | PP 21 | |
| | | Medium | PP 22 |  |
| | | Large | PP 23 | |
| PINK | Round | Small | P 11 | |
| | | Medium | P 12 | |
| | | Large | P 13 | |
| | Long | Small | P 21 | |
| | | Medium | P 22 |  |
| | | Large | P 23 |  |
| BLUE DARK GREY | Round | Small | GB 11 | |
| | | Medium | GB 12 | |
| | | Large | GB 13 | |
| | Long | Small | GB 21 | |
| | | Medium | GB 22 | |
| | | Large | GB 23 | |
| BLACK | Round | Small | BL 11 |  |

| COLOUR | SHAPE | SIZE | CODE |
|--------|-------|--------|-------|
| BLACK | Round | Medium | BL 12 |
| | | Large | BL 13 |
| | Long | Small | BL 21 |
| | | Medium | BL 22 |
| | | Large | BL 23 |



BI-COLOUR

Constant mottled

BI . . M.



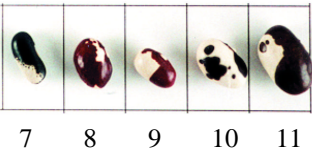
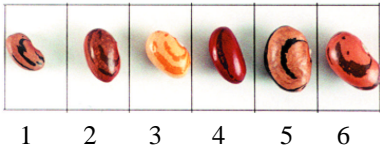
Pinto Type

BI . . P.




Broad striped

BI . . S.





| COLOUR | SHAPE | SIZE | CODE |
|-------------------|-------|------|---|
| TRI-COLOUR | | | TC . . S. |
| | | |  |
| | | | <div>1</div> <div>2</div> <div>3</div> <div>4</div> <div>5</div> |

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QUANTITATIVE TRAITS FOR THE EVALUATION OF *Phaseolus* GERMPLASM

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1. Introduction

The value observed when a quantitative character is measured on an individual, is the phenotypic value.

The phenotypic value is divided into genotypic and environmental components. An important objective is to assess the relative importance of the genotype *versus* environment. Hence, information about genetic parameters, such as heritability and constancy, is relevant to decide which are the most suitable quantitative traits to be used in germplasm evaluation focussed on prebreeding and breeding. Unfortunately, it is difficult to relate and interpret results on genetic parameters from different studies and researches. However, the published data could show the general trend of genetic and environmental variation in some quantitative traits, in spite of the fact, the estimates of these parameters depend on the allelic frequencies of each studied population. The heritability expresses the proportion of the total variance that is attributable to the average effects of genes, and this is what determines the degree of resemblance between relatives.

The heritability has a predictive role expressing the reliability of the phenotypic value as a guide to the breeding value. Only the phenotypic behaviour of individuals can be directly evaluated by breeders, but it is the breeding value what determines its influence on the next generation.

The heritability, in broad sense (H_{BS}), measures the degree to which phenotypic variance is due to variation in genetic factors from a single population. It estimates the proportion of observed variation in the phenotype is attributed to genetic factors, related to environmental factors. The heritability is useful as a measure of potential response to selection and it can also be defined in

narrow sense (H_{NS}). In this case, it measures the degree to which additive genetic variance contributes to phenotypic variance. An equivalent meaning of the heritability is the regression of the breeding value on the phenotypic value (H_{NR})

The correlation between characters is a measure of the degree to which characters vary together or a measure of the intensity of association. Knowledge of the correlation between characters is useful in order to avoid the use of some traits.

2. Heritability of quantitative traits

The chosen quantitative traits are those which are directly related with important characters for bean crop.

2.1. Phenological traits

Emergence

Emergence in bean has been recorded, in experimental plots, as number of days from sowing until 50% of the seedlings have emerged.

The heritability in broad sense (H_{BS}) estimates for emergence ranged from 0.25 to 0.38 (Casquero, 1997; Escibano *et al.*, 1994).

Flowering

Several characters have been recorded related to flowering as days to flowering and period of flowering. Days to flowering can be defined as the number of days from sowing until 50% of the plants have, at least, one opened flower. Period of flowering is the number of days from beginning of flowering until 100% plants had flower abscission.

H_{BS} ranged from 0.57 to 0.98 for days to flowering (Casquero, 1997; Davis and Evans, 1977a; Escibano *et al.*, 1994; Joshi and Mehra, 1983; Samal *et al.*, 1997; Scully *et al.*, 1991). However, the values for period of flowering ranged from 0.09 to 0.33 (Casquero, 1997; Escibano *et al.*, 1994; Santalla, 1995).

H_{NS} ranged from 0.09 to 0.83 for period of flowering (Cerna and Beaver, 1990; Chung and Stevenson, 1973; Davis and Evans, 1977a; Ortega, 1971; Singh and Urrea, 1994; Urrea and Singh, 1989). Beaver and Rosas (1998) reported narrow-sense heritabilities for length of the reproductive period that ranged from 0.43 to 0.83.

Maturity

Fresh pod maturity

Number of days from sowing until 50% of the plants have at least one pod with the optimal stage for fresh consumption.



H_{BS} ranged from 0.33 to 0.56 (Casquero, 1997; Joshi and Mehra, 1983; Santalla, 1995).

Dry seed maturity

Number of days from sowing until 90% of the plants have reached the physiological maturity.

H_{BS} ranged from 0.44 to 0.96 (Casquero, 1997; Conti, 1982; Scully *et al.*, 1991)

H_{NS} ranged from 0.31 to 0.81 (Cerna and Beaver, 1990; Singh *et al.*, 1990; Singh and Urrea, 1994; Singh *et al.*, 1999)

In general, the phenological traits present heritability values which could be considered from low to moderate.

2.2. Plant traits

Plant height

Distance from the ground to the top trifoliate leaf at maturity.

H_{BS} ranged from 0.34 to 0.96 (Conti, 1982; Davis and Evans, 1977b; Joshi and Mehra, 1983; Radkov, 1976; Radkov and Mitranov, 1983; Santalla, 1995; Santos and Vencovsky, 1986).

Inter node length

Santos and Vencovsky (1986) measured internode length in the main stem and the H_{BS} value was 0.88. Davis and Evans (1977b) reported a H_{BS} value of 0.86 for basal internode length. Santalla (1995) measured the distance from ground to the first node and the H_{BS} value was 0.33.

Number of nodes

Number of nodes can be evaluated on the main stem or on the total branches of the plant (total number of nodes per plant).

H_{BS} was 0.92 for nodes in main stem and 0.86 for total number of nodes per plant (Davis and Evans, 1977b; Santos and Vencovsky, 1986).

H_{NS} ranged from 0.63 to 0.69 for nodes in main stem (Chung and Stevenson, 1973; Paniagua and Pinchinat, 1976; Santos and Vencovsky, 1986).

The heritability values for plant traits could be considered moderate.

2.3. Pod traits

Pod traits are recorded when pods have an optimal stage for fresh consumption, when pods have little fiber.

Pod weight

Weight of five or ten green pods.

H_{BS} ranged from 0.44 to 0.55 (Casquero, 1997; Escribano *et al.*, 1994; Santalla, 1995).

Pod length

Exterior distance from the pod apex to the peduncle.

H_{BS} ranged from 0.56 to 0.94 (Casquero, 1997; Davis and Evans, 1977a; Escribano *et al.*, 1994; Joshi and Mehra, 1983; Mitranov, 1983; Natarajan and Amurugan, 1979; Polignano, 1983; Samal *et al.*, 1997; Santalla, 1995) and H_{NS} from 0.53 to 0.70 (Paniagua and Pichinat, 1976; Singh *et al.*, 1994).

Pod width

Distance at right angles to the sutures, at the level of the second seed, from the apex.

H_{BS} ranged from 0.40 to 0.72 (Casquero, 1997; Escribano *et al.*, 1994; Santalla, 1995).

Pod thickness

Diameter of the pod or distance between pod sides at the level of the second and the third seed from the apex.

H_{BS} ranged from 0.32 to 0.73 (Casquero, 1997; Escribano *et al.*, 1994; Natarajan and Amurugan, 1979; Santalla, 1995).

Pod curvature

Relation between pod length and suture string.

H_{BS} ranged from 0.30 to 0.96 (Casquero, 1997; Escribano *et al.*, 1994; Santalla, 1995).

Fresh pod yield

Fresh pod yield determined as number of pods per plant x pod weight of each pod.

H_{BS} ranged from 0.14 to 0.80 (Casquero, 1997; Escribano *et al.*, 1994; Joshi and Mehra, 1983; Santalla, 1995; Singh *et al.*, 1994).

Heritability values for pod traits could be considered moderated.



2.4. Seed yield components and seed yield

Seed yield components

Number of pods per plant

H_{BS} ranged from 0.16 to 0.95 (Atuahene-Amankwa and Michaels, 1997; Casquero, 1997; Davis and Evans, 1977b; Escribano *et al.*, 1994; Joshi and Mehra, 1983; Natarajan and Amurugan, 1979; Petrova, 1985a and 1985b; Quiñones, 1968; Radkov, 1976; Radkov and Mitranov, 1983; Santalla, 1995; Sarafi *et al.*, 1976; Savova, 1985; Singh *et al.*, 1994) while H_{NS} ranged from 0.06 to 0.42 (Atuahene-Amankwa and Michaels, 1997; Chung and Stevenson, 1973; Nienhuis and Singh, 1988; Paniagua and Pinchinat, 1976; Sarafi, 1978).

Number of seeds per pod

H_{BS} varied from 0.30 to 0.94 (Atuahene-Amankwa and Michaels, 1997; Casquero, 1997; Conti, 1985; Davis and Evans, 1977b; Escribano *et al.*, 1994; Natarajan and Amurugan, 1979; Petrova, 1985a y 1985b; Quiñones, 1968; Radkov, 1976; Radkov and Mitranov, 1983; Santalla, 1995; Samal *et al.*, 1997; Sarafi *et al.*, 1973).

H_{NS} ranged from 0.38 to 0.76 (Atuahene-Amankwa and Michaels, 1997; Conti, 1985; Nienhuis and Singh, 1988; Paniagua and Pichinat, 1976; Sarafi, 1978).

Seed weight

Seed weight has been measured either by weighting 50, 100 or 1000 seeds.

H_{BS} ranged from 0.42 to 0.99 (Atuahene-Amankwa and Michaels, 1997; Casquero, 1997; Davis and Evans, 1977b; Escribano *et al.*, 1994; Joshi and Mehra, 1983; Petrova, 1985a y 1985b; Polignano, 1982; Quiñones, 1968; Radkov, 1976; Radkov and Mitranov, 1983; Santalla, 1995; Sarafi *et al.*, 1976; Savova, 1985). Conti (1982) reported estimates for climbing and dwarf bean of 0.29 and 0.93, respectively.

H_{NS} varied from 0.36 to 0.86 (Atuahene-Amankwa and Michaels, 1997; Chung and Stevenson, 1973; Nienhuis and Singh, 1988b; Paniagua and Pinchinat, 1976; Singh and Urrea, 1994; Singh *et al.*, 1990; Singh *et al.*, 1999; Welsh *et al.*, 1995).

Dry seed yield

Seed yield has been determined as the weight of the total seeds of the plant or the weight of the total seeds per plot.

H_{BS} ranged from 0.05 to 0.94 (Atuahene-Amankwa and Michaels, 1997; Casquero, 1997; Davis and Evans, 1977b; Escribano *et al.*, 1994; Joshi and Mehra, 1983; McPerson, 1983; Mutschler and Bliss, 1981; Petrova, 1985a and

1985b; Polignano, 1983; Quiñones, 1968; Radkov 1976, Radkov and Mitranov, 1983; Sarafi *et al.*, 1976; Santalla, 1995; Savova, 1985; Scully *et al.*, 1991; Zimmerman *et al.*, 1984).

H_{NS} ranged from 0.19 to 0.80 (Atuahene-Amankwa and Michaels, 1997; Singh, 1995; Singh *et al.*, 1990; Singh and Urrea, 1994; Singh *et al.*, 1999; Welsh *et al.*, 1995;)

Heritability for seed yield components and seed yield vary from low to high.

2.5. Seed traits

Size traits

Length

The highest measure parallel to the hilum.

H_{BS} ranged from 0.87 to 0.93 (Casquero, 1997; Conti, 1982; Escribano *et al.*, 1994; Santalla, 1995).

Width

Measure from the hilum to the opposite side.

H_{BS} ranged from 0.78 to 0.95 (Casquero, 1997; Conti, 1982; Escribano *et al.*, 1994; Santalla, 1995).

Thickness

The lowest measure parallel to the hilum.

H_{BS} ranged from 0.65 to 0.85 (Casquero, 1997; Escribano *et al.*, 1994; Santalla, 1995).

Heritability values for the seed size traits could be considered high.

2.6. Culinary and nutritional seed traits

Physical quality traits

Hardness

Resistance to the penetration determined on cooked seeds by a penetrometer.

H_{BS} ranged from 0.25 to 0.28 (Escribano *et al.*, 1994; Santalla, 1995).

Texture

It is determined over a fixed volume of cooked seeds by a tenderometer H_{BS} ranged from 0.33 (Escribano *et al.*, 1994).



Water absorption

Amount of water that dry seeds absorb (%) during soaking and determined by soaking 100 dry seeds for several hours in water at environmental temperature. The difference in weight before and after soaking is divided by the dry weight of the 100-seed sample.

H_{BS} ranged from 0.46 to 0.61 (Casquero, 1997; Escribano *et al.*, 1994; Santalla, 1995).

Physical quality seed traits present, in general, low heritability values, except for water absorption, which could be considered a moderate value.

2.7. Nutritional quality traits

Crude protein

H_{BS} ranged from 0.19 to 0.71 (Escribano *et al.*, 1994; Kelly and Bliss, 1975; Leleji *et al.*, 1972; Mutschler and Bliss, 1981; Polignano, 1982; Santalla, 1995).

H_{NS} ranged from 0.05 to 0.89 (Kelly and Bliss, 1975; Leleji *et al.*, 1972; Mutschler and Bliss, 1981).

Crude fat

H_{BS} ranged from 0.31 to 0.36 (Escribano *et al.*, 1994; Santalla, 1995).

Total sugars

H_{BS} ranged from 0.32 to 0.53 (Escribano *et al.*, 1994; Santalla, 1995).

Starch content

H_{BS} ranged from 0.10 to 0.18 (Escribano *et al.*, 1994; Santalla, 1995).

Heritability value for nutritional quality seed traits vary from low to moderate.

3. Correlation among quantitative traits

Phenotypic and genotypic correlations have been computed by calculating the appropriate components of covariance and variance. Correlation coefficient provides a measure of the associations between characters. Knowledge of the correlations among characters is useful in order to avoid duplication in the record and use of some traits. These values would permit to identify characters that have little or no importance in the selection program. The negative genotypic correlations observed between characters may result in a reduction of the rate of improvement to what it could be obtained if the correlation is positive or non-existing. However in some cases these negative correlations can be challenging in the breeding programs.

Correlation coefficients for phenological, pod and seed traits and yield are presented in tables from 3.1 to 3.4, respectively.



Table 3.1. Phenological traits. Correlation coefficients significantly different from zero at 0.05 level (“+” positive; “-” negative).

| CHARACTERS | | REFERENCES |
|---------------------|----------------------------|--|
| Days to flowering | + Days to maturity | Cerna and Beaver, 1990; Escribano, 1992; Santalla, 1995; Scully <i>et al.</i> , 1991 |
| | + Number of pods per plant | Vaid <i>et al.</i> , 1986 |
| | - Period of flowering | Cerna and Beaver, 1990 |
| Period of flowering | + Number of pods per plant | Escribano, 1992 |
| | + Dry seed yield | Escribano, 1992 |
| Days to maturity | + Dry seed yield | Welsh <i>et al.</i> , 1995 |
| Green pod yield | + Number of pods per plant | Arya <i>et al.</i> , 1999; Escribano, 1992; Korla <i>et al.</i> , 1996; Mehta <i>et al.</i> , 1997; Nandi <i>et al.</i> , 1995; Nandi <i>et al.</i> , 1999; Santalla, 1995; Thakur <i>et al.</i> , 1997; Vaid <i>et al.</i> , 1986 |
| | + Days to first flowering | Mehta <i>et al.</i> , 1997; Thakur <i>et al.</i> , 1997 |
| | + Plant height | Arya <i>et al.</i> , 1999; Thakur <i>et al.</i> , 1997 |
| | + Pod lenght | Nandi <i>et al.</i> , 1999; Singh <i>et al.</i> , 1994 |
| | + Pod weight | Singh <i>et al.</i> , 1994 |
| | + Seed weight | Arya <i>et al.</i> , 1999 |

Table 3.2. Pod traits. Correlation coefficients significantly different from zero at 0.05 level (“+” positive; “-” negative).

| CHARACTERS | | REFERENCES |
|--------------------------|-----------------------------|---|
| Number of pods per plant | + Number of seeds per plant | Leleji <i>et al.</i> , 1972; Vasic <i>et al.</i> , 1997 |
| | + Plant height | Arya <i>et al.</i> , 1999 |
| | - Crude protein | Leleji <i>et al.</i> , 1972 |
| | - Pod length | Mehta <i>et al.</i> , 1997 |
| Pod weight | + Number of seeds per pod | Escribano, 1992 |
| | + Fresh pod yield | Santalla, 1995 |
| | + Total sugars | Escribano, 1992 |
| Pod length | + Fresh pod yield | Santalla, 1995 |
| | + Pod weight | Escribano, 1992; Santalla, 1995 |
| | + Pod width | Vaid <i>et al.</i> , 1986 |
| Pod thickness | + Pod weight | Santalla, 1995 |
| | + Pod width | Santalla, 1995 |
| Pod width | + Seed width | Escribano, 1992 |
| | - Total sugars | Escribano, 1992 |
| Pod curvature | + Total sugars | Escribano, 1992 |
| Pod texture | + Crude fat | Escribano <i>et al.</i> , 1997 |
| | - Total sugars | Escribano <i>et al.</i> , 1997 |



Table 3.3. Yield . Correlation coefficients significantly different from zero at 0.05 level (“+” positive; “-” negative).

| CHARACTERS | | REFERENCES |
|----------------|--|---|
| Dry seed yield | + Number of pods per plant or m ² | Anlarsal <i>et al.</i> , 2000; Atuahene-Amankwa and Michaels, 1997; Chand, 1999; Coimbra <i>et al.</i> , 1998; Escribano, 1992; Leleji <i>et al.</i> , 1972; Samal <i>et al.</i> , 1995; Santalla, 1995; Nienhuis and Singh, 1986; Singh <i>et al.</i> , 1995; Vasic <i>et al.</i> , 1997; Welsh <i>et al.</i> , 1995 |
| | + Number of seeds per pod | Atuahene-Amankwa and Michaels, 1997; Chand, 1999; Coimbra <i>et al.</i> , 1998; Mebrahtu <i>et al.</i> , 1991; Nienhuis and Singh, 1986; Samal <i>et al.</i> , 1995; Singh <i>et al.</i> , 1995 |
| | + Fresh pod yield | Escribano, 1992; Santalla, 1995 |
| | + Number of seeds per plant | Anlarsal <i>et al.</i> , 2000; Mebrahtu <i>et al.</i> , 1991; Nienhuis and Singh, 1986; Leleji <i>et al.</i> , 1972; Vasic <i>et al.</i> , 1997; Samal <i>et al.</i> , 1995 |
| | + Plant height | Mebrahtu <i>et al.</i> , 1991 |
| | + Seed size | Mebrahtu <i>et al.</i> , 1991; Singh <i>et al.</i> , 1995 |
| | + Seed weight | Chand, 1999; Coimbra <i>et al.</i> , 1998 |
| | - Seed size | White and Gonzalez, 1990 |
| | - Crude protein | Leleji <i>et al.</i> , 1972 |
| | - Seed weight | Nienhuis and Singh, 1986 |

Table 3.4. Seed traits. Correlation coefficients significantly different from zero at 0.05 level (“+” positive; “-” negative).

| CHARACTERS | | REFERENCES |
|---------------------------|-------------------------------------|---|
| Number of seeds per plant | - Seed weight | Nienhuis and Singh, 1986 |
| Seed width | + Seed length | Santalla, 1995 |
| | + Total sugars | Escribano, 1992 |
| Seed thickness | + Seed length | Santalla, 1995 |
| | + Seed width | Escribano, 1992; Santalla, 1995 |
| | + Pod width | Escribano, 1992 |
| | + Seed weight | Escribano, 1992 |
| | + Total sugars | Escribano, 1992 |
| Seed weight | + Seed length | Escribano, 1992 |
| | + Seed width | Escribano, 1992 |
| | + Seed water absorption | Ghaderi <i>et al.</i> , 1984; |
| | + Seed texture | Ghaderi <i>et al.</i> , 1984; Escribano, 1992 |
| | - Number of pods per m ² | Nienhuis and Singh, 1986 |
| Seed length | + Pod length | Escribano, 1992 |
| Seed texture | + Seed weight | Escribano, 1992 |
| | + Seed length | Escribano, 1992 |
| | + Seed width | Escribano, 1992 |
| | + Starch content | Escribano <i>et al.</i> , 1997 |
| Seed water absorption | + Crude fat | Escribano <i>et al.</i> , 1997 |
| | + Crude fiber | Escribano <i>et al.</i> , 1997 |
| | - Total sugars | Escribano <i>et al.</i> , 1997 |
| Percentage protein | - Number of seeds per plant | Leleji <i>et al.</i> , 1972 |
| Crude fat | - Total sugars | Escribano <i>et al.</i> , 1997 |
| Total sugars | - Starch content | Escribano <i>et al.</i> , 1997 |

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NUTRITIONAL AND SENSORIAL TRAITS*

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A significant part of the human world population relies on legumes as a staple food for subsistence, particularly in combination with cereals. Legumes are often advocated in Western diets because of their beneficial nutritional effects and because they are a low cost source of protein. Therefore, more information is needed about the potential nutritional implications of legume-based diets.

- Beans are rich in protein compared to other starchy plant products like cereals, they have an average 23 % of crude protein content (nearly 26 % of the pulse dry content). The beans protein are poor in sulphur-containing amino acids (cystine and methionine) as well as in tryptophan. However, they are quite rich in lysine. Storage proteins provide a source of nitrogen and carbon for developing seeds during germination. The major storage proteins are vicilin although are present other proteins, legumins (11S) and albumins (2S), with important functional properties (solubility, foaming, emulsion, gelation and viscosity).

- Dry beans are particularly rich in carbohydrates and specially in starch, which represents from 60 % to 80% of total carbohydrates. Other carbohydrates are soluble sugars and dietary fiber which components are: hemicellulose (water soluble and water insoluble), cellulose and lignin.

- Lipids represent a minor component of dry beans, 1 to 3 %. The neutral lipids and phospholipids are the predominant classes. Regarding the fatty acid composition beans are rich in unsaturated fatty acids (oleic, linoleic and linolenic) and palmitic acid.

- Beans are a good source of several minerals including Ca, Fe, Cu, Zn, P, K and Mg. Phosphorus in beans is largely present in phytic acid.

- Dry beans are relatively good sources of water- soluble vitamins especially thiamin, riboflavin, niacin and folacin. There is a great variability in vitamin

* See the PHASELIEU publication "Handbook on common bean related laboratory methods", H. J. Jacobsen, M. Múzquiz, A. Hassa (Eds.), 2001.

contents of beans, such variability of the data may be attributed to the differences in analytical methods. There are not enough studies about the bioavailability of vitamins of cooked legumes and their interactions with other food components.

On the other hand, legumes are under-used because of the content of antinutrient compounds, such as enzyme (trypsin, chymotrypsin, α -amylase) inhibitors, phytic acid, flatulence factors, saponins and toxic factors (lectins) and the need for prolonged cooking.

- Legumes are well known inducers of intestinal gas (flatulence) because of the presence of oligosaccharides of the raffinose family. Animals and man are not able to digest such oligosaccharides because of the absence of α -1,6-galactosidase in their intestinal mucosa. Consequently the raffinose oligosaccharides pass into the colon and they are fermented by intestinal bacteria with considerable production of gas. The most abundant α -galactosides are: raffinose, stachyose and verbascose.

- Phytic acid binds trace elements and macroelements such as zinc, calcium, magnesium and iron, in the gastrointestinal tract making dietary minerals unavailable for absorption and utilisation. It can also form complexes with proteins, proteases and amylases of the intestinal tract, thus inhibiting proteolysis. Moreover, the phosphorus in phytate has been considered to be largely unavailable to the organism because of the limited capacity of monogastric species to hydrolyse phytate in the small intestine. Proportion of IP₆ and other inositol phosphates partially dephosphorylated need to be determined.

- Other antinutritional factors are the saponins which are composed of a steroidal or triterpene aglycone linked to one or three saccharide chains of variable size and complexity via ester and ether linkages. Among the better-known biological effects of saponins is their capacity to cause lysis of erythrocytes and to make the intestinal mucosa permeable.

- The main toxic components in *P. vulgaris* are lectins, sugar-binding proteins which bind and agglutinate red blood cells. As lectins react with the surface epithelium of the digestive tract, they can cause antinutritional, mild allergic or other subclinical effects in higher animals and human, particularly when consumed in large quantities. Phytohemagglutinin (PHA) constitute around 10 % of total protein in the seed.

- The polyphenols are known to occur in food legumes. These are mostly present in the seed coats with a low or negligible amount in the cotyledons. The content is particularly high in seeds with colored seed coat. Their main antinutritive effect is because of their ability to complex proteins making them unavailable and inactivating some enzymes.

- Protease inhibitors are proteins which have the ability to inhibit proteases. Trypsin inhibitors are widely found in legume seeds and because they are extensively used in animal and human nutrition it is important to use adequate heat treatments before their consumption to inactivate them and to provide adequate protein digestion.



Sensory analysis of beans

The objective is to define the sensory quality of the beans, by means of a protocol for the preparation of samples and a tasting sheet for the texture profile. The first methodological aspect is the setting up of a tasting panel, the organisation of sessions, the pre-selection of descriptive factors and the final list. It ends with a tasting sheet with the descriptive factors, in order of perception, and with a structured scale. The second methodological aspect is the training of the judges, with an evaluation of agreed criteria and the consistency and the ability of the team to reproduce results as well as their sensory evaluation of the varieties, by explaining the differences that exist between them.

Preparation of the sample

The different steps in the preparation of the sample are: soaking conditions, boiling procedure, optimum boiling point and preparation of the samples for the tasting. For the boiling conditions the following parameters were studied: type of casserole, cooker, quantity and quality of water, and quantity of salt.

Elaboration of the sensory profile

The main aspects are the following ones:

- The recruitment and selection of the judges.
- The training.
- The tasters selection.
- In this case the experimental plan was developed according to a complete blocks model balanced with repetitions.
- Descriptors selection: visual aspect (it is evaluated the aspect of the seed coat in particular and of the grain as a rule, that it can be presented entire or broken. Also it is evaluated the loos of seed coat), characteristic of surface (it is evaluated the feeling that produces the seed coat in touch with the tongue and the palate, rough, smooth or rugged), behavior of the product to the deformation inside the mouth (it is evaluated the hardness of the seed coat and the albumen, with appraisals of hard, mellow, soft or firm), characteristics of structure (they are evaluated in terms related to the albumen as buttery, mealy, granular or clotty), and other feelings during the mastication (they are evaluated residual feeling aspects as astringency or stickiness).
- Descriptors definition: whole grain (no broken grains), loos of seed coat, smooth surface grain, seed coat and albumen hardness, buttery albumen, granular albumen and mealy albumen.

The sheet of tastes is built as a bipolar scale structured in five points:

- To evaluate the grain integrity, it is observed the aspect of the sample in the plate, scoring from 1 (broken) to 5 (whole).

- To evaluate the texture of the seed coat, the sample is introduced into the mouth, and without biting, is analyzed with the tongue, according to a scale from 1 (smooth surface) to 5 (rough surface).
- To evaluate seed coat and albumen hardness, the sample is introduced into the mouth, and removing albumen. The value is assigned according to a scale from 1(soft) to 5 (hard).
- To evaluate the characteristics of buttery, granular, mealy, stickiness and astringency the sample is introduced into the mouth and is assigned according to a scale from 1 (nothing) to 5 (much), which is appreciated during the mastication.



USE OF GERmplasm IN BREEDING

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Introduction

The genus *Phaseolus* originated in the Americas (Maréchal *et al.*, 1978; Westphal, 1974). After its introduction in Europe, the common bean (*P. vulgaris* L.) not only established itself as an important food crop but also from there it spread to Africa, Asia, and other parts of the world (Gepts & Bliss, 1988). Yet, the short-day species originating from the tropical and subtropical regions (White & Laing, 1989) was subjected to severe selection pressure when introduced to the long summer days of Europe. Consequently, most unadapted germplasm was discarded in the search for desirable cultivars with specific adaptation. Selection for adaptation coupled with extensive use by northern hobby-breeders and researchers of common bean and related germplasm in genetics and breeding studies for more than a century developed new cultivated bean forms. This is evident in the most popular market classes of snap (or stringless green-podded) and dry bean cultivars (see below). This paper provides an overview of the most popular beans in Europe, their production constraints, with breeding and selection strategies that maximize use of germplasm diversity for genetic improvement. Readers interested in details may refer to other publications (e.g., Allavena, 1984; Evans, 1980; Gepts, 1988; Graham & Ranalli, 1997; Laing *et al.*, 1984; Maiti, 1997; Schoonheven & Voysest, 1991; Schwartz & Pastor-Corrales, 1989; Singh, 1992, 1999).

The Common Bean in Europe

Two major groups of common bean are grown and consumed in Europe: snap (or green) and dry beans. Snap bean cultivars possess a thick succulent mesocarp with reduced or no fiber in pod walls and sutures. Green pods are harvested for fresh, frozen, and canning purposes. Snap bean market classes are largely determined based on pod shape (flat, cylindrical or oval), color (dark green, light green or yellow), and length (or sieve size). There is an increasing demand for small, thin and dark green, cylindrical shaped snap bean cultivars. Today, France is by far the largest producer although snap beans are grown in most European countries

including Bulgaria, Netherlands, and Spain. Moreover, there is substantial import of snap beans by the European countries.

Large variation among dry beans is found in Europe. The three major market classes are: medium to large flat rhombohedric or kidney shaped white, resembling the great northern market class in North America; large cylindrical and kidney shaped white; and large oval shaped cream mottled, similar to the cranberry market class in North America. Much greater and often unique variability is found in European collections for these market classes than in their American centers of origin (particularly the two large white dry bean classes). This is despite the fact that two world wars were fought in this century and considerable germplasm diversity might have been lost (Zeven *et al.*, 1999).

Cultivation of great northern types is to be concentrated in Greece, Bulgaria, and other Balkan countries, totaling more than 250,000 ha. Popular cultivars are of indeterminate type III growth habit, requiring 100 to 120 days to maturity. Extremely large cylindrical (e.g., Faba Granja), and large cylindrical (e.g., Alubia) and kidney (e.g., Riñon) shaped white beans are popular in Spain, Portugal, and France. In France, white -greenish colored cultivars (with green cotyledons at harvest) are also preferred. Cranberry (Borloto or Borlotti) beans are more popular in Italy although these are also grown in other countries. The European area for each of these market classes may not exceed 50,000 ha. Thus, there is substantial annual import of dry beans of these and other market classes including small white or navy and large red kidney beans.

Production problems

Biotic stresses are often more important than abiotic stresses causing heavy yield losses in dry beans in Europe. Among these, viruses causing bean common mosaic (BCM), yellow mosaic, and probably cucumber mosaic, are more widespread than in North America. Similarly, bacterial diseases such as halo blight [caused by *Pseudomonas syringae* pv. *phaseolicola* (Burkh.) Young *et al.*], and common bacterial blight [caused by *Xanthomonas campestris* pv. *phaseoli* (Smith) Dye] are serious problems in many areas if susceptible cultivars of snap and dry beans are grown. Root rots caused by a group of fungi including species of *Fusarium*, *Pythium*, *Rhizoctonia*, and *Sclerotinia*, among others, are endemic in most bean-growing regions. Anthracnose [caused by *Colletotrichum lindemuthianum* (Sacc. & Magn.) Bri. & Cal.], a problem until the 1970's (e.g., in Netherlands and France), has been virtually eradicated by use of resistant cultivars and pathogen-free seed. Nonetheless, in humid regions, such as in the Principado de Asturias, Spain the disease continues to take a heavy toll on susceptible cultivars (e.g., Faba Granja). Similarly, bean rust [caused by *Uromyces appendiculatus* (Pers.) Ung.] can be a problem in cool and wet regions (e.g., Galicia and Asturias, Spain).

Pathogen causing most of the above mentioned diseases are seed- transmitted. And survive for long periods on plant residues, alternate hosts, and in the soil. Thus, use of disease resistant cultivars, of clean seed, and appropriate cultural



practices are essential for long- term sustainable and integrated management of bean pests.

Breeding and selection strategies

Although several important collections exist in Europe, systematic collection and evaluation of available diversity for agronomic traits has not been conducted, with some notable exceptions (e.g., Bannerot, 1965). Similarly, the genetic base of most European market classes may be rather narrow because of the crop history, stringent quality requirements, and conservative breeding strategies typically employed by breeders. The increasing demand for organically grown or pesticide-free food products, growing concern for natural resources conservation, and reduction of production costs contribute to a need for an integrated genetic improvement. The challenge for European bean researchers is that introduced germplasm from centers of diversity are poorly adapted to European environment. Thus, an understanding of the organization of genetic diversity and evolution during domestication within *Phaseolus* species is essential for sustained crop improvement. Breeding and selection strategies discussions therefore require a brief review of the organization of diversity in *Phaseolus* beans. Extensive germplasm utilization for maximizing genetic gains from selection accumulates favorable alleles in elite parents from the crop's cultivated races, gene pools, and wild populations forming its primary, secondary, and tertiary gene pools (i.e., parental development). Such parents are used for simultaneous improvement of the maximum number of agronomic traits for cultivar development in dry and snap bean market classes.

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Patterns of Diversity in *Phaseolus* Beans and Useful Traits

The genus *Phaseolus* sensu stricto comprises more than 30 species (Debouck, 1991, 1999; Delgado Salinas, 1985; Maréchal *et al.*, 1978). However, only five are domesticated (Gepts & Debouck, 1991) with the common bean (*P. vulgaris*) occupying more than 85% area sown to *Phaseolus* species worldwide. The primary gene pool of each cultivated species comprises both cultigens and wild populations. The species *P. coccineus*, *P. costaricensis*, and *P. polyanthus* compose the secondary gene pool while the tertiary gene pool of common bean comprises *P. acutifolius* and *P. parvifolius* (Debouck, 1999; Debouck & Smart, 1995; Singh *et al.*, 1997). More than 29, 000 cultivated and 1,300 wild accessions of common bean; and 1, 000 of the secondary and 350 of tertiary gene pools are available at CIAT, Cali, Colombia (Debouck, 1999). Embryo rescue is essential for crossing common bean to the tertiary gene pool (Mejía-Jiménez *et al.*, 1994; Thomas & Waines, 1984) while the secondary gene pool is crossed to common bean unassisted.

There are two major gene pools within the cultivated and wild populations of the common bean: Andean and Middle American (Becerra Velásquez & Gepts, 1994; Evans, 1973; Gepts & Bliss, 1985; Khairallah *et al.*, 1990; Koenig & Gepts, 1989a; Singh *et al.*, 1991b). The cultivated gene pools are further divided into six races: Andean races are Chile, Nueva Granada, and Peru; and Middle American races are Durango, Jalisco, and Mesoamerica (Singh *et al.*, 1991a). Useful genes

for most agronomic traits are distributed across races and gene pools of the common bean (Singh, 1989; Singh *et al.*, 1991a) and its related cultivated and wild species (e.g., Schoonhoven *et al.*, 1983; Schuster *et al.*, 1983; Singh & Muñoz, 1999; Thomas *et al.*, 1983). Table 1 summarizes useful traits found in related species that are deficient in common bean. Large differences in combining ability (Nienhuis & Singh, 1986, 1988; Singh *et al.*, 1992) or breeding incompatibilities occur in distantly related crosses within common bean (Koinange & Gepts, 1992; Kornegay *et al.*, 1992; Singh & Gutiérrez, 1984; Singh & Molina, 1996; Welsh *et al.*, 1995).

Table 1. Resistance traits deficient in common bean and present in closely related *Phaseolus* species.

| Production problems | Source species | References |
|---------------------|-------------------------|----------------------------------|
| Bruchid | Wild <i>P. vulgaris</i> | Schoonhoven <i>et al.</i> , 1983 |
| Anthracnose | <i>P. coccineus</i> | Hubbeling, 1957 |
| Bean yellow mosaic | <i>P. coccineus</i> | Baggett, 1956 |
| Cold | <i>P. coccineus</i> | Bannerot, 1979 |
| Root rots | <i>P. coccineus</i> | Wilkinson, 1983 |
| White mold | <i>P. coccineus</i> | Hunter <i>et al.</i> , 1982 |
| Ascochyta blight | <i>P. polyanthus</i> | Schmit & Baudoin, 1992 |
| Common blight | <i>P. acutifolius</i> | Singh & Muñoz, 1999 |
| Drought | <i>P. acutifolius</i> | Parsons & Howe, 1984 |

Evolution Under Domestication

Knowledge of crop evolution (Evans, 1980; Gepts & Debouck, 1991; Koenig & Gepts, 1989b; Smartt, 1969, 1988) is useful for introgression of genes from the wild populations and alien species. Qualitative and quantitative genes involved in domestication of the common bean have been identified and placed within linkage maps (Freyre *et al.*, 1998; Gepts, 1999; Gu *et al.*, 1998; Koinange *et al.*, 1996). These traits are growth habit (*fin*), photoperiod insensitivity (*ppd*, *hr*), fiber content in pods (*St*), seed dormancy, and seed weight, among others. Marker-assisted introgression of these genes into selected wild populations may facilitate their use in cultivar development. Moreover, working in genetically diverse interracial, inter-gene pool, and dry x snap bean populations, these markers can be used for indirect selection.

Breeding and Selection Strategies

Multiple breeding and selection strategies for germplasm utilization in common bean are available (e.g., Beaver & Kelly, 1994; Bliss, 1993; Fouilloux & Bannerot, 1988; Gutiérrez & Singh, 1992; Haghighi & Ascher, 1988; Mejía-Jiménez *et al.*, 1994; Singh, 1994, 1998; Singh & Terán, 1998; Singh *et al.*, 1998a, 1999; Urrea & Singh, 1994, 1995). Morphological, biochemical, and DNA-based markers in common



bean are routinely used for indirect selection of qualitative and quantitative traits (Kelly & Miklas, 1998). These are combined with direct selection to facilitate germplasm improvement (Singh, 1994, 1998; Singh *et al.*, 1998a; Tanksley *et al.*, 1996). However, no single breeding method is suitable for all circumstances. Instead, breeders use different selection methods or combine two or more methods to suit their needs for each objective.

For integrated genetic improvement, Kelly *et al.* (1998) suggested a three-tiered approach. This involves: 1) introgression of individual genes/traits from alien germplasm, 2) pyramiding two or more complementary genes from different sources for parental development for specific traits, and 3) simultaneous selection for multiple agronomic traits for cultivar development.

Gene Intro gression from Alien Germplasm

Introgression of useful genes from each major distantly related cultivated race, gene pool, wild population, and alien species from the secondary and tertiary gene pools must be accomplished separately. Differences in genetic distance between alien *Phaseolus* species and *P. vulgaris* (Debouck, 1999; Debouck & Smartt, 1995), and between gene pools and races within the common bean cultigens (Gepts & Bliss, 1985; Singh, 1989; Singh *et al.*, 1991a) dictate specific breeding methods and strategies. The frequency of useful genotypes recovered reduces with increasing genetic distance between parents, thus requiring a tailored approach to optimize the probability of success.

In general, there is good complementation and positive combining ability between different races within the Middle American gene pool. Thus, gene manipulation among and across the three races (Durango, Jalisco, and Mesoamerica) is relatively easy when parents have minimal differences in photoperiod response and phenological traits. However, photoperiod and phenology differences can be overcome by growing parents under shorter daylengths (approximately 12 hr photoperiod) for hybridization. Elaborate backcrossing is usually unnecessary unless one parent is an early maturing determinate and the other is a highly photoperiod sensitive or a late maturing extreme climber. A more efficient strategy uses a three-way or modified-double cross (Singh, 1982) to ensure >70% genetic contribution of the parents of the same race and market class under improvement. Moreover, Kelly & Adams (1987) used recurrent selection to introgress upright plant type from race Mesoamerica to race Durango.

Introgressing desirable alleles between Andean and Middle American gene pools often disrupts adaptation, yield, and seed quality characteristics of both common bean market classes. Biparental crosses followed by pedigree (Kornegay *et al.*, 1992), single seed descent (Welsh *et al.*, 1995), or mass selection (Singh *et al.*, 1989) are poor methods for extracting adapted cultivars from Andean x Middle American populations. Similarly, dry or snap bean cultivar selection from single crosses between the two groups is improbable. More elaborate programs of recurrent or congruity inbred-backcrossings (Bliss, 1993; Urrea & Singh, 1995) and recurrent

selection (Beaver & Kelly, 1994; Kelly and Adams, 1987; Singh *et al.*, 1999) are required. Moreover, bridging-parents may be required (Singh & Gutiérrez, 1984), if *Dl-1* and *Dl-2* incompatibility alleles occur in the divergent crosses.

Except for *Dl-1* and *Dl-2* genes (Koinange & Gepts, 1992), there are no known barriers for transferring genes from wild populations of Andean and Middle American gene pool into cultigens. The F_1 hybrids between cultivated x wild, as well as their progenies in subsequent generations, are fully fertile. Thus, studies based on wild x cultivated crosses have resulted in better understanding of the inheritance of seed size (Motto *et al.*, 1978) and yield potential (Singh *et al.*, 1995). Such crosses have also helped map major genes involved in domestication (Koinange *et al.*, 1996) and to transfer resistance to bruchids (*Zabrotes subfasciatus* Boheman) (Cardona *et al.*, 1990).

Crosses of common bean with the three species of the secondary gene pool are effected without embryo rescue (Baggett, 1956; Camarena & Baudoin, 1987; Cheng *et al.*, 1981; Park & Dhanvantari, 1987; Singh *et al.*, 1997), especially with *P. vulgaris* as the maternal parent. However, hybrid progenies may be partially sterile and it may be difficult to recover stable phenotypes of common bean (Wall, 1970). There is a tendency to revert to the maternal genotype, and recombinants are often unstable. Thus, developing true breeding common bean lines with desirable traits from the secondary gene pool is difficult.

Researchers have successfully introgressed common bacterial blight resistance from *P. coccineus* to common bean (Miklas *et al.*, 1994; Park & Dhanvantari, 1987). Recurrent and congruity backcrossing were used for crosses with *P. acutifolius*, using embryo rescue (Haghighi & Ascher, 1988; Mejía- Jiménez *et al.*, 1994). Also, high resistance to common bacterial blight was introgressed from *P. acutifolius* (McElroy, 1985; Scott & Michaels, 1992; Singh & Muñoz, 1999).

Production of large interspecies hybrid progenies from plant-to-plant paired pollination at each step of crossing is advisable. This follows development of a large number of inbred lines. Appropriate screening of those lines helps overcome some of difficulties associated with introgression of traits from distantly related alien germplasm.

Pyramiding Genes and P arental Development

Pyramiding complementary genes broadens the genetic base of cultivars, maximizes gains from selection, and increases the durability of resistance to diseases caused by variable pathogens (e.g., anthracnose, halo blight, and rust diseases). Gene pyramiding should build within and across cultivated races and gene pools and wild populations of common bean, and from its secondary (*P. coccineus*, *P. costaricensis*, and *P. polyanthus*) and tertiary (*P. acutifolius* and *P. parvifolius*) gene pools. While it may be feasible to achieve simultaneous introgression and gene pyramiding (Singh & Muñoz, 1999), often gene pyramiding into a common bean genotype follows after the successful introgression of single genes from alien germplasm. Pyramiding genes for specific traits also requires



simultaneous selection for adaptive features, growth habit, maturity, and seed characteristics of the target market class. For example, for improvement of great northern types from race Durango, pyramiding of useful genes should be accomplished in those types. On the other hand, for large-seeded cranberry from race Nueva Granada, it is advisable to develop elite parents with pyramided genes for specific traits that are similar to the respective seed type, growth habit, and adaptation.

Gene pyramiding has been achieved for seed yield (Singh *et al.*, 1989, 1993) and drought tolerance (Singh, 1995). The dominant *I*, the recessive *bc-3*, and other bean common mosaic resistance genes (Drijfhout, 1978) were pyramided, using molecular markers (Haley *et al.*, 1994; Johnson *et al.*, 1997; Melotto *et al.*, 1996) (J.D. Kelly & R. Stavely, personal communication, 1999). The Andean *Ur-4* and Middle American *Ur-3*, *Ur-6*, and/or *Ur-11* genes for rust resistance were pyramided in pinto and great northern market classes (J.R. Stavely, personal communication, 1999). Interspecific pyramiding of resistance to common bacterial blight was accomplished using recurrent and congruity backcrossings (Singh & Muñoz, 1999).

Cultivar Development

For each market class, commercial cultivars, elite lines, and donor parents of useful genes are selected based on their adaptation, performance, and combining ability. When the necessary genes for each trait are found in separate parents, a few multiple-parent crosses are preferred over a large number of single crosses. This allows production of recombinants with favorable alleles for multiple traits, something that is not possible through single crosses. For example, if simultaneous selection for resistance to BCM, common bacterial blight, bean rust, and root rots is sought, a four-way cross involving all four donor parents is made first. The double-cross F_1 hybrid thus developed then serves as the pollinator parent for the cultivar or elite lines to be improved. Often it is advisable to assure > 10% genetic contribution of each donor-parent of useful genes in a final multiple-parent cross. Moreover, a large number of plant-to-plant pollinations are made at each step of multiple-parent cross development to assure adequate sampling of gametes and genetic contribution of each parent involved in the final crosses. If this procedure is followed, there will be enough (>30 plants) selected F_1 plants for subsequent evaluations and line development.

Gamete selection (Singh, 1994, 1998), using dominant and codominant morphological, biochemical, and DNA-based markers (Kelly & Miklas, 1998) in heterogametic and heterogeneous crosses accumulates necessary alleles early in selection. This reduces population sizes and provides opportunity for subsequent selection of qualitative and quantitative traits. Development of high-quality high-yielding superior cultivars possessing the maximum number of desirable traits should follow from this strategy of tandem selection in each successive breeding cycle (Singh & Terán, 1998; Singh *et al.*, 1998a). But the F_1 selection would not work for traits controlled by recessive genes.

Conclusions and future prospects

Since the introduction of the common bean in Europe, important and unique market classes of dry and snap bean cultivars evolved. Thus, the recent work of systematic regional germplasm collection and characterization (e.g., Bannerot, 1965; Casquero *et al.*, 1997; Escribano *et al.*, 1990, 1997, 1998; Ron *et al.*, 1991; Zeven *et al.*, 1999) needs to be intensified. Moreover, additional germplasm from CIAT (Debouck, 1999) and elsewhere should be introduced if necessary.

Preliminary germplasm evaluation indicates that most regional cultivars and landraces are deficient in many traits including resistance to bean common mosaic, bean yellow mosaic, common bacterial blight, halo blight, and root rots, among others. Resistance genes for these diseases have been identified in tropical and subtropical germplasm of common bean, in its wild populations, and related species in the secondary (*P. coccineus*, *P. costaricensis*, and *P. polyanthus*) and tertiary (*P. acutifolius* and *P. parvifolius*) gene pools (Table 1). For accumulation of favorable alleles from these various sources a comprehensive, integrated, genetic improvement program is warranted for each major market class of dry and snap bean. Three interdependent major breeding activities namely, (1) introgression of useful genes from alien germplasm, (2) pyramiding of favorable alleles for specific traits for parental development, and (3) cultivar development for specific market classes of beans are essential for integrated genetic improvement. Germplasm recombination and selection methods will vary depending upon the genetic distance between parents, breeding needs, and available resources. The availability of an efficient and repeatable transformation system for *P. vulgaris*, integrated linkage maps, use of the knowledge of genetics of domestication and evolution, and development and use of marker-assisted selection should expedite and facilitate *Phaseolus* germplasm use for common bean improvement.

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