Evaluation of Nicaraguan Common Bean (*Phaseolus vulgaris* L.) Landraces

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Abstract

Common bean landraces are an important component of the cropping systems of the Nicaraguan small-scale farmers. They have also been used in breeding programs for the development of improved cultivars. The knowledge about these landraces is, however, incomplete as regards adaptive, physiological and molecular traits. Neither their genetic diversity and its distribution nor the way in which this genetic diversity has changed over time has been well studied. The general purpose of this work was to complement the knowledge already available about the Nicaraguan bean landraces in order to generate additional information with the aim of better utilization of them. More specifically the aims were to: 1) study the yield formation of landraces and bred cultivars; 2) determine their variation in adaptation; 3) determine the landrace genetic diversity and its structure; and 4) monitor changes in the diversity stored *ex situ*. The plant material consisted of red-seeded landraces currently in cultivation, landraces maintained *ex situ* in genebanks and two bred cultivars. Molecular and phenotypic evaluation of the plant material was done in contrasting environments. The landraces yielded less than the bred cultivars at the sites with the poorest environments but similarly to them at the other sites. The bred cultivars had longer leaf area duration during the seed filling period and a longer growth cycle all-together compared with the landraces. One bred cultivar (INTA-Masatepe) and two landraces (V16 and V29) showed good yield stability according to the AMMI analysis. The landraces varied in the amount of within-population genetic diversity. High proportion of it was distributed between populations. In general adaptive traits did not differ between the *ex situ* and *in situ* populations but they differed at the molecular level: the former had a lower genetic diversity than the latter. In conclusion, yield of the landraces varied but some performed as well as the bred cultivars. The main drawback of the landraces was their susceptibility to some biotic stress factors; however, not all of them were affected. In the landraces studied the genetic diversity was distributed within as well as among populations. It was also found that changes have occurred in the genetic diversity of the populations stored *ex situ*.

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Papers I-IV

This thesis is based on the following four papers, which will be referred to in the text by their respective roman numerals:

I. Gómez, O.J., Blair, M.W., Frankow-Lindberg, B.E. & Gullberg, U. 2004. Molecular and phenotypic diversity of common bean landraces from Nicaragua. *Crop Science 44*, 1412-1418.

II. Gómez, O.J., Blair, M.W., Frankow-Lindberg, B.E. & Gullberg, U. 2004. Comparative study of common bean (*Phaseolus vulgaris* L.) landraces conserved *ex situ* in genebanks and *in situ* by farmers. *Genetic Resources and Crop Evolution* (In press).

III. Gómez, O.J., & Frankow-Lindberg, B.E. Yield formation in Nicaraguan landraces of common bean (*Phaseolus vulgaris* L.) compared to bred cultivars (Submitted).

IV. Gómez, O.J., Frankow-Lindberg, B.E. & Gullberg, U. Yield and yield stability of Nicaraguan landraces and bred cultivars of the common bean when grown in contrasting environments (Manuscript).

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Introduction

General aspects

Among major food legumes the common bean (*Phaseolus vulgaris* L.) is the third most important worldwide, superseded only by soybean [*Glycine max* (L.) Merr.] and peanut (*Arachis hypogea* L.). Among the pulses (i.e., annual leguminous food crops that are harvested for dry seeds) the common bean is by far the most important (Singh, 1999). The genus *Phaseolus* is of American origin and comprises over 30 species (Debouck, 1991). Five of them, namely, *P. acutifolious* A. Gray (tepary bean), *P. coccineus* L. (runner or scarlet bean), *P. lunatus* L. (lima, butter or madagascar bean), *P. polyanthus* Greenman (year-long bean), and *P. vulgaris* L. (common bean, haricot, navy, French or snap bean) were domesticated (Debouck, 1999). Among these species the common bean is the most widely distributed and has the broadest range of genetic resources (Singh, 1999). It is mostly used as food crop throughout the world, especially in Latin America and Africa.

Organisation of bean diversity

The bean genetic diversity is large. There are about 65 000 accessions of *Phaseolus* beans in major germplasm banks, of which more than 90% are *P. vulgaris*. The Centro Internacional de Agricultura Tropical (CIAT) collections, the largest in the world, includes over 40 000 entries, of which 26 500 are cultivated *Phaseolus vulgaris*, about 1 300 are wild types of the common bean, and the rest are distant relatives of the common bean (CIAT, 2001). Intra-specific organization of genetic variation in *Phaseolus vulgaris* has been well studied. Two major gene pools, namely, the Mesoamerican and the Andean South American were the first to be recognized. The evidence for the existence of these two gene pools was supported by phaseolin seed proteins (Gepts *et al*, 1986), allozymes (Singh, Nodari & Gepts, 1991), morphological traits (Singh *et al*., 1991), and DNA markers (Becerra Velásquez & Gepts, 1994). Later, a third gene pool in the northern Andes (Ecuador and northern Peru) was described (Debouck *et al*., 1993), and this one is now considered as the nucleus of bean diversity from which wild beans dispersed both northwards and southwards to form the two geographically distinct gene pools in Mesoamerica and Andean South America (Gepts, 1998). Singh, Gepts & Debouck (1991) further divided the Mesoamerican and Andean South American gene pools of cultivated beans into six races (three within each centre), which represent the different cultivated forms that developed as a consequence of domestication. Each race has its own characteristics, ecological adaptation, and agronomic traits (Beebe *et al*., 2000). Cultivated forms of common bean also called landraces are often highly variable in appearance, but they can be identified and usually have local names. They have particular properties or characteristics (i.e. early or late maturing), a reputation for adaptation to local climatic conditions and cultural practices, and resistance or tolerance to diseases and pests (Harlan, 1992). As a result of that landraces are thought to show high yield stability and intermediate yield level under a low input agricultural

system (Zeven, 1998). The genetic diversity of landraces is thought to be the most economic valuable part of global biodiversity and is considered of paramount importance for future world production (Wood & Lenné, 1997). As reservoirs of useful genetic diversity landraces need to be conserved. This may be achieved by the combination of different conservation strategies, which can be classified into two groups, namely, *in situ* (on-farm) and *ex situ* (Maxted, Ford-Lloyd & Hawkes, 1997). The implementation of either one or the other or both strategies will depend on the objectives of the conservation and the characteristics of the fraction of diversity that is to be conserved (Baena, Jaramillo & Montoya, 2003). If the purpose is the conservation of specific genotypes or traits, then the *ex situ* approach is convenient, while if we are interested in the conservation of the species continuously evolving in its environment then the *in situ* method should be the most suitable. An additional benefit of the *in situ* method is the combination of conservation and utilization of landraces. The best results, however, may be reached by the utilization of both strategies in a complementary way (Shand, 1997; Piergiovanni & Laghetti, 1999).

Botanical characteristics

Most beans are herbaceous annuals, although, under tropical conditions, some beans (such as large limas) may behave as short-lived perennials. They may be of determinate or indeterminate growth habit, with pinnately compound trifoliolate leaves. The common bean flower has an elongated twisted keel containing the style and ten stamens. Inside the flower the anthers drop pollen on to the style in the evening before it opens. The next day, if pressure is applied to the flower (by an insect for example) the style and stigma protrude from the keel and retract when the pressure is released. The style leaves pollen at the opening of the keel. Cross-pollination is possible if the stigma contacts a pollen-coated bee when it is extended. Otherwise the stigma will be self-pollinated when it retracts and contacts its own pollen at the opening of the keel. Self-pollination is thus the norm in the common bean, and it probably occurs automatically at or before the flower opens in the morning. However, it takes 8-9 hours for the pollen tube to grow and fertilize the ovules, during which time honey bees and bumble bees can visit the flower and crosspollinate it. Tubes of foreign pollen probably grow faster than the plant's own pollen, so crossing is likely when the plant is cross-pollinated (Free, 1993). For the grower, there is no yield or other economic advantage of cross-pollination. For the bean breeder, cross-pollination is actually a hazard to maintaining the purity of a cultivar. To reduce unwanted hybridizing, one can separate cultivars by at least two meters or by a tall dense barrier.

Beans show a high variation in growth habit, which appears to be continuous from determinate bush to indeterminate, extreme climbing types although for simplicity, Singh (1982) classified the bean growth habits into four major classes (type I = determinate upright or bush; type II = indeterminate upright bush; type III = indeterminate postrate, nonclimbing or viny semiclimbing and type IV = indeterminate strong climbers) and suggested a key for their identification based on the type of terminal bud (vegetative vs. reproductive), stem strength (weak vs. strong), climbing ability (nonclimber vs. strong climbers), and fruiting patterns

(mostly basal vs. along entire stem length or only in the upper part). Comparisons between this and another classifications of bean cultivars by their growth habit can be found in Voysest (2000).

Growth and development of common bean is divided into vegetative and reproductive stages. The vegetative stages (V) are defined on the basis of the number of nodes on the main stem, whereas the reproductive (R) stages are defined on the basis of pod and seed characteristics in addition to nodes (Fageria, Baligar & Jones, 1997). Common bean has an epigeal germination, which is complete in 7-8 days after planting (DAP). Its flowering may be initiated as early as ten DAP (Wallace, 1985) although it usually begins between 28 and 40 DAP. Amongst climbing varieties grown at high elevation, it can occur significantly later (Graham & Ranally, 1997). These authors also mention that flowering in cultivars with growth habit type I may occur during a very short period of time, usually between 5 and 6 days, while in indeterminate cultivars it may last from 15 to 30 days. As regards physiological maturity it can occur between 58 and 68 DAP in early cultivars or it can continue up to 200 DAP amongst the climbing varieties that are used in cooler upland elevations (Graham & Ranally, 1997).

Ecology of common bean

In general, the common bean is a warm-season crop. However, high temperatures (>30ºC) can cause flower blasting (dropping of buds and flowers; Fageria, Baligar & Jones, 1997), which reduces yield. In general, flower and pod abortion rates in bean may vary between 60-80% (White & Izquierdo, 1991). The common bean requires moderate amounts of water (300-600 mm). Adequate amounts early in the season is essential, but particularly so during the pod-filling stage (during and immediately after flowering); during this stage the soil should not hold less than 60% of field capacity to insure proper moisture availability. Dry weather is desirable for the maturation of the crop and for harvesting. Late rains may also discolor the beans and lower their grade and market value (Free, 1993).

Common bean is cultivated under different cropping systems from the highly mechanized, irrigated and intensive production of monocropped bush beans to complex associations of indeterminate or climbing beans with maize, other cereals, sugarcane, coffe, or plantain (Schoonhoven & Voysest, 1991). In these multiple-cropping systems yield can range from less than 500 kg ha^{-1} in parts of Latin America and Africa to as much as 5000 kg ha^{-1} under experimental conditions. Furthermore, the diversity of edaphic and and climatic conditions as well as the highly specific local preferences for particular grain types or colours complicate the genetic improvement of common bean (Rao, 2001). Despite this, significant advances have been done in improving genetic adaptation to major biotic and abiotic constraints, which are described below, although progress in improving genetic yield potential [i.e. the yield of a cultivar when grown in environments to which it is adapted, with nutrients and water nonlimiting and with pest, diseases, weeds, lodging, and other stresses effectively controlled (Evans & Fisher, 1999)] *per se* has been limited (Rao, 2001). Both physiological and morphological characteristics of the bean plant are considered to play an important

role in determining yields. Nienhuis & Singh (1988) studied several morphological traits of common bean in relation to yield. They found that yield was positively correlated with pods m^{-2}, seeds pod^{-1}, and most architectural traits (branches, nodes on main stem and branches, stem and internode lenghts) except branches per plant. Furthermore, Scully & Wallace (1990) evaluated several accessions of common bean for variation in phenological and physiological characteristics and found that the growth rate, biomass and pod filling duration were linearly and positively related to yield. The understanding of these characteristics closely related to yield has led to the development of different morpho-physiological plant models in order to increase the yield potential of common bean. For example, White & Izquierdo (1991) discussed physiological processes that determined yield and applied that information to analyze limitations to yield potential and stress tolerance. They proposed some traits, which might confer common bean general stress adaptation. There were the ability for recuperative growth, presumably resulting from remobilization of carbohydrate or nitrogen reserves and indeterminate grow habit, good competitive ability, high tissue concentration of phenolic compounds with inhibitory effects on a broad range of pathogens or pest organisms, greater partitioning to root growth and buffering ability for adequate pod retention and seed filling.

Production constraints

Diseases and insect pests represent the major hazards that farmers (large or small-scale) confront in the Central American countries. At higher altitudes (>1000 m.a.s.l.) anthracnose and rust are important limiting factors. Bean golden mosaic virus (BGMV), common bacterial blight rust and web blight are important bean diseases at lower altitudes. Of these, BGMV is the single most important disease in Central America (Broughton *et al.*, 2003). Lately, angular leaf spot has become an important bean disease at both lower and higher altitudes. Bean common mosaic virus (BCMV) is also an important widespread disease. Pathogens causing most of the above-mentioned diseases are seed-transmitted or survive for long periods on plant residues, alternate hosts, or in the soil. Thus, the use of disease resistant cultivars and of clean seed, in combination with appropriate cultural practices are essential for the management of bean pests (Singh, 2001). More than 150 species of insects and other invertebrates have been listed as pests of dry common beans but only a few are recognized as being economically important (Cardona *et al.*, 1995).

As regards abiotic stresses, drought stress is a problem that farmers frequently face. It is estimated that up to 73% of the total Latin America production takes place in conditions that have moderate to severe water-deficits at some time during the cropping season. Soil problems due to toxic compounds and/or nutritional deficiencies limit productivity. Common beans are frequently grown on acid soils that are low in available phosphorus (P) and/or have high P-fixing capacities. Over 50% of the bean-growing areas in Latin America are thought to be critically deficient in P. Such soils are also often high in aluminium (Al) and beans are affected by Al toxicity. A major portion of Latin America is also affected by Manganese (Mn) toxicity and low availability of nitrogen (N) in soil. Small-scale

farmers do not have the capital to solve edaphic limitations through inputs (Broughton *et al.*, 2003).

Common bean in Nicaragua

Tapia (1987) reported the presence of three species of the genus *Phaseolus*, namely *P. vulgaris*, *P. acutifolius* and *P. lunatus* in Nicaragua. Of these three species, *P. vulgaris* is distributed in all agroecological zones of the country, while *P. acutifolius* is found in areas of low elevation mainly in the western, central and northern parts of Nicaragua. The areas sown with this species are very small. The third species, *P. lunatus*, grows wild and may be found in areas between 300 and 500 meters above sea level (m.a.s.l.) with moderate temperatures and abundant precipitation. There are a large number of bean landraces in Nicaragua. Numerous collection missions have been carried at different points in time (1952, 1960, 1980´s, 1990´s) and most of the landraces collected are stored in genebanks at different international and national institutions. All the bean accessions (*c*. 450) stored in the Nicaraguan genebank have had preliminary characterization and evaluation. High variability for morphological traits was found in bean landraces collected in areas of high elevation (>800 m.a.s.l.) with moderate temperatures. Among these areas are the districts named Estelí, Madrid, Nueva Segovia, Jinotega and Matagalpa. Other areas with moderate levels of genetic diversity are the districts of Masaya, Granada, Carazo and Rivas (Tapia, 1987). Some particular characteristics of the Nicaraguan landraces are their earliness, a growth habit of type II or III (Singh, 1982), susceptibility to diseases and pests, seeds of small size and different colours (Barrera & Alvarez, 1998). The last two attributes are the most important from the Nicaraguan consumers' point of view (IICA-USAID, 2003).

The yearly production of common beans in Nicaragua has increased steadily from 135 197 to 221 790 Mt during the last five years (BCN, 2003) and it is the second most important crop after maize. The number of farms in Nicaragua has been estimated to *c*. 200 000 (INEC, 2003). The sizes of these varies from <0.4 to more than 350 hectares (ha). In 58% of them beans are sown as a part of the cropping systems. Most of the bean cultivation is carried out on farms with sizes less than 70 ha (which constitutes 91% of the total number of farms in Nicaragua) by small-scale farmers (INEC, 2003) without irrigation and with little use of agrochemicals. In this thesis the term small-scale farmers refers to two group of farmers: 1) those who produce beans only for their subsistence and, 2) those who cultivate beans for their subsistence but who may also produce an excess for the market. The per capita bean consumption in Nicaragua is *c*. 14 and 18 kg year^{-1} in the urban and rural areas, respectively (IICA-USAID, 2003). Beans are grown practically everywhere in the country, from sea level to more than 900 m.a.s.l. in the central parts of Nicaragua (Voysest, 2000). It has been estimated that the area suitable for bean production in Nicaragua is around 720 000 ha, however, the area sown is only *c*. 275 000 ha (MAG-FOR, 2004). Its adaptation to the sites where it is cultivated may vary from optimal to marginal (Table 1), which depends on the environmental conditions at each site.

Table 1. *Adaptation of the common bean to different zones in Nicaragua based on their climatic and edaphic characteristics*

Adaptation	Altitude (m.a.s.l.)	Temperature (C°)	Precipitation Accumulated (mm)	Period (month)	Soil Depth (cm)	Slope (%)	Drainage	pH	Al (%)
Optimal	450-800	17-24	200-450	6	>60	<15	Good	6.5	20
		17-20							
Intermediate	200-450	23-27	450-700	4	40-60	15-30	Moderate	6.0	50
Marginal	100	<17	>700	<4	<40	>30	Imperfect	5.5	>50
		>27	<200	>6					

Small red beans [<25 g, 100-seed weight^{-1} (Voysest, 2000)] are the preferred seed type in Nicaragua, although other seed types (cream-, brown-, pink-seeded) are also locally favoured. Beans are often planted in monoculture at the beginning of the rainy season (late May to early June, called 'primera'). This crop is grown for food security and with the purpose to increase seed stocks for the second bean-growing season. The second growing season (from September to December, called 'prostrera') is the most important in Central America and 60% of the total volume of beans are then produced. In the humid areas of Nicaragua, however, there is a third growing season called "apante" (from November to March), which contributes significantly more than the other growing seasons to the national production (PASA-DANIDA, 2004). The cultivared areas allocated to the different growing seasons in Nicaragua in 2003 was 18, 31 and 51% of the total area sown (MAG-FOR, 2004). Beans can also be intercropped with maize, which is a traditional practice of small-scale farmers in the northern part of the country (Tapia & Camacho, 1988).

In this thesis the term landrace is used to refer to populations that never have been improved by formal breeders and that have been cultivated locally by farmers for many years. Landraces have played a significant role in the improvement of the common bean in Nicaragua. Up to 1953 all beans sown in Nicaragua were local landraces. After that period the National Bean Program, which was established in 1942 (Tapia, 1987), began the collection of local bean landraces and started to introduce bred bean cultivars. These mainly came from Mexico, Costa Rica and Colombia (Araya *et al.*, 1992). Until the 80's, the main purpose of the bean improvement program was to increase the yield. Other traits, such as brightness and seed colour were ignored. During the 80's six black-seeded and one dark-red-seeded cultivar, which outyielded the bean landraces, were developed (Tapia, 1987). The higher yield of the improved cultivars was mainly due to their resistance to BCMV and a good performance when they were intercropped with maize. However, their acceptance by consumers was quite low. Later, other attributes such as seed colour and earliness were gradually incorporated into the improved cultivars through crosses between selected cultivars and bean landraces (Tapia, 1987).

In Nicaragua, common bean landraces still represent an important genetic resource used directly by small-scale farmers, and their contribution to the total bean production in the country is significant. In 2001, approximately the 81% of

the area cultivated with main staple grains (beans, maize, rice and sorghum) was sown with seeds of landraces (INEC, 2003). In the case of the common bean most of the cultivated areas are sown with seeds obtained from landraces (Figure 1). In the past few years the use of bred cultivars has increased somewhat, mainly as a response to a governmental policy which aims at the promotion of the use of certified seeds of bred cultivars.
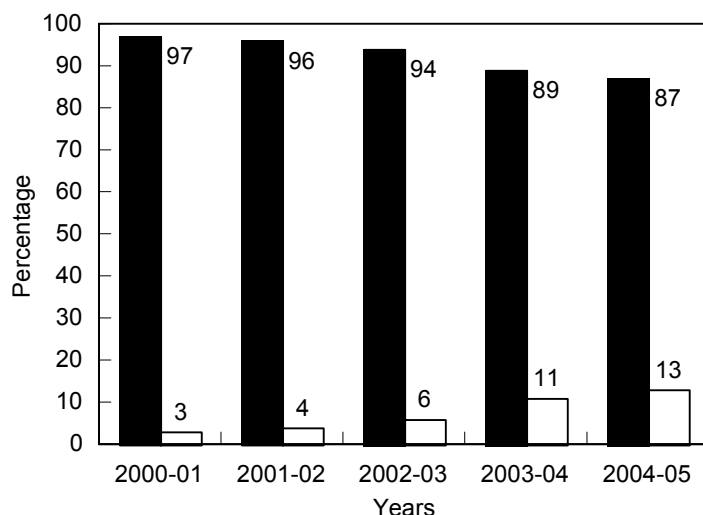

Figure 1. Percentage of commercial areas sown with seeds of (■) landraces and (□) bred cultivars of common bean in Nicaragua (the figures for 2004-05 are estimates).

Research objectives

The importance of bean landraces in the Nicaraguan agriculture cannot be neglected. They are used as sowing material by most of the small-scale farmers because they possess some important trait (e.g. earliness) and they are well suited both to the crop production systems and the socio-economic situation of small-scale farmers (Tapia, 1987). These landraces yield poorly in comparison with new bred cultivars and are in general susceptible to biotic stress factors, although some authors have claimed that under specific conditions they may produce both as well as, or better, than some of the bred cultivars (Barrera & Alvarez, 1998). Thus, a thorough understanding of those factors, which might affect the yield formation processes in both bean landraces and bred cultivars in the Nicaraguan environment is important and may serve as a basis for their rationale utilization and improvement.

Due to their cultivation in a wide and varied range of soils and under contrasting climatic conditions (Llano, Viana & Munguía, 1998) in combination with different management practices, the Nicaraguan bean landraces show a high phenotypic variation in adaptive traits (Tapia, 1987). These traits, however, are strongly affected by environmental conditions (Brown, 2000). This last aspect has at least two important consequences. The first consequence is that the diversity maintained in these landraces as determined only on the basis of phenotypic traits

may be either over- or under-estimated. Thus, the use of alternative markers such as molecular ones may either complement or highlight new features of the variation in bean landraces. Since most of the bean diversity studies that have been carried out in Nicaragua has used phenotypic markers it was decided to include a set of molecular markers in order to study the diversity at molecular level. This has not been done before in the currently cultivated Nicaraguan bean landraces. The second consequence is that a thorough evaluation of phenotypic traits requires multi-location and multi-year trials to account for environmental effects and genotype-by-environment interactions (Gepts & Papa, 2003). The evaluation of bean landraces that has been carried out in Nicaragua has generally been done at only one (usually at an experimental station) or at a few sites. Thus, the information about their adaptability is scarce. Landraces are generally considered to be adapted to their local environments (Collins & Hawtin, 1999); other authors, however, have argued that this is an assumption that should be demonstrated (Wood & Lenné, 1997). The performance of landraces in particular environments as well as their potential as donors of important genes, which depends on their variation in adaptation, need thus to be investigated.

It is well known that the genetic diversity of a crop is shaped by several factors, namely, its population biology, the physical environment where it grows (soil, climate, diseases etc.) and the human management (Jarvis *et al.*, 1998). Thus, one can expect that the genetic variation will change continuously and therefore the picture obtained at the time of collection is just partial and is not necessarily representative of the level of variation in the presently cultivated bean landraces. Furthermore, the threat of genetic erosion in farmers' fields and in genebanks (Serwinski, 1999) is real. As a result of a recent natural disaster (flooding due to the Hurricane Mitch in 1998) in Nicaragua, several small-scale farmers completely lost their crops. More recently, a governmental program called "Libra por Libra" (Pound per Pound) was launched, which aims to replace all bean landraces by bred cultivars. These events suggest that some bean landraces may already have been lost forever. Moreover, it is not known whether the diversity of the bean landraces that are stored *ex situ* are affected by the conditions under which they are maintained and multiplied. Thus, efforts must be addressed to the conservation and utilization of present and "do novo" generated diversity.

The general purpose of this thesis work was to complement the knowledge already available about the Nicaraguan bean landraces and to generate new information in order to promote their rational utilization, either directly by farmers or by bean breeders, and consequently their conservation for the future. More specifically, the aims of this thesis were the following:

1. To compare the yield performance of the Nicaraguan landraces with that of the bred cultivars of common bean to shed some light on the possible factors which might affect their productivity.
2. To determine the variation in adaptation of bean landraces across sites in comparison with recently introduced bred cultivars.
3. To evaluate possible changes in bean landraces stored *ex situ* as compared with the same landraces currently cultivated by farmers.

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In this work we hypothesized that (1) currently grown bean landraces show a better performance than the bred cultivars in farmers' fields and are more stable than the bred cultivars, (2) have similar phenotypic and molecular diversity patterns and that they are not genetically differentiated, and (3) are more genetically variable than the same landraces collected in the past and stored *ex situ* in genebanks.

Materials and methods

This thesis consists of four papers where a detailed description of the experimental sites, methods and measurements carried out are described. This part briefly summarizes the materials and methods used in each experimental work.

Paper I

Seventeen common bean landraces presently cultivated by farmers at different sites in four distinct agroecological zones were used to study the genetic variation at the molecular level. Of these nine red-seeded landraces were chosen for a field study of phenotypic variation. For the molecular evaluation DNA was extracted from trifoliolate leaves of young seedlings grown in a greenhouse using the method described by Dellaporta, Wood & Hicks (1983) and González *et al*. (1995) with minor modifications. In order to select the most polymorphic microsatellites, DNA from 12 individuals from each of the 17 landraces was pooled and tested with 20 microsatellites. The seven most polymorphic microsatellites were chosen for further assays on the full set of individuals. Molecular diversity measures as the number of alleles per locus, observed heterozygosity (H_o) and gene diversity (H_e) were calculated according to Nei (1987). All calculations were realized using the computer programs Fstat V2.9.3 (Goudet, 2002) and Genepop 3.3 (Raymond & Rousset, 2001). The partitioning of variation within and among landraces and agroecological zones was calculated using an analysis of molecular variance (AMOVA) computed using the program Arlequin (Schneider, Roessli & Excoffier, 2000). Wright's F-statistics were calculated according to Weir & Cockerham (1984) using the software Fstat V2.9.3 (Goudet, 2002). The phenotypic evaluation of the nine landraces was conducted at San Marcos ("La Compañia" experimental station) and at San Pedro (in a farmers´ field,) both in the department of Carazo, Nicaragua. The experiments were carried out during the second cropping season (September-December) in 2000. Crop management was similar at both sites with the exception of soil preparation. At San Marcos, it was done with conventional tillage, while at San Pedro, with oxen. The sites of the experiments are located 40 km apart from each other and differ in soil and climatic variables (Table 2, Paper I). The experimental layout was a randomised complete block design with four replicates. Fourteen agromorphological traits (Table 3, Paper I) were determined according to Muñoz, Giraldo & De Soto (1993). Frequencies of each class were calculated for the three qualitative traits. For the analysis of growth habit a square root transformation ($\sqrt{X + 0,5}$) was carried out.

Data were subjected to the analysis of covariance considering the number of harvested plants as a covariable using the software JMP v. 4.05 (SAS Institute, 2000). The mean separation was done using Tukey-Kramer HSD at 5% level. For the comparison of population frequencies for qualitative traits (wing color, standard color) a Fisher's exact test was used and calculated using the PROC FREQ procedure in SAS software packages (SAS Institute, 1997).

Paper II

The genetic material used consisted of two landraces (a red- and a cream-seeded) stored in two *ex situ* collections at the Genetic Resource Program of the National Agrarian University of Nicaragua (REGEN) and at the CIAT, Colombia. Passport information was used to identify the localities where these two landraces were originally grown by farmers. The same localities were revisited in 1999 in order to get a seed sample of the same populations, which have been continuously used by farmers. For the red-seeded populations three separate samples were collected from three farmers at the same location (Table 1, Paper II). Since 1970 when the seed samples of the two landraces came to CIAT they have been regenerated more than 10 times at different times of the year and in different localities (O.Toro, personal communication) and the seeds used in the present work represent the material regenerated in 1999 by the Genetic Resource Unit of CIAT. The red- and cream-seeded accessions managed by the Nicaraguan genebank were stored as original samples in 1991 and 1992, respectively, and had not been regenerated since. Due to the small number of seeds obtained from the accessions stored in CIAT and REGEN genebanks, they were multiplied in the first part of the growing season (May-August) in 1999 and 2000, respectively. The sample size of the populations collected directly from farmers was *c*. 450 g and these were not multiplied. Throughout this thesis the accessions stored *ex situ* in genebanks are referred to as CIAT and REGEN populations and the present populations recently collected from farmers as *in situ* populations. The DNA extraction was done using the same methods as in Paper I. The DNA from 16 individuals of each CIAT and REGEN population and 12 from the *in situ* ones (a total of 112 individuals) was separately analysed using the seven microsatellite markers which were selected for the study in Paper I. The genetic diversity at molecular level was measured by means of the number of alleles per locus, the observed heterozygosity (H_o) and the gene diversity (H_e) indices calculated per locus combination and across all loci according to Nei (1987). The contribution of the different sources of variation (landrace and conservations methods) to the total variation was also determined by means of the analysis of molecular variance (AMOVA). Calculations were carried out using the computer programs Arlequin (Schneider, Roessli & Excoffier, 2000) and Fstat V2.9.3.2 (Goudet, 2002). The field evaluation of the two landraces used in this study was done at San Marcos ("La Compañia" experimental station). The experimental design consisted in a randomised complete block design with three replicates. Samples of five plants from each plot were taken for growth analysis at 29, 34 and 64 DAP. Each plant was separated into leaf, stem and rest (petioles, branches, flowers, pods) of plant components. The part of the stem below the cotyledonary node together with the root system was discarded. Total leaf area,

leaf dry matter, dry matter of the rest of plant components and total dry matter were among the variables measured. Phenological, morphological and agronomic traits were also recorded (Table 2, Paper II). Univariate and multivariate (for repeated measurements) analyses of variance were carried out using the statistical software program for personal computers JMP v 4.05 (SAS Institute, 2000). Mean comparison was done by Tukey-Kramer HSD test at 5% level of probability.

Paper III

The plant material consisted of four red-seeded landraces currently cultivated by farmers. These landraces were selected on the basis of contrasting yield potentials and the genetic diversity levels observed in Paper I. Two bred populations, DOR-364 and INTA-Masatepe, which were developed by CIAT in Colombia were also included. The field evaluation was carried out at San Marcos ("La Compañia" experimental station). The experimental plots were fertilized, weeded by hand and sprayed regularly with agrochemicals (Metamidofos and Benomyl) to protect the crop against pests and diseases until harvest. All populations were seeded in six-row plots each 5 m long with a within-row spacing of 10 cm and with 50 cm between rows. The first and sixth rows from each plot were used for periodic collections of primary data for growth analysis; yield and yield components were determined from the remaining rows. The seed yield rate [seed yield (days to final harvest)$^{-1}$] was also calculated. A sample of five randomly distributed plants per plot were harvested on seven different occasions (data of some traits from the 6th sequential harvest were eliminated due to practical problems). The plants were cut at ground level. Traits measured were stem diameter at the cotiledonary node, number of nodes on stem (data were collected until the 5th sequential harvest), number of pods per plant and leaf area. After recording these traits the plants were separated into leaves, stems plus petioles and reproductive organs (buds, flowers and pods of different sizes) and dried for at least 72 hours in a forced air oven at 80ºC before weighing. At the final harvest, which was done at 69 and 77 DAP for landraces and bred materials, respectively, a random sample of ten plants per plot was taken for the determination of yield components. Data of the experiment were analyzed as a repeated measures design in a randomized complete block design with four replicates by PROC MIXED option of the SAS program. When the interactions were statistically significant simple main effects were calculated using the LSMEANS statement with the SLICE option (SAS Institute, 2001). Yield, yield components and seed yield rate were subjected to the analysis of covariance considering the number of harvested pants plot^{-1} as a covariate. All traits were analyzed according to the model described above but without considering the harvest time and interaction effects using the procedure PROC GLM from SAS (SAS Institute, 2001). As a measure of precision of the treatment means the average of their individual standard errors was used. Finally Pearson correlation analysis between yield area^{-1} and pods plant^{-1} with physiological traits based on individual observations of the populations studied was carried out.

Paper IV

The six populations (two bred cultivars and four red-seeded landraces of common bean) described in Paper III were also used in this study. They were evaluated in six contrasting environments: one experiment was established at San Marcos ("La Compañia"experimental station) and the rest were sown in farmers´ fields representing a wide range of agroecological zones. The sites were Dulce Nombre (DN), Estelí (Est), Jinotega (Jin), Masaya (Mas), and Santo Tomas (ST) (Table 1, Paper IV). Soil preparation was carried out with conventional tillage at the experimental station, while at the other sites it was done with oxen. All the other farming practices were similar at all sites including fertilization. The experimental design was a randomised complete block design with four replicates. Each plot consisted of six rows, 4 m long and 0.4 and 0.5 m apart in farmers´ fields and at the experimental station, respectively. Within row distance between plants was 10 cm. All the experimental sites were regularly sprayed against pests and diseases when considered necessary. The middle four rows from each plot were harvested to determine seed yield, which was adjusted to 14% seed moisture content. Yield components were measured on a random sample of 10 plants per plot just previous to the final harvest. All traits were registered at maturity. Data for the traits measured were subjected to a single site and a combined analysis of variance across sites. The analyses were applied to the full data set, and to two subsets of the data. The environment at the experimental station is not typical for small-holder farms and this site was therefore excluded in one subset (on-farm trial I). There was also a serious disease attack at one on-farm site (DN) affecting some of the populations heavily (Table 2, Paper IV) and therefore this site was also excluded in another subset of the data (on-farm trial II). REML estimates of the variance components for the different sources of variation were also calculated considering all effects as random. In order to determine the nature of the population x site (PxS) interaction, it was further partitioned into two parts, that associated with heterogeneous variances over sites and that due to deviations from a perfect positive correlation (imperfect correlation) of genotypic rankings among environments by the method described by Muir, Nyquist & Xu (1992). The Additive Main Effects and a Multiplicative Interaction (AMMI) analysis (Gauch, 1992) was used to study the contribution of populations and sites studied to the PxS interaction. In this study we consider a population or site as being stable relative to the others (type 2 stability) if its contribution, determined by the AMMI analysis was small. Perfect stability according to the type 2 stability concept implies an observed or modelled yield response that is always parallel to the environmental mean yield (i.e. zero interaction, Annicchiarico, 2002). Furthermore, a population will be considered as having specific or wide adaptation on the basis of the consistence of its mean yield across the site studied. In addition, the criterion used for specifying whether an experimental site had a favourable environment for bean production or not was the overall mean yield of all populations at that site. The variance components and the AMMI analyses were performed in SAS (SAS Institute, 2001) using the VARCOM procedure and the program developed by Hernández & Crossa (2000), respectively.

Results

Diversity of currently cultivated common bean landraces

Population differentiation and genetic structure

The bean landraces showed a strong genetic differentiation according to the Wright's F statistics. The F_{ST} indicated that on average 34% of the genetic variation was explained by differences among landraces. The AMOVA analysis showed that the contribution of the agroecological zones to the total variation at molecular level was zero while 36.5% and 63.5% of that variation was attributable to differences among landraces within agroecological zones and differences within landraces, respectively.

Molecular diversity

An average of 5.7 alleles were identified per microsatellite locus with a range of 2 to 13 distinct alleles in the full array of individuals per landrace depending on the individual microsatellite (Table 4, Paper I). Some of these alleles were unique to individual landraces. The observed heterozygosity within populations was low (average = 0.01) although for some landraces, particularly those from the agroecological zone "I", and for some microsatellites (J04555 and BM114) higher values than that were observed (Table 5, Paper I). The expected heterozygosity (gene diversity) value within populations averaged over all loci was 0.35 while the total gene diversity (H_T) averaged 0.51 across all individuals across all analyzed loci. In general, the populations collected in the agroecological zones "B", "F"and "H" showed higher within population gene diversity averaged over all loci than zone "I".

Phenotypic diversity

The ANOVA analysis indicated that the agroecological zones where the landraces were collected and the experimental sites had a great impact on the majority of the traits studied. Interactions between these factors were also significant for some traits (100-seed weight, leaf surface area and growth habit). Additionally, landraces within agroecological zones differed in growth habit and phenological traits. Landraces from the agroecological zone "B" yielded more than landraces from other agroecological zones although they were only significantly superior to landraces from the agroecological zone "I" (Table 6, Paper I). They also flowered and matured earlier than all the landraces studied. Within agroecological zones landraces differed mainly by phenological traits, standard color, and growth habit (Table 7, Paper I). The differences were more marked between the two landraces from the agroecological zone "H" than among landraces from the another ones.

The growing conditions at San Pedro (in a farmer's field) were poorer than at San Marcos ("La Compañia" experimental station). This resulted in a significant reduction of growth traits, which was reflected by reduced stem length and leaf surface area, and total bean yield which was 26% lower at San Pedro The site

conditions affected the landraces collected from the agroecological zone "H" more than the landraces from the other zones (Table 8, Paper I). These differences were mainly observed in 100-seed weight and in the proportion of plants with indeterminate bush (Type II according to Muñoz, Giraldo & De Soto, 1993) growth habit.

Changes in two landraces managed *ex situ* in genebanks and *in situ* by farmers

Molecular diversity

An average of 4.3 alleles were identified per micosatellite locus across all the populations studied. The mean values of alleles per population in cream- and red-seeded landraces varied between 1.3 to 2.7 and from 1.1 to 1.9, respectively (Table 3, Paper II). In both landraces the average number of alleles was lower in both *ex situ* populations (CIAT and REGEN) than in the *in situ* populations. *Ex situ* populations also had a lower gene diversity averaged over loci than *in situ* ones. Overall the gene diversity within populations was twice as high in the cream- compared with the red-seeded landrace (H_S = 0.26 and 0.12, repectively) although in their total gene diversity they were similar (H_T = 0.43 for both). The observed average heterozygosity (H_O) across all loci in both landraces was very low (0.01).

The AMOVA analysis showed that most of the variation (39.1%) at molecular level was attributed to differences among the three conservation methods (CIAT, REGEN and *in situ*), the remainder was distributed among populations within conservation methods (35.7%) and within populations (25.2%).

Phenotypic diversity

According to the results of the ANOVA and MANOVA analyses, the conservation methods and landrace effects were important sources of variation for most of the phenotypic traits measured. The CIAT populations yielded significantly less, had lower 100-seed weight, tended to mature earlier and together with the REGEN populations had smaller leaf area than the *in situ* populations in both cream- and red-seeded landraces, although the differences were more marked in the cream-seeded landrace (Table 7 and Figure 1, Paper II). For the remaining traits no significant differences between *ex situ* and *in situ* populations were detected. Concerning the two landraces studied the cream-seeded populations were significantly differentiated from the red-seeded for all traits studied. They had a higher yielding ability, flowered earlier, had heavier seeds, taller plants, a larger leaf area (Figure 1, Paper II) and, leaf dry matter than the red-seeded ones.

Yield formation in Nicaraguan landrace populations as compared with two bred cultivars

In general, the maximum leaf area was observed at 41 DAP, thereafter it decreased in all bean populations but, with the exception of V6, more so in the landraces (Figure 1a, Paper III). Both bred cultivars showed significantly larger leaf areas than the landraces at 62 DAP. No differences in total above-ground dry matter among populations were observed up to 62 DAP. The leaf dry matter accumulation was similar for all populations but plants of the four landraces began losing their leaves earlier than the bred cultivars did (Figure 1b, Paper III). Differences among populations in their relative allocation patterns were also observed. With one exception the landraces, partitioned proportionately more dry matter to reproductive organs compared with the bred cultivars although a larger proportion of this investment was later lost (Figure 1c, Paper III). As regards morphological traits, the landraces tended to produce more nodes than the bred cultivars during the early growth stages but this effect was only significant at 27 DAP (Figure 2a, Paper III). The final number of nodes produced was, however, the same for all populations. The bred cultivars had thicker stems than the landraces and continued the increase in stem thickness for a longer period of time (Figure 2b, Paper III). The populations studied differed in their rate of pod formation but not in the final number of pods per plant (Figure 2c, Paper III). Significant differences between populations for yield and yield components were observed. The bred cultivars yielded a little more than three of the landraces while one landrace was intermediate in yield (Table 1, Paper III). This was a result of both more pods per plant and a greater 100-seed weight in the bred cultivars. The seed yield rate did not differ among the populations. No correlation was found between yield and the total above-ground dry matter (Table 2, Paper III) but there was a correlation of yield with leaf area and leaf dry matter at the last sequential harvest (62 DAP). These two traits were also significantly and positively correlated with the number of pods per plant from 41 DAP (pod formation stage) and onwards until the final sequential harvest.

Yield and yield stability

The yield superiority of the bred cultivars over the landraces was evident at Dulce Nombre, Jinotega and Masaya (= sites with the poorest environments). At the remaining sites either one or all of the landraces performed similarly to the bred cultivars. The bred cultivars tended to have more pods per plant and heavier seeds than the landraces. In general, differences among populations were more marked at the most stressful sites. Further, population differences were particularly high and significant for 100-seed weight at all sites studied. The combined analysis of variance (Table 3, Paper IV) indicated that most of the total variation for all traits, with the exception of 100-seed weight which depended most on the population component, was due to the site effect. The PxS interaction components were, with few exceptions, significant for all traits studied. Generally the PxS component explained more of the variation than the population component when the non-diseased data set was used for the analysis. Further partitioning of the PxS

interaction sum of squares revealed that a large proportion of this interaction was in part due to due heterogeneous genetic variances across environments but largely due to imperfect correlation of environmental ranking among the genotypes (Table 4, Paper IV). This effect was particularly strong for 100-seed weight, the number of seeds per pod and yield independently of which data set was analysed. The bred cultivar DOR-364 and the San Marcos, Dulce Nombre and Jinotega sites contributed most to the PxS interaction irrespective of which data set was used for the analysis (Figure 2, Paper IV). When all sites where included in the AMMI analysis INTA-Masatepe was the most stable population (Figure 2a, Paper IV), but when data from San Marcos or from Dulce Nombre and San Marcos together were excluded from the analyses the landraces V16 and V29 were just as stable as INTA-Masatepe (Figure 2b and 2c, Paper IV). The bred cultivars yielded, on average, and across all sites, significantly more and had higher values for some of the yield components than the landraces.

Discussion and conclusions

The yield performance of the landraces varied but for some it was equivalent to that of the bred cultivars irrespective of evaluation site. Thus, the assumption about the superiority of bean landraces when grown in farmers' field was not true. This finding indicates that advances in the genetic improvement of yield *per se* of the bean cultivars for the Nicaraguan environment have not been large but that the enhancement of resistance to major diseases such as BGMC, BCMV, bacterial blight, angular leaf spot and web blight in the cultivars released is the main characteristic which contributes to an improvement of yield in the Nicaraguan environment. The main drawback of the Nicaraguan bean landraces is their poor resistance to biotic stress factors; however, there were differences between the landraces studied in their tolerance of different diseases (Paper IV). This may indicate that there are valuable genes within the landraces that confer a certain "rusticity" (generalized stress tolerance). For example, White & Izquierdo (1991) reported that this was the case of the bean cultivar Porrillo Sintetico, which was evaluated in one of the International Bean Yield and Adaptation Nurseries. This cultivar showed a relatively better performance than other similarly yielding cultivars particularly at poor sites that were affected by several stress factors.

Environmental conditions (soil, climatic, crop management etc.) differed greatly between the experimental station and the farmers' fields, which resulted in very different yield performances of all of the populations studied. The conclusion that can be drawn from this is that for a better utilization of the yield potential of some of the Nicaraguan bean landraces two things are necessary: 1) the genetic improvement of certain characteristics (i.e. resistance to biotic and abiotic stress factors) in order to confer a better adaptation to the environments in which they are cultivated, and 2) enhancement of the production practices.

In general yield is correlated with time to maturity and selection for lateness has been one strategy for increasing the yield of common bean (White & Izquierdo, 1991). Such a correlation was found in the study reported in Paper III. However, it

is worth mentioning that the performance of one landrace (V9) was as good as that of the bred cultivars despite the fact it reached maturity earlier than the bred cultivars. The mechanism behind this finding should be evaluated more carefully in future studies. This is especially true since the currently available bred cultivars are not well accepted by the farmers due to their longer growth cycle as compared with landraces. Another characteristic that should be altered is the seed coat colour if a better acceptance of bred cultivars is to take place. In the cultivars available it is dark-red which is too dark for the Nicaraguan consumers' taste. These last two aspects have in part impeded the full adoption of new bean cultivars in Nicaragua (Singh, 1999).

High yield stability is an important attribute of any cultivar, particularly in environments subject to several biotic and abiotic stress factors. There are two concepts of stability frequently used in relation to cultivar performance: static (biological) and dynamic (agronomic). The former implies that a cultivar has a stable performance across environments and there is no among-environmental variance (Annicchiarico, 2002). The latter implies that a cultivar has a stable performance but, for each environment, its performance corresponds to the estimated level or predicted level (i.e. a cultivar is stable if its response to the environments is parallel to the mean response of all cultivars in a test; Kang, 2002). The static and dynamic concepts of stability are equivalent to the Lin & Binns's (1994) type I and type II stability, respectively

On the basis of the AMMI analysis which was carried out in the study reported in Paper IV, which measures the type 2 stability, the bred cultivar INTA-Masatepe was found to be the most stable population while the other bred cultivar (DOR-364) was the least stable of all the populations studied. However, this result must be interpreted with caution since the classification of a cultivar as being stable or not depends on the other cultivars considered in a study. The problem with biological stability is that it usually is associated with relatively low yields as was the case in the study reported in Paper IV. On the basis of this measure, which is determined by the means of the environmental variance (data not shown), the landraces V16 and V26 were the most stable, but they yielded (on average across all sites) less than the two bred cultivars but more than the other two landraces. Thus, on the basis of the results and analysis carried out it seems that agronomic stability is not a typical landrace trait although biological stability is.

It was expected to find a geographical pattern in the distribution of genetic diversity of the bean landraces evaluated in Paper I. However, the results differed depending on the kind of marker used. There was no pattern of a geographical distribution of genetic diversity when molecular marker data was used, although patterns were seen when phenotypical trait data was used. Based on phenotypical trait data there was a clear differentiation between landraces from different agroecological zones. The contrast between the supposedly neutral or near-neutral nature of microsatellites (Jarne & Lagoda, 1996; Li, Rossnagel & Scoles, 2000) and the adaptive value of phenotypic traits (Hill, Becker & Tigerstedt, 1998) might in part explain this apparent discrepancy. Some authors (Reed & Frankham, 2001; McKay & Latta, 2002) have found only a weak correlation between data on molecular and phenotypic diversity. They state that molecular markers may not

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provide results equivalent to phenotypic traits due to lack of correspondence in gene action between phenotypic traits (additive, dominance or epistatic actions) and molecular markers (indirect measure of additive gene action), differences in heritability (high for molecular markers, low to high for phenotypic traits), different mutation rate (high for polygenic phenotypic traits, low for molecular markers) or environmental effects (phenotypic traits are environment dependent whereas molecular markers are not). Another possible reason is that the statistical power differs between molecular markers and phenotypic traits. Lewontin (1984) pointed out that differences between species or populations in quantitative characters can not be compared with differences in gene frequencies at individual loci, because the power of statistical tests to discriminate populations are vastly different for the two kind of characters. Only a few polymorphic microsatellite loci were analysed in the study reported in Paper I, and therefore no biological explanation to the observed discrepancy in geographic pattern between molecular and phenotypic markers can be given at this time.

Within agroecological zones, however, a strong genetic differentiation among landraces for both molecular and phenotypic markers was detected. It can be assumed that there is low gene flow among populations as a result of the self-pollinated mating system of the common bean (Ibarra-Pérez, Ehdaie & Giles Waines, 1997), which has contributed to the interpopulation differentiation of the bean landraces studied. Another reason could be due to the founder effect since it is common practice among farmers to conserve small amounts of the seeds of a crop for sowing in the next growing period. This genetic phenomenon may also occur when a farmer obtains small quantities of seeds from other farmers in order to replenish his own stocks or to replace it for a new one. In both cases random changes in the diversity of these populations will occur. The founder effect is more likely to occur in small populations (Jarvis & Hodgkin, 2000). The common bean landraces differed in phenotypic traits and it is possible that this was due to the effect of natural or human selection pressures focused on the adaptation of landraces to new environments or cropping systems. The presence of a structure for the genetic diversity at molecular and phenotypic levels may be useful in the design of sampling protocols.

An aspect considered in this thesis was to monitor the occurrence of possible changes in the diversity of bean landraces conserved *ex situ* in genebanks. It was found that genetic diversity at the molecular level was lower in the gene bank material than in the populations maintained by farmers. These changes were thought to be due to the conditions under which the populations were maintained and multiplied (regeneration process). A reduction in the genetic diversity in the genebanks could be due to the low sample sizes used when an accession is regenerated since this can cause a random loss of alleles (genetic drift). Zeuli, Sergio & Perrino (1995) and Parzies, Spoor & Ennos (2000) reported that this was the case for wheat and barley, respectively, while Del Rio, Bamberg & Huaman (1997) and Richard (1998) working with potato and ryegrass accessions did not observe any changes in their genetic diversity. Khlestkina, Huang & Quenum (2004) studied the genetic diversity of wheat accessions of the Gatersleben genebank, which were repeatedly collected from different geographical areas over an interval of 40-50 years. They found that the genetic diversity has been

maintained, although there was clear evidence of qualitative changes. However, even if most of the phenotypic traits of adaptive value were generally not affected by the conservation strategies studied here, it is worthwhile to notice that some adaptational changes did occur (Table 7, Paper II) which was mainly observed in populations from CIAT. We do not have an obvious explanation to the lower yield of the populations maintained in CIAT, but we observed a somewhat larger incidence of viral infections in these populations than in the others (data not shown). This may indicate that the removal of landraces from the environment in which they are cultivated also isolated them from exposure to the diseases, which are prevalent in the farmers' fields. It can be concluded that *ex situ* conservation in general has been successful in keeping most of the adaptations found in the landraces studied. However, the loss of genetic diversity at the molecular level suggests that protocols for the regeneration process must be carefully worked out if most of the alleles are to be preserved for the future. Furthermore, the qualitative changes that may occur in the landraces which continue to be cultivated by farmers indicate the necessity of new collections for the exploitation of the new allelic variation together with that already existing in the *ex situ* collections.

In conclusion yield performance of the bean landraces studied varied but in some it was just as good as in the bred cultivars. One of the main drawbacks of the landraces is their poor resistance to some biotic stress factors; however, they may harbour specific adaptive traits to cope with different stress factors. Agronomic stability was not a typical landrace trait but they showed biological stability. The bred cultivars showed good yield performance but since they lack some specific traits (earliness, seed coat colour), which are valued by farmers and consumers they have a poorer market value. This study found that the diversity of the current bean landraces was distributed within as well as among populations. This must be considered in the design of sampling protocols. Finally, it was found that conservation of the diversity harboured in the Nicaraguan bean landraces is threatened by a total reliance on *ex situ* conservation.

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