



## Morphological characterization of accessions of *Phaseolus vulgaris* L. in the GeneBank of INIFAT

### Caracterización morfológica de accesiones de *Phaseolus vulgaris* L. en el Banco de Germoplasma del INIFAT

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**ABSTRACT:** The common bean collection (*Phaseolus vulgaris* L.) from the Genebank of Institute for Fundamental Research in Tropical Agriculture “Alejandro de Humboldt” (INIFAT), has the potential to be used as genetic material in breeding programs for the crop, in the development of strategies for the conservation of local varieties, for confronting climate change, among other functions. The optimal exploitation of the genetic potential of this collection is only achieved through characterization. The following work aims to characterize the genetic variability of a sample of 35 accessions of common bean (*Phaseolus vulgaris* L.) through the use of 31 morphological descriptors. The selected sample presented less variability in terms of maturity and harvest times, also presented a significant correlation between the traits related to the architecture of the plant. Principal component analysis indicated that first two components explained 63.8 % of total variance. The type and size of grains have an important role in the grouping of the accessions. Several genotypes evaluated have potential as basic breeding lines.

**KEY WORDS:** agronomic traits, germplasm, common beans.

**RESUMEN:** La colección de frijol común (*Phaseolus vulgaris* L.) del Banco de Germoplasma del Instituto de Investigaciones Fundamentales en Agricultura Tropical “Alejandro de Humboldt” (INIFAT) tiene potencial para ser utilizada en programas de mejoramiento genético del cultivo, en el desarrollo de estrategias de conservación de variedades locales, para el enfrentamiento al cambio climático, entre otras funciones. La óptima explotación del potencial genético de dicha colección solo se alcanza mediante la caracterización. El siguiente trabajo tuvo como objetivo caracterizar la variabilidad genética de una muestra de 35 accesiones de frijol común (*Phaseolus vulgaris* L.) mediante el empleo de 31 descriptores morfológicos. La muestra seleccionada presentó menor variabilidad en cuanto a los tiempos de madurez y cosecha y una significativa correlación entre los caracteres relacionados con la arquitectura de planta. El análisis de componentes principales indicó que los dos primeros componentes explicaron el 63,8 % de la varianza total. El tipo de grano y su tamaño tuvieron un papel importante en el agrupamiento de las accesiones. Varios genotipos evaluados presentan potencial como líneas básicas de mejoramiento.

**Palabras clave:** características agronómicas, germoplasma, frijol.

## INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is among the most cultivated and consumed legumes in the world, representative of tropical regions; although also in temperate regions, beans play a fundamental role in the

diet of millions of people. It is a bean that contains about 15 essential minerals and is an important source of protein (1-6). *Phaseolus vulgaris* has a Mexican origin and expanded, through different migration events, to different regions of the continent. The species is characterized by

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three eco-geographic gene flows: Mesoamerica and the Andes, the two largest gene flows that include both wild and domesticated species, and a third flow to the north of Peru-Ecuador with a more limited distribution and includes only wild forms (6-8).

In the case of Cuba, beans are considered one of the most important crops in the staple diet. Bean planting has decreased in recent years; in 2020, the figure of 73 096 hectares of the bean was reached, almost half of the figures reached in 2016. Yields have also been affected with respect to previous years with decreases of up to 0.22 tons per hectare of grain, between 2016 and 2020 (9). Evidently, these figures are not enough to meet the national demand; it has been raised that the country needs to import around 60 thousand tons of the grain, which represents more than 52 million dollars. Bean production in Cuba faces problems of low soil fertility, drought, weeds and diseases, which are the main limiting causes (10). The availability of germplasm with greater genetic variability and tolerance to the aforementioned limiting factors can contribute to the increase of crop productivity under the conditions of the country.

The systematic selection and breeding of crops has resulted in the loss of genetic diversity and the replacement of traditional varieties, and *Phaseolus vulgaris* is no exception. Genetic diversity, represented by locally adapted traditional varieties, is considered vital to safeguard the productivity of different crops for the future (7,11). Germplasm banks promote the conservation of the world's agricultural diversity by preventing the effect of highly productive and homogeneous new varieties on genetic erosion (2,12).

The Germplasm Bank of the Fundamental Institute of Tropical Agriculture "Alejandro de Humboldt". (INIFAT) has a collection of more than 700 accessions of common bean, collected in different parts of the island and introduced foreign materials. Although this collection has been used as a source of genes for plant breeding of the species, currently, there is little information about its genetic constitution and there are still gaps on the variability of many of its accessions. Considering that beans are considered one of the most diverse crops in terms of variations in growth habit, plant height, pod characteristics, maturation, seed weight and size (13), the characterization of the collections of the species acquires greater importance.

The objective of this work is to characterize the genetic variability of a sample of 35 accessions of common bean (*Phaseolus vulgaris* L.), using 31 morphological descriptors.

## MATERIALS AND METHODS

### Plant material and description

The sample selected for the study came from the common bean collection, conserved at 80C, at the INIFAT Germplasm Bank, located between 23°58'39"N and 82°22'41"W, in Boyeros municipality, Havana, Cuba. For

this purpose, 35 accessions (Table 1) collected during the years 2008-2010 were selected. Planting was carried out in the 2016-2017 winter season in the INIFAT areas, using a completely randomized design with 35 treatments (n=30). The 9.6 m<sup>2</sup> experimental unit consisted of five 1.5 m long furrows, spaced 0.6 m apart, with 8 cm between plants.

### Analysis of morphological characters

A total of 21 qualitative and 10 quantitative characters were evaluated, corresponding to different phenological stages of the plant and related to yield and its components: three at the seedling stage, thirteen at flowering, six at the physiological maturity stage and nine at harvest time (Table 2). The descriptors were selected according to the list developed by the International Center for Tropical Agriculture (CIAT) (14).

For quantitative traits, the assumptions of normality and homogeneity of variances were tested by Analysis of Variance (ANOVA) using the Shapiro-Wilks W statistic to accept or refute the experimental hypothesis (data not shown). The mean, minimum, maximum, standard deviation and coefficient of variation were calculated for each variable. The degree of association between pairs of quantitative variables was estimated using Pearson's correlation coefficient. A Principal Component Analysis (PCA) was also developed to determine the similarities between the different accessions. For qualitative traits, these were transformed into a matrix of binary attributes; similarities between accessions were calculated using Jaccard's similarity coefficient. All statistical analyses were performed with InfoStat version 2020 (National University of Córdoba, Argentina).

## RESULTS AND DISCUSSION

### Analysis of quantitative characters

As recorded in Table 3, the most variable trait was PS where Variado Chileno showed the highest values followed by Velazco largo Mejorado and accession P-3793; while the lowest values for this trait corresponded to accession P-3609. Other highly variable characters were LxA, LTP and DF. The characters related to CD and DMF were the least variable, the accessions that took the least time to mature were Variado Chileno, P-3337, P-3309 and P-3614 with 59 days, while accession P-3805 required more time for maturation with 77 days. The accessions that required the fewest days to harvest (DC) were P-3793, Variado Chileno, California and Maní with 77 days; while P-3802 was the accession that took the longest with 92 days.

The mean values, coefficient of variation (CV) and minimum (Min.) and maximum (Max.) values are analyzed for each character.

Many of the accessions studied are the result of several years of natural and artificial selection, depending on the adaptation to the local characteristics of each region where the material was collected. This explains many of the results obtained, such as the relatively low variation in

**Table 1.** Nomenclature and characteristics of the 35 common bean accessions

No.	Accession	Grain color (14)	Origin place
1	P-3207	Golden yellow	Habana
2	P-3289	Black	Guantánamo
3	P-3293	Black	Guantánamo
4	P-3306	Black	Guantánamo
5	P-3309	Black	Guantánamo
6	P-3310	Reddish brown mottled in beige	Guantánamo
7	P-3334	Red	Guantánamo
8	P-3337	Brown	Guantánamo
9	P-3606	Red	Artemisa
10	P-3609	Black	Artemisa
11	P-3611	White	Artemisa
12	P-3613	Brown	Artemisa
13	P-3614	Light brown mottled in red	Artemisa
14	P-3785	Light brown mottled in red	Unknown
15	P-3787	Black	Guantánamo
16	P-3788	Red mottled red	Guantánamo
17	P-3790	Reddish brown	Guantánamo
18	P-3791	Reddish brown mottled beige	Guantánamo
19	P-3793	Red	Guantánamo
20	P-3794	Black	Guantánamo
21	P-3796	Reddish brown mottled in beige	Guantánamo
22	P-3797	Sulphur yellow mottled in brown	Guantánamo
23	P-3798	Dark brown mottled in red	Guantánamo
24	P-3802	Black	Artemisa
25	P-3805	Black	Guantánamo
26	P-3806	Black	Artemisa
27	P-3809	Black	Artemisa
28	P-3810	Black	Artemisa
29	P-3811	Black	Artemisa
30	Velazco largo Mejorado (Variety)	Red	Villa Clara
31	Variado Chileno	Red	Villa Clara
32	California (Variety)	Red	Unknown
33	Caujerí 2170 (Variety)	Black	Villa Clara
34	Rayado 2258 (Variety)	Brown mottled in red	Villa Clara
35	Maní (F3)	Red	Villa Clara

characters such as Days to Physiological Maturity (DMF) and Days to Harvest (DC), which, as previously mentioned, are characters highly valued by producers, as well as yield. Long cycles coincide with the driest periods of the country, so short cycles could avoid such periods, as well as guarantee several crop cycles in one season. Similar results have been described by other authors (15).

Correlation coefficient analysis (Table 4) shows that several traits are positively correlated with each other. Based on the results, DE correlates with DA ( $r=0.176$ ), DMF ( $r=0.151$ ) and DC ( $r=0.102$ ); the character Days to anthesis DA correlates positively with DMF ( $r=0.459$ ) and DC ( $r=0.431$ ). The data reveal a significant correlation between L with A ( $r=0.803$ ) and LxA ( $r=0.911$ ), as well as between DMF and DC ( $r=0.658$ ).

Negative relationships were observed between different characters (Table 4), mainly between PS and DA ( $r=-0.678$ ), DMF ( $r=-0.649$ ) and DC ( $r=-0.612$ ) where the highest values were obtained. Likewise, negative interrelationships were

observed between physiological maturity traits such as LTP, L, A and LxA and those related to harvest, DMF and DC.

Principal Component Analysis for quantitative traits suggests that 63.8 % of the total variance is explained by the first two components (Table 5). The results in Tables 6 and 7 indicated that the first component allowed distinguishing the accessions according to the characters LTP, L, A and LxA, characters related to plant architecture. DE, DA, DF, DMF and DC had a negative contribution to this component. The PS trait had a positive contribution, but to a lesser degree.

The second principal component contributed 23.9 % of the total variance explained (Table 5). According to the coefficients of the second eigenvector (Table 6) and correlation coefficients (Table 7), the variable that contributed the most was PS related to yield. The variables DE and DF have a positive contribution to this component, but of lesser importance.

**Table 2.** Morphological descriptors used in the characterization of common bean accessions

Qualitative variables		Quantitative variables	
<b>CCot</b>	Predominant color of cotyledons	<b>DE</b>	Days to emergence
<b>Chip</b>	Predominant color of hypocotyl	<b>DA</b>	Days to anthesis
<b>CNH</b>	Predominant color of primary leaf veins	<b>DF</b>	Duration of flowering
<b>CA</b>	Predominant color of wings	<b>LTP</b>	Length of main stem (cm)
<b>CE</b>	Predominant color of banner leaf blade	<b>L</b>	Leaf length
<b>DCL</b>	Leaf blade color distribution	<b>A</b>	Leaf width
<b>VLE</b>	Veinlets	<b>LxA</b>	Leaf area
<b>CV</b>	Predominant color of the veins	<b>DMF</b>	Days to physiological maturity
<b>CCE</b>	Predominant color of the neck of the standard	<b>DC</b>	Days to harvest
<b>CCal</b>	Predominant color of calyx	<b>PS</b>	Mass of 100 seeds
<b>Hab</b>	Predominant stem growth habit		
<b>CTP</b>	Predominant color of main stem		
<b>Ram</b>	Predominant type of branching		
<b>A</b>	Lodging		
<b>CPS</b>	Primary seed color		
<b>PDCS1</b>	Primary seed color distribution pattern		
<b>PDCS2</b>	Secondary seed color		
<b>Testa</b>	Predominant aspect of testa		
<b>VS</b>	Predominant type of branching		
<b>CHilo</b>	Presence of color around the yarn		
<b>FS</b>	Predominant seed shape		

**Table 3.** Variation of quantitative traits in the 35 accessions of common bean (*Phaseolus vulgaris* L.)

Characters	Mean	CV (%)	Min.	Max.
DE	4.83	14.82	4.00	7.00
DA	36.58	13.06	29.00	46.00
DF	25.61	26.23	14.00	56.00
LTP (cm)	34.48	30.41	10.00	59.00
L (cm)	9.87	18.39	5.00	16.30
A (cm)	8.07	17.49	3.40	12.80
LxA (cm <sup>2</sup> )	80.91	34.95	5.00	185.82
DMF	64.90	6.86	59.00	77.00
DC	84.16	4.65	77.00	92.00
PS (g)	32.75	42.12	17.90	61.79

**Table 4.** Genotypic correlation coefficient (Pearson) for the ten quantitative traits evaluated in *Phaseolus vulgaris* L. accessions

	DE	DA	DF	LTP	L	A	LxA	DMF	DC	PS
<b>DE</b>	1.000									
<b>DA</b>	0.176	1.000								
<b>DF</b>	-0.129	-0.184	1.000							
<b>LTP (cm)</b>	-0.179	-0.160	-0.130	1.000						
<b>L (cm)</b>	-0.180	-0.170	-0.010	0.406	1.000					
<b>A (cm)</b>	-0.131	0.044	-0.070	0.307	0.803	1.000				
<b>LxA (cm<sup>2</sup>)</b>	-0.182	-0.067	-0.032	0.371	0.924	0.911	1.000			
<b>DMF</b>	0.151	0.459	0.030	-0.297	-0.144	-0.007	-0.062	1.000		
<b>DC</b>	0.102	0.431	0.024	-0.382	-0.183	-0.035	-0.098	0.658	1.000	
<b>PS (g)</b>	-0.137	-0.678	-0.050	0.299	0.124	-0.160	-0.033	-0.649	-0.612	1.000

Days to emergence (DE), Days to anthesis (DA), Flowering duration (DF), Main stem length (LTP), Leaf length (L), Leaf width (A), Leaf area (LxA), Days to physiological maturity (DMF), Days to harvest (DC), 100-seed weight (PS) ( $P \leq 0.001$ )./ Days to emergence (DE), Days to anthesis (DA), Flowering duration (DF), Main stem length (LTP), Plant leaf length (L), Plant leaf width (A), Leaf area (LxA), Days to physiological maturity (DMF), Days to harvest (DC), Weight of 100 seeds (PS) ( $P \leq 0.001$ )

**Table 5.** Eigenvalues associated with the Principal Components, proportion of absolute and relative variance (%) referenced for the 10 quantitative descriptors evaluated in the 35 common bean accessions of the INIFAT Germplasm Bank

Lambda ( $\lambda$ )	Eigenvalues	Proportion of total variance explained	
		Absolute (%)	Accumulated (%)
1	3.988	39.9	39.9
2	2.395	23.9	63.8
3	1.371	13.7	77.5
4	0.752	7.5	85.1
5	0.556	5.6	90.6
6	0.415	4.1	94.8
7	0.274	2.7	97.5
8	0.178	1.8	99.3
9	0.043	0.4	99.7
10	0.028	0.3	100.0

**Table 6.** Eigenvectors of the first two principal components in the characterization of the 35 common bean accessions of the Germplasm Bank

Variables	Main Components	
	e1	e2
DE	-0.249	0.018
DA	-0.244	-0.389
DF	-0.060	0.083
LTP	0.384	-0.091
L	0.444	-0.236
A	0.352	-0.416
LxA	0.411	-0.337
DMF	-0.276	-0.365
DC	-0.297	-0.379
PS	0.271	0.463

**Table 7.** Correlation between the original variables and the first two principal components in the characterization of the 35 common bean accessions of the Germplasm Bank

Variables	Main Components	
	CP 1	CP 2
DE	-0.497	0.027
DA	-0.487	-0.602
DF	-0.120	0.128
LTP	0.767	-0.140
L	0.887	-0.365
A	0.703	-0.643
LxA	0.821	-0.521
DMF	-0.552	-0.564
DC	-0.592	-0.586
PS	0.542	0.717

Figure 1 graphs the above, with the details of accessions. The first component groups similar accessions in terms of plant architecture, being P-3309 very similar to P-3606; P-3293, P-3797, P-3794 and Caujerí 2170, also, show similarities in terms of architecture independently of grain color. The second component allows grouping varieties in terms of the character 100-seed mass (PS), the accessions with the largest kernel size were Variado Chileno, P-3337, P-3793, California, the breeding line known as Maní (all with brown or red kernels) and the accession P-3306, the only one with black kernel, but of large size.

Results in the literature suggest that the characterization of common bean germplasm requires to consider between 10 and 20 descriptors because the use of a higher number of descriptors is laborious, expensive and does not provide important information; however, the most informative and variable descriptors for some genotypes are not necessarily so for the rest of the accessions of *Phaseolus vulgaris* of the Germplasm Bank (16). In this sense, different studies show that the greatest variability in beans can be recorded between 1 and 4 components (16,17); however, in the case of the sample studied, two components were able to explain 64 % of the total variance.

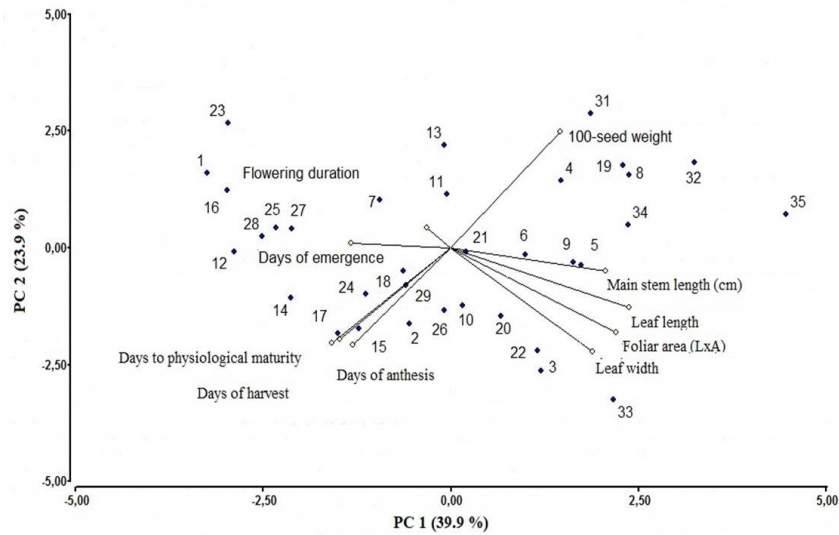
Traits such as DMF and DC allowed grouping some accessions (Figure 1). Of these, P-3785, P-3787, P-3790,

P-3613, P-3802 and P-3289 prevailed because they had the longest periods, so they could be considered long-cycle, a trait not appreciated by breeders and producers who prefer short-cycle varieties. As a result of the domestication process, a large number of varieties show differences in quantitative traits such as bean size, bean quality and vegetative growth periods in bean cultivation. Such variation has been extensively used in breeding programs or genetic diversity studies (2).

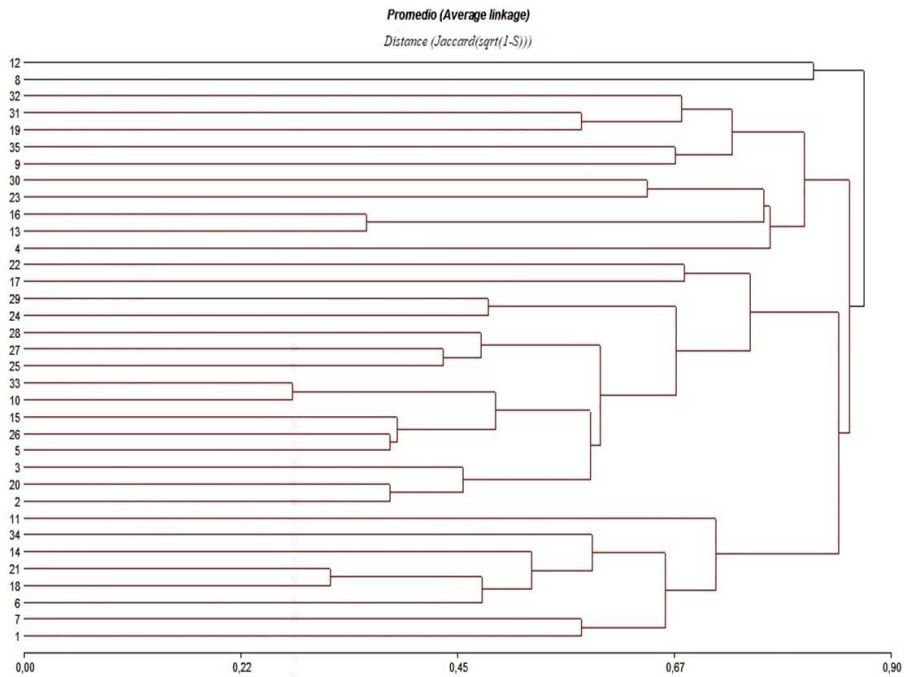
### Qualitative trait analysis

The qualitative polymorphic data allowed grouping the accessions into four groups (Figure 2). Group I consisted of two accessions with brown grains; group II consisted of 10 accessions with mostly red grains; group III consisted of 15 accessions, mainly represented by black grains; group IV consisted of eight accessions with a wide variety of colors (Figures 2 and 3). The coefficient of co-phenetic correlation was 0.877.

Thus, it is inferred that the type of grain plays an important role in the identification of genotypes. Precisely, traits related to grain color are considered highly heritable traits and, therefore, of importance for breeding programs (1). Seed color, shape and size are of particular interest to consumers. Black and red kernels are the most consumed



**Figure 1.** Distribution of the original accession variables on the first and second principal components (PC1 and PC2) in the characterization of the 35 common bean accessions



**Figure 2.** Dendrogram showing the differences according to the analysis of qualitative variables evaluated in the 35 common bean accessions

by the Cuban population, despite the wide range of colors, also the predominant appearance of the testa can be correlated by the Cuban population with cooking times or the hardness of the kernel. Shiny kernels generally present a cover that absorbs water more slowly and, therefore, require longer cooking times than opaque kernels (18).

Growth habit (Hab) is another factor of importance to breeders. Plants with a more compact architecture are used in crosses for the pursuit of upright position and earliness, although this type of genotype exhibits lower yield potential compared to those of the prostrate type. Indeterminate

growth cultivars develop higher yields than defined growth cultivars, this is due to the progress of vegetative development through the production of new flower buds, which generate flowers. However, type I (determinate) and II (indeterminate) growth habits are ideal for mechanized harvesting (18).

Cluster analysis based on qualitative traits showed a significant number of similarities between some accessions, this is the case of genotypes 33 and 10, 21 and 18, 26 and 5, and 20 and 2 (Figure 2). Although some of these accessions were collected in different places (Table 1), it



**Figure 3.** Bean characteristics of the 35 common bean accessions, represented by equal numbers in Figure 2

could be thought that they constitute duplicates or that their presence in different parts of the island denotes a continuous flow of exchange of materials. However, other types of analysis, preferably molecular, are recommended to determine duplicates with greater precision (11). The presence of duplicates in *Phaseolus vulgaris* collections from different genebanks has been frequently described in the literature (1,19,20).

Related to the origin, purple colors in the grain are typical of the Andean flow, as well as large seeds (> 40 g 100 seeds<sup>-1</sup>); while pink, brown, and black colors are predominantly associated with the Mesoamerican flow together with small grains (< 40 g 100 seeds<sup>-1</sup>) (4,8,21). Beige, yellow, and red colors in grains have been identified for both flows (2,18). In this study, it was not possible to distinguish the evolutionary origin of the accessions with the selected qualitative characters.

## CONCLUSIONS

The results of this study show that the common bean collection held in the INIFAT Germplasm Bank includes a wide variability of genotypes in terms of agro-morphological traits. Characters of interest such as short growing cycles showed less variation than the rest and coincided with the accessions of larger bean size. The greatest variation detected was explained by variables related to plant architecture and 100-seed mass. Grain characteristics allowed grouping individuals, independently of the origin of the collection. Several genotypes evaluated show potential as basic breeding lines.

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