

## MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF COMMON BEAN LANDRACES CULTIVATED IN THE SEMI-ARID MEXICAN HIGH PLATEAU

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**Summary:** The objective of the current research was to characterize the common bean in agricultural fields planted with common bean landraces from the semi-arid Mexican high plateau using both morphological and amplified fragment length polymorphism (AFLPs) data. The morphological traits were discriminating and exhibited the clustering of 150 accessions based on the geographic origin and seed coat color. AFLP primer combinations exhibited a polymorphic range between 0.292 (E-AGG + M-ACT) and 0.375 (E-ACA + M-AGA). The frequency and distribution of the polymorphic fragments allowed the detection of a larger number of rare fragments in accessions 121 and 111 (Flor de Mayo and black common beans, respectively). The analysis of genetic relationships, analysis of molecular variance (AMOVA), and Powell's diversity index confirmed a broad genetic basis for the germplasm of the common bean from the semi-arid Mexican high plateau. The clustering and principal coordinate analyses demonstrated a strong genetic relationship among the collected common bean landraces based on the similarity in the variety name, origin, and seed coat color, demonstrating the influence of different cultivation practices in the two regions and the adaptation of *P. vulgaris* to the agroclimatic conditions of the semi-arid Mexican high plateau.

**Key words:** AFLP, Genetic variation, Landraces, *Phaseolus vulgaris* L.

**Resumen:** Caracterización morfológica y molecular de las variedades locales de frijol común cultivado en el altiplano mexicano semi-árido. El objetivo de este trabajo fue caracterizar el frijol común de parcelas agrícolas cultivadas con variedades criollas de frijol común de la región semiárida del altiplano mexicano utilizando datos morfológicos y de Polimorfismos en la Longitud de los Fragmentos Amplificados (AFLPs). Las características morfológicas fueron discriminantes y mostraron la agrupación de las 150 accesiones en función del origen geográfico y el color de la testa de la semilla. La combinación de iniciadores AFLP (amplified fragment length polymorphism) mostraron un rango polimórfico entre 0,292 (EM-ACT AGG +) y 0,375 (M + E-ACA-AGA). La frecuencia y distribución de los fragmentos polimórficos permitió la detección de un mayor número de fragmentos raros en las accesiones 121 y 111 (Flor de Mayo y frijol común Negro, respectivamente). El análisis de las relaciones genéticas, varianza molecular (AMOVA), y el índice de diversidad de Powell confirmaron amplia base genética del germoplasma de frijol común del altiplano semi-árido mexicano. El análisis de coordenadas principales y de agrupación demostraron fuerte relación genética entre las variedades colectadas con base a la similitud en el nombre de la variedad, el origen y el color de la testa de la semilla, lo que demuestra la influencia de las diferentes prácticas de cultivo en las dos regiones y la adaptación de *P. vulgaris* a las condiciones agroclimáticas de la región semiárida del altiplano mexicano.

**Palabras clave:** AFLP, criollos, *Phaseolus vulgaris* L., variación genética.

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## INTRODUCTION

The common bean (*Phaseolus vulgaris* L.) is an ancient crop that originated on the American continent. The origin of the common bean is corroborated by the cultivation methods implemented by a wide variety of settlers and farmers, the uses of the crop, and the range of environments to which the common bean has adapted (Broughton *et al.*, 2003). The common bean has been growing in Mexico since the pre-Hispanic period, and Mexico is known as the main center of domestication for the common bean when regarding the Mesoamerican genetic pool of this species, which exhibits a high genetic diversity (Gepts & Debouck, 1991), and as a center of origin for the common bean (Bitocchi *et al.*, 2011).

The genetic diversity of the common bean in Mexico is safeguarded at the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP, Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias). INIFAP has used this diversity to generate improved varieties using cultivated strains, landraces, and wild samples as progenitors (Acosta *et al.*, 1999). This practice has led to the development of new and improved commercial classes of common beans, such as the Azufrado and Peruano varieties (Rosales-Serna *et al.*, 2004).

In rural areas of Mexico, the common bean landraces are still cultivated and are valued only in local markets. These landrace germplasms of common bean are classified by the farmer based on the seed color and flavor and are identified using specific names. It is common for commercial common bean varieties grown in Mexico to be identified by the specific names of their landrace ancestors because the systematic identification of these varieties using specific names is an important aspect of the management of these commercial varieties within local agricultural systems. It is not clear if these varieties maintain their genetic identity, if the genetic variation is influenced by the distribution patterns, or if the names associated with specific phenotypes can be used to evaluate the genetic diversity of the common beans available in a particular region.

Until now, all research conducted in the semi-arid Mexican high plateau based on common bean genetic resources has been based on morphological

and phenological characterization (Rosales-Serna *et al.*, 2003), and the phenotypic evaluation has demonstrated high value. Molecular techniques, such as amplified fragment length polymorphism (AFLP) marker technology (Vos *et al.*, 1995), have been effective tools for studying the genetic relationships among common bean germplasms (Rosales-Serna *et al.*, 2005; Kumar *et al.*, 2008; Persegui *et al.*, 2011) and are being used extensively for studies on plant genetic diversity (Tatikonda *et al.*, 2009; Pecina-Quintero *et al.*, 2011; Boczkowska *et al.*, 2012). The objective of the current research was to investigate the status of common bean landrace genetic resources grown in agricultural parcels of the semi-arid Mexican high plateau based on morphological and AFLP data. This research was also motivated by the assumption that genetic diversity in common bean is prone to change; therefore, there is a possibility for a decrease or increase in the genetic diversity of common bean because of the constant movement of materials throughout Mexico.

## MATERIALS AND METHODS

### *Study area and collections*

The study area consisted of three states located in the semi-arid Mexican high plateau (Chihuahua, Durango, and Zacatecas) in the north of Mexico. For comparative purposes, landrace samples from the states of Hidalgo, Puebla, and Guanajuato were included. In 2006, 10 individual plants were collected from each of 12 agricultural parcels sown with common bean landraces in the states of Durango (4), Chihuahua (4), Zacatecas (4), Hidalgo (1), Puebla (1), and Guanajuato (1) (Table 1).

### *Morphological analysis*

In 2007, agronomic characterization was carried out following specific design protocols that were adopted from previously used field trial outlines; the data scoring protocol was adapted from the standard evaluation protocol of the Mexican National Seed Inspection and Certification Service (SNICS, Sistema Nacional de Inspección y Certificación de Semillas, 2001) for the common bean. Ten plants from each accession were planted in rows (5 m long) with three replications in the Campo Experimental Valle del Guadiana in Durango state, which belongs

**Table 1.** Relationship of common bean (*Phaseolus vulgaris* L.) landraces analyzed in the semi-arid Mexican high plateau.

State	Seed type	Locality	Collection number
Durango	Canario	Ricardo Flores Magon	1-10
	Canario	Antonio Amaro	11-20
	Black	Poanas la Joya	21-30
	Flor de Junio	Poanas la Joya	31-40
Chihuahua	Landrace Pinto	Lazaro Cardenas	41-50
	Ojo de Cabra	Lazaro Cardenas	51-60
	Landrace Pinto	Lazaro Cardenas	61-70
	Flor de Mayo Media Oreja	Ejido Benito Juarez	71-80
Zacatecas	Bayo	Rio Grande	81-90
	Flor de Junio	Rio Grande	91-100
	Black	González Ortega	101-110
	Black	Flores Garcia	111-120
Hidalgo <sup>†</sup>	Flor de Mayo	Alvaro Obregon	121-130
Guanajuato <sup>†</sup>	Flor de Mayo	San Luis de la Paz	131-140
Puebla <sup>†</sup>	Palacio	Puebla	141-150

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to the Mexican National Institute of Forestry, Agriculture, and Livestock Research (INIFAP).

In total, 51 qualitative and quantitative traits of the leaves, pod, seed, and architecture of each plant were measured, as was the reaction of the common bean plants to disease (Table 2). The traits evaluated in each accession were classified into the following five categories: 1) phenological traits, 2) plant architecture and yield components (International Board for Plant Genetic Resources, 1982), 3) color of the recently harvested dry seed (van Schoonhoven & Pastor-Corrales, 1987), 4) seed quality (van Schoonhoven & Pastor-Corrales, 1987), and 5) reaction to diseases that are common and cause destructive damage in the test site. These diseases included anthracnose disease [*Colletotrichum lindemuthianum* (Sacc. and Magn.) Scribner], halo blight [*Pseudomonas syringae* pv. *phaseolicola* (Burkholder)], common bacterial blight [*Xanthomonas campestris* pv. *phaseoli* (Smith) Dye], bean common mosaic virus, root rot (*Rhizoctonia solani* Kühn), and angular leaf spot [*Phaeoisariopsis griseola* (Sacc.) Ferr.]. The damage analysis was performed during the

reproductive phase (flowering of each accession) and in accordance with the standard grades provided by the International Center for Tropical Agriculture (CIAT, Centro Internacional de Agricultura Tropical) (van Schoonhoven & Pastor-Corrales, 1987), which includes nine destructive grades (1-9) in which 1 = no symptoms and 9 = greater than 75% of the plant exhibits disease symptoms. The values from 1 to 3, 4-6, and 7-9 are classified as indicating resistance reactions, intermediate reactions, and susceptibility to the pathogen, respectively.

Germplasm was classified by geographic origin (state) and commercial-seed class (color) (Table 1). In Mexico, there are approximately 16 commercial classes of common beans according to the seed color: red mottled (Cacahuatate); purple (Morado); black (Negro); marbling (Jaspeado); yellow (Amarillo); cream (Bayo); brown-striped (Ojo de Cabra); cream mottled (Pinto); white (Blanco); white mottled (Vaquita); light purple (Manzano); brown (Café); pink (Flor de Mayo); red (Rojo); gray (Gris); pink-striped (Flor de Junio) (Bellón *et al.*, 2009; Rosales-Serna *et al.*, 2004; SNICS, 2001; Voysest, 2000; Cárdenas, 1984).

**Table 2.** Morphological qualitative traits evaluated in the sample of semi-arid Mexican high plateau common bean (*Phaseolus vulgaris* L.) landraces.

Descriptors	Measurement scale
Immature seed color	White; Light green
Longitudinal seed section	Circular; Circular to elliptical; Elliptical; Kidney; Cubic; Truncated
Seed coat aspect	Shiny; Intermediate; Opaque
Seed color	Single color; Two or more
Main seed color	White; Gray; Yellow; Greenish; Beige; Brown; Pink; Red; Purple; Black; Cream; Other
Secondary seed colors	Absent; One; More than one
Main secondary seed color	White; Gray; Yellow; Greenish; Beige; Brown; Pink; Red; Purple; Black; Cream; Other
Seed color pattern	Uniform; Veined; Striped; Speckled; Stippled
Secondary color distribution	Around the hilum; In stripes; Half the seed; Variegated
Seed veins in dry state	Weak; Medium; Strong
Presence of hilum	Absent; Present
Hilum color	White; Yellow; Beige; Brown; Pink; Red; Purple; Black; Absent
Hypocotyl	Absent; Present; Intermediate
Climbing start	Early; Intermediate; Delayed
Seed prominence	Absent; Light; Medium; Pronounced; Very pronounced
Seed coating during filling period	Absent or very weak; Weak; Medium; Strong; Very strong
Seed coating in dry stage	Weak; Medium; Strong; Very strong
Pod fibers	Absent; Present
Pod surface texture	Soft; Medium rough; Rough
Pod distribution	Low; High; Dispersed
Transversal pod section	Flat; Pear; Elliptic to ovate; Cordate; Round; Eight-shaped
Surface color pattern	Uniform; Veined; Stained; Spotted; Other
Primary pod color	Yellow; Green; Red; Purple; Brown; Other
Pod pigmentation	Absent; Tenuous; Intermediate; Dense; Very dense
Pod pigmentation color	Red; Purple; Absent
Secondary pod color	Yellow; Green; Red; Purple; Brown; Other; colorless
Degree of pod curvature	Straight; Slightly curved; Curved; Recurved
Shape of pod apex	Straight; Slightly upwards; Slightly downward; Variable
Color intensity	Weak; Medium; Strong
Roughness	Smooth; Crumpled
Shape of terminal leaflet	Lanceolate; Deltoid; Cordate; Rhombic
Apex of terminal leaflet	Acute; Acuminate; Cuspidated; Obtuse
Base of terminal leaflet	Obtuse; Oblique; Cordate; Cuneate; Attenuated
Bracteole shape	Small; Medium; Large
Banner color	White; Cream; Pink; Violet; Purple; Lilac
Wing color	White; Cream; Pink; Violet; Purple; Lilac

With the existence of a physical support in the field experiment to allow the plants to climb and conform to a type IV (climbing) habit of growth, growth habit types were classified differently from those reported by Debouck & Hidalgo (1982): Ia, bushy determined, stem and branches erect and strong; Ib, bushy determined, weak stem and branches; IIa, erect indeterminate or undeveloped short guides; IIb, erect indeterminate guides, medium to long, non-climbing; IIIa, indeterminate prostrate, non-climbing short guides; IIIb, indeterminate prostrate long guides, climbers; IVa, climber indeterminate, pods distributed throughout the plant; IVb, climber indeterminate, pods concentrated at the top of the plant. Quantitative traits were measured as follows: The number of seeds per pod was scored as low ( $\leq 4$ ), medium (5-6), or high ( $\geq 7$ ); the flowering period was very early ( $<40$  days), early (40-45 days), mid (45-50 days), delayed (50-60 days), or very late ( $> 60$  days); the 100-seed weight was small ( $<25$  g), medium (25-40 g), or large ( $> 40$  g); plant height was low ( $<30$  cm), medium (30-50 cm), or high ( $> 50$  cm); pod length was very short ( $<4$  cm), short (4-7 cm), medium (7-10 cm), long (10-13 cm), or very long ( $> 13$  cm); pod width was small ( $<6$  mm), medium (6-10 mm), or large ( $> 19$  mm); and leaflet tip size was small ( $<5$  cm), medium (5-9 cm), or large ( $> 9$  cm). Intervals were used for quantitative traits to adjust the morphological characterization guidelines of the International Union for the Protection of New Varieties of Plants (UPOV) for application to *P. vulgaris*.

#### *AFLP analysis*

#### *Plant material*

All common bean landraces were selected to investigate variation within and between farmer's materials (Table 1). Five seedlings were obtained from each of the 10 individual plants from each of the 15 agricultural parcels and bulked together. Their DNA was extracted, resulting in 150 DNA samples in total.

#### *Molecular procedures*

To obtain young tissue from the accessions, the seeds were germinated in trays using a sterile substrate. Two weeks after emergence, the samples were collected. The total genomic DNA was isolated using the method described by Dellaporta

*et al.* (1983). The analysis of the polymorphisms in the length of the amplified fragments was performed as described by Vos *et al.* (1995). First, the genomic DNA was digested using 5 U *EcoRI* and *MseI* (Roche®) at 37 °C for 3 hours. Next, the digested DNA samples were incubated at 70 °C for 15 minutes to deactivate the restriction enzyme. Finally, the adapters [5 pmol *EcoRI* and 50 pmol *MseI*] were added to the digested DNA fragments along with the ligation buffer (1x T4 DNA ligase) and 1 U T4 DNA ligase and incubated at 37 °C overnight. The AFLP loci were amplified using four primer pair combinations (E-AGG + M-ACT, E-ACT + M-CTA, E-ACA + M-AGA, and E-ACC + M-AGA). The selective pre-amplification was performed using complementary primers for the *EcoRI* and *MseI* adapters, using two selective nucleotide bases (adenine and cytosine). The PCR was performed in a Px2 thermocycler (Thermo Electron Corporation, Milford, MA, USA). The selective amplification was performed using *EcoRI* and *MseI* primers labeled with fluorescent dye (IRD700 and IRD800) and three selective nucleotides. Each of the PCR products was electrophoresed on denaturing polyacrylamide gels (6 %). The PCR products were separated in a sequencing system (Li-COR IR<sup>2</sup>; LI-COR, Inc. Lincoln, NE, USA) equipped with an infrared laser with the capacity to read wavelengths between 700 and 800 nm. The profile of the obtained fragments was analyzed using Cross Checker V.2.9 software (Buntjer, 1999).

#### *Statistical analysis*

#### *Morphological data*

To visualize the existing relationships between the accessions of the common bean from the semi-arid Mexican high plateau and the external sample groups, the morphoagronomic data were used to create a matrix of quantitative and standardized qualitative data. This matrix was used to build a dendrogram based on the similarity coefficient for multistate variables described by Rogers & Tanimoto (1960) and the unweighted pair group method with arithmetic mean algorithm. To corroborate the interrelationships between the common bean samples, a tridimensional graphical representation was built using principal coordinate analysis (PCoA) with the similarity coefficient of

Rogers & Tanimoto (1960). The analyses were performed using NTSYSpc statistical software V2.2 (Rohlf, 2009).

#### *AFLP data*

To determine the most informative combination of AFLP primers, parameters such as the polymorphic information content (PIC), marker index (MI), and resolution power (RP) were analyzed (Laurentin & Karlovsky, 2007). The PIC value for each AFLP primer pair was calculated as described by Roldan *et al.* (2000);  $PIC = 2f_i(1 - f_i)$ , where PIC is the polymorphic information content of marker  $i$ ,  $f_i$  is the frequency of markers present, and  $1 - f_i$  is the frequency of absent markers. The MI was calculated using the formula from Varshney *et al.* (2007):  $MI = PIC \times EMR$ , where EMR is the effective multiplex ratio ( $E$ ), defined as the total number of loci products/number of fragments per primer ( $n$ ) multiplied by the fraction of polymorphic loci/number of fragments ( $\beta$ ) ( $E = n\beta$ ). The RP of each primer was calculated according to Prevost and Wilkinson (Prevost & Wilkinson, 1999) as follows:  $RP = \sum I_b$ , where  $I_b$  represents the informativity of the fragment. The  $I_b$  term contains values from 0 to 1 and is determined by the formula  $I_b = 1 - [2 \times |0.5 - p|]$ , where  $p$  is the proportion of the 150 samples contained the fragment.

#### *Diversity index*

The germplasm genetic diversity index was calculated using the formula described by Powell *et al.* (1996);  $DI = 1 - \sum P_i^2$ , where  $P_i$  is the frequency of the  $i^{\text{th}}$  allele, and each individual allele is considered a unique amplified fragment.

#### *Intra and Inter-cultivar Variation and Genetic Identification*

The common bean landraces were selected according to the seed coat color and agricultural parcel origin for the analysis of variation among and between the farmer samples (Table 1). A binary matrix was used to calculate the molecular variation analysis (AMOVA) (Huff *et al.*, 1993) based on the following hierarchical ordering: groups (Chihuahua, Durango, Zacatecas, Hidalgo, Guanajuato, and Puebla) and populations within groups (common bean types within states). The number of AMOVA significance test permutations

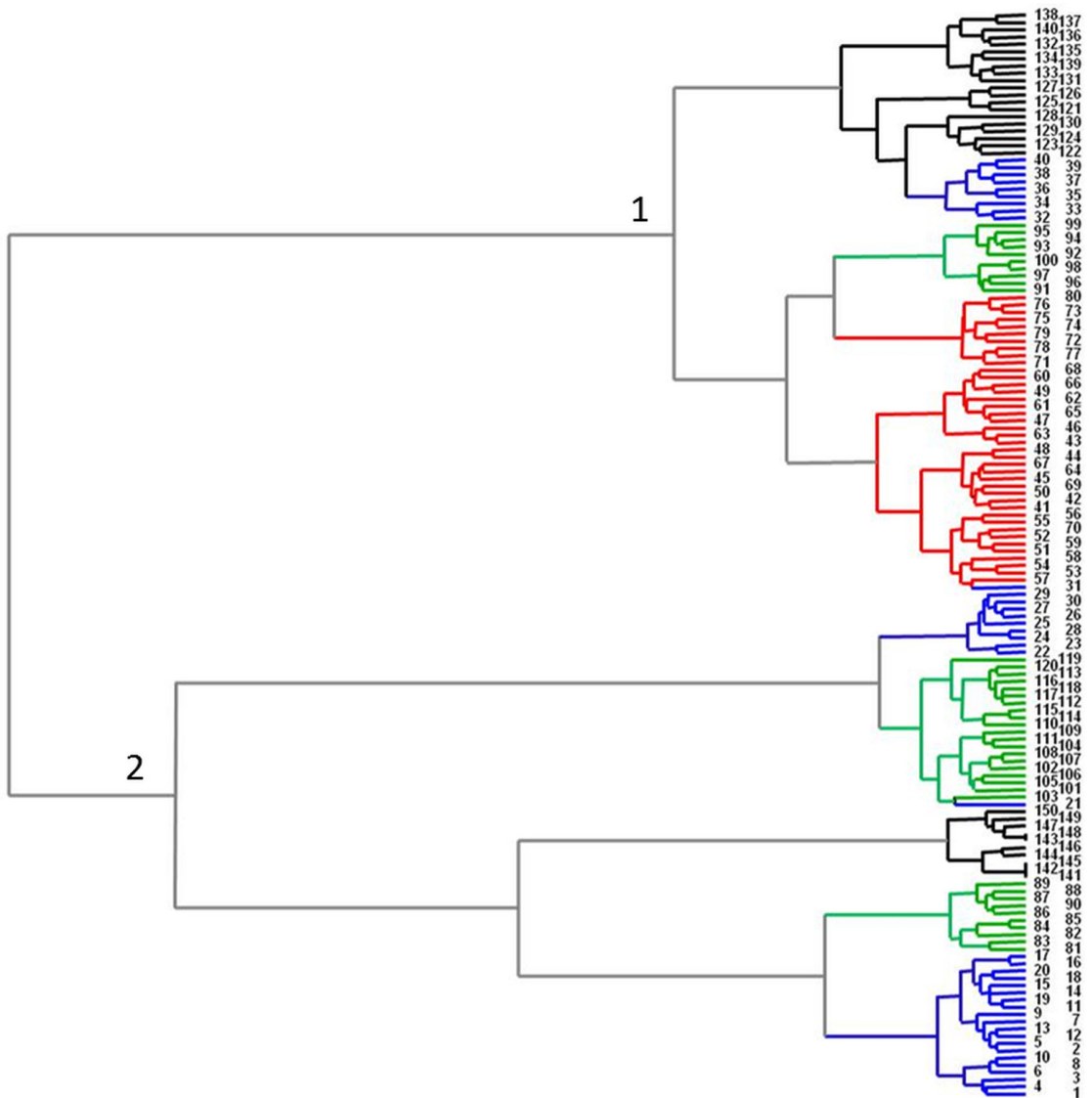
was 1,023 (Guo & Thompson, 1992). AMOVA was performed using the Arlequin statistical program V3.5 (Excoffier & Lischer, 2010). To determine existing genetic relationships between the 150 common bean samples analyzed, a binary matrix was created (where 1 denotes the presence and 0 the absence of a locus) for each pair of AFLP primers. A dendrogram was calculated using the Jaccard similarity coefficient (Nei & Li, 1979) and the weighted neighbor-joining algorithm and jackknife method for corroboration. This resampling method was applied to determine the robustness of the dendrogram. A resampling of 10,000 repetitions was performed based on the original data from the 150 common bean accessions. To corroborate the interrelations, a tridimensional graphical representation was generated, and PCooA was performed using the Jaccard similarity coefficient (Nei & Li, 1979). The clustering analysis was performed using the DARWIN V5 statistical software (Perrier & Jacquemoud, 2006), and the NTSYSpc statistical program V2.2 (Rohlf, 2009) was used for the PCooA.

## RESULTS

#### *Morphological traits*

The UPGMA clustering analysis of the common bean landraces from the semi-arid Mexican high plateau demonstrated two main germplasm groups (Fig. 1). Group 1 consisted of the Flor de Mayo (external group) common bean variety from the agricultural parcels in Hidalgo and Guanajuato states, Flor de Mayo Media Oreja from the parcels located in Chihuahua, Flor de Junio common bean variety from the parcels in Zacatecas, and cream-colored common beans with brown speckles (Pinto and Ojo de Cabra common bean landraces) collected in the agricultural parcels in Chihuahua. The second group consisted of black common beans from the parcels in Durango and Zacatecas, Bayo variety common beans from Zacatecas, Canario variety common beans from the two parcels in Durango, and Palacio variety common beans from Puebla state.

The germplasm was represented by a scatter plot using the results of the PCooA. The following three principal groups were observed (Fig. 2):



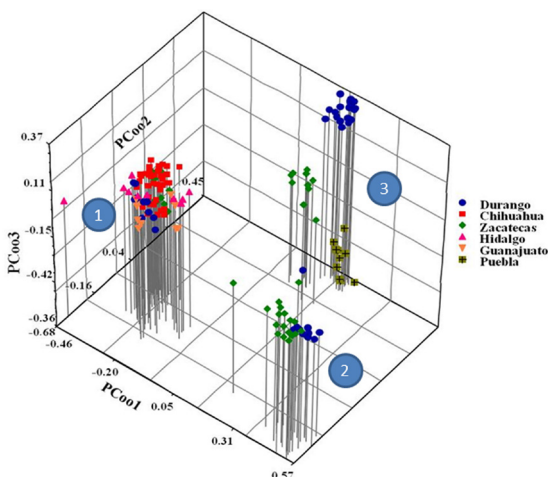
**Fig. 1.** Graphical representation of genetic similarities based on the Roger and Tanimoto similarity coefficient for the germplasm of the common bean in from the semi-arid Mexican high plateau, based on 51 morpho-agronomic traits. The numbers 1 and 2 refer to the clusters discussed in the text.

Group 1 included pink and cream common beans from parcels located in Chihuahua [Pinto (two parcels), Ojo de Cabra, and Flor de Mayo Media Oreja] and Durango (Flor de Junio), and Flor de Mayo from agricultural parcels in Hidalgo and Guanajuato. Group 2 consisted of only black common beans from Durango and Zacatecas (two parcels). Group 3 included Canario common beans from Durango (two parcels), Bayo common

beans from Zacatecas, and Palacio common bean landraces from Puebla.

#### *AFLP analysis*

In this study, 4 different AFLP primer pairs were used, and each AFLP primer pair generated information on the 120 common bean landrace accessions collected from the different agricultural parcels located in the semi-arid Mexican high



**Fig. 2.** Scatter plot representation of common bean germplasm from the semi-arid Mexican high plateau analyzed based on PCoA and 51 morpho-agronomic traits. The blue shape contains material from Durango state; red, from Chihuahua state; green, from Zacatecas state; pink, from Hidalgo state; melon, from Guanajuato state; and pistachio-green plus cross, from Puebla state. The numbers 1, 2 and 3 refer to the clusters discussed in the text.

plateau and the 30 landrace accessions collected from parcels located in the states of central Mexico. The number of products amplified per combination varied from 61 to 130. In total, the 4 combinations of selected primers amplified 406 products, of which 382 (94 %) were polymorphic.

The 382 polymorphic fragments were classified as unique and rare fragments. Unique fragments are specific to an accession by AFLP combination, and five unique fragments were observed. The accessions with identification numbers 55 and 151 for Ojo de Cabra and Palacio from Chihuahua and Puebla, respectively, demonstrated two unique alleles in different AFLP combinations (E-ACA + M-GTA and E-ACC + M-AGA). The number of rare fragments was determined and defined as the fragments present in only 10 % of accessions via AFLP combination. In total, 50 fragments were observed in 150 common bean landrace samples, with an average of 12.5 per AFLP combination. A larger number of rare fragments (22) were observed in the E-AGG + M-ACT combination, whereas the E-ACA + M-AGA combination demonstrated only fragments. Of the

150 common bean landrace accessions analyzed, the Flor de Mayo accessions 121 and 128 from Hidalgo, and the black common bean accession 111 from Zacatecas had the largest number of rare fragments.

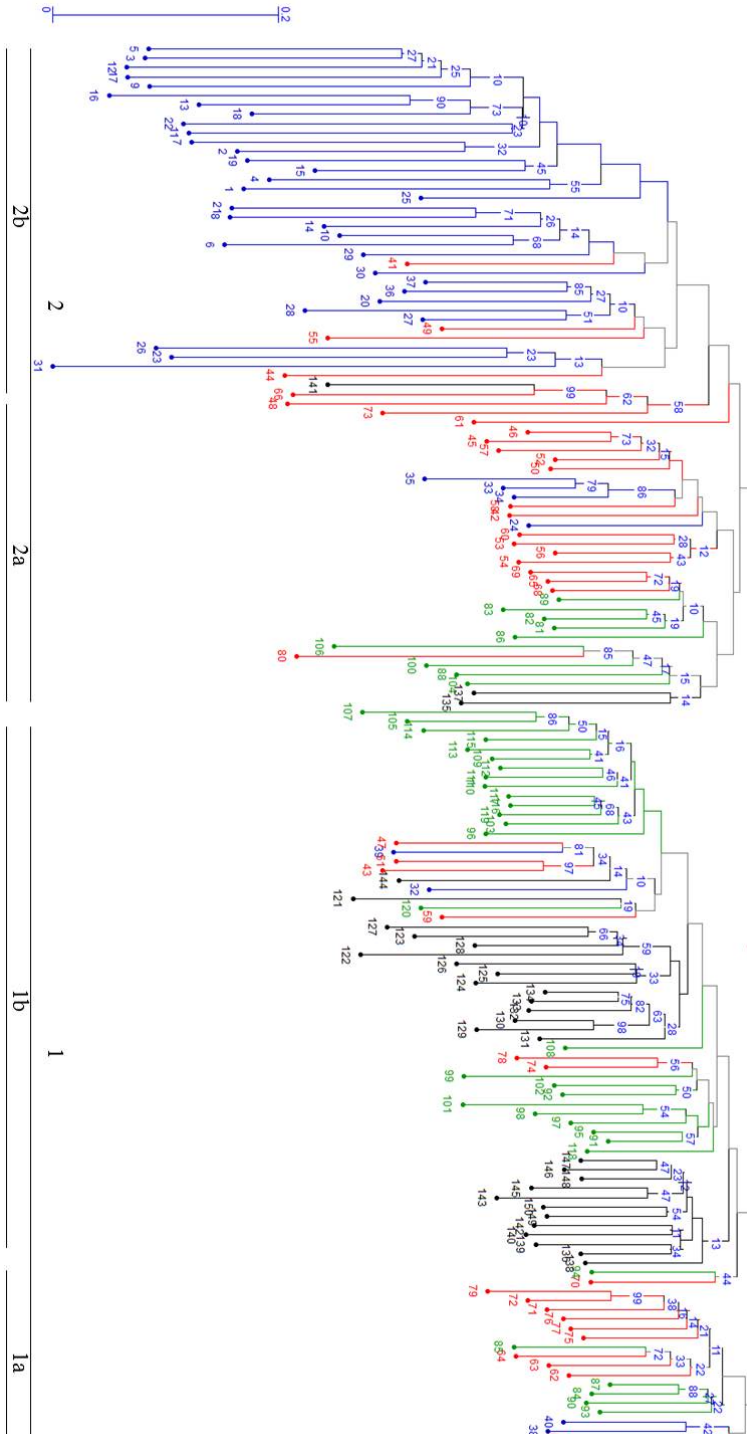
To identify the most informative AFLP primer combinations, the PIC was determined, exhibiting a range per combination between 0.292 (E-AGG + M-ACT) and 0.375 (E-ACA + M-AGA), with an average of 0.336 per AFLP combination. EMR ranged from 77 to 116, with an average of 95.3 per AFLP combination. The E-ACC + M-AGA and E-ACT + M-CTA combinations demonstrated the highest and lowest values, respectively. MI ranged from 28.56 to 36.37 with a median of 31.81 per combination. The E-ACC + M-AGA combination demonstrated the highest value and E-ACT + M-CTA the lowest value. To evaluate the discriminatory power between AFLP combinations, the RP was determined, which ranged from 41.3 to 57.2 with an average of 49.8. The E-ACA + M-AGA combination demonstrated the highest value and E-AGG + M-ACT the lowest value. The Powell genetic diversity index varied from 21% to 29% per combination with an average of 24%. The E-ACA + M-AGA combination demonstrated the highest value, and the E-ACC + M-AGA and E-AGG + M-ACT the lowest value.

#### *Intra- and Inter-cultivar Variation and Genetic Identification*

The AMOVA of the AFLP data demonstrated that 71.2% of the genetic variation occurred within populations (accessions), 17% occurred between populations within regions, and 11.7% between regions. Therefore, the highest proportion of genetic variation was demonstrated within populations and not between regions. The dendrogram (Fig. 3) shows the formation of two principal accession groups. Although there was a tendency to form groups by geographic origin or bean type (seed coat color), the dendrogram consisted of groups of different regions, which suggests a broad genetic base for the common bean in the semi-arid Mexican high plateau.

Group 1 was the most heterogeneous and was subdivided into two subgroups (1a and 1b). Subgroup 1a was made up of 17 accessions: Pinto variety common bean landraces (accessions 62, 63, 64, and 67) from Lázaro Cárdenas; Flor de Mayo





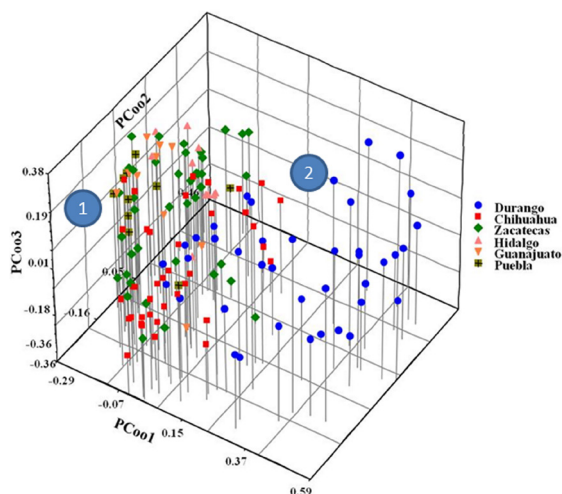
**Fig. 3.** Graphical representation of genetic similarities of common bean germplasm from the semi-arid Mexican high plateau based on the Jaccard similarity coefficient and AFLP markers. The values in the nodes indicate bootstrap values after 10,000 repetitions. The numbers 1, 2 and 1a, 1b and 2a, 2b refer to the clusters and subclusters discussed in the text.

Media Oreja common beans (accessions 71, 72, 75, 76, 77, and 79) from Benito Juárez, Chihuahua; and Bayo common beans (accessions 84, 85, 87, and 90) from Rio Grande, Zacatecas. Subgroup 1b had the highest number of accessions (62 in total). This subgroup combined common beans from the external collections from central Mexico, such as Flor de Mayo and Palacio (from parcels in Guanajuato, Puebla, and Hidalgo) and common beans from Zacatecas (4 locations; Bayo, black, and Flor de Junio common beans). This subgroup also included accessions from Lázaro Cárdenas (Pinto common bean landrace accessions 43 and 47 and Ojo de Cabra common bean accessions 57 and 59); Namiquipa, Chihuahua (Flor de Mayo Media Oreja common bean accessions 74 and 78); and Poanas la Joya, Durango (Flor de Junio common bean accessions 32 and 39).

Group 2 was more homogenous and comprised two subgroups (subgroup 2a and 2b). Subgroup 2a was formed by 30 accessions, mostly comprising Pinto variety common beans from the Lázaro Cárdenas community in Chihuahua; Bayo and Negro variety common beans from Rio Grande and González Ortega in Zacatecas, respectively; Flor de Junio and Negro variety common beans from Poanas la Joya in Durango; and two accessions (135 and 137) from an external group of samples of Flor de Mayo variety common beans from San Luis de la Paz in Guanajuato. Subgroup 2b contained the largest number of samples (accession 41), mostly Canario, black, and Flor de Junio common bean landraces collected from agricultural parcels in Durango (Ricardo Flores Magon, Antonio Amaro, and Poanas la Joya), Pinto and Flor de Mayo Media Oreja samples from Lázaro Cárdenas and Namiquipa in Chihuahua, and accession no. 141 Palacio bean variety from Puebla. The clustering analysis results were confirmed using PCoOA analysis as shown in Figure 4, which demonstrates that 71% of the genotypic variation was explained by the spatial separation of the common bean samples.

## DISCUSSION

For most characters, great diversity was found among the accessions. This diversity is largely continuous, with a clear separation in the three-



**Fig. 4.** Scatter plot representation common bean germplasm from the semi-arid Mexican high plateau analyzed based on PCoOA and AFLP markers. The blue shape contains material from Durango state; red, from Chihuahua state; green, from Zacatecas state; pink, from Hidalgo state; melon, from Guanajuato state; and pistachio-green plus cross, from Puebla state. The numbers 1, 2 refer to the clusters discussed in the text.

dimensional and dendrogram representation (Figs. 1 and 2). The growth habit trait showed the greatest number of classes in the architecture of the plant and was predominantly indeterminate, with non-climbing shoots. These results agree with those obtained in similar studies by Rosales-Serna *et al.* (2001) and Garcia *et al.* (1997). The primary pod color was yellow in all of the accessions, but the secondary pod was green, red, or colorless. The predominant seed types were Flor de Mayo, Pinto and black, with differing intensities (Table 2). These are the preferred colors of common beans by farmers and consumers in Mexico (Bellon *et al.*, 2009). Most black bean accessions came from the center region of Mexico. This fact is influenced by the preference for black common beans in this region. In a recent study, Andean seed colors were distributed as 23% cream, 17% black, 11% yellow, and 49% other (Blair *et al.*, 2009). As expected, all accessions with purple flowers had black seeds, in accordance with Bassett (1995), who reported that purple and pink flowers confer black seed coats. Many farmers select seeds for sowing based on the

seed size and color (Papa & Gepts, 2003).

This study adds new insights into the picture of diversity recently drawn for the semi-arid Mexican high plateau common bean landraces (Beebe *et al.*, 2000). First, the sample of common bean landraces presented significant diversity in its morphology, with very diverse seed colors, architecture, phenology, flowers and leaves. However, not all morphologies reported by IBPGR (1982) for the species had a wide variety of classes in this study; for example, the growth habit was only present in five of eight possible classes in this sample. These results are in agreement with a previous study performed in the same region with landrace samples (Rosales-Serna *et al.*, 2001; Beebe *et al.*, 2000).

Second, interesting traits were identified related to the nomenclature for common bean landraces in the semi-arid Mexican high plateau. Among the accessions of common bean landraces with shared common names in the present study, duplicates were only observed in outside reference accessions, for example, accessions 143 and 148 and accessions 141, 142, and 145 of Palacio common beans of Puebla (Fig. 1 and Table 1). Most of the accessions within the same varietal class (as indicated by a shared name) presented clear morphological, agronomic, and/or genetic differences, for example, in flower color (wing and standard), seed color, determinacy, and disease resistance. Vargas-Vazquez *et al.* (2006) also evaluated the morphological diversity among accessions in a common bean gene bank from INIFAP and observed that within the same varietal classes, there were no duplicates. Despite the fact that some diversification may have occurred within the same landrace type over a long period of cultivation, there is also the aspect of uncertainty of landrace names chosen by small farmers.

Third, the morphological diversity of the semi-arid Mexican high plateau group was either equal to or slightly higher than the diversity in outgroups materials, although the second was less numerous. With regard to agronomic traits, both groups in our sample of landraces also showed clear differences in their susceptibility and resistance to important common bean diseases. For example, both groups presented a low proportion of accessions susceptible to anthracnose, halo blight, root rot, and bean common mosaic virus resistance evaluated in field conditions. For angular leaf spot and common bacterial blight resistance, the semi-arid Mexican

high plateau group (common bean Canario, Flor de Junio and Flor de Mayo types) included fewer resistance accessions than the out-group's materials (common beans Flor de Mayo and Palacio types), but both groups included greater amounts of materials with intermediate values of resistance to both pathogens.

The above observations indicate that the landrace samples in the northern region of the country were introduced from the central Mexican states and adapted to the environmental conditions of the semi-arid high plateau, which emphasizes the domestication of the Mexican common bean and the distribution of the species by the farmers and breeders. Similarly, as shown by Lopez *et al.* (2005), landraces and pre-improved forms of *P. vulgaris* demonstrate a lower range of environmental adaptation than the domesticated varieties managed by farmers and breeders, which has modified important traits of the plant, such as biological cycle, reaction to diseases, and plant structure. These adaptations have led to the development of a greater diversity in the environments and local consumer preferences, allowing the consumption of Pinto, Bayo, Flor de Mayo, and Flor de Junio commercial varieties in the semi-arid Mexican high plateau and, to a lesser extent, the black and Ojo de Cabra varieties (Acosta *et al.*, 2002).

AFLP analysis allows for improved estimates of the genetic relationships between closely related individuals. In this study, the AFLP analysis detected high levels of polymorphism (94%) between 150 samples of common beans landraces. The analysis of the polymorphic fragment frequency and distribution demonstrated five specific fragments present in three accessions and 50 rare fragments present in up to 10% of the accessions. Genetic improvement programs for the common bean in Mexico often use landrace germplasm to obtain better results by contributing agronomically important and productive traits to existing commercial cultivars (Acosta *et al.*, 1996; Acosta *et al.*, 1999). Thus, this information may be useful for genetic improvement programs for common beans because it would aid the selection of materials that would increase variability or take advantage of heterosis (Acosta *et al.*, 1999). A potential use for the analysis of the intra-population genetic variation of common beans using AFLPs is that the AFLP combinations demonstrating

a higher number of rare and unique fragments (E-AGG + M-ACT and E-ACC + M-AGA) could be used to increase differences between accessions from the same populations. The highest number of rare and unique fragments were exhibited by the Pinto common beans (accession 55, Ojo de Cabra common bean landrace), Flor de Mayo (accessions 121 and 128), and black common beans (accession 111). The presence of these polymorphisms (unique and rare fragments) in one accession in particular would allow the development of specific sequences for the development of markers linked to quantitative trait loci of agronomic importance in *P. vulgaris*. For example, accession 55 (Ojo de Cabra common bean type) is highly resistant to anthracnose, halo blight, root rot, and bean common mosaic virus but is moderately tolerant to angular leaf spot and common blight, and it has a determinate growth habit and short production cycle (98 days to physiological maturity).

The PIC is used widely in genetic diversity studies. In the case of dominant markers (biallelic), such as AFLPs, the maximum expected value for the PIC is 0.50. In this study, the AFLP combinations demonstrated an average PIC value of 0.25. Based on the total PIC value (0.32), the use of the E-ACA + M-AGA combination is recommended for *P. vulgaris* landrace germplasm analysis because it differs from that reported by Rosales-Serna *et al.*, (2005). The obtained MI value corroborated the usefulness of the E-ACA + M-AGA AFLP combination and demonstrated that the E-ACC + M-AGA combination might be of use in common bean germplasm analysis because these combinations demonstrated MIs of 33.38 and 36.37, respectively. Whereas the PIC has been used more widely for studying organisms of economic interest (Varshney *et al.*, 2007; Gupta *et al.*, 2008). Prevost & Wilkinson (1999) implemented the concept RP for evaluating the discriminatory effect of AFLP combinations. Therefore, the E-ACT + M-CTA and E-ACA + M-AGA combinations with high RP values (56.7 and 57.2, respectively) are more informative for differentiating related accessions (Prevost & Wilkinson, 1999).

Compared with the observed genetic diversity in other regions of Mexico (Rosales-Serna *et al.*, 2005), the 150 landrace samples of common bean collected in the parcels from the semi-arid Mexican high plateau demonstrated medium to high levels of

genetic diversity. These data confirm the presence of high genetic diversity in the landraces common bean germplasm in semi-arid Mexican high plateau region (Gepts & Debouck, 1991). Farmers from the semi-arid Mexican high plateau often grow different common bean types, which include the three or four *P. vulgaris* races (e.g., the Durango, Jalisco, Mesoamerican, and New Granada races) found in the region. These small-scale growers use plant seeds of two or more common bean landraces that have been carefully selected, sometimes mixing different seeds for cultivation (Delgado-Salinas *et al.*, 2006). The type of cultivation practiced in the area is used mainly to mitigate the adverse effects of drought in the area, to increase harvest security (Beebe *et al.*, 2000), and to improve or ensure the simultaneous adaptability of different pre-improved and improved materials to the semi-arid high plateau agro-ecosystem for sustainable common bean production (Bellon *et al.*, 2009). These practices also improve disease management, especially when different levels of resistance are present in different cultivated common bean varieties (Cárdenas *et al.*, 1996).

Whereas the common bean is predominantly an autogamous species, none of the accessions analyzed using AFLPs were identical (Fig. 3). The causes of the intra-variety variation in the landrace cultivated by the farmers possibly include a cross between common bean types from the same breed or common bean variety; a cross between different common bean breeds, which generated the existing genetic diversity in the semi-arid Mexican high plateau region; or spontaneous mutations (Ko *et al.*, 1994). The genetic relationship between the common bean type grown by farmers of the different localities and states provides new information on the practices of variety identification by the farmers. A number of common bean types with the same name were grouped genetically, including those collected in different localities. Therefore, the traditional names of the common bean landrace types in the semi-arid Mexican high plateau might be a good indicator of the state of the genetic diversity in the landrace common bean in the northern region of the country. This hypothesis is consistent with a report by Appa Rao *et al.* (2002), which demonstrated that the names of varieties offer clues to the genetic diversity of rice in specific regions.

The results of the AFLP analysis demonstrated the genetic similarity between the common bean landraces with names identical to those in other regions. Farmers in different regions exchange materials (Cárdenas, 2000). The genetic similarity of common bean landraces with similar names demonstrates the migration effects of germplasm, and the genetic behavior may be because of similar cultivation practices used by the farmers in the different regions analyzed. The main differences observed were in the planting systems, cultural practices (such as crop density), level of variety mixtures, and mixed cultivation techniques (Cárdenas, 2000). These differences in cultural practices have apparently influenced the development of the common bean landrace germplasm, even those germplasms coming from the same region that change their genetic profile after growth in separate areas. This observation is consistent with reports by Teshome *et al.* (1999), in which crop management practices were shown to change the morphological and genetic identity of sorghum. The clustering and PCoA analyses (Figs. 3 and 4) demonstrated the formation of two principle accession groups, including external evaluation materials. These data suggest an intra-cultivar genetic base for the common bean germplasm in the semi-arid high plateau of northern Mexico. This germplasm base contained materials that may have few, and likely unique, genetic changes. These changes may be the result of the evolution of *P. vulgaris* in various environments or via the adaptation by farmers and breeders to the environmental conditions of the northern Mexican dry lands (Blair *et al.*, 2011).

By integrating the morphological, agronomic, and molecular characterization into the diversity assessment of a Mexican germplasm collection of common beans from the semi-arid Mexican high plateau, we were able to improve our understanding of the organization of this diversity in this region of northern Mexico. The integration of these different types of data into this assessment also allowed for the identification of important differences among the two genetic groups analyzed, such as for agronomic and genetic traits. Our results emphasize the importance of such an integrated approach in the diversity assessment for the conservation of resources, as a clue to promote the use of genetic resources of farmers, such as landrace germplasm

collections. Finally, it is important to note that the two types of data exhibited interesting common bean landrace germplasms with the potential for use in the genetic improvement of the *P. vulgaris* in the northern region of Mexico.

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