

Collection and characterization of wild species of *Phaseolus* (Fabaceae) in northeastern Mexico for ex situ conservation

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Background and aims – The northeastern region of Mexico is recognized for its diversity of wild species of *Phaseolus* (Fabaceae). However, seed samples for species characterization and ex situ conservation are scarce. This study aimed to (1) determine the growing sites of wild species of *Phaseolus* in northeastern Mexico, (2) collect seed samples for ex situ conservation, and (3) characterize morphologically the species found in the region.

Methods – During 2010–2013, explorations were undertaken in sixteen municipalities in the states of Nuevo Leon and Tamaulipas. Each growing site was geo-referenced, and soil and vegetation characteristics were registered. Seed samples and wild bean plants at flowering stage were collected for taxonomic identification and conservation.

Key results – Eleven wild species were found: *Phaseolus neglectus* Herm., *P. leptostachyus* Benth. var. *leptostachyus*, *P. pedicellatus* Benth., *P. altimontanus* Freytag & Debouck, *P. albiflorus* Freytag & Debouck, *P. novoleonensis* Debouck, *P. maculatifolius* Freytag & Debouck, *P. zimapanensis* A. Delgado, *P. vulgaris* L., *P. coccineus* L. subsp. *coccineus* var. *splendens* Freytag, and *P. glabellus* Piper. *Phaseolus vulgaris* exhibited the greatest morphological variation.

Key words – Wild beans, wild bean distribution, wild bean seeds, wild bean morphology, Mexican beans.

INTRODUCTION

The genus *Phaseolus* includes 75 taxa, most of which are wild forms (Rodríguez Cabrera et al. 1987, Freytag & Debouck 2002, Estrada et al. 2004, Peña-Valdivia et al. 2012). The majority of known wild species of *Phaseolus* grow in Mexico (Delgado-Salinas 2012), which is considered as the main diversity center (Gepts et al. 1986, Gepts & Debouck 1991, Delgado-Salinas 2012). They are distributed mainly in the central region, in the Sierra Madre Occidental and the Eje Neovolcánico (from Jalisco state to México state), and also in the southern region (Chiapas state) (Freytag & Debouck 2002). In other regions, such as the Sierra Madre Oriental, the number of species is smaller, but not less important (Acosta-Gallegos 2012). The majority of these species are endemic (Delgado-Salinas 1985, Delgado-Salinas 2012, Porch et al. 2013).

In the second half of the last century, monumental efforts were devoted to the collection, conservation, and use of phylogenetic resources; consequently, the genetic variability of cultivated forms of dry beans is well represented in germplasm banks. However, this cannot be said of wild species, which represent less than ten percent of the total accessions (Cárdenas et al. 1996).

Northeastern Mexico, recognized for its diversity of wild *Phaseolus* species (Rodríguez Cabrera et al. 1987, Freytag & Debouck 2002), has been the focus of several botanical expeditions, during which wild bean plants were collected and identified (Rodríguez Cabrera et al. 1987, Freytag & Debouck 2002, Estrada et al. 2004). Just recently, *Phaseolus novoleonensis* was discovered and characterized as a new species (Salcedo et al. 2006). Nevertheless, according to information in the *Phaseolus* Germplasm Bank Catalog of the National Research Institute of Forestry, Agriculture,

and Livestock (INIFAP), the genetic bean bank contains seed samples of only a few wild *Phaseolus* species (Cárdenas et al. 1996) from among the fifteen species reported in the region (Rodríguez Cabrera et al. 1987, Freytag & Debouck 2002, Salcedo et al. 2006). Also in the seed bank of the National Service of Seed Inspection and Certification (SNICS), seed samples of only seven wild species have been reported from the state of Nuevo Leon (Acosta-Díaz et al. 2014), a factor which has limited the study of other species not available at the seed bank (Acosta et al. 1996). Other problems are the restricted access to seed banks that have seeds of some species and the difficulty in reaching some growing areas where there is a greater abundance of seeds (Acosta-Gallegos et al. 2007).

In view of this situation and the increasing reduction in genetic variability due to anthropocentric intervention, several researchers of INIFAP, with the economic support of the National System of Phytogenetic Resources for Food and Agriculture (SINAREFI) through SNICS, an agency of the Mexican Agricultural Ministry (SAGARPA), undertook the present study (1) to determine growing sites of wild species of *Phaseolus* in northeastern Mexico, (2) to collect seed samples of these species for their ex situ conservation, and (3) to characterize morphologically the species found in the region.

MATERIALS AND METHODS

The methodology was adapted from the guidelines of SINAREFI (2005) and from various studies (e.g. Jaramillo & Baena 2000, Gold et al. 2004, Villanueva-Ávalos et al. 2012).

Study area

Located in the extreme northeastern corner of the Mexican Republic, the study area lies between parallels 22°30' and 25°45'N latitude and 99°00' and 100°30'W longitude (fig. 1). The predominant climates are:

- (1) semi-warm, sub-humid with an annual mean temperature of > 18°C, temperature of < 18°C in the coldest month and > 22°C in the hottest month; an annual precipitation of 500–2500 mm, with 0–60 mm during the driest month and 5–10.2% of the annual rain during summer;
- (2) temperate, semi-humid with an annual mean temperature between 12 and 18°C, temperature of -3–+18 °C during the coldest month and < 22°C during the hottest month; an annual precipitation of 200–1800 mm, with 0–40 mm during the driest month and 5–10.2 % of the annual rain during summer (Köppen classification modified by García 1973).

Low hills (500–700 m a.s.l.) and mountain peaks (> 3000 m a.s.l.) are characteristic of the study area. The dominant vegetation is coniferous, oak and pine-oak forest, plains with grassland and desert scrubland (INEGI 2013). The majority of the soil types are lithosols, rendzinas, calcareic regosols and chromic vertisols (INIFAP-CONABIO 1995).

The study area includes the protected natural areas of Parque Nacional Cumbres de Monterrey in the state of

Nuevo Leon, and Altas Cumbres and the El Cielo Biosphere Reserve in Tamaulipas.

Preparations for field observation and species collection

Prior to the field trips, interviews with other researchers who have collected in the study area were arranged, and herbarium specimens of *Phaseolus* species were reviewed at two herbaria of the Universidad Autonoma de Nuevo Leon (UANL): the Faculty of Forest Sciences Herbarium (CFNL) and the Biological Science Faculty Herbarium (UNL). Information derived from herbarium specimens and provided by local in-

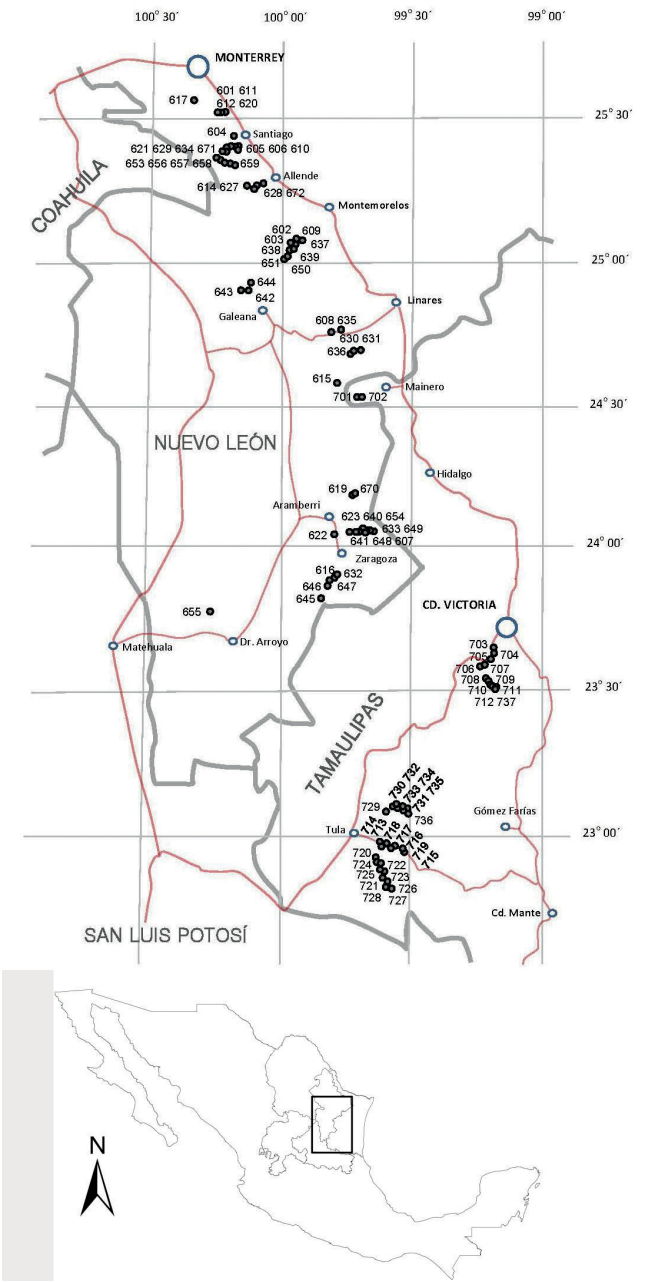


Figure 1 – Study area and numbers of seed sample accessions of wild bean species (*Phaseolus* spp.) collected during 2010–2013 in northeastern Mexico.

habitants was used to determine periods when the *Phaseolus* species had flowers/pods, considering that the phenology of a species varies from year to year due to climatic fluctuations (Gold et al. 2004). Potential distribution maps (climatic niche models) were reviewed for *P. neglectus*, *P. glabellus*, *P. zimapanensis*, *P. coccineus*, *P. leptostachyus*, *P. pedicellatus* and *P. vulgaris* (Ramírez-Villegas et al. 2010). Google Earth was also used to locate collection sites and to define areas and collection routes.

A literature review was made, considering inventories and collection reports at local and regional levels and published studies on the distribution and growing sites of collected *Phaseolus* species (e.g. Rodríguez Cabrera et al. 1987, Moreno-Limón 1993, Freytag & Debouck 2002, Estrada et al. 2004, Estrada 2007, García-Morales 2009, Velasco-Macias 2009, Salinas-Rodríguez 2012).

For the collection of seeds and plant specimens, permits were obtained from the federal and state governments.

Field trips

From 2010 to 2013, several field trips were undertaken to locate the growing sites of the wild species of *Phaseolus* in northeastern Mexico. Twenty-four routes (table 1) with transversal orientation to the Sierra Madre Oriental mountain range were defined. Eighteen of these routes extended across ten municipalities in the state of Nuevo Leon, while six covered four municipalities in the state of Tamaulipas. All these municipalities have a history of significant diversity of wild species of *Phaseolus*.

Each year, two field trips were undertaken: first, in August, September and October; and then, in November and December. During the first trip, growing sites were located when the different bean species were in the vegetative-reproductive stage. This permitted the observation of leaves, flowers and pods and the recording of traits that are characteristic and distinctive of each species. During these trips, the various wild bean species growing among weeds, shrubs, and trees were easily located, and they were identified by inspecting their flower structures. Plant samples, especially of the lesser known or recently identified species, were obtained and prepared for conservation in herbaria.

Collection and post-harvest management of seed samples

Seed samples were collected during the second expedition, when plants at previously identified sites were at physiological maturity, especially at sites having greater populations for each species. Through random sampling, at least 100 pods of various plants in close proximity in the collecting site were gathered before dehiscence.

Seed samples were treated with methyl bromide to protect them from insect damage. The seeds were dried to less than 15% humidity and then sealed in labeled glass containers. Duplicates of seed samples were sent to the seed germplasm banks at the Centro de Conservación Región Occidente of the Universidad de Guadalajara and the Centro de Conservación Región Norte of the Universidad Autónoma Agraria Antonio Narro for their ex situ conservation.

Collection data

Accession data were registered for identification in two separate series: the 600 series for the state of Nuevo Leon and the 700 series for the state of Tamaulipas. Each growing site was geo-referenced, and general information concerning the characteristics of the soil, vegetation and distinctive features of the plant was registered on a Passport Data sheet provided by the Bean Network of SINAREFI. The information may be requested from the Director of SINAREFI through the website www.sinarefi.org.mx. Digital pictures showing the main characteristics of the in-situ growing species were also taken (Guarino & Friis-Hansen 1995, Hawkes et al. 2000).

Species identification

Wild bean plants for the herbaria were collected at flowering stage for their taxonomic identification, based on published taxonomic criteria (Delgado-Salinas 1985, Freytag & Debouck 2002) and through direct identification of some plants by Dr. Alfonso Delgado-Salinas of the Biology Institute of UNAM (Universidad Autónoma de México). For their conservation, specimens were deposited in the herbaria of CFNL (UANL), MEXU (Biology Institute, UNAM) and IBUG (Instituto Botánica de la Universidad de Guadalajara) (table 1).

RESULTS AND DISCUSSION

Growing sites

Along the Santa Catarina–Puerto del Conejo Road (Huasteca Canyon) (altitude 600–2550 m a.s.l.), near Santa Catarina, Nuevo Leon (route 1), no growing sites were recorded, even though previous studies had mentioned the presence of at least *Phaseolus neglectus* and *P. leptostachyus* in the area (Rodríguez Cabrera et al. 1987, Estrada et al. 2004). Along this route, the predominant features included small hills and the Santa Catarina River, which had undeveloped riversides and no running water for most of the year, thus providing easy access for cattle. These circumstances could explain the results obtained along this route. Cattle grazing and low available soil moisture may have significantly reduced *Phaseolus* populations, making them difficult to find.

A total of 93 growing sites were recorded, each having one or more species. The same number of seed samples (93) was accessioned, representing eleven different species. Routes 5, 8, 12, 20, 21 and 22 showed great diversity and high frequency of species and large sizes of populations. Each of these routes had the presence of seven or more growing sites of wild species of *Phaseolus* (table 1).

Among species having the greatest presence and distribution in the northeastern region of Mexico were the following: *Phaseolus albiflorus*, with 25 sites; *P. leptostachyus*, with 21 sites; *P. pedicellatus*, with eleven sites; and *P. zimapanensis*, with ten sites. Also of considerable importance was the presence of *P. neglectus*, *P. coccineus* and *P. maculatifolius*, with eight, six and five sites, respectively. The presence of *P. vulgaris*, *P. novoleonensis* and *P. altimontanus* was relatively low, with three, two sites and one site, respectively.

The previously reported collection sites of *P. glabellus* in Xicotencatl and Aldama in Tamaulipas state (Freytag &

Table 1 – Routes for exploring growing sites of wild species of *Phaseolus* spp. in northeastern México during 2010–2013.

The 600 series correspond to the state of Nuevo Leon and the 700 series to the state of Tamaulipas. Superscripts indicate specimens deposited in the following herbaria: A: MEXU, B: CFNL, C: IBUG.

Route number	Accession number	Route description	Altitude (m a.s.l.)
1		Santa Catarina Road – “El Conejo” Port (“Huasteca Canyon”), Santa Catarina. Nuevo Leon	600 to 2550
2	617	San Pedro Garza Garcia Road – Chipinque Park, San Pedro Garza Garcia. Nuevo Leon.	1284
3	601, 611, 612, 620	Federal Highway 85 – “La Estanzuela” Park, Monterrey. Nuevo Leon.	665 to 895
4	604	Cieneguillas Road – San Francisco, Santiago. Nuevo Leon.	675
5	605, 606 ^B , 610, 621, 629, 634 ^B , 653 ^B , 671	Federal Highway 85 – Laguna de Sánchez, Santiago. Nuevo Leon.	800 to 1913
6	656, 657, 658, 659	Federal Highway 85 – Potrero Redondo, Santiago. Nuevo Leon.	846 to 1350
7	614, 627, 628, 672	Federal Highway 85 – La Trinidad, Montemorelos. Nuevo Leon.	878 to 1136
8	602 ^B , 603, 609, 637, 638, 639, 650, 651 ^B	Federal Highway 85 – Rayones, Rayones. Nuevo Leon.	1000 to 1291
9	630, 631, 636	State Highway 58 – Iturbide (“El Encino” Ranch), Linares. Nuevo Leon.	1200 to 1360
10	608 ^B , 635 ^B	State Highway 58 – Iturbide. Nuevo Leon.	800 to 900
11	619 ^B , 670 ^B	Aramberri Road – Lampacitos, Aramberri. Nuevo Leon.	1210
12	607, 623, 633, 640, 641, 648 ^B , 649, 654 ^B	Aramberri Road – Agua Fria, Aramberri. Nuevo Leon.	1450 to 2195
13	622	Aramberri Road – Zaragoza, Aramberri, Nuevo Leon.	1160
14	616, 632, 646, 647 ^B ,	Zaragoza Road – La Encantada, Zaragoza. Nuevo Leon.	2170 to 2700
15	655	Route 61 – Las Jarillas Community, Dr. Arroyo. Nuevo Leon.	1990
16	645 ^B	Route 61 – La Siberia, Dr. Arroyo.	2625
17	642, 643	18 de Marzo Community Road – Cerro del Potosí, Galeana. Nuevo Leon.	2337 to 2820
18	644 ^B	18 de Marzo Community Road – Las Placetas Community, Galeana. Nuevo Leon.	2277
19	615, 701, 702,	Villa Mainero Road, Minero, Tamaulipas – Camarones, Iturbide, Nuevo Leon.	1028 to 1270
20	713, 714 ^{ABC} , 715, 716, 717, 718, 719, 736 ^A	Route 66, Tula – Ocampo, Tula. Tamaulipas.	1342 to 1473
21	720, 721 ^{AB} , 722, 723, 724 ^{ABC} , 725, 726, 727, 728	Route 66 – Carrera Torres, Tula. Tamaulipas.	1097 to 1433
22	729, 730 ^{AB} , 731 ^{ABC} , 732 ^{ABC} , 733 ^A , 734, 735	Route 66 – Mesa de la Libertad Community, Tula. Tamaulipas.	1185 to 1685
23	703, 704 ^{ABC} , 705, 706 ^{ABC} , 707	San Luis Potosi Route 101 - Ciudad Victoria (Altas Cumbres) and Camino Real, Jaumave - Victoria. Tamaulipas.	861 to 1180
24	708, 709 ^A , 710, 711, 712, 737	San Luis Potosi Route 126 – El Huizachal Community, Victoria. Tamaulipas.	1005 to 1405

Debouck 2002) may have disappeared due to anthropogenic activities (CONABIO & SEMARNAT 2009), including the introduction of crops such as sugar cane and grasses. The species was not found either in Gomez Farias (Tamaulipas state), another previously reported site (Schmit et al. 1996, Freytag & Debouck 2002), although its continuing presence there is possible since the area is part of the El Cielo Biosphere Reserve. Freytag & Debouck (2002) mentioned that the habitat of *P. glabellus* seemed to be restricted to the east-

ern slope of the Sierra Madre Oriental in Tamaulipas; however, in the present study, the species was found on the western slope (fig. 1).

The discovery of wild *P. vulgaris* in the state of Tamaulipas was unexpected. There had been only one previous collection reported; it was in Sierra between Jaumave and Victoria on 14 Oct. 1931 by Von Rozynski (Freytag & Debouck 2002). This is only the second time that *P. vulgaris* is reported growing in Tamaulipas. The plants with accession num-

bers 725 and 726 grew on bushes, with an estimated stem length of three to five feet, while accession 724 grew to nine or ten feet on a tall tree. *Phaseolus vulgaris* and *P. glabellus* are apparently at the northern limit of their main distribution area (Freytag & Debouck 2002, Schmit et al. 1996), which explains the low presence observed in this study.

Specimens of the cultivated form of *P. coccineus* were collected at the limits of cropland and within native vegetation at Tula in Tamaulipas (accessions 718, 719, 727, 729, 730 and 731 in table 1). The collection sites were close to Cueva de Ocampo, where the oldest remains of cultivated *P. coccineus* had been found, dating between 5500 and 7000 B.C.E. (Kaplan & McNeish 1960 cited by Delgado Salinas et al. 1988). Local inhabitants reported that about thirty years ago, they regularly consumed this wild bean species, but now they consume it only when there is a shortage of food or money.

It is important to point out that there were no observed growing sites of *P. vulgaris*, *P. coccineus* and *P. glabellus* in the state of Nuevo Leon. Rodríguez Cabrera et al. (1987), Freytag & Debouck (2002) and Estrada et al. (2004) likewise did not mention the presence of these species in that Mexican state.

Reflecting the affinity between altitude and wild bean species in northeastern Mexico, *P. albiflorus* showed the widest altitudinal distribution, ranging from 675 m a.s.l. (route 4) to 2170 m a.s.l. (route 14). The distribution of *P. leptostachyus* ranged from 714 m a.s.l. (route 4) to 1990 m a.s.l. (route 12). Of singular importance is the fact that *P. vulgaris* was discovered in an area ranging from 1097 to 1234 m a.s.l.

along route 21 in Tula, Tamaulipas. This species displayed significant phenotypic variability in flower colour, size and diameter of pods, grains per pod, and size and colour of seeds (table 2), as similarly reported by Gepts & Debouck (1991) and Lépiz et al. (2004a, 2004b) for the western region of México.

Another species found across different altitudes in northeastern Mexico was *P. maculatifolius*, observed from 1005 m a.s.l. along route 24 (accession 709) in Victoria, Tamaulipas, to 1836 m a.s.l. along route 12 (accession 654) in the municipality of Aramberri, Nuevo Leon.

P. neglectus also showed wide altitudinal distribution. It was detected in low and moist regions within the state of Tamaulipas, ranging from 861 m a.s.l. (route 23, accession 703) to 1405 m a.s.l. (route 24, accession 711) in the municipality of Victoria.

P. pedicellatus was unique in showing broad altitudinal distribution at higher altitudes of the Sierra Madre Oriental. In the state of Nuevo Leon, it was found in areas ranging from 1990 to 2820 m a.s.l. (accessions 648 and 643). This species was found near hills in an oak and pine forest, in a half-shade humid environment.

Salcedo et al. (2006) reported *P. novoleonensis* as a new species, collected only in the state of Nuevo Leon. Plants of this species were collected in Iturbide, Nuevo Leon, in 1986 (Debouck & Muruaga 2061 and E. Estrada Castillon 633). It was also collected in 2005 by Dr. E. Estrada Castillón (Universidad Autónoma de Nuevo León, Mexico, pers. comm.), who mentioned an abundant population at the same site. In the present study, however, plants of this species were not

Table 2 – Morphological characteristics of eleven wild species of *Phaseolus* collected during 2010–2013 in northeastern Mexico.

Species	Growth habit	Flowering time	Flower colour	Seeds/pod	Weight of 100 seeds (g)	Seed colour
<i>P. albiflorus</i>	Climbing	01 Sep.–15 Nov.	White	5–8	4.1–5.3	Gray background with brown spots
<i>P. leptostachyus</i>	Prostrate	20 Apr.–30 Sep.	Light lilac	3–5	1.5–1.8	Gray background with black spots
<i>P. pedicellatus</i>	Climbing	01 May–30 Sep.	Light to dark lilac	3–4	7.2–8.5	Gray background with black spots
<i>P. novoleonensis</i>	Prostrate	01 May–22 Sep.	Dark lilac	3–4	9.8–10.9	Cream background with brown spots
<i>P. altimontanus</i>	Prostrate	01 Aug.–30 Sep.	White to light purple	4–5	4.4–4.5	Dark brown background with black spots
<i>P. maculatifolius</i>	Climbing	15 Sep.–15 Oct.	Dark lilac	4–6	7.5–8.3	Gray background with black and brown spots
<i>P. zimapanensis</i>	Climbing	10 Aug.–30 Sep.	Lilac	6–8	1.2–1.4	Dark gray background with black spots
<i>P. neglectus</i>	Climbing	1 Oct.–15 Nov.	White to light violet or light blue	5–7	3.6–3.9	Light gray background with brown spots
<i>P. coccineus</i>	Climbing	10 Sep.–15 Oct.	Red	5–7	12.5–14.2	Light gray background with brown spots
<i>P. vulgaris</i>	Climbing	15 Sep.–15 Oct.	Lilac, white or light purple	5–6	8.6–11.7	Gray background with black and yellow spots
<i>P. glabellus</i>	Climbing	10 Sep.–15 Oct.	Red	5–6	2.9–3.6	Gray background with black spots

found at that site, possibly because of intensive grazing by goats and sheep in the area. Instead, they were found in two growing sites in Rayones, Nuevo León (accessions 650 and 651).

Previous botanical explorations in northeastern Mexico reported the presence of fifteen wild bean species (Rodríguez Cabrera et al. 1987, Freytag & Debouck 2002, Estrada et al. 2004, Salcedo et al. 2006). In the present study, however, only eleven species were found in the same region: *Phaseolus neglectus*, *P. leptostachyus*, *P. pedicellatus*, *P. albiflorus*, *P. novoleonensis*, *P. maculatifolius*, *P. zimapanensis*, *P. altimontanus*, *P. vulgaris*, *P. coccineus* and *P. glabellus*.

The species *P. polymorphus* was not found even though Hinton & Hinton (1995) had been able to collect plants in Zaragoza and Galeana (Nuevo León) in 1969 and 1993 that were identified as *P. polymorphus*. These plants were subsequently deposited in the GB Hinton Herbarium (17245, 23421 and 23424).

Regarding *P. plagiocylix*, Freytag & Debouck (2002) mentioned that it was very rare on rocky, limestone soil found in the western mountains of Monterrey (Nuevo León, Mexico), and only a few collections were known. They referred to two herbarium specimens collected from Nuevo León: (1) Monterrey, Bishop Hill, 12 Oct. 1895, *Seler* 1042; and (2) 2.6 mi N of Grutas de García, 5 Sep. 1967, *Weedon* 4074. The first was collected nearly 120 years ago from a site that is now in the center of the city, where vegetation has been disturbed by anthropocentric activity. Field trips were made to the site where the second specimen had been collected, but no plants of this species were found, possibly also because of anthropocentric activity and heavy grazing by goats. Estrada et al. (2004) reported the species for Nuevo León; however, their statements seemed to suggest that the studied specimens were from herbaria. More explorations in

areas close to the reported collection sites are needed as the possible presence of the species in those areas cannot be discounted.

Collected seeds

A total of 93 seed samples were accessioned, representative of eleven different species of *Phaseolus* in northeastern Mexico: *Phaseolus albiflorus*, *P. leptostachyus*, *P. pedicellatus*, *P. altimontanus*, *P. novoleonensis*, *P. maculatifolius*, *P. zimapanensis*, *P. neglectus*, *P. vulgaris*, *P. coccineus*, and *P. glabellus*. Fifty-six accessions were representative of the state of Nuevo León, and 37 were representative of the state of Tamaulipas (table 1, fig. 1).

An important consideration shows up when following the route along an approximately east-to-west corridor in the Sierra Madre Oriental region of Mexico: This area is home to numerous species with larger populations at medium to high altitudes. In general, this region is characterized by vigorous development of vegetation (weeds, shrubs, and trees), and during the autumn-winter season, it receives less solar radiation. These two factors favor greater availability of soil moisture at the end of the growing cycle of the *Phaseolus* species and probably explain the greater density of the species at the intermediate and high altitudes of the Sierra Madre Oriental.

Another observation deserves mention. Populations of the different wild bean species are mainly located in areas where wood production is not practiced and where cattle have no access. This indicates that bean plants: (1) survive in sites with difficult access, such as areas characterized by steep slopes having boulders or old roads with stony walls and (2) survive by climbing on trees and spiny shrubs. These observations show that wild bean species, in most cases, are 'refuged' in sites with difficult access, where human activi-



Figure 2 – Wild species of *Phaseolus* (Fabaceae) collected during 2010–2013 in northeastern Mexico: A, *Phaseolus zimapanensis*; B, *P. neglectus*; C, *P. pedicellatus*; D, *P. maculatifolius*; E, *P. glabellus*; F, *P. leptostachyus*; G, *P. altimontanus*; H, *P. albiflorus*; I, *P. novoleonensis*; J, *P. coccineus*; K, *P. vulgaris*. Measuring rod = 2 cm.

ties have negligible impact. On the other hand, areas available for forest use and intensive livestock grazing serve as an indicator of reduced populations of wild bean.

In northeastern Mexico, the wild species of the genus *Phaseolus* are adversely impacted by the historical affinity for cattle and goat production. These animals are often indiscriminately managed at or near hilly areas, and this practice can cause serious damage to native flora. The maintenance of some communication roads by local associations can also negatively affect this species, as in the case of two small populations of *P. novoleonensis* found along route 8 at altitudes ranging from 1150 to 1291 m a.s.l. Additionally, wild bean populations are being adversely affected by other factors, such as climate change, devastation of natural environments and increasingly intensive agriculture and livestock production, which contribute to the loss of populations (Brush 2000, Acosta-Gallegos 2012).

The collection of wild bean accessions in northeastern Mexico has been irregular, especially for some species, due to climate conditions prevailing each year. For example, in 2010, seed of *P. maculatifolius* (accession 672) was collected along route 7, at 1025 m a.s.l.; since 2011, however, seed has not been collected again along the same site because of unfavorable climate conditions, with early frosts and intermittent droughts throughout the year. However, in 2012 and 2013, seeds (accession 671) were collected along route 5, at 1400 m a.s.l. Also, in 2013, seeds were collected along route 12, at 1836 m a.s.l. (accession 654), and along route 24, at 1005 and 1090 m a.s.l. (accessions 709 and 710).

Morphological characteristics

The eleven species collected are perennials and have indeterminate growth habits. Eight of them are climbing species: *P. neglectus*, *P. pedicellatus*, *P. albiflorus*, *P. maculatifolius*, *P. zimapanensis*, *P. vulgaris*, *P. coccineus* and *P. glabellus*, while three have prostrate habits: *P. novoleonensis*, *P. altimontanus* and *P. leptostachyus*. A climbing variant of *P. leptostachyus* was also found (accessions 619, 715, 717 and 723).

Thickness of the root system was observed in all species reported in this study, except *P. vulgaris*. This morphological characteristic endows plants with the capacity to develop new sprouts each year if the climatological conditions are favorable. This type of root system can grow 40 centimeters or more, as in *P. pedicellatus*. In general, wild beans have fibrous or fleshy roots somewhat similar to *Pachyrhizus erosus* (L.) Urban, and from their crowns, herbaceous stems grow, which at times are somewhat woody (Delgado-Salinas 2012). These woody stems can remain from one year to another, and when the conditions are favorable, they show new sprouts; this is common in *P. maculatifolius* and has been observed in *P. albiflorus*.

The species varied in flower colour, flowering period, number of seeds per pod and seed weight per 100 seeds (table 2). The morphological characteristic of pods and seeds also displayed variations among species (fig. 2). In general, seed shape was elliptic to truncated elliptic, with opaque to intermediate seed coat. Seed colour varied from light brown to brown with dispersed black spots; the colour around the

seed-hilum was black and no seed veins were present. *Phaseolus novoleonensis* and *P. maculatifolius* were the exceptions, with seed colour varying from cream to light brown, with brown around the seed-hilum and with the presence of seed veins (fig. 2).

CONCLUSIONS

The genetic diversity of wild species of *Phaseolus* in northeastern Mexico is evident in eleven species found in 93 growing sites in Nuevo Leon and Tamaulipas: *P. neglectus*, *P. leptostachyus*, *P. albiflorus*, *P. maculatifolius*, *P. novoleonensis*, *P. zimapanensis*, *P. pedicellatus*, *P. coccineus*, *P. altimontanus*, *P. vulgaris* and *P. glabellus*.

Species with greater distribution and larger populations were *P. albiflorus*, *P. leptostachyus*, *P. pedicellatus* and *P. neglectus*. The species with least distribution were *P. glabellus* and *P. altimontanus*, found at only one site each. *Phaseolus vulgaris* was the species exhibiting the greatest morphological variation.

Some loss of wild bean germplasm is possible, thus necessitating continued explorations and collections of other wild populations in the region, particularly in areas not covered by this study. Also, further studies are needed to characterize the populations of wild bean species in the region in order to determine their genetic potential.

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Appendix – Wild species of *Phaseolus* in northeastern Mexico with accession numbers and georeference and altitude data of collection sites.

***P. albiflorus*:** accessions 601 (25°31'59"N, -100°16'32"W; 895 m a.s.l.), 602 (25°5'27"N, -99°58'10"W; 1082 m a.s.l.), 603 (25°05'58"N, -99°59'42"W; 1242 m a.s.l.), 604 (25°23'21"N, -100°09'59"W; 675 m a.s.l.), 605 (25°21'11.76"N, -100°11'35"W; 1480 m a.s.l.), 606 (25°21'25"N, -100°11'39"W; 1600 m a.s.l.), 607 (24°3'51"N, -99°44'17"W; 1944 m a.s.l.), 608 (24°44'34"N, -99°49'13"W; 900 m a.s.l.), 611 (25°32'23"N, -100°16'25"W; 760 m a.s.l.), 612 (25°33'18"N, -100°16'00"W; 665 m a.s.l.), 614 (25°13'18"N, -100°05'12"W; 1005 m a.s.l.), 615 (24°31'54"N, -99°44'21"W; 1270 m a.s.l.), 616 (23°56'23"N, -99°47'29"W; 2080 m a.s.l.), 617 (25°37'2"N, -100°22'38"W; 1284 m a.s.l.), 627 (25°13'13"N, -100°05'03"W; 1136 m a.s.l.), 628 (25°13'13"N, -100°4'24"W; 878 m a.s.l.), 629 (25°21'42"N, -100°9'46"W; 800 m a.s.l.), 630 (24°42'39"N, -99°44'51"W; 1204 m a.s.l.), 631 (24°42'27"N, -99°45'2"W; 1360 m a.s.l.), 632 (23°55'35"N, -99°48'0.4"W; 2170 m a.s.l.), 633 (24°3'23"N, -99°44'14"W; 2120 m a.s.l.), 656 (25°19'41"N, -100°09'43"W; 846 m a.s.l.), 657 (25°17'09"N, -100°08'16"W; 1124 m a.s.l.), 659 (25°17'34"N, -100°08'26"W; 952 m a.s.l.), and 701 (24°31'43"N, -99°43'52"W; 1028 m a.s.l.); ***P. leptostachyus*:** accessions 610 (25°21'25"N, -100°11'39"W; 1600 m a.s.l.), 619 (24°12'56"N, -99°42'59"W; 1210 m a.s.l.), 620 (25°32'31"N, -100°16'19"W; 714 m a.s.l.), 621 (25°20'25"N, -100°15'38"W; 1913 m a.s.l.), 622 (24°04'14"N, -99°48'40"W; 1160 m a.s.l.), 623 (24°03'42"N, -99°45'30"W; 1450 m a.s.l.), 634 (25°21'3.6"N, -100°11'30"W; 1400 m a.s.l.), 635 (24°45'1"N, -99°48'09"W; 800 m a.s.l.), 636 (24°42'30"N, -99°44'54"W; 1230 m a.s.l.), 637 (25°06'09"N, -99°58'32"W; 1020 m a.s.l.), 638 (25°5'30"N, -99°58'44"W; 1061 m a.s.l.), 639 (25°04'44"N, -100°0'19.7"W; 1200 m a.s.l.), 640 (24°03'43"N, -99°44'50"W; 1616 m a.s.l.), 641 (24°03'43"N, -99°44'13"W; 1990 m a.s.l.), 658 (25°17'25"N, -100°8'23"W; 975 m a.s.l.), 702 (100°08'23"N, -99°43'52"W; 1028 m a.s.l.), 712 (23°33'04"N, -99°12'38"W; 1256 m a.s.l.), 715 (22°56'52"N, -99°36'12"W; 1473 m a.s.l.), 717 (22°55'14"N, -99°34'29"W; 1381 m a.s.l.), 723 (22°55'26"N, -99°37'50"W; 1364 m a.s.l.), and 734 (23°01'26"N, -99°32'13"W; 1403 m a.s.l.); ***P. pedicellatus*:** accessions 642 (24°53'18"N, -100°11'58"W; 2337 m a.s.l.), 643 (24°53'20"N, -100°12'45"W; 2820 m a.s.l.), 644 (24°55'10"N, -100°12'19"W; 2277 m a.s.l.), 645 (23°49'19"N, -99°52'51"W; 2625 m a.s.l.), 646 (23°55'35.9"N, -99°47'55"W; 21799'47'55"0 m a.s.l.), 647 (23°54'47"N, -99°47'57"W; 2700 m a.s.l.), 648 (24°03'43"N, -99°44'13"W; 1990 m a.s.l.), 649 (24°03'22"N, -99°44'8.1"W; 2195 m a.s.l.), 706 (23°35'36"N, -99°12'33"W; 1108 m a.s.l.), 707 (23°35'11"N, -99°12'44"W; 1038 m a.s.l.) and 736 (22°55'59"N, -99°35'18"W; 1425 m a.s.l.); ***P. altimontanus*:** accession 653 (25°21'23"N, -100°11'37.8"W; 1555 m a.s.l.); ***P. novoleonensis*:** accessions 650 (25°05'48"N, -99°59'15"W; 1150 m a.s.l.) and 651 (25°05'31"N, -99°59'37"W; 1291 m a.s.l.); ***P. maculatifolius*:** accessions 654 (24°3'55"N, -99°44'13"W; 1836 m a.s.l.), 671 (25°21'03"N, -100°11'30"W; 1400 m a.s.l.), 672 (25°13'15"N, -100°04'55"W; 1025 m a.s.l.), 709 (23°34'01"N, -99°13'36"W; 1005 m a.s.l.) and 710 (23°33'40"N, -99°13'11"W; 1090 m a.s.l.); ***P. zimapanensis*:** accessions 655 (23°46'21"N, -100°15'54"W; 1990 m a.s.l.), 670 (24°12'56"N, -99°42'59"W; 1210 m a.s.l.), 713 (22°55'51"N, -99°35'9"W; 1410 m a.s.l.), 714 (22°56'52"N, -99°36'12"W; 1473 m a.s.l.), 716 (22°57'04"N, -99°36'23"W; 1457 m a.s.l.), 720 (22°57'34"N, -99°38'54"W; 1320 m a.s.l.), 721 (22°55'06"N, -99°37'34"W; 1335 m a.s.l.), 722 (22°52'07"N, -99°36'39"W; 1135 m a.s.l.), 728 (22°55'56"N, -99°38'14"W; 1433 m a.s.l.) and 733 (23°01'23"N, -99°31'26"W; 1662 m a.s.l.); ***P. neglectus*:** accessions 609 (25°05'57"N, -99°59'42"W; 1242 m a.s.l.), 703 (23°37'53"N, -99°11'53"W; 861 m a.s.l.), 704 (23°37'42"N, -99°12'18"W; 1070 m a.s.l.), 705 (23°36'53"N, -99°12'12"W; 1181 m a.s.l.), 708 (23°34'01"N, -99°13'36"W; 1005 m a.s.l.), 711 (23°32'50"N, -99°12'32"W; 1405 m a.s.l.), 735 (23°01'24"N, -99°31'37"W; 1659 m a.s.l.) and 737 (23°33'40"N, -99°13'11"W; 1090 m a.s.l.); ***P. vulgaris*:** accessions 724 (22°54'22"N, -99°37'06"W; 1234 m a.s.l.), 725 (22°52'07"N, -99°36'39"W; 1135 m a.s.l.), 726 (22°50'37"N, -99°35'42"W; 1097 m a.s.l.); ***P. coccineus*:** accessions 718 (22°55'14"N, -99°34'29"W; 1382 m a.s.l.), 719 (22°54'54"N, -99°33'45"W; 1342 m a.s.l.), 727 (22°55'26"N, -99°37'50"W; 1364 m a.s.l.), 729 (23°01'55"N, -99°34'16"W; 1389 m a.s.l.), 730 (23°01'37"N, -99°30'43"W; 1685 m a.s.l.), 731 (23°1'13"N, -99°32'25"W; 1184 m a.s.l.) and ***P. glabellus*:** accession 732 (23°01'16"N, -99°32'22"W; 1189 m a.s.l.).