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## **Advances in Genetic Diversity Analysis of *Phaseolus* in Mexico**

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### **1. Introduction**

Mexico is a centre of origin, domestication and diversity of major crops worldwide, such as maize (*Zea mays* L.) and beans (*Phaseolus* spp). It is known that five species of *Phaseolus* have been domesticated: *P. vulgaris* L. (common bean), *P. coccineus* L. ('ayocote' bean), *P. lunatus* L. ('lima' bean), *P. acutifolius* Gray ('tepary' bean) and *P. polyanthus* Greenm. (= *P. polyanthus* McFad.) ('acalete' bean) [1]. The five species are well distributed through Mexico as wild, semi-domesticated, and domesticated forms [2]. Beans are economically, socially, biologically and culturally important [3]. Owing to the importance of beans for Mexico and the world [4] extensive programmes focusing on the conservation, management and characterization of genetic resources of *Phaseolus* have been implemented in some countries [5] and international institutions such as Centro Internacional de Agricultura Tropical (CIAT) in Cali, Colombia; the Grupo de Mejoramiento de Leguminosas de la Misión Biológica de Galicia and Consejo Español de Investigación Científica (MBG-CSIC) in Spain; the USDA/ARS Western Regional Plant Introduction Station at USA, and the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) of Mexico who include bean collections in their germplasm banks, including all forms [6, 7].

Collections at germplasm banks are usually classified as base, active, for the work, and core [5]. Core collections include the highest levels of genetic diversity of one species (from 70 to 80%) as *Phaseolus* spp. into the lower number of accessions and due its size offers an easy and cheaper management and improves and efficient the germplasm use [8]. In this sense researchers need to characterize and to evaluate the core collections. Traditional strategies were based on the use of high-heritability morpho-agronomic traits such as growth habit, flower colour; the use of biochemical markers as seed reserve proteins as phaseolins, etc. Recent advances in molecular biology have improved the capability of plant genetic resource characterization using methodologies based on DNA analysis such as dominant (random amplified polymorphic DNA, or RAPDs, and amplified fragment length polymorphisms, or AFLPs) and co-dominant (restriction fragment length polymorphisms, or RFLPs, and simple sequence repeats, or SSRs) molecular markers [9]. The sequencing of the common bean genome has been concluded and this information broadens our perspective about some facts and challenges related to origin, domestication, diversity patterns and breeding of common beans [10-13].

## 2. Beans in Mexico

The common bean is the second legume crop worldwide, behind soybeans [*Glycine max* (L.) Merr] [4]. Despite the major anthropocentric use of beans being their green pods and dry grains, in some Latin American and African countries people consume young leaves and flowers as fresh vegetables [14]. By 2013, more than 1.8 million hectares were cultured with beans, and grain yields were 0.74 t ha<sup>-1</sup> [15]. Common beans are well adapted, and grow through the different agroecosystems of Mexico and different seasons [7], depending upon the genetic diversity of the native germplasm and breed cultivars developed for each region [3].

Beans are a basic food due to being a major source of proteins, minerals, fibre, carbohydrates and vitamins in the Mexican daily diet for most people, but mainly for those with low economic resources [3]. Beans are the perfect complement to the Mexican diet based on maize as 'tortillas' in order to substitute animal protein for proper nutrition [4, 6]. Clinical studies showed that bean consumption prevents or improves both cholesterol and glucose levels in the blood [16]. However, the consumption of common beans has some problems, since they contain anti-nutritional compounds such as polyphenols (condensed tannins and anthocyanins), and inhibitors of proteases as trypsin, lectins and phytic acid [17, 18]. These compounds limit bean consumption and prevent them need breeding programmes and/or industry treatments such as cooking.

Economic conditions and customer preferences cause a variation in bean consumption *per capita* between countries, and between regions within countries. In Mexico, black beans are preferred in the southern regions, while 'Flor de Mayo' and 'Flor de Junio' beans are preferred in the central western regions. Finally 'pinto', 'bayo' and 'azufrado' beans are mostly consumed in the northern regions. While Mexican bean *per capita* consumption is 11 kg per year, some African countries report more than 40 kg [18].

### 3. Cultivated species of *Phaseolus*

As has been listed before, only five *Phaseolus* species have been domesticated, and wild and cultivated forms are well known: *P. vulgaris* L., *P. coccineus*, *P. lunatus*, *P. acutifolius*, and *P. dumosus* from over 70 *Phaseolus* species, subspecies and varieties reported for Mexico (Tables 1 and 2). Most *Phaseolus* species are well distributed through Mexico with the exception of cold humid temperate and extremely hot arid tropic climates. The species *P. coccineus* and *P. leptostachyus* are distributed in the largest numbers of climatic types [19].

The cultivated form of *P. vulgaris* is grown across Mexico, but the highest producers are the states of Zacatecas, Durango, Sinaloa, San Luis Potosí, Guanajuato and Chiapas. Common beans represent 95% of national bean consumption. Common beans show an annual cycle and exhibit broad variability in growth habits, biological cycle duration, adaptation to different altitudes and soil conditions, as well as a large variation in seed colour and shapes. The last decades the common bean crop has migrated from central and southern regions to the north. Bean crop growth has also changed from common cultivation association with maize or pumpkin to monoculture using cultivars with determinate growth habits. Wild populations of *P. vulgaris* are mainly distributed through major mountain systems: Sierra Madre Occidental, Sierra Madre del Sur, Eje Neovolcánico, and Sierra Madre Oriental from 760 to 2,250 m above sea level. This species has a climbing growth habit, thin pods and small seeds where greyish-spotted seed coat colours are common, although there are other colours including black, 'bayo', yellow, brown, etc. Pods are dehiscent when mature, and seeds can be latent due to seed coat hardness. In this species it is common to find segregant populations derived from the spontaneous crossing between wild plants and cultivated forms, due to both types co-habiting or co-existing by variable lengths of time or seasons [7, 20-22].

Ayocote beans, *P. coccineus*, are known as 'patol' or 'patola'. They are a perennial species that develop tuber roots, as well as being a vigorous species, with climbing plants. Ayocote beans are commonly grown at the highlands of the central states of Puebla, Tlaxcala, Hidalgo and Mexico. *P. coccineus* is allogamous due to incompatibility problems, and landraces and wild plants show a great level of genetic variability in seed colours, sizes and shapes. The species shows short-width pods with a low number of seeds per pod; the seeds are large and have thick coats. The cultivated form has an impermeable seed coat. The wild form is distributed in temperate regions from the highlands and mountains: Sierra Madre Occidental, Eje Neovolcánico and Sierra Madre Oriental, between 1,800 and 3,000 m above sea level [23-26].

Lima beans, *P. lunatus*, are named as 'comba' at the Balsas River depression (western Mexico) or 'ibes' at the Península of Yucatán (southeastern Mexico). As ayocotes, lima beans are perennial and climbing species, vigorous and with a late biological cycle. Seeds of cultivated populations are variable in colour, shape and size. The species tolerates high temperatures, drought stress and some insect pests. Lima beans are frequently inter-cropped with maize, but most of their vegetative development and reproductive phase is completed after the maturity of maize when high levels of sun radiation are available. The wild form is perennial and climbing, and is distributed in the lower-coastal lands of Mexico near the Gulf of Mexico, Pacific Ocean and the Peninsula of Yucatán. Some wild populations have been found at the

Balsas River depression (states of Michoacán, Jalisco, Colima and Nayarit) at altitudes of up to 1,600 m above sea level (masl) [27, 28].

The poor surface cropped with *P. acutifolius* is located mainly in northern and northeastern Mexico at altitudes of up to 1,800 masl. Tepary bean is an annual species with some landraces with an undetermined growth habit for monoculture. Despite the cultivated form having larger seeds than the wild population, some varieties conserve grain traits similar to their ancestors. Wild populations are distributed at Sierra Madre Occidental from the northern states of Sonora and Chihuahua to the central-eastern state of Michoacán [27].

Finally, the less cultivated *Phaseolus* species, *P. dumosus* (= *P. polyanthus*), named ‘acalete’ shows vigorous climbing growth habits, and is commonly cultured in the state of Oaxaca, associated with maize. Acalete seeds are medium to large and are highly variable in colour, shape and size. The seed coat is thick, as in *P. coccineus*, but is permeable to water. The wild form is inferred to be dispersed through the state of Oaxaca [19, 27].

Section	Species	Mexican states of distribution
A. Acutifolii	<i>P. acutifolius</i> A. Gray var. <i>acutifolius</i>	Jalisco, Nayarit, Baja California, Chihuahua, Durango, Sinaloa, Sonora
	<i>P. acutifolius</i> A. Gray var. <i>latifolius</i>	Nayarit, Chihuahua, Coahuila, Durango, Sinaloa, Querétaro, Sonora
	<i>P. acutifolius</i> A. Gray var. <i>tenuifolius</i>	Colima, Jalisco, Baja California, Chihuahua, Coahuila, Colima, Durango, Jalisco, Sinaloa, Sonora.
	<i>P. parvifolius</i> Freytag	Baja California, Chiapas, Chihuahua, Durango, Guerrero, Jalisco, Michoacán, Nayarit, Oaxaca, Sonora
B. Phaseoli	<i>P. vulgaris</i> L.	Chiapas, Durango, Guanajuato, Guerrero, Jalisco, Mexico, Michoacán, Morelos, Nayarit, Oaxaca, Puebla, Querétaro, Sinaloa, Tamaulipas, Veracruz
	<i>P. costaricensis</i>	In Costa Rica and Panamá, Central America
	<i>P. dumosus</i> Macfady	Chiapas
	<i>P. albescens</i> McVaugh ex R. Ramírez & A. Delgado	Jalisco, Michoacán
C. Coccinei	<i>P. coccineus</i> L. subsp. <i>coccineus</i> (12 varieties: <i>coccineus</i> , <i>parvibracteolatus</i> , <i>griseus</i> , <i>lineatibracteolatus</i> , <i>tridentatus</i> , <i>splendens</i> , <i>strigillosus</i> , <i>semperbracteolatus</i> , <i>condensatus</i> , <i>pubescens</i> , <i>argenteus</i> , <i>zongolicensis</i> ).	Higher regions with temperate or cold climatic conditions from Chiapas, Oaxaca, Guerrero, Morelos, Puebla, Veracruz, Tlaxcala, Edo de Mexico, Hidalgo, Guanajuato, Michoacán, Jalisco, Nayarit, Zacatecas, Durango, Nuevo León, Tamaulipas, Sinaloa,

Section	Species	Mexican states of distribution
	<i>P. coccineus</i> L. subsp. <i>striatus</i> ( 7 varieties: <i>striatus</i> , <i>minuticatricatus</i> , <i>guatemalensis</i> , <i>purpurascens</i> , <i>rigidicaulis</i> , <i>pringlei</i> , <i>timilpanensis</i> )	Idem Subsp. <i>coccineus</i>
	<i>P. glabellus</i> Piper	Chiapas, Hidalgo, Oaxaca, Puebla, San Luis Potosí, Tamaulipas, Veracruz
D. Paniculati Sub-section I, Volubili.	<i>P. lunatus</i> L.	Baja California, Campeche, Chiapas, Colima, Guerrero, Jalisco, Mexico, Michoacán, Morelos, Nayarit, Oaxaca, Sinaloa, Tabasco, Tamaulipas, Veracruz, Yucatán
	<i>P. polystachyus</i> (three subspecies)	Only in the United States of America
	<i>P. salicifolius</i> Piper	Durango, Sinaloa, Sonora
	<i>P. maculatifolius</i> Freytag & Debouck	Nuevo León
	<i>P. dasycarpus</i> Freytag & Debouck*	Veracruz
	<i>P. longiplacentifer</i> Freytag	Veracruz
D. Paniculati Sub-section II, Lignosi	<i>P. jaliscanus</i> Piper	Jalisco, Nayarit, Sinaloa, Michoacán
	<i>P. scrobiculatifolius</i> Freytag	Michoacán
	<i>P. nudosus</i> Freytag & Debouck	Jalisco
	<i>P. albinervus</i> Freytag & Debouck	Chihuahua
	<i>P. marechalii</i> Delgado	Mexico, Morelos, Puebla
	<i>P. rotundatus</i> Freytag & Debouck	Jalisco, Michoacán
	<i>P. acinaciformis</i> Freytag & Debouck	Oaxaca
	<i>P. xolocotzii</i> Delgado	Guerrero, Mexico, Oaxaca
	<i>P. sonorensis</i> Standl	Chihuahua, Sinaloa, Sonora
	<i>P. juquilensis</i> Delgado	Oaxaca
E. Bracteati	<i>P. macrolepis</i> and <i>P. talamancensis</i>	Only in Guatemala and Costa Rica, Central America
F. Minkellersia	<i>P. pluriflorus</i> Maréchal, Mascherpa & Stainer	Distrito Federal, Durango, Mexico, Jalisco, Michoacán, Morelos, Nayarit, Sinaloa
	<i>P. nelsonii</i> Maréchal, Mascherpa & Stainer	Chiapas, Jalisco, Mexico, Michoacán, Oaxaca, Zacatecas
	<i>P. perplexus</i> Delgado	Jalisco, Mexico, Michoacán
	<i>P. plagiosylix</i> Harms	Nuevo León



Section	Species	Mexican states of distribution
	<i>P. amblyosepalus</i> (Piper) Morton	Durango, Michoacán, Sinaloa
	<i>P. tenellus</i> Piper	Mexico, Michoacán, Zacatecas
	<i>P. parvulus</i> Greene	Chihuahua, Durango, Nayarit, Sinaloa, Sonora, Zacatecas
	<i>P. anisophyllus</i> (Piper) Freytag & Debouck	Chihuahua, Durango
	<i>P. amabilis</i> Standl	Chihuahua
	<i>P. pausiflorus</i> Sessé & Mociño	Chihuahua, Distrito Federal, Durango, Guerrero, Jalisco, Mexico, Michoacán, Morelos, Nayarit, Sinaloa, Sonora, Zacatecas
G. Zanthotricha	<i>P. xanthotrychus</i> Piper	Chiapas
	<i>P. hintonii</i> Delgado	Durango, Jalisco, Mexico, Nayarit
	<i>P. zimapanensis</i> Delgado	Durango, Hidalgo, Nuevo León, Querétaro, San Luis Potosí, Tamaulipas
	<i>P. gladiolatus</i> Freytag & Debouck	Hidalgo, San Luis Potosí
	<i>P. magnilobatus</i> Freytag & Debouck	Durango, Jalisco
	<i>P. esquincensis</i> Freytag	Chiapas
H. Revoluti	<i>P. leptophyllus</i> G. Don	Guerrero
I. Digitati	<i>P. neglectus</i> Hermann	Nuevo León, Tamaulipas
	<i>P. albiflorus</i> Freytag & Debouck	Coahuila, Nuevo León, Tamaulipas
	<i>P. albiviolaceus</i> Freytag & Debouck	Tamaulipas, Nuevo León
	<i>P. trifidus</i> Freytag	Nuevo León
	<i>P. altimontanus</i> Freytag & Debouck	Nuevo León
J. Rugosi	<i>P. filiformis</i> Benth.	Baja California, Chihuahua, Coahuila, Sonora
	<i>P. angustissimus</i> A. Gray	Sonora
	<i>P. carteri</i> Freytag & Debouck	Baja California
	<i>P. microcarpus</i> Mart	Chiapas, Guanajuato, Durango, Guerrero, Jalisco, Michoacán, Oaxaca, Puebla
K. Falkati	<i>P. micranthus</i> Hook & Arn	Jalisco, Michoacán, Nayarit, Sinaloa, Sonora
	<i>P. leptostachyus</i> Benth. (five varieties: <i>leptostachyus</i> , <i>intusus</i> , <i>pinnatifolius</i> , <i>nanus</i> , <i>lobatifolius</i> )	Chiapas, Chihuahua, Colima, Distrito Federal, Durango, Guanajuato, Guerrero, Hidalgo, Jalisco, Mexico, Michoacán, Morelos, Nayarit, Nuevo León, Oaxaca, Puebla, Querétaro, San Luis Potosí, Sinaloa, Sonora, Tamaulipas, Veracruz, Zacatecas
	<i>P. opacus</i> Piper	Tamaulipas, Veracruz

Section	Species	Mexican states of distribution
	<i>P. persistentus</i> Freytag & Debouck	Guatemala
	<i>P. macvaughii</i> Delgado	Baja California, Colima, Guerrero, Jalisco, michoacán, Sinaloa
L. Brevilegumeni	<i>P. oligospermus</i> Piper	Chiapas
	<i>P. campanulatus</i> Freytag & Debouck	Nayarit, Jalisco
	<i>P. tuerckheimii</i> Donnell-Smith	Chiapas
M. Pedicellati	<i>P. pedicellatus</i> Benth	Distrito Federal, Guanajuato, Guerrero, Hidalgo, Mexico, Morelos, Nuevo León, Querétaro, San Luis Potosí, Tamaulipas, Veracruz, Jalisco, Michoacán
	<i>P. oaxacanus</i> Rose	Oaxaca
	<i>P. esperanzae</i> Seaton	Hidalgo, Mexico, Michoacán, Puebla
	<i>P. polymorphus</i> S. Wats (two varieties: <i>polymorphus</i> , <i>albus</i> )	Aguascalientes, Coahuila, Durango, Guanajuato, Jalisco, Nuevo León,
	<i>P. palmeri</i> Piper	Zacatecas
	<i>P. purpusii</i> Brandegees	San Luis Potosí
	<i>P. grayanus</i> Woot	Chihuahua, Coahuila, Durango, San Luis Potosí, Sonora, Zacatecas
	<i>P. scrabellus</i> Benth.	Durango, Sinaloa, Sonora
	<i>P. teulensis</i> Freytag	Durango, Zacatecas
	<i>P. pyramidalis</i> Freytag	Chihuahua
	<i>P. laxiflorus</i> Piper	Hidalgo, Mexico, Veracruz
N. Chiapasana	<i>P. chiapasanus</i> Piper	Chiapas, Oaxaca
O. Coriacei	<i>P. maculatus</i> Scheele subsp. <i>maculatus</i>	Aguascalientes, Chihuahua, Coahuila, Durango, Guanajuato, Hidalgo, Puebla, Querétaro, San Luis Potosí, Sonora, Tlaxcala, Zacatecas
	<i>P. maculatus</i> Scheele subsp. <i>ritensis</i>	Chihuahua, Durango, Jalisco, Nayarit, Sinaloa, Sonora, Zacatecas
	<i>P. venosus</i> Piper	Aguascalientes, Durango, San Luis Potosí, Jalisco, Zacatecas
	<i>P. reticulatus</i> Freytag & Debouck	Durango

Adapted from Freytag and Debouck [29] and López-Soto et al. [19].

**Table 1.** Species of *Phaseolus* in Mexico and their distribution.



Taxon	Distribution	Altitudinal distribution (masl)
Section <i>Chiapasana</i>	Oaxaca, Veracruz, Chiapas	1000-1500
<i>P. chiapasanus</i>		
Section <i>Phaseolus</i>		
<i>P. angustissimus</i>	Chihuahua, Coahuila	1600-1750
<i>P. filiformis</i>	BCN, BCS, Sonora, Sinaloa, Chihuahua, Durango	200-1350
<i>P. leptostachyus</i>	Most of the country	500-2200
<i>P. vulgaris</i>	Most of the country	700-2000
<i>P. acutifolius</i>	BCS, BCN, Sonora, Sinaloa, Chihuahua, Durango, Zacatecas, Nayarit, Coahuila, Oaxaca, Guerrero, Morelos	20-2300
<i>P. microcarpus</i>	Jalisco, Oaxaca, Puebla, Veracruz, Guerrero, Morelos, Durango, Nayarit	20-1600
<i>P. lunatus</i>	Mexican coastal states, Puebla, Morelos	10-1200
<i>P. neglectus</i>	Tamaulipas, Nuevo León, Coahuila	1200-1600
<i>P. coccineus</i>	Medium to highlands of most of the country	700-2900
<i>P. salicifolius</i>	Durango, Sinaloa	1400-1900
<i>P. maculatus</i>	Chihuahua, Durango, Sonora, Zacatecas, Sinaloa, Aguascalientes, Guanajuato, Querétaro, Hidalgo, Jalisco, Nuevo León	1400-2200
<i>P. polystachyus</i>	Veracruz, Morelos	1200-1600
<i>P. xolocotzii</i>	Oaxaca, Morelos	1500-1800
<i>P. ritensis</i>	Chihuahua, Durango	1800-2100
<i>P. marechalii</i>	Hidalgo, Tlaxcala, Mexico	2100-2600
<i>P. pedicellatus</i>	Chihuahua, Durango, Aguascalientes, Zacatecas, SLP, Hidalgo, Oaxaca, Querétaro	1500-2800
<i>P. sonorensis</i>	BCS, BCN, Sonora, Chihuahua, Durango	40-1600
Section <i>Minkelersia</i>		
<i>P. pauciclorus</i>	Durango, Michoacán, Jalisco, Mexico, Oaxaca	1700-2000
<i>P. nelsonii</i>	Oaxaca, Mexico, Jalisco	1900-2200
<i>P. parvulus</i>	Chihuahua, Durango	2100-2500
<i>P. pluriflorus</i>	Mexico, Michoacán, Oaxaca, Jalisco	2000-2500
Section <i>Xanthotrichus</i>		
<i>P. xanthotrichus</i>	Hidalgo, Chiapas	1500-1700
<i>P. hintonii</i>	Oaxaca, Morelos, Michoacán	1500-1700

SLP = San Luis Potosí, BCN = Baja California Norte, BCS = Baja California Sur.

**Table 2.** Distribution of wild species of *Phaseolus* in Mexico based on specimens of INIFAP's herbarium.

#### 4. Why must common bean diversity be conserved?

Mexico is broadly recognized as the centre of origin, domestication and diversification of major crops including avocado, amaranth, cocoa, pumpkin, maize, beans, and others. These species have been well dispersed and cultivated worldwide and constitute a major source of economic input for many countries. One major Mexican institution advocated to the collection, study, documentation, preservation and promotion of Mexican genetic resources is the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) which created the Genetic Resources Unit, and recently a new National Bank of Germplasm (CNRG, INIFAP) located in Tepatitlán, Jalisco, where *Phaseolus* represents one of the most important species to be preserved and studied. Special emphasis must be given in further works to those species not properly represented in Mexican Germplasm Banks, without seed samples preserved under proper conditions or species only represented as herbarium samples (Table 3). The recent explorations and collections of *Phaseolus* species across Mexico (Fig. 1) conducted by Muruaga-Martinez et al. (unpublished data, 2010-2012), showed the evident genetic erosion to be abnormally high in some regions. At these regions genetic erosion is so fast that can not will compensate by new genetic variability 'normally created' inside each ecosystem. Genetic erosion is significantly increased by human perturbation of agroecosystems. In addition, economical financing of a new re-collection of *Phaseolus* genetic resources must be revalued and consistently supported, assuming this involves future investment for genetic resource preservation and utilization for bean improvement.

Species	With seed available		Without seed available	
	Accessions registered in herbarium	Accessions with seeds	Species	Accessions registered in herbarium
<i>P. acutifolius</i> (three subspecies)	30	74	<i>P. acinacifolius</i>	1
<i>P. amblyosepalus</i>	5	2	<i>P. albescens</i>	2
<i>P. coccineus</i> (two subspecies)	123	282	<i>P. albiflorus</i>	8
<i>P. chiapasanus</i>	5	1	<i>P. albinervus</i>	1
<i>P. esperanzae</i>	12	1	<i>P. albiviolaecus</i>	2
<i>P. glabellus</i>	12	7	<i>P. altimontanus</i>	2
<i>P. grayanus</i>	25	8	<i>P. amabilis</i>	1
<i>P. hintonii</i>	5	1	<i>P. anisophyllus</i>	2
<i>P. jaliscanus</i>	9	4	<i>P. angustissimus</i>	2
<i>P. leptostachyus</i> (5 subspecies)	106	203	<i>P. campanulatus</i>	1
<i>P. lunatus</i>	43	91	<i>P. carteri</i>	4
<i>P. maculatus/maculatus</i>	25	28	<i>P. dacicarpus</i>	1
<i>P. maculatus/ritensis</i>	22	17	<i>P. esquincensis</i>	1

Species	With seed available		Without seed available	
	Accessions registered in herbarium	Accessions with seeds	Species	Accessions registered in herbarium
<i>P. marechalii</i>	3	1	<i>P. filiformis</i>	33
<i>P. micranthus</i>	11	1	<i>P. gladiolatus</i>	2
<i>P. microcarpus</i>	31	7	<i>P. laxiflorus</i>	3
<i>P. neglectus</i>	3	7	<i>P. juquilensis</i>	1
<i>P. nelsonii</i>	12	4	<i>P. leptophyllus</i>	1
<i>P. oaxacanus</i>	5	3	<i>P. longiplacentifer</i>	1
<i>P. oligospermus</i>	6	10	<i>P. maculatifolius</i>	1
<i>P. parvulus</i>	14	4	<i>P. macvaughii</i>	10
<i>P. pedicellatus</i>	34	13	<i>P. magnilobatus</i>	3
<i>P. pluriflorus</i>	15	11	<i>P. nudosus</i>	1
<i>P. polymorphus</i>	19	2	<i>P. opacus</i>	1
<i>P. scabrellus</i>	3	1	<i>P. palmeri</i>	1
<i>P. tuerckheimii</i>	6	13	<i>P. parvifolius</i>	17
<i>P. vulgaris</i>	32	58	<i>P. pauciflorus</i>	30
<i>P. xanthotrichus</i>	2	8	<i>P. perplexus</i>	4
			<i>P. plagyosilix</i>	2
			<i>P. purpusii</i>	1
			<i>P. pyramidalis</i>	2
			<i>P. rotundatus</i>	1
			<i>P. salicifolius</i>	3
			<i>P. scrobiculatifolius</i>	2
			<i>P. sonorensis</i>	2
			<i>P. tenellus</i>	3
			<i>P. teulensis</i>	2
			<i>P. trifidus</i>	1
			<i>P. venosus</i>	3
			<i>P. xolocotzii</i>	3
			<i>P. zimapanensis</i>	12

**Table 3.** Species of *Phaseolus* geo-positioned, with herbarium registers and seed samples available and species without seed samples [29, 30].

In this sense, traditional strategies for germplasm analysis can be improved and better understood by the use of DNA-based strategies as molecular markers. Breeding programmes underutilize the genetic diversity available because of the necessity of pre-breeding exotic germplasm. The hybridization between wild and domesticated types of *Phaseolus* from the same gene pools offer greater potential for enhancing crop variation due the partial reproductive isolation between Andean and Mesoamerican domesticated gene pools. Former evaluations of wild and semi-wild *Phaseolus* accessions have shown resistance to insects and diseases [21,22, 31-33] and higher N, Fe, and Ca content in seeds, which could contribute to the improvement of nutritional grain quality and grain yield [34]. Acosta-Gallegos et al. [34] suggested *Phaseolus* pre-breeding based on the use of information on gene pool origins, domestication syndrome traits, molecular diversity, and mapping data of the wild forms; the indirect screening for biotic and abiotic stresses; and marker-assisted selection [35, 36].



**Figure 1.** Locations of exploration and collection of *Phaseolus* species in Mexico by Muruaga-Martínez et al. (unpublished data, 2010-2012).

## 5. Analysis of genetic diversity in domesticated *Phaseolus* species

### 5.1. Common beans

One of the pioneer works on the study of *Phaseolus* genetic diversity was conducted by Debouck et al. [37] who described the ecological adaptations and geographical locations of

cultivated and wild species at northwestern South America (Colombia, Ecuador, Perú). Germplasm (12 wild and 36 cultivated accessions) collected from 1985 to 1990 was analysed on the basis of phaseolin and isozyme patterns. Wild beans showed a discontinuous distribution that, compared with bean distribution in Mexico, Central America and the Andean region, was classified as narrow. Later, Cerón et al. [38] analysed 151 bean accessions from the CORPOICA germplasm bank of Mosquera, Colombia. The data suggested higher morpho-agronomic variation in Mesoamerican germplasm than Andean accessions. Rodiño et al. [39] reported the analysis of 388 bean landraces from Spain and Portugal on the basis of the use of morpho-agronomic traits and phaseolin patterns. Germplasm was classified as Andean (74.7%), Mesoamerican (16.8%) and mixed (8.4%). The data indicated that only 52 accessions should constitute one representative core collection from Spain and Portugal.

In Mexico, the core collection of common beans structured by INIFAP includes 200 accessions, and this was characterized using morpho-agronomic traits and AFLP molecular markers. The data indicated a high level of genetic variability and no duplicity of accessions (non-shared haplotypes) into the core collection, becoming itself in a representative sample of *P. vulgaris* variation through Mexico [7, 40]. Rossi et al. [41] detected higher genetic diversity in the wild germplasm of beans from the Andean and Mesoamerican gene pools, while domesticated populations showed the largest linkage disequilibrium. Recent works of the analysis of *P. vulgaris*' genetic diversity have been published, where germplasm from different origins are analysed and broad genetic diversity ascertained into the genus and intra- and inter-specific and inter- and intra-population levels [42-48].

## 5.2. Ayocote beans

The genetic diversity of ayocote beans has been previously studied using morphological, agronomical and molecular markers, mainly using 'European' germplasm in order to detect high-yielding parents [49], highly tolerant germplasm to low temperatures [50], as well to characterize genetic relationships [51]. Spataro et al. [52] found clear differentiation between ayocote accessions [(wild, landraces, and *P. dumosus* (= *P. coccineus* ssp. *dumosus*)] as well as reduced gene diversity due the introduction of ayocote beans to Europe. Vargas-Vázquez et al. [24-26] reported that 80% of 798 *P. coccineus* accessions from Mexico originated from neovolcanic Axis and Eastern Sierra Madre with humid or semi-arid temperate climates, from 1500-2000 m above sea level and at 500-1000 mm of annual precipitation. Ayocote bean germplasm can be separated into two groups: late accessions adapted to minimum temperatures (2-5°C), with large seeds and pods, and early accessions adapted to 0-2°C, with small seeds and pods. Analysis of European domesticated *P. coccineus*, including botanical varieties *albiflorus*, *bicolour* and *coccineus* and domesticated and wild accessions from Mesoamerica using cpSSRs, nuclear SSRs and phenotypic traits, suggested a moderate-to-strong cytoplasmic bottleneck that followed the expansion of species into Europe and multiple domestication events into the species. An adaptive population differentiation was also found, suggesting that selection led to the diversification of *P. coccineus* in Europe [53].



### 5.3. Lima beans

The genetic diversity of *P. lunatus* from the Yucatán Peninsula was assessed based on morphological and phenological characters, and then related to ethnobotanical information obtained about intraspecific diversity recognized by farmers, their selection criteria, agronomic management, production purpose and percentage of cultivated area. Ethnobotanical and morpho-phenological data indicated 30 putative distinct landraces, two wild, and two weedy variants from 149 seed samples of *P. lunatus* germplasm, suggesting gene flow among them. Richness and diversity estimates were greatest, and evenness lowest, where there was minimal agricultural intensification, wild and weedy populations, and greater persistence of traditional culture [28]. Afterwards, Martínez-Castillo et al. [54] determined genetic diversity, structure and gene flow of 11 wild populations of *P. lunatus* in four regions of traditional agriculture in the Yucatán Peninsula, Mexico, using SSR loci. Strong genetic differentiation was found among populations due to isolation among agricultural regions, as well as low long-term gene flow and low rates of recent migration among populations. Positive correlation between agricultural intensification and increased diversity was found because wild populations are favoured by the intensification of disturbance in situations involving at least three years of fallow.

Recent low gene flow at both intraregional and interregional levels into the wild-weedy-domesticated complex of *P. lunatus* under traditional agricultural conditions was found in four regions on the Yucatán Peninsula, Mexico, while gene flow from domesticated to wild populations was three times higher than in the opposite direction. This asymmetry was explained by regional agricultural practices and seed selection criteria. Domesticated alleles were shown to be entering wild populations of different agricultural regions, suggesting exchange of domesticated seeds between farmers of different regions. Thus, *P. lunatus* on the Yucatán Peninsula has a predominantly domesticated to wild gene flow, leading to genetic assimilation of the wild lima bean by its domesticated counterpart [55]. Afterwards, *P. lunatus* accessions collected in 1979 were compared with accessions collected in 2007 using SSR markers. The germplasm from 1979 was more diverse than that from 2007, suggesting the presence of a 'bottleneck' effect since alleles detected at each year of collection were different, as well as demonstrating allele drift due to the introduction of breed cultivars or changes in the selection criteria of germplasm [56].

Two wild Mesoamerican (MI and MII) gene pools with contrasting geographical distributions have been found in relation to *P. lunatus*. While the MI gene pool occurs in central western Mexico, including the Pacific coastal range, the MII gene pool is widespread and occurs towards the Gulf of Mexico, the Yucatán Peninsula, and Central and South America. Mesoamerican landraces clustered together with wild accessions from the MI gene pool (L haplotype), suggesting a unique domestication event in central western Mexico. The most likely domestication region is an area of the states of Nayarit–Jalisco or Guerrero–Oaxaca, and not areas such as the Peninsula of Yucatán where the crop is currently widespread and diverse. A strong founder effect due to domestication has been detected, and several recently diversified haplotypes identified [57]. The analysis of 67 wild populations of *P. lunatus* from Mexico with ten microsatellite loci confirmed not only the presence of the two gene pools (MI and MII), but



also the possible existence of two subgroups within MI (MIa and MIb). While MI and MII are mainly divergent geographically, MIa and MIb overlap in their distribution. Thus, the genetic structure of the wild lima bean in Mexico is more complex than previously thought, and the presence of three gene pools (MIa, MIb, and MII), each one possessing relatively high levels of genetic diversity, is proposed [58]. Other work, including *P. lunatus* populations from different areas of America and germplasm, was analysed using two intergenic spacers of chloroplast DNA: *atpB-rbcL* and *trnL-trnF*. Three groups (AI, MI, MII) of genotypes were found, confirming the existence of Mesoamerican and Andean gene pools and multiple origins of domestication for the MA gene pool. For MI, western central Mexico was proposed as the domestication area, and for MII this was between Guatemala and Costa Rica [59].

#### 5.4. Tepary beans

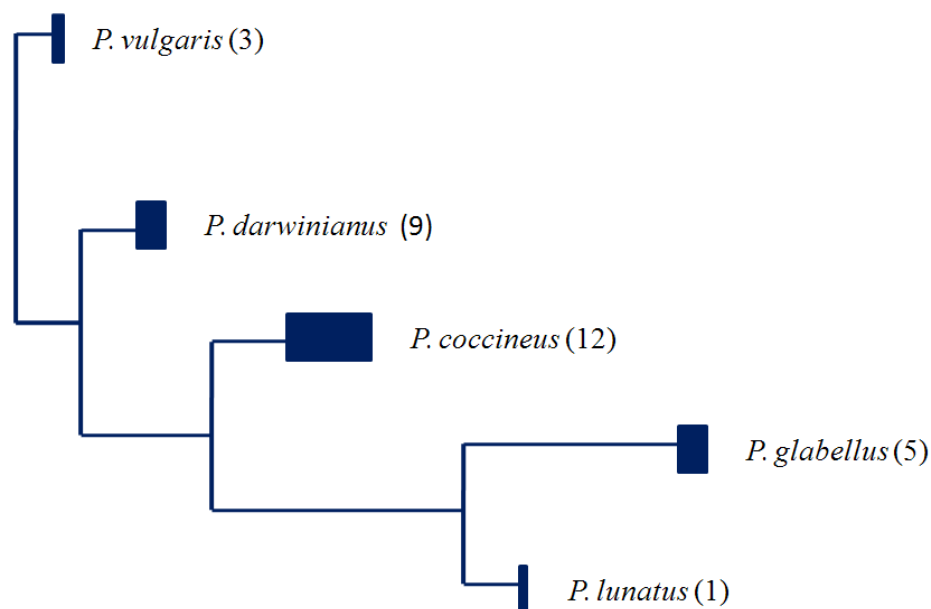
Since few genetic tools have been developed or tested for tepary bean, Blair et al. [60] validated one set of gene-derived and non-gene simple sequence repeat or microsatellite markers from the common bean in tepary bean cultivars and wild relative accessions. They then evaluated the genetic diversity and population structure of tepary bean accessions to determine if leaf morphology variants are valid as separate subgroups of wild tepary beans; if *P. parvifolius* was a separate variant or species; and if cultivated tepary beans originated from one domestication event or several events. The analysis of 140 tepary bean genotypes showed that a single domestication was likely as the cultivars were most closely related to accessions from Sinaloa and northern Mexico, and that diversity was much higher in the wild genotypes compared to the cultivated ones. *P. parvifolius* was classified as a separate species by population structure analysis while the variants *P. acutifolius* var. *acutifolius* and var. *tenuifolius* were admixed and inter-crossed. *P. latifolius* was not a valid species or variant of *P. acutifolius*, but represents a group of cultivars within the tepary bean. Other recent work was focused on the analysis of the agro-morphological variation of *P. acutifolius* germplasm in Botswana, where low genetic diversity was found [61].

#### 5.5. Acalete beans

Total seed protein variability in a sample of 163 entries of year bean (*P. polyanthus*), including wild, feral and cultivated forms of the whole range of distribution in Latin America, was studied using 1-dimensional SDS/PAGE and 2-dimensional IEF-SDS/PAGE. Ten different patterns were observed in this crop. Eight of these are found in the Mesoamerican materials, the other two of those in the northern Andes. The highest diversity is found in the wild ancestral forms present in central Guatemala with six patterns. The 'b' pattern predominant in all Mesoamerican cultivated materials and is also present at low frequency in Colombia. The 'k' pattern, predominant in the northern Andes, is present in Costa Rica. These results, together with information on indigenous names for the crop, suggest that there is a single gene pool domesticated from a wild ancestor still present in Guatemala, and distributed afterwards to the northern Andes, but with a clinal genetic drift from Mesoamerica to the Andean region [62].

## 6. Comparisons of genetic diversity among bean species

The diversity and relationships among species of *Phaseolus* complex were analysed using chloroplast DNA. Restriction patterns were used to identify polymorphisms and assess the type of mutations detected, and identify regions of high variability (Fig. 2). There is high cpDNA variability within *P. coccineus* but other species as *P. vulgaris* and *P. coccineus* subsp. *glabellus* show a very distinct cpDNA genotype compared to the former species. These evidences strongly suggests that *P. coccineus* subsp. *glabellus* belongs to a different but as yet undetermined section of the genus. In *P. coccineus* subsp. *darwinianus* (= *P. polyanthus*), the cpDNA lineage was in disagreement with data obtained from nuclear markers, and suggested a reticulated origin by hybridization between *P. coccineus* as the male parent and an ancestral *P. polyanthus* type, closely allied to *P. vulgaris*. Molecular markers are an important strategy for elucidating phylogenetic relationships; in addition, accurate phylogenies will require analyses of both nuclear and cytoplasmic genomes [63].



**Figure 2.** Dendrogram of five *Phaseolus* species analysed by cpDNA restriction patterns. Adapted from data of Llaca et al. [63].

Hamann et al. [64] identified 18 species from 90 genotypes using SSR markers, where the species *P. vulgaris*, *P. lunatus*, *P. coccineus*, *P. acutifolius* and *P. polyanthus* showed four specific (GATA) sequence patterns that help to clearly separate auto-pollinated (*P. vulgaris* and *P. lunatus*) from allogamous species as *P. coccineus*, but this species was found to have lower intra-specific variation. Gaitán-Solís et al. [65] later isolated, cloned and sequenced genomic DNA fragments into three gene libraries and then evaluated the polymorphisms of 68 SSRs. Markers were capable of separating germplasm on the basis of *Phaseolus* species: *P. coccineus*, *P. polyanthus*, *P. acutifolius*, and *P. lunatus*. Blair et al. [66] used genic and genomic microsatellites

to analyse allele diversity and heterozygosity in *P. vulgaris* and *P. acutifolius*. Genic sequence SSRs were more polymorphic than genomic SSR. SSR distinguished between Mesoamerican and Andean gene pools, and separated genetic races into each gene pool and into wild from cultivated germplasm. Andean germplasm was more polymorphic at both inter- and intra-population levels. Contrasting results were found by Benchimol et al. [67], who analysed Mesoamerican and Andean *P. vulgaris* accessions and reported low values of polymorphisms, likely due to the domestication process [68].

In Italy, 66 genotypes representing 14 local varieties of *P. vulgaris* and nine of *P. coccineus*, collected through regions of Marche using ISSR markers, SSRs and cpSSRs, were analysed. Farmers' selection and adaptability to variable environments have provoked bean preservation and diversification. A total of 71% of local varieties of *P. vulgaris* come from Andean origins [69]. Chacón et al. [70] analysed 31 accessions of *Phaseolus* (27 from *P. coccineus* including both *P. coccineus* subspecies *darwinianus* and *glabellus*; three from *P. vulgaris* and one from *P. lunatus*) using restriction patterns of cpDNA. Molecular analysis clearly differentiated between *Phaseolus* species, and *P. coccineus* showed the highest molecular polymorphism values in both wild and cultivated accessions compared with all other species [71, 72]. In Mexico, Ramírez et al. [73] characterized 107 common bean populations, 42 ayocote beans and one acalete bean on the basis of morpho-agronomic traits. Ayocote beans showed more diverse seed coat colours than common beans (54.8% purple, 26.2% black, 19.0% white and brown).

## 7. Our modest advances

### 7.1. About the origin, domestication and classification of *Phaseolus*

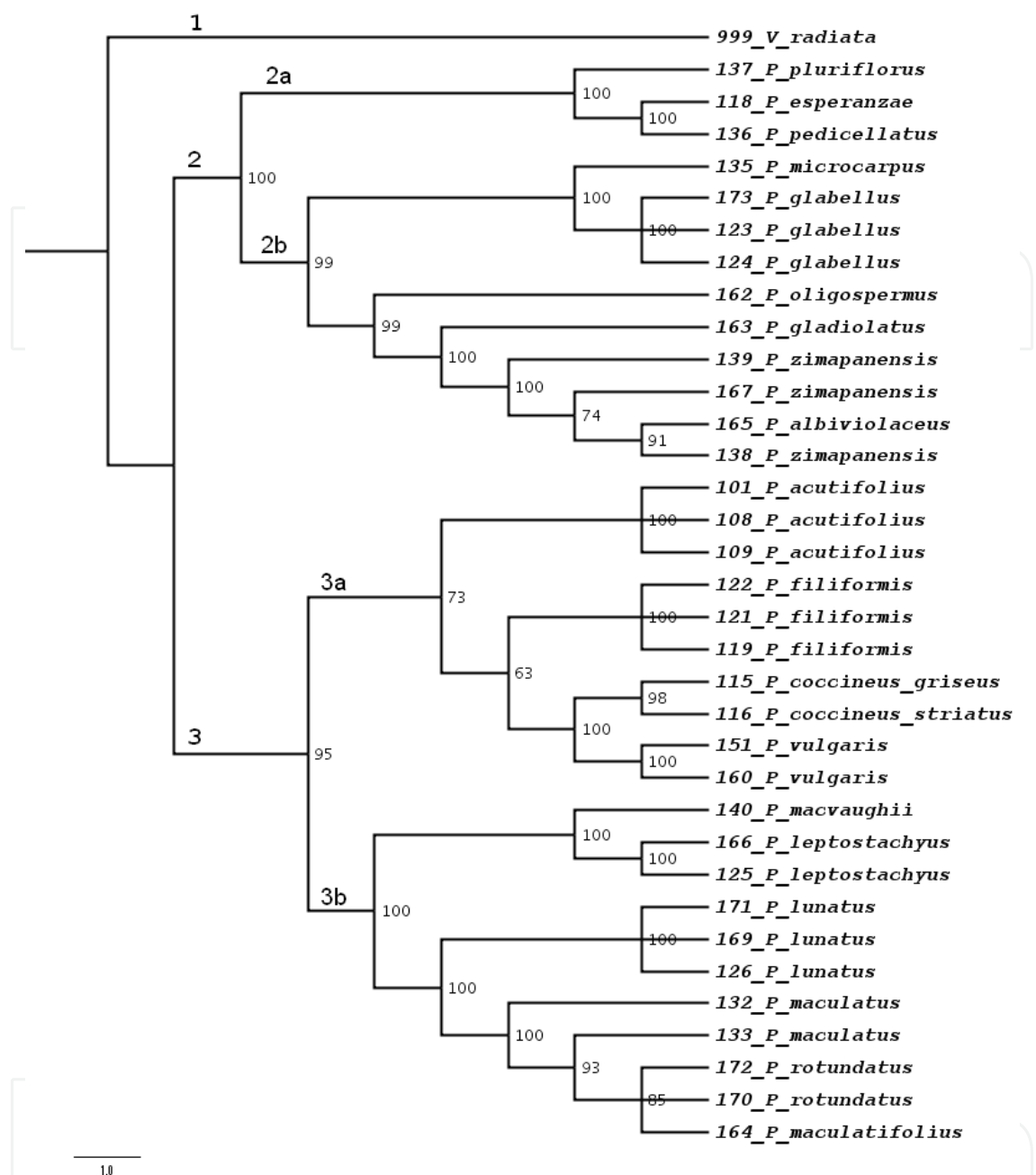
Hernández-López et al. [74] published a review paper that analysed classic works focused on determining and locating the centres of origin and domestication of *P. vulgaris*, assuming that these areas are major sources of populations carrying useful genes for breeding, and because such populations can improve our understanding of the evolution, diversification and conservation of the species. Despite the broad and abundant information published over decades, new information is consistently published, new evidence found, and new strategies such as genomic and genetic techniques based on DNA analysis applied in these studies. The accumulated knowledge derived from varied sources including archaeology, agronomy, ethno-linguistic, ethnobotany, molecular biology, biochemistry, physics etc. is currently being applied in order to study and clarify origins, domestication and diversification patterns, phylogeographical relations, among others. Therefore, the use of tools based on molecular technologies and genomics should give definitive evidence on the origin, domestication and genetic diversity of *Phaseolus* [11, 74, 75].

As has been described by Muruaga-Martínez et al. (unpublished data), recent re-collection tours have been conducted in order to clarify the real and current state of genetic resources of *Phaseolus* across Mexico. One major problem for taxonomy, phylogeny or systematics studies in *Phaseolus* is that most specimens belong to herbariums and no 'fresh' plants are available. Thus, our work group assumed the necessity to re-collect *Phaseolus* specimens. We then

subjected the germplasm to genetic analysis using molecular marker strategies and reproduced them under controlled conditions for future works, and to preserve endangered species and specimens. The first expeditions (2010-2012) yielded the collection of more than 100 samples (seeds), which comprised 19 species, including two subspecies of *P. coccineus* (*P. coccineus griseus* and *P. coccineus striatus*). Three species in this collection (*P. albiviolaceus*, *P. maculatifolius* and *P. rotundatus*) had not been studied before. Villarreal-Villagrán et al. [76] analysed them by using five *trnT-trnL*, *trnL-trnF*, *rpl16*, *rpoC1-rpoC2*, *rps14-psaB* non-coding regions of chloroplastic DNA amplified by PCR (polymerase chain reaction). Cluster analysis confirmed with strong bootstrap support that the genus *Phaseolus* is a monophyletic group that can subdivide itself into two major lineages: one includes *P. pluriflorus*, *P. esperanzae*, *P. pedicellatus*, *P. microcarpus*, *P. glabellus*, *P. oligospermus*, *P. gladiolatus*, *P. zimapanensis* and *P. albiviolaceus*; and the other includes *P. filiformis*, *P. acutifolius*, *P. vulgaris*, *P. coccineus striatus*, *P. coccineus griseus*, *P. macvaughii*, *P. leptostachyus*, *P. lunatus*, *P. maculatus*, *P. maculatifolius* and *P. rotundatus*. The topology of the dendrogram obtained agreed with the topology of *Phaseolus* recognized to this date, which was obtained using only the ribosomal ITS and chloroplast *trnK* locus [77]. The exception was *P. albiviolaceus*, a species not studied before, that according to traditional morphological criteria, belongs to the *Pedicellatus* group, but which in this study appeared with the *Tuerckheimii* group. The other two species that were characterized for the first time in a molecular phylogeny are *P. maculatifolius* and *P. rotundatus*, both of which were clustered within the *polystachios* group (Fig. 3).

## 7.2. About the genetic diversity analyses of *Phaseolus*

The analysis of Mexican common bean core collection using SSR and AFLP markers revealed that the highest genetic diversity is found in central Mexico and Chiapas, which seems to be an important diversity centre in the south. SSR analysis indicated a reduced number of shared haplotypes among accessions and core collection has no duplicated accessions [40]. Hernández-López et al. [21, 22] evaluated the diversity and genetic relations of one collection of bean populations produced after the random crossing among wild and domesticated or cultivated bean genotypes throughout Mexico to assess its usefulness for *Phaseolus* breeding due the detection of six SCAR markers associated with common blight (*Xanthomonas axonopodis* pv. *phaseoli*) resistance, as well as four for anthracnose (*Colletotrichum lindemuthianum*). The results indicated significant morphological variability in the common bean germplasm. AFLP marker analysis revealed high genetic diversity in those germplasms from north-central and central Mexico. Germplasm from Morelos, Guanajuato, Querétaro, Durango, and Tamaulipas showed the highest genetic diversity indexes. Cluster analysis was not consistent with classification forms and their distribution based on geographical or agro-ecological origin. Germplasm from Guanajuato and Tlaxcala showed the highest SCAR frequencies for both diseases. Genetic diversity and SCAR detection for resistance to anthracnose and common blight was analysed in two *P. coccineus* collections, one originating from the 'Huasteco Karst' located mainly in the state of Puebla, Mexico. The other group of ayocote accessions comes from the state of Veracruz. Analysis of germoplasm from Huasteco Karst revealed great genetic variability among and within accessions as well as high genetic differentiation among germplasm. Resistance to anthracnose was more frequent into the germplasm [31, 32]. Ayocote beans from



**Figure 3.** Dendrogram of 19 *Phaseolus* species analysed by gene sequencing and maximum parsimony method. Numbers indicate percentages of replication of node topology assessed by robustness method [76].

the state of Veracruz are highly variable, as those from Puebla; the domestication could have happened under reproductive isolation conditions and thus clearly differentiated lineages were produced based on the origin of each population. Since the ayocote beans analysed here include some accessions with high genetic variability, they are candidates for conservation and exploitation for *Phaseolus* breeding. Seed exchange in locations where ayocotes are cultivated through consumers and producers increase genetic diversity. Additionally, *P. coccineus* shows moderate open pollination (14.7%), which helps high genetic variation. The



results indicated high genetic variability among and within accessions of ayocote beans from the states of Puebla and Veracruz; the data also suggest them to be an important source of allele useful for inclusion in breeding programmes of *P. coccineus* or *P. vulgaris* [33].

## 8. Concluding remarks

In order to understand genetic variation patterns and to reinforce the richness and genetic potential of *Phaseolus*, it is necessary to preserve, characterize and take advantage of Mexican germplasm. Knowledge of genetic diversity in the common bean could give us a better view for conservation as well as management and use of these plants' genetic resources. Despite significant effort and financial support by the Mexican government, this is not enough. Last year a new national centre for genetic resources was constructed and financed (Centro Nacional de Recursos Genéticos, CNRG, INIFAP) as well as initiatives to preserve not only beans but also other major Mexican crops such as maize, amaranth, cocoa, pumpkin, agave, avocado, etc. (SINAREFI-SAGARPA, Sistema Nacional de Recursos Fitogenéticos para la Alimentación y la Agricultura). We suggest that this financial support is maintained or improved, and research consistently supported through mid- and long-term projects.

Collection tours have demonstrated fast genetic erosion in most regions where wild types of *Phaseolus* were sought. Unfortunately, the social and economic situation in Mexico has provoked the migration of farmers and rural people, who have originally maintained and preserved genetic resources and their diversity in order to improve our lives. Thus, basic crops are leaving and are frequently changed by 'highly profitable' crops. The growth of urban populations has increased pollution and should have consequences through the alteration of natural reservoirs of natural populations inside 'Reservas de la Biósfera' or 'protected areas'. The Mexican government offers incipient economic support to those farmers that preserve genetic diversity *in situ* on their land. This strategy is appropriate for genetic resource conservation, but is currently not enough.

Genetic diversity observed in *Phaseolus* germplasm collections represent an important sample of total genetic variability contained in the genus. Molecular marker strategies and other recent and advanced techniques such as sequencing, genomics, proteomics and other '-omics' could help us to better understand genetic structure, genetic relations, genetic patterns of dispersion, and variation, promising germplasm to be used as parents or in breeding programmes based on their outstanding traits, etc. Biotechnology will not substitute traditional strategies of germplasm characterization or the taking advantage of *Phaseolus* germplasm. Biotechnology should be one allied or even more strengthen traditional breeding to accelerate and improve breeding methods and analysis strategies of genotypes.

Finally, we suggest that genetic diversity is a major challenge for botanists and taxonomists, biotechnologists and breeders, as well as to the governmental institutions of Mexico, towards maintaining natural populations both *in situ* and *ex situ*, to avoiding their loss, to increasing the strategies for their use, and to exploiting their benefits.



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