RFLP diversity of common bean (*Phaseolus vulgaris*) in its centres of origin

VIVIANA L. BECERRA VELÁSQUEZ AND PAUL GEPTS
Department of Agronomy and Range Science, University of California, Davis, CA 95616-8515, U.S.A.

Corresponding Editor: R. Kemble
Received May 11, 1993
Accepted November 2, 1993

BECERRA VELÁSQUEZ, V.L., and GEPTS, P. 1994. RFLP diversity of common bean (*Phaseolus vulgaris*) in its centres of origin. Genome, **37**: 256–263.

Eighty-five wild and cultivated accessions of common bean (*Phaseolus vulgaris* L.), representing a wide geographic area in the centres of domestication were tested for restriction fragment length polymorphisms (RFLPs). Genomic DNA was digested with one of three restriction enzymes (*Eco*RI, *Eco*RV, and *Hin*dIII) and hybridized to 12 probes distributed throughout the common bean genome. Accessions could be classified into two major groups with a distinct geographical distribution in Middle America and the Andes. Within each gene pool, cultivated accessions clustered together with wild forms from the same geographical area supporting the multiple domestications hypothesis for this crop. Estimates of Nei's genetic distances among the cultivated races from the two different gene pools varied from 0.12 to 0.56 and among races from the same gene pool from 0.04 to 0.12, suggesting that the divergence in *Phaseolus vulgaris* has reached the subspecies level. The level of genetic diversity (H_t = 0.38) was twice the value obtained with isozyme analysis. Genetic diversity within races (H_s = 0.27) was four to five times higher compared with isozymes, but genetic diversity between races (D_{st} = 0.11) was similar for both categories of markers. These results corroborate previous studies on the characterization of genetic diversity in common bean that clearly showed two distinct gene pools, Middle American and Andean. Moreover, RFLP markers are superior to isozymes because they provide better coverage of the genome and reveal higher level of polymorphisms.

Key words: common bean, restriction fragment length polymorphism, domestication, genetic diversity.

BECERRA VELÁSQUEZ, V.L., et GEPTS, P. 1994. RFLP diversity of common bean (*Phaseolus vulgaris*) in its centres of origin. Genome, **37** : 256–263.

Quatre-vingt cinq accessions indigènes ou cultivées de haricot commun (*Phaseolus vulgaris* L.), représentant une grande surface géographique dans des centres de domestication, ont été testées pour le polymorphisme des longueurs de fragments de restriction (PLFR). L'ADN génomique a été digéré par l'une ou l'autre des trois enzymes de restriction : *Eco*RI, *Eco*RV et *Hin*dIII, puis hybridé avec 12 sondes réparties à travers le génome du haricot commun. Les accessions ont pu être classées en deux groupes principaux, ayant une distribution géographique distincte dans le « Middle America » et les Andes. À l'intérieur de chaque pool de gènes, des accessions cultivées se sont regroupées avec des formes indigènes de la même région géographique, ce qui appuie l'hypothèse des domestications multiples pour cette espèces cultivée. Des estimés de distances génétiques, selon Nei, entre les races cultivées de deux pools géniques différents ont varié de 0,12 à 0,56 et, entre les races d'un même pool génique, de 0,04 à 0,12, ce qui suggère que la divergence chez *Phaseolus vulgaris* a atteint le niveau de sous-espèce. Le niveau de diversité génétique (H_t = 0,38) a été le double de sa valeur obtenue par les analyses des isozymes. La diversité génétique à l'intérieur des races (H_s = 0,27) a été de quatre à cinq fois plus élevée par comparaison aux isozymes, mais la diversité entre les races (D_{st} = 0,11) a été similaire pour les deux catégories de marqueurs. Ces résultats corroborent les études antérieures sur la caractérisation de la diversité génétique chez le haricot commun, laquelle a clairement démontré l'existence de deux pools géniques distincts, soit celui de « Middle America » et celui des Andes. De plus, les marqueurs de PLFR sont supérieurs aux isozymes, parce qu'ils fournissent une meilleure couverture du génome et révèlent un niveau plus élevé de polymorphisme.

Mots clés : haricot commun, polymorphisme des longueurs de fragments de restriction, domestication, diversité génétique.

[Traduit par la rédaction]

Introduction

Common bean (*Phaseolus vulgaris* L.; $2n = 2x = 22$) is an annual leguminous crop that originated in the America and is now grown worldwide. It is especially important in Latin America and eastern Africa, regions that account for 47 and 24%, respectively, of global production of dry bean (Pachico 1989). The wide variety of environments under which this species is grown has led to substantial phenotypic variation, especially for growth habit, seed type, phenology, and photoperiod sensitivity (Wallace 1985; Debouck 1991; Voysest and Dessert 1991). This high level of morphological and physiological diversity, together with the widespread geographic distribution, had until recently obscured the actual pattern of domestication and genetic relatedness among cultivars. Phenologically similar genotypes may not be genotypically closely related and, conversely, phenotypically distinct cultivars may actually be closely related.

Recent investigations in common bean using seed proteins and isozymes have provided insights into the organization of the genetic diversity in this species. Both seed proteins and isozyme analysis conducted in a sample of wild bean and landraces from Latin America, show that common bean was domesticated separately in Middle America and the southern Andes (Gepts et al. 1986; Gepts and Bliss 1986; Sprecher 1988; Koenig and Gepts 1989; Singh et al. 1991*c*; Khairallah et al. 1990, 1992). Domestications in these two areas led to two distinct groups of cultivars (Gepts 1990, 1993*b*; Gepts and Debouck 1991) that could each be further subdivided into three races (Singh et al. 1991*a*).

Printed in Canada / Imprimé au Canada

TABLE 1. Cultivated and wild bean germplasm analyzed for restriction length polymorphisms

Identification				
CIAT	Other	Name	Country[a]	Race[b]
	Cultivated accessions: Middle American gene pool			
G02511	PI313590	Boyacá 22	CLB	J
G10945	DGD78/016A	Flor de Mayo	MEX	J
		Cacahuate criollo	MEX	J
		Canario regional	MEX	J
		Flor de Mayo	MEX	J
G14027		Orgulloso	MEX	J
G14914		Azufrado	MEX	J
G01764	PI309712	Apetito	MEX	J
		Jalisco 20	MEX	J
G01344	PI208776		NCA	J
G13625	X16775	Flor de Mayo	MEX	J
		Rosa de Castilla	MEX	J
G13686	CP125	Naranja coral	MEX	J
		Garbancillo zarco	MEX	J
		Frijola	MEX	J
		Pinto nacional	MEX	D
G11010	DGD78/066A	Bayo regional	MEX	D
G04399		Tamaulipas 9	MEX	D
G011012	DGD78/068	Ojo de liebre	MEX	D
		Sutter Pink	USA	D
		Mexico 222	MEX	D
		Bayo Durango	MEX	D
G10982	DGD78/046B	Pinto	MEX	D
		Pinto U.I. 114	MEX	D
G10971	DGD78/035G	Bayo	MEX	D
G02618	PI313755	Col. #168	MEX	D
		ICA-Pijao	CLB	M
G04830		Rio Tibagi	BZL	M
	PI152326	Negro redondo	ECD	M
		Black Turtle Soup	USA	M
		Sal	USA	M
G03807	I1098	Brasil 2	BZL	M
G04017	PI54	Carioca	BZL	M
		Rim de porco	BZL	M
G04495		Porrillo Sintético	ELS	M
G02997		Rabia de gato	GTA	M
	Cultivated accessions: Andean gene pool			
		California Dark Red Kidney	USA	N
G01326	PI207300	Guanajuato 4	MEX	N
G02488	PI313471	Michoacán 61	MEX	N
G04520		Estrada rosado	CLB	N
G04717		Valle 18	CLB	N
G05708		Sangretoro	CLB	N
G12368	CAT0784	Canario alargado	ECD	N
		Bagajo	BZL	N
G12720		Calabozo	CLB	N
G14660	CAT0310	Uribe	CLB	N
	PI15020	Burrito	CLE	C
		Frutilla corriente	CLE	C
G04474	PI151023	Coscorrón corriente	CLE	C
		Tórtola corriente	CLE	C
		Cocacho	PER	P
G12327	CAT0668	Cargamanto ecuatoriano	ECD	P
G12438	CAT0963	Bolón amarillo	ECD	P
G12421	CAT0913	Bolón rojo	ECD	P
G12207	CAT0004	Canario	ECD	P
	PG0128	Nuña coneja	PER	P
G12587	PG0138	Nuña callashina	PER	P
G00111	PI153714	Huasca Huallaga colorado	PER	P
G12229	CAT0246	Bola	ECD	P
G12407	CAT0839	Bolón bayo	ECD	P
G12709	Sañudo-0045	Mortiño	CLB	P
G05702		Cargamanto	CLB	P
	Wild accessions: Middle American gene pool			
G12868	PI318699		MEX	
G12851	PI201011		GTA	
G12922	PI417683		MEX	

TABLE 1 (*concluded*)

Identification				
CIAT	Other	Name	Country[a]	Race[b]
G9989A	HM735bulk		MEX	
G12957	PI417786		MEX	
G11056	DGD78/148		MEX	
G12873	PI325678		MEX	
G10008	NI401		MEX	
	X634		CLB	
G11055	DGD78/147		MEX	
G12866	PI313697		MEX	
	Wild accessions: Andean gene pool			
G21245	DGD1962		PER	
G10025			ARG	
G19902	DGD650		ARG	
G21195	DGD632		ARG	
G21197	DGD1711		ARG	
G19894	DGD636		ARG	
G19896	DGD639		ARG	
G21199	DGD1713		ARG	
G21200	DGD1715		ARG	
G21194	DGD621		ARG	
G21198	DGD1712		ARG	
G12856B			PER	

[a]ARG, Argentina; BZL, Brasil; CLE, Chile; CLB, Colombia; ECD, Ecuador; ELS, El Salvador; GTA, Guatemala; MEX, Mexico; NCA, Nicaragua; PER, Perú; USA, United States of America.
[b]C, Chile; D, Durango; J, Jalisco; M, Mesoamerica; N, Nueva Granada; P, Perú.

In the work reported here, we extend these observations based on seed protein and isozymes to an analysis of RFLP levels and geographic distribution. A sample of wild and cultivated accessions from the Middle American and Andean groups was analyzed with 12 homologous probes. Our results confirm the high levels of RFLP and the divergence between Middle American and Andean genotypes observed previously in common bean (Nodari et al. 1992).

Materials and methods

Plant material

Eighty-five common bean accessions were screened for RFLP diversity (Table 1). Germplasm accessions were selected among cultivated genotypes and wild relatives to represent the range of genetic diversity based on phaseolin and isozyme diversity data (Gepts et al. 1986; Koenig and Gepts 1989; Koenig et al. 1990; Singh et al. 1991*c*) and geographical distribution of the common bean primary gene pool.

The 63 cultivated accessions included 36 genotypes from the Middle American gene pool and 26 from the Andean gene pool. The Middle American gene pool included 15 accessions of race Jalisco, 11 of race Durango, and 10 of race Mesoamerica. The Andean gene pool was represented by 10 accessions of race Nueva Granada, 4 of race Chile, and 12 of race Peru (Table 1). The sample of wild genotypes consisted of 22 accessions, half of which originated in the Middle American gene pool and half in the Andean gene pool. The seed samples (Table 1) were kindly provided by Drs. S. Singh and M. Iwanaga (Centro Internacional de Agricultura Tropical, CIAT, Cali., Colombia).

Clones

Ten low-copy number clones of unknown function were chosen at random from a *Pst*I *Phaseolus vulgaris* genomic library (Nodari et al. 1992): D1003, D1020, D1026, D1028, D1031, D1032, D1041, D1042, D1094, and D1163. Two clones of known function were also used: chalcone isomerase (pCHI1; Mehdy and Lamb 1987) and chalcone synthase (pCHS1; Ryder et al. 1984).

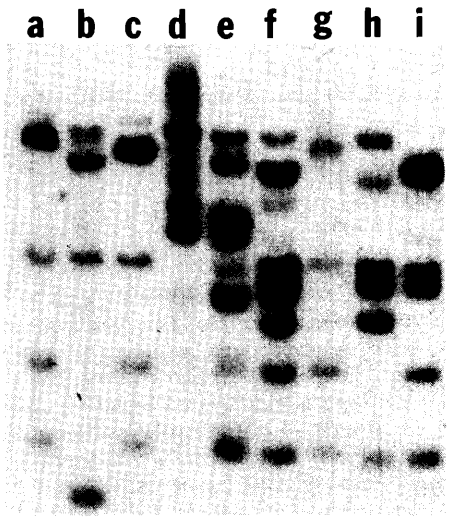

FIG. 1. Genomic DNA of a sample of cultivated *Phaseolus vulgaris* germplasm digested with *Hin*dIII and hybridized to *Pst*I clone D1031. Marks on the left side of the figure indicate the migration distances of the 23.3-, 9.6-, 6.6-, 4.4-, 2.3-, and 2.0-kb fragments of λ digested with *Hin*dIII. Lane a, ICA-Pijao; lane b, California Dark Red Kidney; lane c, Naranja Coral; lane d, Garbancillo; lane e, Frijola; lane f, Bayo Durango; lane g, G10982; lane h, Pinto UI 114; and lane i, Porrillo Sintético.

DNA isolation, digestion, and electrophoresis

Four or more plants from each accession were grown in the greenhouse for DNA extraction. Samples of young and healthy leaves were harvested before flowering, bulked, and frozen at −70°C for future DNA extraction. Approximately 5 g of leaf tissue from each accession were used to isolate DNA as described earlier (Nodari et al. 1992; Gepts et al. 1992). Digestion with restriction enzymes and agarose gel electrophoresis were performed according to Nodari et al. (1992) and Gepts et al. (1992).

DNA Southern transfer and hybridization

The DNA fragments were transferred to Zetabind membranes following the protocol of the manufacturer (AMF-CUNO, Meriden, Connecticut), as were the Southern hybridizations. Thirty to 40 ng of the bean genomic DNA insert was labeled with [^{32}P]dCTP using the random primer method (Feinberg and Vogelstein 1983). Labeled DNA was separated from unincorporated nucleotides by chromatography on a Sephadex G-50 column. Membranes were hybridized for 20 h at 42°C. After hybridization, membranes were rinsed twice with 2× SSC, 0.1% SDS, and 0.1× SSC at room temperature for 15 min and once with 0.1× SSC, 0.1% SDS at 60°C for 1 h. X-ray film was exposed to the blots in the presence of intensifying screens at −70° for 2–3 days.

Scoring of polymorphisms

Fragment lengths in autoradiography were scored by measuring the distance of migration from the wells. These distances were plotted and regressed to a standard curve of lambda DNA digested with *Hin*dIII, which included fragments from 125 bp to 23 kbp. To facilitate consistent scoring, two control accessions, ICA-Pijao from the Middle American gene pool and California Dark Red Kidney from the Andean gene pool, were included on all membranes. Data were scored as the presence or absence of bands (0, absence; 1, presence).

Statistical analysis

To calculate genetic distances, a single, randomly chosen band per probe was analyzed to avoid weighting results in favor of probes revealing multiple bands. Individual analyses of restriction fragment diversity following digestion of genomic DNA by each of the three restriction enzymes were conducted as was a joint analysis with the three data sets.

Estimates of total gene diversity (H_t), as well as the partitioning into intrapopulation (H_s) and interpopulation (D_{st}) gene diversity, were calculated from the allele frequencies of restriction fragments in each cultivated race and in each wild type according to Nei (1987). Dendrograms based on Nei's genetic distance were constructed based on the unweighted paired group method (Sneath and Sokal 1973) with the SAHN program of NTSYS, version 1.6 (Rohlf 1990). In addition, a principal component analysis was conducted using the PRINCOMP program of SAS Institute Inc. (1988) based on the covariance matrix of RFLP frequencies in the eight groups considered in this study (wild Middle American, three Middle American races, wild Andean, and three Andean races).

Results

Restriction fragment patterns and polymorphisms

Genomic DNA in this study was digested with three restriction enzymes (*Eco*RI, *Eco*RV, and *Hin*dIII) that had revealed the highest level of polymorphism in previous studies (Nodari et al. 1992). Hybridization was performed with 12 probes. Ten of these probes originated from a *Pst*I genomic library (Nodari et al. 1992) and two were sequences of known functions, chalcone isomerase (CHI; Mehdy and Lamb 1987) and chalcone synthase (CHS; Ryder et al. 1984). The map location of the majority of the probes had been determined earlier. Probes D1003, D1020, D1026, D1028, D1031, D1032, CHI, and CHS were located on linkage groups D10, D3, D2, D2, D5, D1, D7, and D2, respectively, whereas probe D1163 remained unassigned (Nodari et al. 1993; Gepts 1993*a*; Gepts et al. 1993). Therefore, with the exception of probes D1026, D1028, and CHS, all probes were unlinked and characterized different regions of the bean genome.

Several probes revealed multiple bands after hybridization to genomic DNA, e.g., CHS and D1031 (Fig. 1). Other probes gave patterns with one to three bands, e.g., CHI, D1003, D1028, D1041, D1163, D1020, and D1042 (Fig. 2). Multiple bands could be due to the existence of restriction sites within the sequence homologous to the probe or to gene duplication. Although it was beyond the scope of this investigation to distinguish between these two possibilities, independent information available shows that the genes for chalcone synthase constitute a multigene family of six to eight members (Ryder et al. 1987), which appear to map to a single locus on linkage group D2 (Nodari et al. 1993; Gepts et al. 1993). Multiple bands revealed by probes D1031 also mapped to a single locus on linkage group D5 (Nodari et al. 1993; Gepts et al. 1993).

The level of polymorphism observed in the entire data set was 70% when considering all probe–enzyme combinations. Polymorphism among the Middle American genotypes was somewhat higher than among the Andean genotypes (76 vs. 63%). Compared with the results of Nodari et al. (1992), between-gene-pool polymorphism was lower in this study, but within-gene-pool diversity was higher. In addition, restriction enzymes showed differences in their efficiency of detecting polymorphisms. The highest level of polymorphism among the 85 accessions was detected when genomic DNA was digested with *Eco*RV (75% of the probes showed a polymorphism), followed by *Hin*dIII (70%) and *Eco*RI (62%). These observations confirmed the results of Nodari et al. (1992). Some probe – restriction enzyme combinations failed to reveal any polymorphism in the sample analyzed here: e.g., D1032 with *Eco*RI and *Eco*RV.

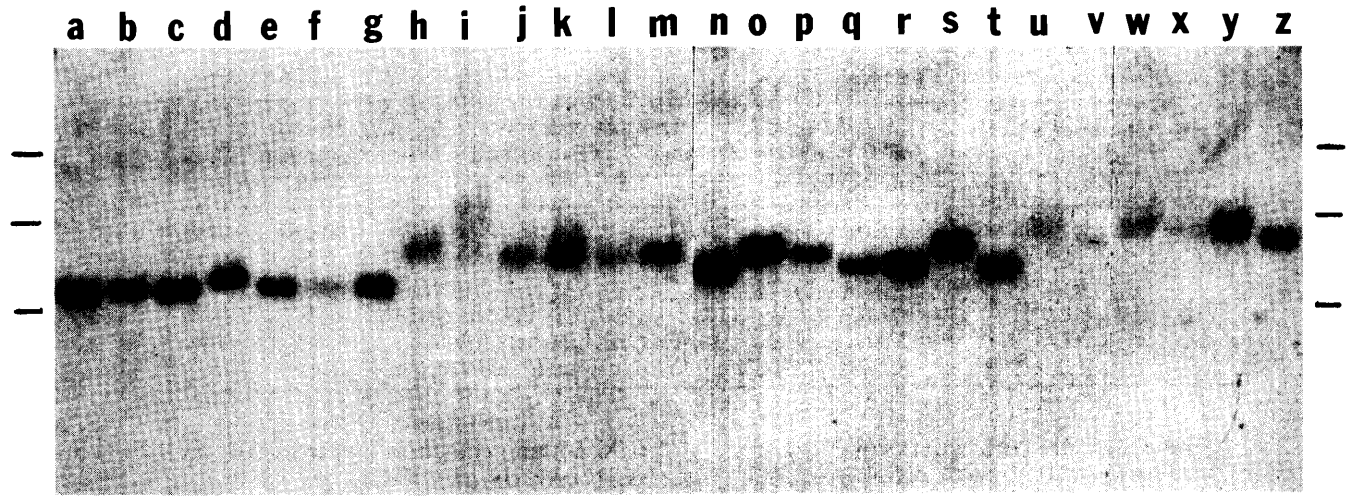

FIG. 2. Genomic DNA of a sample of *Phaseolus vulgaris* germplasm digested with *Eco*RV and hybridized to *Pst*I clone D1028. Marks on the left and right sides of the figure indicate the migration distances of the 9.6-, 6.6-, and 4.4-kb fragments of λ digested with *Hin*dIII. Lanes a–m are cultivated genotypes and n–z are wild genotypes. Based on previous isozyme and phaseolin analyses, lanes a–h and n–t are Middle American genotypes, lanes i–m and v–z are Andean genotypes, and lane u is a genotype intermediate between the two groups. Lane a, Cacahuate criollo; lane b, Jalisco 20; lane c, Pinto Nacional; lane d, Rio Tibagi; lane e, Rosa de Castilla; lane f, Ojo de Liebre; lane g, Naranja Coral; lane h, Burrito; lane i, Calabozo; lane j, Cocacho; lane k, Cargamanto Ecuatoriano; lane l, Bolón amarillo; lane m, Canario; lane n, G12868; lane o, G12922; lane p, G09989A; lane q, G11056; lane r, G12873; lane s, G10008; lane t, X634; lane u, DGD1962; lane v, G10025; lane w, G19896; lane x, DGD1713; lane y, DGD1715; lane z, G12856B.

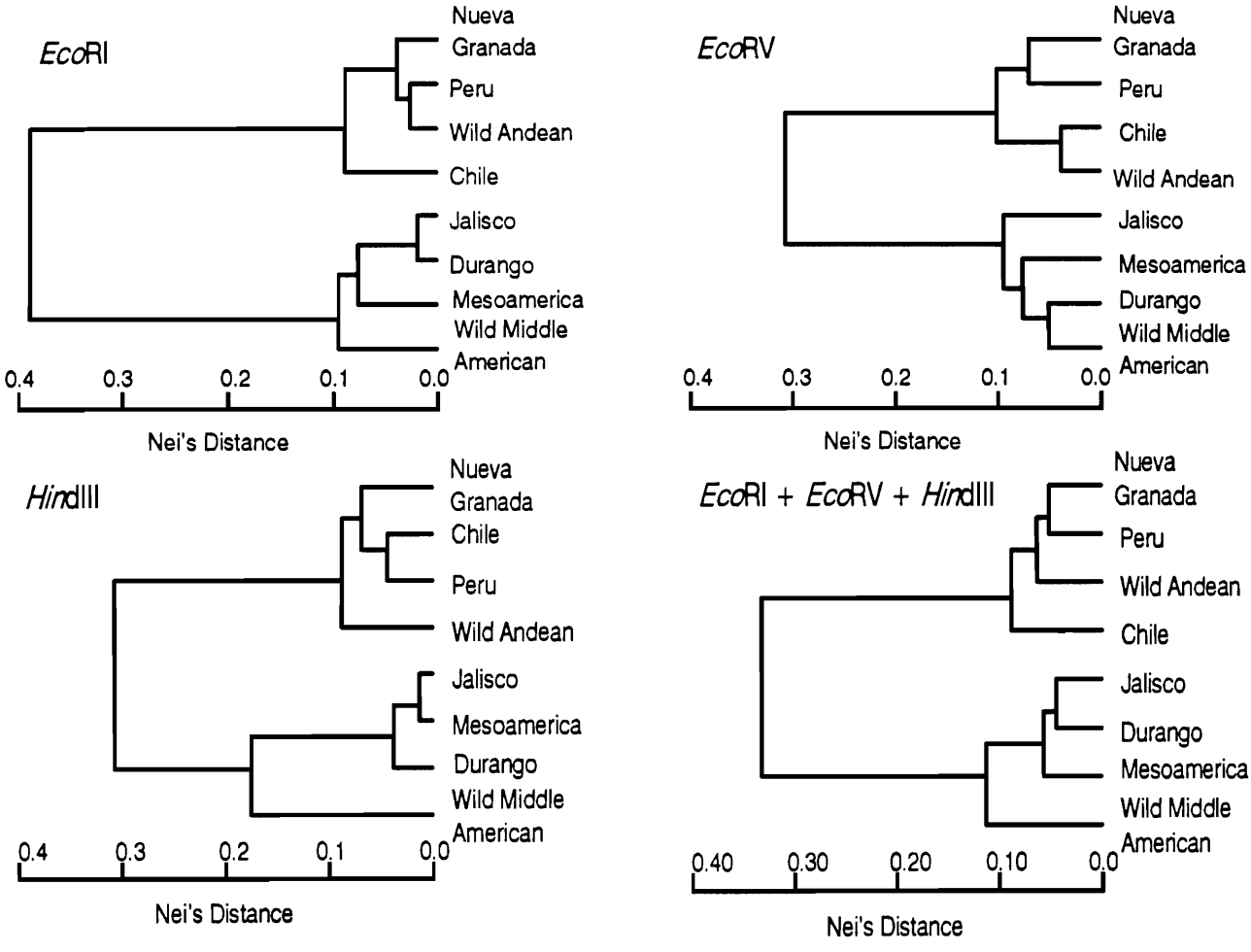

FIG. 3. UPGMA dendrogram based on Nei's distances derived from RFLPs among cultivated races and wild germplasm of *Phaseolus vulgaris*. *Eco*RI, *Eco*RV, and *Hin*dIII represent data sets based on digestion of genomic DNA with each enzyme. *Eco*RI + *Eco*RV + *Hin*dIII represents a joint data set resulting from pooling of the three preceding data sets.

TABLE 2. Nei's distances for RFLPs of 12 single copy nuclear DNA sequences among cultivated races and wild forms of common bean (*Phaseolus vulgaris* L.) after digestion of genomic DNA with *Eco*RV

		Middle America			Andes			
		Jalisco	Durango	Mesoamerica	Wild	Nueva Granada	Chile	Peru
Middle America	Wild	0.12	0.05	0.06	0.33	0.31	0.34	0.22
	Jalisco		0.07	0.07	0.25	0.28	0.39	0.13
	Durango			0.08	0.50	0.50	0.56	0.30
	Mesoamerica				0.24	0.22	0.25	0.12
Andes	Wild					0.05	0.06	0.04
	Nueva Granada						0.06	0.07
	Chile							0.08

TABLE 3. Total (H_t), within (H_s), and between (D_{st}) genetic diversity in common bean (*Phaseolus vulgaris* L.) detected by RFLP (after *Eco*RV digestion of genomic DNA) and isozyme markers

Marker type	H_t	H_s	D_{st}
Isozymes[a]	0.19	0.06	0.13
RFLPs	0.38	0.27	0.11

[a]Data of Koenig and Gepts (1989) and Singh et al. (1991*c*).

Genetic distances

Phenetic dendrograms of genetic distances were calculated for four data sets corresponding to the three individual restriction digestions and the pooled data. The four dendrograms clearly showed the existence of two major groups of genotypes with a distinct geographical distribution (Fig. 3). In general, cultivated genotypes clustered with wild accessions from their respective geographic areas. Wild accessions in the upper branch included material from Mexico, Central America, and Colombia. Cultivated accessions in the upper branch included cultivars from Mexico, Central America, Colombia, and Brazil. Wild accessions in the lower branch included materials from Peru and Argentina. Cultivated accessions in this branch included accessions from the Andean region (Colombia, Ecuador, Peru, and Chile) and Brazil. Exceptions to this pattern included two cultivars that had been classified as Andean based on phaseolin and isozyme data but were classified as Middle American based on RFLP data: 'Nuña callashina' from Peru and 'Cargamanto' from Colombia. Conversely, accessions 'Rabia de Gato' from Guatemala, 'Sal' from the U.S.A., 'Negro redondo' from Ecuador, and G10008 and G11056 from Mexico were classified as Andean genotypes based on RFLP data, although they had previously been classified as Middle American materials based on phaseolin and isozyme data.

Within the two major branches, clustering of the three races and their respective wild progenitors differed among the four data sets. This observation suggested that the distinctions among these groups are not as marked as the differences between the Middle American and Andean gene pools. Comparisons among races belonging to different gene pools showed genetic distances ranging from 0.12 to 0.56 (Table 2). Genetic distances within gene pools ranged from 0.04 to 0.12. Conversely, genetic identities ranged from 0.89 to 0.96 within gene pools and from 0.61 to 0.89 betwen gene pools (not shown).

Genetic diversity

The total observed gene diversity (H_t) for RFLPs based on the *Eco*RV data set was twice the value obtained for isozyme markers (Koenig and Gepts 1989; Singh et al. 1991*c*) (Table 3). Similar values were obtained for the data sets based on the *Eco*RV, *Hin*dIII, and joint data sets.Variation within cultivated races and the two wild bean groups (H_s) was four to five times higher for RFLP markers than for isozymes, but RFLP variation between races and the two wild bean groups (D_{st}) was similar to that of isozyme markers (Table 3). RFLP analyses indicated that the cultivated races of Middle America and the Andes had levels of diversity generally lower than those in their respective wild progenitors (Table 4). An exception was the higher diversity observed in race Peru compared with the Andean wild beans.

Discussion

Genetic distances

Cluster analysis and calculations of Nei's distances and identities clearly confirmed the divergence between Middle American and Andean genotypes into two gene pools. This divergence had been observed previously using phaseolin (Gepts et al. 1986; Gepts and Bliss 1986), isozymes (Koenig and Gepts 1989; Singh et al. 1991*c*), RFLPs for nuclear sequences (Nodari et al. 1992) and mtDNA (Khairallah et al. 1990, 1992), morphological data (Singh et al. 1991*b*), and F_1 hybrid weakness (Gepts and Bliss 1985; Koinange and Gepts 1992).

Within each gene pool, cultivated accessions clustered with wild forms of their respective geographic areas similar to that observed previously for phaseolin (Gepts et al. 1986; Koenig et al. 1990) and isozymes (Koenig and Gepts 1989; Singh et al. 1991*c*). This parallel pattern of geographic variation between wild and cultivated common bean suggests a pattern of multiple domestication along the distribution area of wild common bean in the highlands of Middle America and the Andes (Gepts 1990, 1993*b*; Gepts and Debouck 1991).

The values of Nei's distances and identities between the Middle American and the Andean gene pools (>0.10 and <0.80, respectively) suggest that the divergence between these gene pools has reached the subspecific level (reviewed in Doebley 1989, 1992). Further evidence supporting this level of divergence is provided by the existence of partial reproductive isolation leading to F_1 hybrid weakness in

TABLE 4. Average heterozygosity for isozyme and RFLPs for single copy nuclear DNA in common bean (*Phaseolus vulgaris* L.) after *Eco*RV digestion of genomic DNA

			Average heterozygosities	
		n	Isozymes[a]	RFLPs for single copy nuclear DNA
Middle America	Wild	11	0.13	0.37
	Jalisco	15	0.06	0.27
	Durango	11	0.04	0.23
	Mesoamerica	10	0.10	0.33
	All cultivated Middle American	36	0.09	0.31
	All Middle American	47	0.10	0.32
Andes	Wild	12	0.07	0.23
	Nueva Granada	10	0.01	0.21
	Chile	4	0.00	0.22
	Peru	12	0.02	0.30
	All cultivated Andean	26	0.03	0.28
	All Andean	38	0.06	0.25

[a]Data of Koenig and Gepts (1989) and Singh et al. (1991*c*).

crosses between Middle American and Andean genotypes (Gepts and Bliss 1985; Koinange and Gepts 1992). The simple genetic control of this reproductive isolation (two complementary genes: Shii et al. 1980) suggests that *P. vulgaris* may be in the process of speciation. By analogy with rice, which has two major gene pools that are partially isolated, *indica* and *japonica*, the two major gene pools of common bean could be labeled *mesoamericanus* and *andinus*.

Within each gene pool, racial subdivisions established previously based on phaseolin, isozyme, morphological, and ecological variability (Singh et al. 1991*a*, 1991*b*) were not as distinct with RFLPs as the divergence between the Middle American and Andean gene pools. There are at least three possible reasons for this observation. First, unlike the Middle American – Andean divergence, which is maintained by marked geographic isolation, races within each gene pool are subject to gene flow, which would tend to blur racial distinctions.

Second, RFLP levels are higher than those observed for phaseolin or isozymes (see below). Independent repeated mutations in different races may lead to accessions that show the same RFLP but do not share an immediate common ancestor. These accessions would show the distinct phenotype characteristic of their respective race, yet be similar for one or more RFLP markers.

Third, races may have a polyphyletic origin. The common phenotype of each race may have resulted from selection operating on several distinct ancestral genotypes. Convergent evolution would then have led to a phenotype characteristic of each race against a diverse molecular background. This hypothesis is supported by the observation that races Durango (Middle American gene pool) and Chile (Andean gene pool) are evolutionarily divergent, yet have a similar phenotype presumably resulting from a common environmental selection pressure, i.e., adaptation to arid environments.

Genetic diversity

The level of gene diversity (Nei 1987) for RFLPs observed in common bean (H_t = 0.38) was lower than that observed for *Brassica campestris* (H_t = 0.68; McGrath and Quiros 1992), *Lens culinaris* (H_t = 0.51; Havey and Muehlbauer 1989), and *Lactuca* sect. *Lactuca* subsect. *Lactuca* (H_t = 0.48; Kesseli et al. 1991). Differences may be due to inherently different levels of sequence variation among these different species, the types of probe used, sampling effects, and the different definitions of species included in the respective studies.

A comparison with isozyme data obtained by Koenig and Gepts (1989) and Singh et al. (1991*c*) shows that RFLPs revealed a substantially higher level of diversity than isozymes. Genetic diversity values among races calculated on the same set of accessions included in both studies were three to four times as high for RFLPs than for isozymes. Variation for RFLPs within races, as defined on the basis of phaseolin, isozymes, morphological, and ecological traits (Singh et al. 1991*a*, 1991*b*), was also higher compared with that for isozymes.

RFLP diversity data in wild and cultivated beans from the two domestication centres did not confirm the marked reduction in diversity suggested by comparable data for the seed proteins phaseolin and arcelin (Gepts and Bliss 1986; Gepts et al. 1986; Koenig et al. 1990; Osborn et al. 1988). They appeared to confirm, however, isozyme diversity data that showed a more limited reduction in diversity during domestication (Koenig and Gepts 1989; Singh et al. 1991*c*). Several hypothesis can account for this discrepancy.

First, incomplete sampling of the wild gene pools, especially in the Andes, may lead to lower estimates of genetic diversity. Lack of available materials at the initiation of this study led to underrepresentation of wild populations from southern Peru and absence of wild populations from Bolivia. In addition, to maintain the experiment within a manageable size, the number of wild bean populations was smaller than the total number of cultivated accessions in the Middle American and Andean gene pools.

Second, seed proteins may have a stronger selective effect than isozymes or the gene products of RFLP loci or genes linked to it. Specific phaseolin types found in cultivars may be better suited as nitrogen and carbon sources for the germinating seed in cultivated environments than in natural environment. Phaseolin types found in cultivars may have a superior nutritional value (i.e., digestibility or sulphur amino acid content) than phaseolin types found in wild forms. RFLPs would likely show a more limited reduction because they would be selectively neutral. Multiple domestications,

gene flow between wild and cultivated forms subsequent to domestication, or frequent mutations at certain loci would have reduced the magnitude of a presumed genetic bottleneck during domestication.

Seed protein loci such as the phaseolin locus may be associated directly, or indirectly through linkage, with an essential element of the domestication syndrome. Hartana (1986) identified a relationship between phaseolin and seed size. This relationship was recently confirmed by quantitative trait loci mapping in two different populations (Nodari 1992; Koinange 1992). In addition, a relationship between the phaseolin locus and seed dormancy was also identified in one of these populations involving a cross between a wild and a cultivated bean. Whether this association is due to pleiotropy or tight linkage, selection for increased seed size and reduced dormancy during domestication may have led to inadvertent selection for specific phaseolin types.

Our results have important consequences for genetic resources conservation and breeding of common bean. The divergence between the Middle American and Andean gene pools suggests that germplasm of both areas should be simultaneously explored and conserved. Because of the divergence between these two gene pools, novel gene combinations could be obtained provided gene transfer and recombination can take place freely. Finally, the high RFLP levels within races suggest that RFLP or other markers such as RAPDs can be used as indirect selection tools in breeding experiments involving more closely related genotypes.

Acknowledgements

This research was funded by the Agency for International Development, Washington, DC, under the PSTC program (grant DHR-5542-G-SS-9020-00). Thanks go to Drs. M. Iwanaga and S. Singh (CIAT) for providing seed samples and to V. Llaca and R. Nodari for advice.

Debouck, D.G. 1991. Systematics and morphology. *In* Common beans: research for crop improvement. *Edited by* A. Van Schoonhoven and O. Voysest. CAB, Wallingford, Oxon, U.K. pp. 55–118.

Doebley, J. 1989. Isozymic evidence and the evolution of crop plants. *In* Isozymes in plant biology. *Edited by* D.E. Soltis and P.S. Soltis. Dioscorides, Portland, Oregon. pp. 165–191.

Doebley, J. 1992. Molecular systematics and crop evolution. *In* Molecular systematics of plants. *Edited by* P.S. Soltis, D.E. Soltis, and J.J. Doyle. Chapman Hall, New York. pp. 202–222.

Feinberg, A.P., and Vogelstein, B. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**: 9–13.

Gepts, P. 1990. Biochemical evidence bearing on the domestication of *Phaseolus* beans. Econ. Bot. **44**(3S): 28–38.

Gepts, P. 1993*a*. Linkage map of common bean (*Phaseolus vulgaris* L.). *In* Genetic maps. *Edited by* S.J. O'Brien. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York. pp. 6.101–6.109.

Gepts, P. 1993*b*. The use of molecular and biochemical markers in crop evolution studies. Evol. Biol. **27**. In press.

Gepts, P., and Bliss, F.A. 1985. F_1 hybrid weakness in the common bean: differential geographic origin suggests two gene pools in cultivated bean germplasm. J. Hered. **76**: 447–450.

Gepts, P., and Bliss, F.A. 1986. Phaseolin variability among wild and cultivated common beans (*Phaseolus vulgaris*) from Colombia. Econ. Bot. **40**: 469–478.

Gepts, P., and Debouck, D.G. 1991. Origin, domestication, and evolution of the common bean, *Phaseolus vulgaris*. *In* Common beans: research for crop improvement. *Edited by* O. Voysest and A. Van Schoonhoven. CAB, Oxon, U.K. pp. 7–53.

Gepts, P., Osborn, T.C., Rashka, K., and Bliss, F.A. 1986. Phaseolin-protein variability in wild forms and landraces of the common bean (*Phaseolus vulgaris*): evidence for multiple centers of domestication. Econ. Bot. **40**: 451–468.

Gepts, P., Llaca, V., Nodari, R.O., and Panella, L. 1992. Analysis of seed proteins, isozymes, and RFLPs for genetic and evolutionary studies in *Phaseolus*. *In* Modern methods of plant analysis (new series): seed analysis. *Edited by* H.-F. Linskens and J.F. Jackson. Springer, Berlin. pp. 63–93.

Gepts, P., Nodari, R., Tsai, R., Koinange, E.M.K., Llaca, V., Gilbertson, R., and Guzmán, P. 1993. Linkage mapping in common bean. Annu. Rept. Bean Improv. Coop. **36**: xxiv–xxxviii.

Hartana, A. 1986. Components of variability for seed protein of common bean (*Phaseolus vulgaris* L.). Ph.D. thesis, Department of Plant Breeding and Plant Genetics, University of Wisconsin, Madison.

Havey, M.J., and Muehlbauer, F.J. 1989. Variability for restriction fragment lengths and phylogenies in lentil. Theor. Appl. Genet. **77**: 839–843.

Kesseli, R., Ochoa, O., and Michelmore, R.W. 1991. Variation at RFLP loci in *Lactuca* spp. and origin of cultivated lettuce (*L. sativa*). Genome, **34**: 430–436.

Khairallah, M.M., Adams, M.W., and Sears, B.B. 1990. Mitochondrial DNA polymorphisms of Malawian bean lines: further evidence for two major gene pools. Theor. Appl. Genet. **80**: 753–761.

Khairallah, M.M., Sears, B.B., and Adams, M.W. 1992. Mitochondrial restriction fragment polymorphisms in wild *Phaseolus vulgaris* — insights in the domestication of common bean. Theor. Appl. Genet. **84**: 915–922.

Koenig, R., and Gepts, P. 1989. Allozyme diversity in wild *Phaseolus vulgaris*: further evidence for two major centers of diversity. Theor. Appl. Genet. **78**: 809–817.

Koenig, R., Singh, S.P., and Gepts, P. 1990. Novel phaseolin types in wild and cultivated common bean (*Phaseolus vulgaris*, Fabaceae). Econ. Bot. **44**: 50–60.

Koinange, E.M.K. 1992. Genetic differentiation between wild and cultivated common bean (*Phaseolus vulgaris* L.). Ph.D. thesis, Department of Genetics, University of California, Davis.

Koinange, E.M.K., and Gepts, P. 1992. Hybrid weakness in wild *Phaseolus vulgaris* L. J. Hered. **83**: 135–139.

McGrath, J.M., and Quiros, C.F. 1992. Genetic diversity at isozyme and RFLP loci in *Brassica campestris* as related to crop type and geographical origin. Theor. Appl. Genet. **83**: 783–790.

Mehdy, M.C., and Lamb, C.J. 1987. Chalcone isomerase cDNA cloning and mRNA induction by fungal elicitor, wounding and infection. EMBO J. **6**: 1527–1533.

Nei, M. 1987. Molecular evolutionary genetics. Columbia University Press, New York.

Nodari, R.O. 1992. Towards an integrated linkage map of common bean (*Phaseolus vulgaris* L.). Ph.D. thesis, Department of Genetics, University of California, Davis.

Nodari, R.O., Koinange, E.M.K., Kelly, J.D., and Gepts, P. 1992. Towards an integrated linkage map of common bean. I. Development of genomic DNA probes and levels of restriction fragment length polymorphism. Theor. Appl. Genet. **84**: 186–192.

Nodari, R.O., Tsai, S.M., Gilbertson, R.L., and Gepts, P. 1993. Towards an integrated linkage map of common bean. II. Development of an RFLP-based linkage map. Theor. Appl. Genet. **85**: 513–520.

Osborn, T.C., Alexander, D.C., Sun, S.S.M., Cardona, C., and Bliss, F.A. 1988. Insecticidal activity and lectin homology of arcelin seed protein. Science (Washington, D.C.), **240**: 207–210.

Pachico, D. 1989. Trends in world common bean production. *In*

Bean production problems in the Tropics. *Edited by* H.F. Schwartz and M.A. Pastor-Corrales. CIAT, Cali, Colombia. pp. 1–8.

Rohlf, F.J. 1990. NTSYS-pc: numerical taxonomy and multivariate analysis. Exeter Software, Setauket, N.Y.

Ryder, T.B., Cremer, C.L., Bell, J.N., Robbins, M.P., Dixon, R.A., and Lamb, C.J. 1984. Elicitor rapidly indues chalcone synthase mRNA in *Phaseolus vulgaris* cells at the onset of the phytoalexin defense response. Proc. Natl. Acad. Sci. U.S.A. **81**: 5724–5728.

Ryder, T.B., Hedrick, S.A., Bell, J.B., Liang, X., Clouse, S.D., and Lamb, C.J. 1987. Organization and differential activation of a gene family encoding the plant defense enzyme chalcone synthase in *Phaseolus vulgaris*. Mol. Gen. Genet. **210**: 219–233.

SAS Institute Inc. 1988. SAS/STAT user's guide, release 6.03 edition. SAS Institute, Cary, N.C.

Shii, C.T., Mok, M.C., Temple, S.R., and Mok, D.W.S. 1980. Expression of developmental abnormalities in hybrids of *Phaseolus vulgaris* L. J. Hered. **71**: 218–222.

Singh, S.P., Gepts, P., and Debouck, D.G. 1991*a*. Races of common bean (*Phaseolus vulgaris* L., Fabaceae). Econ. Bot. **45**: 379–396.

Singh, S.P., Gutiérrez, J.A., Molina, A., Urrea, C., and Gepts, P. 1991*b*. Genetic diversity in cultivated common bean. II. Marker-based analysis of morphological and agronomic traits. Crop Sci. **31**: 23–29.

Singh, S.P., Nodari, R., and Gepts, P. 1991*c*. Genetic diversity in cultivated common bean. I. Allozymes. Crop Sci. **31**: 19–23.

Sneath, P.H.A., and Sokal, R. 1973. Numerical taxonomy. Freeman, San Francisco, Calif.

Sprecher, S.L. 1988. Allozyme differentiation between gene pools in common bean (*Phaseolus vulgaris* L.), with special reference to Malawian germplasm. Ph.D. thesis, Michigan State University, East Lansing, Mich.

Voysest, O., and Dessert, M. 1991. Bean cultivars: classes and commercial seed types. *In* Common beans: research for crop improvement. *Edited by* A. van Schoonhoven and O. Voysest. CAB, Wallingford, Oxon, U.K. pp. 119–162.

Wallace, D.H. 1985. Physiological genetics of plant maturity, adaptation, and yield. Plant Breed. Rev. **3**: 21–167.

This article has been cited by:

1. Boris Briñez, Matthew W. Blair, Andrzej Kilian, Sérgio Augusto Morais Carbonell, Allison Fernando Chiorato, Luciana Benchimol Rubiano. 2012. A whole genome DArT assay to assess germplasm collection diversity in common beans. *Molecular Breeding* **30**:1, 181-193. [CrossRef]
2. Teresa Avila, Matthew W. Blair, Ximena Reyes, Pierre Bertin. 2012. Genetic diversity of bean (Phaseolus) landraces and wild relatives from the primary centre of origin of the Southern Andes. *Plant Genetic Resources* **10**:01, 83-92. [CrossRef]
3. E. Bitocchi, L. Nanni, E. Bellucci, M. Rossi, A. Giardini, P. S. Zeuli, G. Logozzo, J. Stougaard, P. McClean, G. Attene, R. Papa. 2012. PNAS Plus: Mesoamerican origin of the common bean (Phaseolus vulgaris L.) is revealed by sequence data. *Proceedings of the National Academy of Sciences* . [CrossRef]
4. Sujan Mamidi, Monica Rossi, Deepti Annam, Samira Moghaddam, Rian Lee, Roberto Papa, Phillip McClean. 2012. Investigation of the domestication of common bean (Phaseolus vulgaris) using multilocus sequence data. *Functional Plant Biology* . [CrossRef]
5. Andrés J. Cortés, Martha C. Chavarro, Matthew W. Blair. 2011. SNP marker diversity in common bean (Phaseolus vulgaris L.). *Theoretical and Applied Genetics* **123**:5, 827-845. [CrossRef]
6. Phillip E. McClean, Jeff Terpstra, Melody McConnell, Caleb White, Rian Lee, Sujan Mamidi. 2011. Population structure and genetic differentiation among the USDA common bean (Phaseolus vulgaris L.) core collection. *Genetic Resources and Crop Evolution* . [CrossRef]
7. Lucy M. Díaz, Héctor F. Buendía, Myriam C. Duque, Matthew W. Blair. 2011. Genetic diversity of Colombian landraces of common bean as detected through the use of silver-stained and fluorescently labelled microsatellites. *Plant Genetic Resources* **9**:01, 86-96. [CrossRef]
8. Melody McConnell, Sujan Mamidi, Rian Lee, Shireen Chikara, Monica Rossi, Roberto Papa, Phillip McClean. 2010. Syntenic relationships among legumes revealed using a gene-based genetic linkage map of common bean (Phaseolus vulgaris L.). *Theoretical and Applied Genetics* **121**:6, 1103-1116. [CrossRef]
9. Matthew W. Blair, Laura F. González, Paul M. Kimani, Louis Butare. 2010. Genetic diversity, inter-gene pool introgression and nutritional quality of common beans (Phaseolus vulgaris L.) from Central Africa. *Theoretical and Applied Genetics* **121**:2, 237-248. [CrossRef]
10. M. Santalla, A. M. Ron, M. La Fuente. 2010. Integration of genome and phenotypic scanning gives evidence of genetic structure in Mesoamerican common bean (Phaseolus vulgaris L.) landraces from the southwest of Europe. *Theoretical and Applied Genetics* **120**:8, 1635-1651. [CrossRef]
11. G. Igrejas, V. Carnide, P. Pereira, F. Mesquita, H. Guedes-Pinto. 2009. Genetic diversity and phaseolin variation in Portuguese common bean landraces. *Plant Genetic Resources* **7**:03, 230. [CrossRef]
12. Asrat Asfaw, Matthew W. Blair, Conny Almekinders. 2009. Genetic diversity and population structure of common bean (Phaseolus vulgaris L.) landraces from the East African highlands. *Theoretical and Applied Genetics* **120**:1, 1-12. [CrossRef]
13. BARBARA PICKERSGILL. 2009. Domestication of plants revisited ### Darwin to the present day. *Botanical Journal of the Linnean Society* **161**:3, 203-212. [CrossRef]
14. Monica Rossi, Elena Bitocchi, Elisa Bellucci, Laura Nanni, Domenico Rau, Giovanna Attene, Roberto Papa. 2009. Linkage disequilibrium and population structure in wild and domesticated populations of Phaseolus vulgaris L. *Evolutionary Applications* **2**:4, 504-522. [CrossRef]
15. Matthew W. Blair, Lucy M. Díaz, Hector F. Buendía, Myriam C. Duque. 2009. Genetic diversity, seed size associations and population structure of a core collection of common beans (Phaseolus vulgaris L.). *Theoretical and Applied Genetics* **119**:6, 955-972. [CrossRef]
16. S. A. Angioi, F. Desiderio, D. Rau, E. Bitocchi, G. Attene, R. Papa. 2009. Development and use of chloroplast microsatellites in Phaseolus spp. and other legumes. *Plant Biology* **11**:4, 598-612. [CrossRef]
17. Myounghai Kwak, Paul Gepts. 2009. Structure of genetic diversity in the two major gene pools of common bean (Phaseolus vulgaris L., Fabaceae). *Theoretical and Applied Genetics* **118**:5, 979-992. [CrossRef]

18. Xiaoyan Zhang, Matthew W. Blair, Shumin Wang. 2008. Genetic diversity of Chinese common bean (Phaseolus vulgaris L.) landraces assessed with simple sequence repeat markers. *Theoretical and Applied Genetics* **117**:4, 629-640. [CrossRef]
19. Marko Maras, Jelka Šuštar-Vozli#, Branka Javornik, Vladimir Megli#. 2008. The efficiency of AFLP and SSR markers in genetic diversity estimation and gene pool classification of common bean (Phaseolus vulgaris L.). *Acta agriculturae Slovenica* **91**:1, 87-96. [CrossRef]
20. Vishnu Bhat, Deepmala Sehgal, Soom Nath RainaApplicability of DNA Markers for Genome Diagnostics of Grain Legumes 497-557. [CrossRef]
21. Tatjana Kavar, Marko Maras, Marjetka Kidri#, Jelka Šuštar-Vozli#, Vladimir Megli#. 2008. Identification of genes involved in the response of leaves of Phaseolus vulgaris to drought stress. *Molecular Breeding* **21**:2, 159-172. [CrossRef]
22. M. W. Blair, J. M. Díaz, R. Hidalgo, L. M. Díaz, M. C. Duque. 2007. Microsatellite characterization of Andean races of common bean (Phaseolus vulgaris L.). *Theoretical and Applied Genetics* **116**:1, 29-43. [CrossRef]
23. M. I. Chacón S., B. Pickersgill, D. G. Debouck, J. Salvador Arias. 2007. Phylogeographic analysis of the chloroplast DNA variation in wild common bean (Phaseolus vulgaris L.) in the Americas. *Plant Systematics and Evolution* **266**:3-4, 175-195. [CrossRef]
24. L. M. Díaz, M. W. Blair. 2006. Race structure within the Mesoamerican gene pool of common bean (Phaseolus vulgaris L.) as determined by microsatellite markers. *Theoretical and Applied Genetics* **114**:1, 143-154. [CrossRef]
25. M. Z. Galván, M. C. Menéndez-Sevillano, A. M. Ron, M. Santalla, P. A. Balatti. 2006. Genetic Diversity among Wild Common Beans from Northwestern Argentina Based on Morpho-agronomic and RAPD Data. *Genetic Resources and Crop Evolution* **53**:5, 891-900. [CrossRef]
26. M. Maras, S. Sušnik, J. Šuštar-Vozli#, V. Megli#. 2006. Temporal changes in genetic diversity of common bean (Phaseolus vulgaris L.) accessions cultivated between 1800 and 2000. *Russian Journal of Genetics* **42**:7, 775-782. [CrossRef]
27. M. W. Blair, M. C. Giraldo, H. F. Buendía, E. Tovar, M. C. Duque, S. E. Beebe. 2006. Microsatellite marker diversity in common bean (Phaseolus vulgaris L.). *Theoretical and Applied Genetics* **113**:1, 100-109. [CrossRef]
28. M. W. Blair, G. Iriarte, S. Beebe. 2006. QTL analysis of yield traits in an advanced backcross population derived from a cultivated Andean × wild common bean (Phaseolus vulgaris L.) cross. *Theoretical and Applied Genetics* **112**:6, 1149-1163. [CrossRef]
29. Andrea Pedrosa-Harand, Cícero C. Souza Almeida, Magdalena Mosiolek, Matthew W. Blair, Dieter Schweizer, Marcelo Guerra. 2006. Extensive ribosomal DNA amplification during Andean common bean (Phaseolus vulgaris L.) evolution. *Theoretical and Applied Genetics* **112**:5, 924-933. [CrossRef]
30. Emeterio Payró Cruz, Paul Gepts, Patricia Colunga GarciaMarín, Daniel Zizumbo Villareal. 2005. Spatial Distribution of Genetic Diversity in Wild Populations of Phaseolus vulgarisL. from Guanajuato and Michoacán, Méexico. *Genetic Resources and Crop Evolution* **52**:5, 589-599. [CrossRef]
31. Manoj Tiwari, N. K. Singh, Meenal Rathore, Narendra Kumar. 2005. RAPD markers in the analysis of genetic diversity among common bean germplasm from Central Himalaya. *Genetic Resources and Crop Evolution* **52**:3, 315-324. [CrossRef]
32. Shree SinghCommon Bean (Phaseolus vulgaris L.) . [CrossRef]
33. Ho#Jeong Na, Jae#Young Um, Sung#Chul Kim, Kang#Hoon Koh, Woo#Jun Hwang, Kang#Min Lee, Cheorl# Ho Kim, Hyung#Min Kim. 2004. Molecular Discrimination of Medicinal Astragali Radix by RAPD Analysis. *Immunopharmacology and Immunotoxicology* **26**:2, 265-272. [CrossRef]
34. Phillip McClean, Paul Gepts, James Kamir . [CrossRef]
35. K. Tertivanidis, O. Koutita, G. Skaracis, E. Traka-Mavrona, M. Koutsika-Sotiriou. 2003. The Use of RAPD Markers in Monitoring Molecular Changes During a Selection Process in Snap Bean. *Journal of New Seeds* **5**:4, 87-96. [CrossRef]
36. J. De Meaux, I. Cattan-Toupance, C. Lavigne, T. Langin, C. Neema. 2003. Polymorphism of a complex resistance gene candidate family in wild populations of common bean (Phaseolus vulgaris) in Argentina: comparison with phenotypic resistance polymorphism. *Molecular Ecology* **12**:1, 263-273. [CrossRef]

37. Stephen A. Harris, Julian P. Robinson, Barrie E. Juniper. 2002. Genetic clues to the origin of the apple. *Trends in Genetics* **18**:8, 426-430. [CrossRef]

38. HWAN-SUCK CHUNG, JAE-YOUNG UM, MI-SUN KIM, SEUNG-HEON HONG, SAE-MIN KIM, HYEONG-KYUN KIM, SANG-JUN PARK, SUNG-CHUL KIM, WOO-JUN HWANG, HYUNG-MIN KIM. 2002. Determination of the site of origin of Pinellia ternata roots based on RAPD analysis and PCR-RFLP. *Hereditas* **136**:2, 126-129. [CrossRef]

39. Awegechew Teshome, A. H. D. Brown, T. HodgkinDiversity in Landraces of Cereal and Legume Crops 221-261. [CrossRef]

40. Jae-Young UM, Hwan-Suck CHUNG, Mi-Sun KIM, Ho-Jeong NA, Hyun-Jeong KWON, Jeong-Joong KIM, Kang-Min LEE, Seung Jae LEE, Jong Phil LIM, Keum-Rok DO, Woo-Jun HWANG, Yeoung-Su LYU, Nyeon-Hyoung AN, Hyung-Min KIM. 2001. Molecular Authentication of Panax ginseng Species by RAPD Analysis and PCR-RFLP. *Biological & Pharmaceutical Bulletin* **24**:8, 872-875. [CrossRef]

41. M. Le Thierry D'Ennequin, Toupance, Robert, Godelle, Gouyon. 1999. Plant domestication: a model for studying the selection of linkage. *Journal of Evolutionary Biology* **12**:6, 1138-1147. [CrossRef]

42. Valérie Geffroy, Delphine Sicard, Julio C. F. de Oliveira, Mireille Sévignac, Séverine Cohen, Paul Gepts, Claire Neema, Thierry Langin, Michel Dron. 1999. Identification of an Ancestral Resistance Gene Cluster Involved in the Coevolution Process Between Phaseolus vulgaris and Its Fungal Pathogen Colletotrichum lindemuthianum. *Molecular Plant-Microbe Interactions* **12**:9, 774-784. [CrossRef]

43. Craig M. Sandlin, James R. Steadman, Carlos M. Araya, Dermot P. Coyne. 1999. Isolates of Uromyces appendiculatus with Specific Virulence to Landraces of Phaseolus vulgaris of Andean Origin. *Plant Disease* **83**:2, 108-113. [CrossRef]

44. Lucia Lioi, Concetta Lotti, Incoronata Galasso. 1998. Isozyme diversity, RFLP of the rDNA and phylogenetic affinities among cultivated Lima beans,Phaseolus lunatus (Fabaceae). *Plant Systematics and Evolution* **213**:3-4, 153-164. [CrossRef]

45. References 329-369. [CrossRef]

46. D. Sicard, Y. Michalakis, M. Dron, C. Neema. 1997. Genetic Diversity and Pathogenic Variation of Colletotrichum lindemuthianum in the Three Centers of Diversity of Its Host, Phaseolus vulgaris. *Phytopathology* **87**:8, 807-813. [CrossRef]

47. W. Nagl, S. Ignacimuthu, J. Becker. 1997. Genetic engineering and regeneration of Phaseolus and Vigna. State of the art and new attempts. *Journal of Plant Physiology* **150**:6, 625-644. [CrossRef]

48. Rosanna Freyre, Raúl Ríos, Lorena Guzmán, Daniel G. Debouck, Paul Gepts. 1996. Ecogeographic distribution ofPhaseolus spp. (Fabaceae) in Bolivia. *Economic Botany* **50**:2, 195-215. [CrossRef]

49. H. T. Stalker, J. S. Dhesi, and G. Kochert. 1995. Genetic diversity within the species Arachis duranensis Krapov. &W.C. Gregory, a possible progenitor of cultivated peanut. *Genome* **38**:6, 1201-1212. [Abstract] [PDF] [PDF Plus]

50. Guohao He, C. S. Prakash, and R. L. Jarret. 1995. Analysis of genetic diversity in a sweetpotato (Ipomoea batatas) germplasm collection using DNA amplification fingerprinting. *Genome* **38**:5, 938-945. [Abstract] [PDF] [PDF Plus]