Theor Appl Genet (1989) 78:809–817

TAG THEORETICAL AND APPLIED GENETICS

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Allozyme diversity in wild *Phaseolus vulgaris*: further evidence for two major centers of genetic diversity

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Received December 10, 1988; Accepted August 30, 1989
Communicated by A. L. Kahler

Summary. Allozyme analysis was performed on 83 wild *Phaseolus vulgaris* accessions, representing a wide geographical distribution from Mesoamerica to Argentina, to determine levels of genetic diversity and geographic patterns of variability at nine polymorphic isozyme loci. The collection can be divided into two major groups, one consisting of accessions from Mexico, Central America, Colombia and Peru, and the other consisting of accessions from Peru and Argentina. One accession from northern Peru is distinct from the two major groups, and may delineate a transition zone between the two divergent groups. The level of genetic diversity within wild *P. vulgaris* (Ht = 0.132) is comparable with those found in other *Phaseolus* species. There was no significant within-accession gene diversity (Hs = 0.006); however, there is a moderate level of genetic diversity (Dst = 0.126) between accessions. Our results are consistent with previous studies on the genetic diversity of wild *P. vulgaris* using phaseolin, the major seed storage protein of beans.

Key words: Crop evolution – Domestication – Molecular markers – Common bean

Introduction

The common bean, *Phaseolus vulgaris* L. (2n = 2x = 22), is a predominantly self-pollinated crop from the New World. Wild ancestral forms are distributed over a large geographical area which extends from northern Mexico to northwestern Argentina. Extant wild populations have been described in the Andes by Burkart (1941, 1943) and Brücher (1988), in Guatemala by McBryde (1947) and in Mesoamerica by Miranda Colín (1967, 1979), Gentry (1969) and Delgado et al. (1988). Biochemical, physiological and morphological evidence suggests the existence of two major gene pools for the common bean, the Andean region and Mesoamerica (Gepts and Bliss 1985; Gepts 1988). Andean cultivars, on the average, have larger seeds than Middle American cultivars. Bean accessions from Middle America and the Andes have distinctive phaseolin types, with the former exhibiting 'S' or 'M' types, and the latter exhibiting 'T', 'C', 'H', 'A', 'J' or 'I' types (Gepts et al. 1986; Gepts and Bliss 1986; Koenig et al. 1989). On the basis of phaseolin patterns, we have suggested that a geographical transition area between Mesoamerican and southern Andean populations may be located in the northern Peru-Columbia region (Koenig et al. 1990)

A limited number of studies have utilized isozymes to determine genetic diversity and phylogeny relationships in *Phaseolus* species. Bassiri and Adams (1978a) examined the isozyme variability of esterase, acid phosphatase and peroxidase of 13 species within the genus *Phaseolus*. Most species showed unique banding patterns in each isozyme system, with the exception of cultivated *P. vulgaris*, wild *P. vulgaris* and *P. coccineus*, which had similar banding patterns. Bassiri and Adams (1978b) examined genetic diversity in 34 cultivars of *P. vulgaris* belonging to 19 commercial classes. The isozymes peroxidase and esterase were suitable markers for cultivar identification and for estimating the genetic relationships among cultivars of the same class or among classes. Within each isozyme system no pattern was found to be exclusive to any particular commercial class. A banding similarity index was calculated for the cultivars based on the number of common polymorphic bands between each cultivar pair. These indices were highly correlated with genetic distances obtained by principal component analysis. A study of the evolutionary variation of aspartate aminotransferase and superoxide dismutase in 14 wild and cul-

Table 1. Identification, origin, and allozyme constitution of wild *Phaseolus vulgaris*

CIAT no.	Other no.	Country[a]	Province[b]	*Diap-1*	*Lap-3*	*Mdh1*	*Mdh2*	*Me*	*Gpi-2*	*Prx*	*Rbcs*	*Skdh*
	L625	mex	chh	100	100	100	100	100	100	98	100	103
G22199	L13	mex	jal	96	100	100	100	100	100	98	100	100
	P1319441	mex	dgo	96, 100	100	100	102	100	100	98	100	103
G09989A	HM7395bulk	mex	jal	100	100	100	100	102	100	98	100	100
G09998	HM8130-1	mex	jal	96	100	100	100	100	100	100	100	100
G10000	Guerrero924	mex	gue	100, 102	100	100	100, 100	100, 102	100	100	98, 100	100, 103
G10008	NI401	mex	mor	102	100	100	100	98	100	100	100	103
G11050	DGD78/123	mex	mch	96	100	100	102	100	100	100	100	100
G11055	DGD78/147	mex	jal	100	100	100	100, 102	100	98	100	100	100
G11056	DGD78/148	mex	jal	100	100	100	100	98	100	100	100	100
G12861	PI318692	mex	mch	102	100	100	100	100	98	100	100	103
G12863	PI318694	mex	jal	96	100	100	100	100	100	98	100	103
G12864	PI318695	mex	col	96	100	100	100	100	100	98	100	103
G12866	PI318697	mex	jal	100	100	100	102	100	100	98	100	103
G12867	PI318698	mex	nay	95	100	100	100	102	100	98	100	100
G12868	PI318699	mex	dgo	96	100	100	100	100	100	100	100	100, 103
G12869	PI318700	mex	mch	96, 100	100	100	100	98	100	100	100	103
G12872A	PI325677	mex	mor	96, 100	100	100	100	98	100	100	100	100 103
G12873	PI325678	mex	mex	96	100	100	100	98	100	100	100	103
G12877B	PI325683	mex	mor	95, 96	100	100	102	100	100	98, 100	100	103
G12882	PI325690	mex	gue	96, 102	100	100	100	98	100	98	100	103
G12884	PI329247	mex	gue	100, 102	100	100	100	100	100	98	100	103
G12891	PI417624	mex	jal	96, 100	100	100	100	100	100	98	100	100, 103
G12905	PI417641	mex	gua	96	100	100	100	100	100	–	100	103
G12910	PI417653	mex	gua	95	100	100	100	100	100	100	100	103
G12914	PI417661	mex	jal	96, 100	100	100	100	100	100	98	100	100
G12922	PI417683	mex	jal	96	100	100	100	100	100	–	100	100
G12925	PI417686	mex	jal	96	100	100	100	100	100	98	100	103
G12949	PI417775	mex	jal	95	100	100	100	100	100	98	100	100
G12957	PI417786	mex	jal	96, 100	100	100	100	100	100	98	98	103
G13015	V-1396	mex	gue	100	100	100	102	100	100	98	100	103
G13018	V-1438	mex	mor	96	100	100	100	98	100	98	100	103
G13505	NI404	mex	mor	–	100	100	100	98	100	100	100	103
G09997a	M7446a	mex	jal	96	100	100	100	100	100	100	100	100
G09997b	M7446b	mex	jal	100	100	100	100, 102	102	100	100	100	103
G12862a	PI318693a	mex	jal	96	100	100	100	102	100	100	100	103
G12862b	PI318693b	mex	jal	100	100	100	102	102	100	100	100	100
G12851	PI201011	gta	–0–	100	100	100	100	100	100	100	100	103
G19906	DGD1610	gta	–0–	100	100	100	100, 102	98	100	100	100	103
G19907	DGD1611	gta	–0–	100	100	100	100	98	100	98	100	103
G19908	DGD1616	gta	–0–	100	100	100	100	100	100	98	100	103
G19909	DGD1619	gta	–0–	100	100	100	100	98	100	98	100	103
G20559	PI256410	cra	–0–	100	100	100	100	98	100	100	100	103
–0–	NI1926	clb	cun	96	100	100	100	100	100	100	100	103
–0–	NI1928	clb	cun	–	100	100	100	100	100	100	100	103
–0–	NI937	clb	cun	96	–	100	100	100	100	100	100	103
–0–	X634	clb	cun	96	100	100	100	98	100	100	100	103
–0–	X643	clb	cun	96	100	100	100	98	100	100	100	103

Table 1. (continued)

CIAT no.	Other no.	Country[a]	Province[b]	*Diap-1*	*Lap-3*	*Mdh1*	*Mdh2*	*Me*	*Gpi-2*	*Prx*	*Rbcs*	*Skdh*
-0-	X663	clb	cun	100	100	100	100	98	100	100	100	103
G07225	-0-	per	apu	96, 100	103	100	100	100	100	–	98	103
G12856A	PI260405	per	-0-	100	100	100	100	98	100	98	100	100
G12856B	PI260405	per	-0-	100	103	103	100	98	100	98	98	100
-0-	DGD1962	per	caj	100	103	100	100	102	96	100	100	103
G07469	NI29, L326	arg	-0-	100	103	100	100	98	100	100	98	100
G10021	PI266910	arg	-0-	100	103	100	100	98	100	98	98	100
G10024	NI190	arg	-0-	96, 100	103	100	100	98	100	100	98	100
G10025		arg	-0-	100	103	100	100	98	100	98	100	100
-0-	V0909	arg	-0-	100	103	100	100	98	100	100	98	100
	DGD0621	arg	juj	96	103	100	100	100	100	100	100	100
G19887	DGD0622	arg	juj	100	103	100	100	98	100	98	100	100
G19888	DGD0623	arg	juj	100	103	100	100	98	100	98	100	100
G19889	DGD0624	arg	juj	100	103	100	100	98	100	98	100	100
G19890	DGD0626	arg	sal	100	103	100	100	98	100	98	98	100
G19891	DGD0628	arg	sal	100	103	100	100	98	100	98	98	100
G19892	DGD0629	arg	sal	100	103	100	100	98	100	98	98	100
G19893	DGD0630	arg	sal	–	–	100	100	–	100	–	–	100
-0-	DGD0632	arg	tuc	100	103	100	100	98	100	–	100	100
-0-	DGD0634	arg	tuc	100	103	100	100	98	100	–	98, 100	100
G19894	DGD0636	arg	tuc	100	103	100	100	98	100	100	98	100
G19895	DGD0637	arg	tuc	100	103	100	100	98	100	100	98	100
G19896	DGD0639	arg	tuc	100	103	100	100	98	100	100	98	100
G19897	DGD0643	arg	tuc	–	–	100	100	–	100	–	–	100
G19898	DGD0644	arg	tuc	–	–	100	100	–	100	–	–	100
G19899	DGD0647	arg	tuc	100	103	100	100	98	100	100	100	100
G19901	DGD0649	arg	tuc	100, 103	100	100	100	98	96, 100	100	98, 100	100
G19902	DGD0650	arg	tuc	100	103	100	100	98	100	100	100	100
G19903	DGD0651	arg	tuc	–	–	100	100	–	100	–	–	100
-0-	DGD1711	arg	juj	100	103	100	100	98	100	100	98, 100	100
-0-	DGD1712	arg	juj	100	103	100	100	100	100	98	100	100
-0-	DGD1713	arg	juj	100	103	100	100	98	100	100	98	100
-0-	DGD1715	arg	sal	100	103	100	100	98	100	100	98	100
-0-	DGD1716	arg	sal	100	103	100	100	98	100	100	98	100

[a] arg: Argentina; clb: Colombia; cra: Costa Rica; gta: Guatemala; mex: Mexico; per: Peru

[b] apu: Apurimac; caj: Cajamarca; chh: Chihuahua; col: Colima; cun: Cundinamarca; dgo: Durango; gua: Guanajuato; gue: Guerrero; jal: Jalisco; juj: Jujuy; mex: Mexico; mch: Michoacán; mor: Morelos; nay: Nayarit; sal: Salta; tuc: Tucumán

tivated species of *Phaseolus* and *Vigna* by Jaaska and Jaaska (1988) revealed that in the genus *Phaseolus*, *P. vulgaris*, *P. coccineus*, *P. lunatus* and *P. acutifolius* form a homogenous group with only minor isoenzyme variation. However, the genus *Vigna* is heterogeneous for these isozyme characters.

More recently, several studies have examined the genetic control of the polymorphisms detected by isozymes. Weeden (1984a, b, 1986) and Weeden and Liang (1985) determined that the polymorphism for each of the enzymes ribulose biphosphate carboxylase (its small subunit: RBCS), shikimate dehydrogenase (SKDH), cathodal peroxidase (PRX), malic enzyme (ME), glucose phosphate isomerase (GPI), N-acetyl glucosaminidase (NAG) and adenylate kinase (ADK) was controlled by a single gene *Rbcs*, *Skdh*, *Prx*, *Me*, *Gpi-c1*, *Nag*, and *Adk*, respectively. Sprecher (1988) determined that the NADH-dependent diaphorase (DIA) polymorphism was controlled by two tightly linked genes, *Diap-1* and *Diap-2*. Koenig and Gepts (1989) identified two additional polymorphisms for leucine aminopeptidase (LAP) and malate dehydrogenase (MDH), coded by *Lap-3* and *Mdh-1*, respectively. With the exception of the *Diap-1* and *Diap-2* loci (see above) and the *Rbcs* and *Me* loci ($r > 30$ cM), these enzyme loci are unlinked and therefore represent different regions of the genome.

The objectives of this study were: (1) to examine the hypothesis of two gene pools in common bean using allozyme analysis of wild *Phaseolus vulgaris;* (2) to determine the relative amount of genetic variation in wild *P. vulgaris* from the Mesoamerican and Andean regions, and compare this to the variability in phaseolin found in these areas; and (3) to define the genetic transition zone where the two centers of domestication meet, resulting in plant accessions with a combination of Mesoamerican and Andean alleles.

Materials and methods

Plant materials

Eighty-three wild *P. vulgaris* plant accessions representing a wide geographical distribution from Mesoamerica to Argentina were analyzed in this study. The seed samples described in Table 1 were obtained from Drs. D. Debouck, D. Wood and R. Hidalgo at the *Phaseolus* World Collection (Centro Internacional de Agricultura Tropical, Cali, Colombia), Dr. R. Hannan at the Western Regional Plant Introduction Station (Pullman/WA and Dr. J. G. Waines at the University of California, Riverside. Thirty-seven accessions were from Mexico, 5 from Guatemala, 1 from Costa Rica, 4 from Peru, 6 from Colombia and 30 from Argentina.

Isozyme assays

At least four seeds of each accession were sown in vermiculite and plant tissues were analyzed at the first true leaf stage, approximately 10 days after sowing or the V2 stage (Gepts 1987). A crude tissue homogenate was produced by grinding the primary leaf or root apex tissue (depending on the enzymes assayed) in a buffer containing 3.75% reduced glutathion, 1% polyvinylpyrrolidone (PVP), and adjusted to pH 7.6 with 1 *M* TRIS. The homogenate, absorbed onto paper wicks, was loaded on 10% starch gel and subjected to electrophoresis following the procedures of Weeden (1984b). Following preliminary assays with 20 enzyme systems to determine the plant tissue with maximum enzyme expression and reproducibility and the gel medium for optimum clarity and separation, 14 enzyme systems were analyzed in the study (Table 2). The TRIS-citrate lithium borate, pH 8.1 (Selander et al. 1971), or the histidine citrate, pH 6.5, (Cardy et al. 1980), buffer systems were used depending on the enzyme system (Table 2). The stain composition for the enzymes LAP and glucose-6-phosphate-dehydrogenase (G6PDH) is described by Shaw and Prasad (1970), for peptidase (PEP) and RBCS by Weeden (1984a), for fructokinase (FK) by N. F. Weeden (personal communication), for aspartate aminotransferase (AAT), GPI, 6-phosphogluconate dehydrogenase (6-PGDH), MDH, DIA, PRX, SKDH and triosephosphate isomerase (TPI) by Vallejos (1983) and for ME by Brewer (1970). Loci were labeled sequentially with those migrating closest to the anodal end being designated as number 1. The most common allele was designated as 100 and all other allozymes were measured in millimeters from the standard (Koenig and Gepts 1989). In each gel, the cultivars 'ICA-Pijao' and 'California Dark Red Kidney' (CDRK) were included as standards. 'ICA-Pijao' has the following genotype at polymorphic enzyme loci: $Rbcs^{100}$, $Skdh^{103}$, Prx^{98}, Me^{100}, $Mdh\text{-}1^{100}$, $Mdh\text{-}2^{100}$, $Diap\text{-}1^{95}$, $Diap\text{-}2^{105}$ and $Lap\text{-}3^{100}$. CDRK exhibits the following genotype at polymorphic enzyme loci: $Rbcs^{98}$, $Skdh^{100}$, Prx^{98}, $Mdh\text{-}1^{103}$, $Mdh\text{-}2^{103}$, $Diap\text{-}1^{100}$, $Diap\text{-}2^{100}$, and $Lap\text{-}3^{103}$.

Table 2. Enzyme systems assayed, loci and alleles

Isozyme	Buffer[a]	Loci[b]	Alleles	Tissue
Aspartase amino-transferase (E.C.2.6.1.1)	L	*Aat-1**	100	Root
		*Aat-2**	100	Root
Diaphorase (E.C.1.6.4.3)	H	*Diap-1*	95, 96, 100, 102	Root
	H	*Diap-2*	100	Root
Glucose-6-phosphate dehydrogenase (E.C.1.1.1.49)	H	*G6pdh**	100	Root
Glucose phosphate isomerase (E.C.5.3.1.9)	L	*Gpi-c1*	100	Leaves
		*Gpi-c2**	96, 100	
Fructokinase (E.C.2.7.1.4)	H	*Fk**	100	Leaves
Leucine aminopeptidase (E.C.3.4.11.1)	L	*Lap-1**	100	Leaves
		*Lap-2**	100	Leaves
		Lap-3	100, 103	Leaves
Malate dehydrogenase (E.C.1.1.1.37)	L	*Mdh-1*	100, 103	Roots
		*Mdh-2**	100, 102	Roots
Malic enzyme (E.C.1.1.1.40)	L	*Me*	98, 100	Roots
Peptidase (E.C.3.4.–.–)	L	*Pep**	100	Leaves
Peroxidase (E.C.1.11.1.7)	L	*Prx*	98, 100	Root
6-P-Phosphogluconate dehydrogenase (E.C.1.1.1.44)	H	*6Pgdh**	100	Root
Shikimate dehydrogenase (E.C.1.1.1.25)	L	*Skdh*	100, 103	Leaves
Ribulose bisphosphate carboxylase (E.C.4.1.1.39)	L	*Rbcs*	98, 100	Laeves
Triosephosphate isomerase (E.C.5.3.1.1)	H	*Tpi-1**	100	Leaves
		*Tpi-2**	100	Leaves
		*Tpi-3**	100	Leaves

[a] L: Lithium hydroxide TRIS borate, pH 8.1 (Selander et al. 1971); H: Histidine citrate, pH 6.5 (Cardy et al. 1980)
[b] Loci designations marked with an asterisk are tentative, pending identification of polimorphisms and segregation analysis

Genetic diversity analysis

Nei's (1973) genetic diversity statistics were used in the analysis to measure the total gene diversity (Ht) of the allozyme data as well as the intra- (Hs) and inter-population (Dst) differences. Nei's distance (D) was used to compute the pairwise genetic distances. A dendrogram was constructed from the genetic distance data by the unweighted paired group method (Sneath and Sokal 1973), through the use of a computer program provided by Dr. K. Ritland (Department of Botany, University of Toronto, Canada) and with the help of Dr. Richard Kesseli (Department of Vegetable Crops, University of California, Davis).

Results

Wild accessions of *Phaseolus vulgaris* showed allozyme variation in 8 of the 14 enzyme systems analyzed in the study. The monomorphic systems included G6PDH,

PEP, FK, AAT, 6PGDH and TPI; the polymorphic enzyme systems consisted of DIA, LAP, MDH, ME, GPI, PRX, RBCS and SKDH. The genetic control of the polymorphisms has been determined previously (see 'Introduction'), with the exception of a polymorphism for MDH different from the one controlled by *Mdh-1* (Koenig and Gepts 1989). This novel polymorphism corresponds to a band of activity that is more cathodal to that coded by *Mdh-1*, but does not affect the migration of the enzymes coded by the latter gene. Pending genetic confirmation, we tentatively propose the symbol *Mdh-2* for the gene controlling this new polymorphism.

The 14 enzyme systems showed 22 bands of activity, 10 (45%) of which corresponded to polymorphic loci that were used in genetic distance analyses (Table 2). This is consistent with the generalization that in plant populations, approximately 37% of all loci are polymorphic (Hamrick 1983). The total genetic diversity (Ht) was 0.132 for the entire array of accessions included in this study. There was little within-accession gene diversity (Hs = 0.006), but there was moderate between-accession genetic diversity (Dst = 0.126). The coefficient of gene differentiation (Gst = Dst/Ht) was 0.952 and the absolute genetic differentiation (Dm) was 0.127.

The dendrogram constructed from pairwise genetic distances (Fig. 1) revealed the genetic divergence of two distinct groups of accessions. One group – called hereinafter the Mesoamerican group – primarily consisted of accessions from Mexico, Guatemala, Costa Rica, Colombia and southern Peru. The second group – called hereinafter the Andean group – primarily consisted of accessions from Argentina and one accession from southern Peru. The geographical separation between the two divergent groups was nearly complete; there was only one accession from Argentina (DGD621) included in the Mesoamerican group. Accession DGD1962 from northern Peru, although included in the southern Andean group, was distinct from the other accessions constituting this group, indicating that its location of origin perhaps delineates the transition zone between the two divergent groups.

Allelic frequency differences were discernible between the geographical regions and for the two groups. Table 3 gives the frequency of each allele for each geographical region and Fig. 2 divides the frequency data into five geographical regions – Mexico, Central America, Colombia, southern Peru and Argentina. The wild bean accessions from Mexico exhibited the greatest amount of allelic diversity. Accessions from Central America and Colombia exhibited allelic frequencies which were more similar to the Mexican genotypes. Accessions from southern Peru represented a transition area where some alleles are unique (e.g. *Mdh-1*^{103}), some alleles were similar to Argentine material (*Mdh-2*^{100}, *Me*^{98}), some alleles were similar to Mexican material (*Skdh*^{103}) and some alleles were from both geographical regions (*Diap-1*^{100} and *Diap-2*^{95}; *Lap-3*^{103} and *Lap-3*^{100}). The accessions from Argentina exhibited the least amount of genetic diversity (with the exception of Costa Rica, which was represented by a single accession). For example, at each

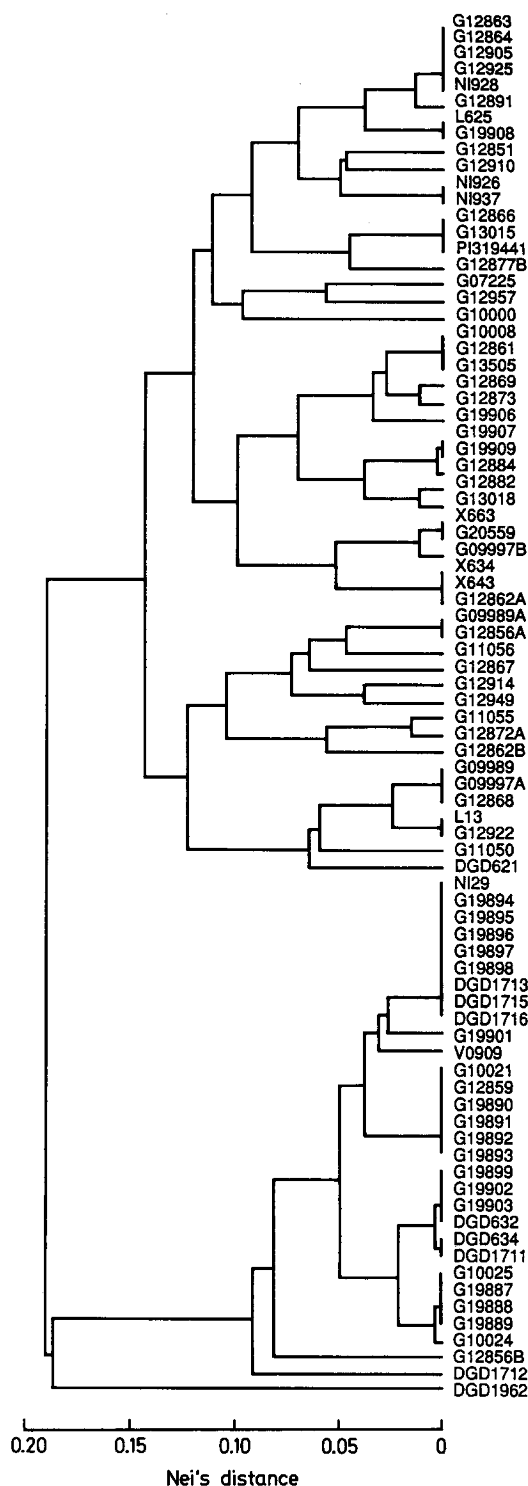

Fig. 1. Dendrogram of 83 wild *Phaseolus vulgaris* accessions based on frequency data at 22 isozyme loci

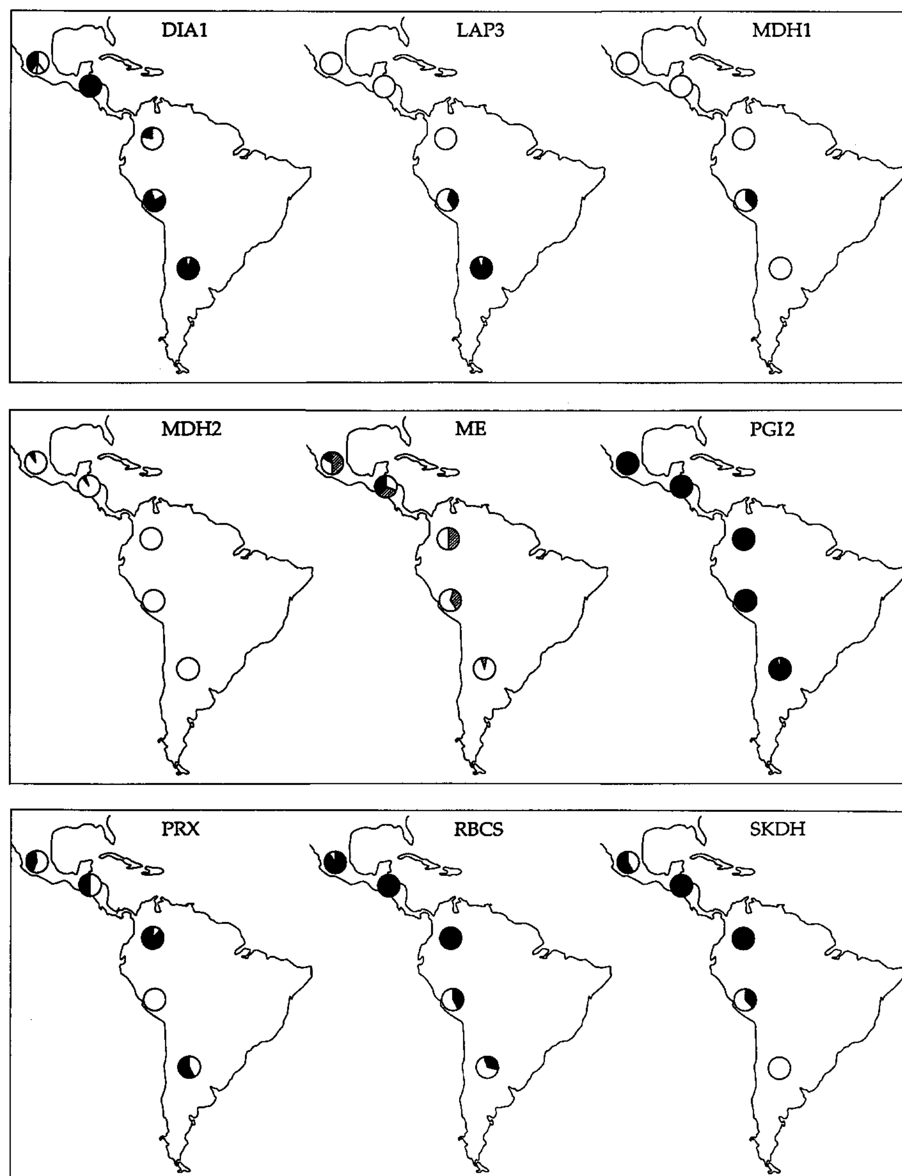

Fig. 2. Distribution of allozymes into five major geographical regions – Mexico, Central America, Colombia, southern Peru and Argentina. DIA1: *solid Diap-1*^{100}, *open Diap.1*^{95}, *shaded* (in Mexico) *Diap-1*^{102} and *Diap-1*^{96}; LAP3: *solid Lap-3*^{103}, *open Lap-3*^{100}; MDH1: *solid Mdh-1*^{103}, *open Mdh-1*^{100}; MDH2: *solid Mdh-2*^{102}, *open Mdh-2*^{100}; ME: *solid Me*^{102}, *shaded Me*^{100}, *open Me*^{98}; PGI2: *solid Gpi-c2*^{100}, *open Gpi-c2*^{96}; PRX: *Prx*^{100}, *open Prx*^{98}; RBCS: *solid Rbcs*^{100}, *open Rbcs*^{98}; SKDH: *solid Skdh*^{103}, *open Skdh*^{100}

of the loci, *Skdh, Lap-3, Mdh-1, Mdh-2, Me* and *Diap-1*, the frequency of the most common allele was over 90%.

A number of allozymes was found only in specific geographical locations. For example, almost all of the accessions from Mexico, Central America and Colombia showed the 100 allele of *Lap-3*, while accessions from Argentina showed the 103 allele of *Lap-3*. Allele 100 of *Diap-1* was found primarily in accessions from Central America, Argentina and southern Peru. Accessions from Mexico and Colombia had a higher frequency of allele *Diap-1*^{95}. All of the accessions from Argentina had the allele 100 of *Skdh*. Accessions from southern Peru and Mexico had contrasting frequencies of the *Skdh*^{103} and *Skdh*^{100}. All of the accessions from Colombia and Central America had the *Skdh*^{103} allele.

Certain allozymes showed a less pronounced geographical pattern. For example, all geographical areas contained accessions with either the 98 and 100 allele for *Prx*. Accessions from Mexico and Central America had all three *Me* alleles, with the 100 allele predominating in Mexico, and similar frequency among all three alleles in Central America. Both the 100 and 98 *Me* alleles were present in the accessions from Colombia, southern Peru and Argentina, with the 98 allele having a higher frequency in southern Peru and Argentina, and similar frequency between alleles in Colombia. Accession DGD1962 from northern Peru had the 102 allele of *Me*, similar to accessions from Mesoamerica.

Certain allozymes were found in a limited number of plant accessions. For example, allele *Gpi-2*^{96} was found

Table 3. Geographic origin and frequency of allozyme variants in wild *P. vulgaris*

Region	No. of acces- sions	*Diap-1*				*Lap-3*		*Mdh-1*		*Mdh-2*		*Me*			*Gpi-2*		*Prx*		*Rbcs*		*Skdh*	
		102	100	96	95	103	100	103	100	103	100	102	100	98	100	96	100	98	100	98	103	100
Mexico	37	0.07	0.39	0.10	0.44	0	1.0	0	1.0	0.14	0.86	0.27	0.55	0.18	1.0	0	0.44	0.56	0.95	0.05	0.61	0.39
Central America	6	0	1.0	0	0	0	1.0	0	1.0	0.04	0.96	0.33	0.33	0.33	1.0	0	0.50	0.50	1.0	0	1.0	0
Northern Peru and Colombia	7	0	0.67	0	0.33	0.83	0.17	1.0	0	1.0	0	0.14	0.43	0.43	0.86	0.14	0.86	0.14	1.0	0	1.0	0
Southern Peru	3	0	0.83	0	0.17	0.33	0.64	0.64	0.33	0	1.0	0	0.33	0.67	1.0	0	0.10	0	0.33	0.66	0.33	0.66
Argentina	30	0	0.96	0	0.04	0.99	0.01	0	1.0	0	1.0	0	0.08	0.92	0.997	0.025	0.59	0.41	0.32	0.68	0	1.0

in only two accessions: DGD1962 from northern Peru and G19901 from Argentina. Allele *Mdh-1*^{103} was found in accession G12856B from southern Peru. A new variant at the *Diap-1* locus was found in a limited number of accessions from Mexico: allele 102 migrated further than the 100 allele described previously (Sprecher 1988). The 'rare' allele (*Diap-1*^{96} or *Diap-1*^{1}) described previously by Sprecher (1988) was found in a limited number of accessions from Mexico (Table 1). None of the wild accessions had the 'unique' genotype (*Diap-1*^{95}, *Diap-2*^{105}) observed in the small black-seeded cultivars from Mesoamerica (Sprecher 1988).

Discussion

The use of allozymes complements the information provided by our analysis of phaseolin diversity (Gepts et al. 1986; Gepts and Bliss 1986; Koenig et al. 1989) and provides a clearer picture of the organization of genetic diversity in wild *P. vulgaris*. The genetic diversity within wild *P. vulgaris* (Ht = 0.132) is lower than that found in other *Phaseolus* species (Wall and Wall 1975); however, our population size, geographical range and number of polymorphic loci examined is larger. The low mean intrapopulation genetic diversity (Hs = 0.006) indicates there is no significant genetic diversity within populations of wild *P. vulgaris*. These results are consistent with those reported by Loveless and Hamrick (1984), who indicated that annual selfing species have fewer alleles per locus and more skewed allele frequencies, a pattern that is indicative of reduced gene flow. The gene differentiation among populations (Gst) measures the proportion of variation among populations relative to the total species diversity (Ht). Our results indicate a moderate level of genetic diversity among populations consistent with the selfing, annual and early successional nature of *P. vulgaris*, whose seeds are primarily dispersed by gravity (Loveless and Hamrick 1984).

Reviews on the factors which account for the amount of genetic diversity within plant populations (Brown 1979; Gottlieb 1981; Hamrick et al. 1979) indicate that the selfing mode of reproduction limits the movement of alleles from one population to another and results in an increase in genetic differentiation among populations. Geographical isolation and selfing mating system are probably two major causes of the differentiation of genes between the Mesoamerican and Andean groups in common bean. Further research on the linkage relationships between isozyme loci and genes conferring adaptation to particular environments is needed to assess the role of natural selection in the genetic divergence of the wild populations.

The dendrogram (Fig. 1) illustrates the separation of two major groups. The first group consists of accessions

from Mexico, Guatemala, Costa Rica, Colombia and southern Peru. the second group consists of accessions from Argentina and southern Peru. Accession DGD1962 from northern Peru is genetically distinct and occupies a position separate from the two major population clusters. Our allozyme diversity data suggesting two geographically separate groups of wild common bean are consistent with previous studies which analyzed phaseolin variability, the major seed storage protein of *P. vulgaris*. Wild beans from Mesoamerica had the 'S' and 'M' types, while those from the Andean region showed 'T', 'C', 'J' or 'I' types (Gepts et al. 1986; Koenig et al. 1989). In Mesoamerica, wild beans exhibited a greater amount of genetic variability in phaseolin protein as compared to Andean material. Wild beans from Colombia showed the 'S', 'CH' and 'B' types, the latter two types being unique to this geographical region. Gepts and Bliss (1986) suggested that this region might be a minor domestication center of the common bean and a geographical meeting place for the Mesoamerican and Andean cultivated forms. Our present allozyme data suggest that the wild *P. vulgaris* populations from the northern Andes (i.e. Colombia) are very similar to Mesoamerican wild *P. vulgaris* populations. The phaseolin pattern of accession of DGD1962 from northern Peru lacks the 52-K high molecular weight subunits of phaseolin and is more similar to Colombian phaseolin types. This accession may, therefore, originate in a geographical transition area between the Mesoamerican and Andean populations.

Our data confirm the findings, based mostly on cultivated common bean germplasm from Malawi, of Sprecher (1988), who also observed that common bean cultivars belong to two major gene pools distinguished by contrasting alleles. In our small sample of crosses (R. Koenig and P. Gepts, unpublished results), we found no evidence for reproductive isolation such as the one observed between Mesoamerican and Andean cultivars (Singh and Gutierrez 1984; Gepts and Bliss 1985; Gepts 1988). However, a more definitive answere will come from extensive crossing involving wild Mesoamerican and Andean genotypes.

The allozyme data confirms phaseolin data indicating that there exists higher genetic variability in accessions from the Mesoamerican gene pool compared to the Andean gene pool. The dendrogram (Fig. 1) divides the Mesoamerican population of 52 accessions into 34 allozyme profiles, while the Andean population of 31 accessions is divided into 11 allozyme profiles. On the other hand, accessions from the Mesoamerican populations (Mexico, Central America and Colombia) have six polymorphic loci with 17 allelic variants, comparable with accessions from the Andean region (Argentina and southern Peru) which have eight polymorphic loci with 16 allelic variants. There are more polymorphic loci in the Andean region, because two of the loci, *Gpi-2* and *Mdh-1*, exhibit rare variants. The genetic variability at these loci is small, but may indicate that these geographical regions might be potential sites for the collection of germplasm which possess rare alleles. The major geographical regions (Mesoamerican and Andean) can be delineated by plant accessions which exhibit specific allozyme patterns for *Lap-3, Diap-1* and *Skdh* (Fig. 2). Accession DGD1962 from northern Peru has allozyme patterns which are either a combination of Mesoamerican and Andean variants or unique, and perhaps delineates a geographical transition area.

The demarcation of a geographical transition zone based on allozyme and phaseolin diversity in wild *P. vulgaris* can be widely defined to the Colombia-Peru region. Additional wild *P. vulgaris* plant collections are needed to more narrowly define the boundaries of a transition region and to determine whether this region may, in fact, be a minor center of genetic diversity and domestication. It also remains to be determined whether this transition zone can be correlated with major changes in the habitat, such as vegetation type.

The limitation of utilizing allozymes as molecular markers to assess genetic diversity in a self-pollinating crop as *P. vulgaris* is that the number of polymorphic loci and alleles per locus are limited. Therefore, it may be appropriate to use restriction fragment length polymorphisms (RFLPs) to give a more comprehensive analysis of the genetic diversity which exists in this species.

Acknowledgements. We thank Drs. D. Debouck, D. Wood and R. Hidalgo (Centro Internacional de Agricultura Tropical, Cali, Colombia), Dr. R. Hannan (Western Plant Introduction Station, Pullman/WA) and Dr. J. G. Waines (University of California, Riverside) for supplying seeds of the accessions used in this study, Dr. R. Kesseli for assisting in the computer analysis and E. Martinelli for typing the manuscript. This research was supported in part by a grant of the C. A. Lindbergh Fund. R. K. is a recipient of a fellowship of the International Agriculture Development Graduate Group, University of California at Davis.

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