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Extensive introgression of Middle American germplasm into Chilean common bean cultivars

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Summary

The genetic diversity of 95 representative Chilean common bean (*Phaseolus vulgaris* L.) landraces was analyzed using phaseolin seed protein and eight isozyme systems as genetic markers. Four types of phaseolin were found, "C", "T", "S" and "H", in decreasing order of frequency. Each type had a different distribution between the Northern and Southern regions of the country. Nei's genetic distance based on isozyme diversity indicated that a high percentage of the total variation found in this sample occurred between landraces and only a small percentage of the variation was detected within populations. Cluster analysis based on Nei's genetic distance and a principal component analysis of isozyme frequencies did not detect a clear association between the geographic distribution of the landraces and their isozyme constitution. However, Nei's genetic distance analysis clustered the bean landraces into two major groups which had a specific isozyme pattern, seed color, and seed size. The genetic analysis also detected a rare polymorphism for the *Mdh-2* locus, a null allele at the *Diap-2* locus, and polymorphism for the *Aco-2* locus. The principal component analysis of isozyme frequencies showed that only 30% of the genotypes analyzed were similar to the Andean check and 5% of the samples were similar to Middle American check. This finding suggests a high frequency of hybridization between the Middle America and Andean gene pools in cultivated common bean from Chile.

Introduction

In Chile, common bean (*Phaseolus vulgaris* L.) is cultivated from Arica (18° S.Lat.) to Chiloé (40° S.Lat.). However, approximately 90% of the commercial dry bean production area is concentrated in the central southern part of the country (INE, 1990/91). Chilean bean production covers an area approximately 100,000 ha, which represents 8 to 10% of the area dedicated to annual crop production. Average bean seed yield varies between 900 to 1000 kg/ha (INE, 1990/91). Common bean is cultivated mainly by medium- and smallholder farmers as a monocrop but some smallholder farmers, especially those located in the provinces of Ñuble and Bío-Bío grow beans in association with maize at very low density in the same row. The level of mechanization, use of fertilizers and certified seed is rather limited, specially when bean is grown by smallholder farmers. However, in the last years, the use of certified seed and other improved technology has been promoted by the Government through the Instituto de Investigaciones Agropecuarias (INIA) and private companies to increase production and export of common bean.

The production of common bean in Chile is mainly devoted to dry beans and only a small area is dedicated to green pods, "granados" (well-developed but immature seeds), and seed production. The main types of beans produced and consumed in Chile are "tórtolas" and "coscorrón", market classes that are unique in the world and belong to gene pool 10 (Singh, 1989) and race Chile (Singh et al., 1991a). In addition to "tórtolas" and "coscorrón", Chile produces other bean market classes such as "small blacks", "navies", "great northern", "pintos", "cranberries" and "kidneys" for export, depending on international market conditions.

Despite the agricultural and economic importance of the species in the country, Chile did not until recently have a complete and well-characterized bean collection. In 1990, the food legume program at INIA with the technical collaboration of the Centro Internacional de Agricultura Tropical (CIAT) and the economic support of the International Board for Plant Genetic Resources (IBPGR), organized and collected more than 1000 landraces throughout the country with the purpose to conserve, characterize and use these genetic resources in breeding programs.

Traditionally, genetic diversity in common beans has been evaluated using morphological and agronomic traits of individual genotypes with little consideration of their evolutionary origin (gene pools) or relationships between and within gene pools. Yet in many cases, morphological characters do not reliably show the genetic diversity present between genotypes because of genotype x environmental interaction (Smith & Smith, 1989) and the presence of only few genes with major effects that control these characters (Gepts, 1990; Smart, 1990). In addition, these major genes represent only a small percentage of the variation present in the genome.

Morphological, ecological, isozyme, phaseolin, DNA data have shown that the Middle American and Andean gene pools can be subdivided into different races (Singh et al., 1991a, 1991b, 1991c; Khairallah et al., 1990; Becerra Velásquez, 1992). Thus, the Middle American gene pool has been divided into the races Mesoamerica, Durango and Jalisco, and the Andean gene pool has been partitioned into races Nueva Granada, Perú and Chile (Singh et al., 1991a).

In *Phaseolus*, phaseolin, the major storage seed protein, has been a useful tool for studying the origin of domestication of the crop (Gepts et al., 1986; Gepts & Bliss, 1986; Gepts, 1988, 1989, 1990; Koenig et al., 1990), dissemination of cultivars from the centers of domestication (Gepts & Bliss, 1988; Gepts et al., 1988), segregation and linkage relationships (Koenig & Gepts, 1989a), the effect of genetic domestication upon genetic diversity (Gepts, 1990, 1993b), genetic variability between genotypes (Mutschler et al., 1980; Brown et al., 1981, 1982; Gepts & Bliss, 1985; Schinkel & Gepts, 1988; Lioi, 1989a, 1989b; Koenig et al., 1990), and genetic manipulation to improve nutritional quality in beans (Bliss & Brown, 1983; Bliss, 1989).

Isozyme variation in *Phaseolus* has been used to confirm the hypothesis of multiple domestication of common bean (Sprecher, 1988; Koenig & Gepts, 1989b; Singh et al., 1991c), to evaluate multilocus associations (Gepts, 1989), measure differential elimination of donor parents in crosses (Wall, 1968), measure genetic diversity between genotypes (West & Garber, 1967; Wall & Wall, 1975; Bassiri & Adams, 1978a, 1978b; Weeden, 1984b; Sprecher, 1988; Koenig & Gepts, 1989b; Singh et al., 1991c), and identify the organization of common bean gene pool into different races (Singh et al., 1991a).

The objectives of the research reported in this paper are: a) to study the genetic organization and amount of variation present in a large sample of Chilean common bean germplasm from the various bean growing regions of the country using phaseolin and isozyme markers, and b) identify a relationship, if any, between the observed genetic variation and its geographic distribution.

Materials and methods

Plant material. The 95 landraces analyzed in this study were obtained from the Food Legume Program of Chile (INIA). These landraces are the product of the national bean collection carried out in 1990 and other local explorations carried out around the Chillán area between 1987 and 1989. These landraces are representative of the geographic distribution of the species in the country. For the purpose of this study, the country was divided into two main geographic areas. The

Northern region, which included the regions I, IV, V and VI, was represented by twenty landraces. The Southern region, which included regions VII, VIII, IX and X, was represented by seventy-five landraces due to the greater importance of the bean production in the area (Table 1).

Phaseolin analyses. Samples of flour from the end of each of the five seed raphe per landrace was taken for phaseolin analyses using one-dimensional sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Brown et al., 1981).

Isozyme analyses. The same five seeds utilized for the phaseolin determination were used for isozyme analysis. Seeds were germinated in petri dishes and treated with the fungicide Rovral (10 mg/l). After germination, seedlings were sown in vermiculite and grow under greenhouse conditions. Samples of leaves or roots were taken when seedlings were at the first true leaf stage, depending on the isozyme system. The preparation of the crude homogenate and electrophoretic conditions for the isozyme analysis were similar to those described elsewhere (Weeden, 1984b; Koenig & Gepts, 1989b). The light isozyme systems used in this study were selected based on the polymorphism reported in previous works (Koenig & Gepts, 1989b; Singh et al., 1991c): diaphorase (DIAP), glucose phosphate isomerase (GPI), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), malic enzyme (ME), shikimate dehydrogenase (SKDH), small subunit of ribulose bisphosphate carboxylase (RBCS) and aconitase (ACO). The genetics of these isozyme systems has been described previously (Weeden, 1984a, 1986; Koenig & Gepts, 1989a), except for the polymorphism at the *Aco*-2 locus, which has not been reported previously in Chilean bean landraces.

In each gel, the cultivars "ICA-Pijao" and "California Dark Red Kidney" (CDRK) were included as Middle American and Andean checks, respectively. "ICA-Pijao" has the following genotype at polymorphic enzyme loci: *Aco-2* ^{100}, *Diap-1* ^{95}, *Diap-2* ^{105}, *Lap-3* ^{100}, *Mdh-1* ^{100}, *Mdh-2* ^{100}, *Me* ^{100}, *Skdh* ^{103}, *Rbcs* ^{100}. CDRK shows the following pattern at polymorphic enzyme loci: *Aco-2* ^{102}, *Diap-1* ^{100}, *Diap-2* ^{100}, *Lap-3* ^{103}, *Mdh-1* ^{103}, *Mdh-2* ^{103}, *Me* ^{98}, *Skdh* ^{100}, *Rbcs* ^{98}.

Statistical analysis of genetic diversity. Total gene diversity (Ht), and its partition into intra (Hs) and inter-population (Dst) diversity, as well as the coefficient of gene diversity (Gst) and absolute gene diversity (Dm) were calculated using Nei's (1973) genetic diversity statistics on allozyme frequency data. A dendrogram was constructed from Nei's distance (D) by the unweighted paired group method using arithmetic averages (Sneath & Sokal, 1973) of 95 landraces using eight polymorphic loci and a computer program provided by Dr. K. Ritland (Department of Botany, University of Toronto, Canada). A principal component analysis, was also performed, using the SAS PROC PRINCOMP program (SAS, 1985) to analyze the pattern of genetic diversity using the isozyme data.

Results

Geographic distribution of phaseolin types. Four electrophoretic types of phaseolin were found among the 95 Chilean common bean landraces analyzed. The "C" phaseolin was present at the highest frequency (59.9%), followed by the "T" type with 11.4%, "S" type with 9.2% and "H" type with 3.9% (Table 2). An analysis of the distribution of phaseolin types in the two major geographic regions (North and South) of Chile indicated that the "C" phaseolin type was the most abundant type in both agroecological regions. The "S" phaseolin type was found in higher proportion in the southern region (13.3%) compared with the northern region (5.0%). On the other hand, only one landrace (Parrón) showed the "T" phaseolin type in the northern region of the country and 19 landraces had this phaseolin type in the southern part of the country. Conversely, the percentage of "H" phaseolin type was higher in the northern region (5.0% vs 2.7%) compared with the southern area in the country (Table 2). The percentage of accessions that were heterogenous for phaseolin type was higher in the northern part of the country than in the southern part of Chile.

Genetic distances. The total genetic diversity (Ht) based on isozyme data was 0.159, the coefficient of genetic diversity (Gst) was 0.978 and the absolute gene differentiation (Dm) was 0.158. The partition of the total genetic variation between (Dst) and

Table 1. Identification (ID), genotypes, origin, phaseolin and allozyme constitution of 95 Chilean landraces

ID	Genotype	Origin	Region	*Phs*	*Skdh*	*Lap-3*	*Rbcs*	*Mdh-1*	*Mdh-2*	*Me*	*Diap-1*	*Diap-2*	*Aco-2*
CH018	Tórtola	Chillán	VIII	C	100	103	100	100	100	98	100	100	100
CH019	Coscorrón	Chillán	VIII	C	100	103	98	100	100	98	100	100	98
CH020	Araucano	Chillán	VIII	C	100	103	98	100	100	98	95	100	100
CH022	Burro Alemán	Chillán	VIII	C	100	103	98	100	100	98	100	100	100
CH023	Abalito	Chillán	VIII	C	100	103	100	100	100	98	100	100	100
CH024	Metro	Chillán	VIII	T	100	—	—	103	100	98	100	100	—
CH025	Payar	Chillán	VIII	S	103	100	100	100	100	100	100	100	98
CH026	Hallados	Chillán	VIII	S	100	103	98	100	100	98	100	100	100
CH027	María	Chillán	VIII	C	100	103	98	100	100	98	100	100	98
CH028	Zeus	Chillán	VIII	T	100	103	98	100	100	98	100	100	98
CH029	Señorita	Chillán	VIII	C	100	103	100	100	100	98	100	100	100
CH030	Traile	Chillán	VIII	C	100	103	100	100	100	98	100	100	98
CH031	Hallados chico	Chillán	VIII	C	100	103	100	100	100	98	100	100	98
CH032	Cabritos	Chillán	VIII	C	100	103	98	100	100	98	100	100	100
CH033	Ganso	Chillán	VIII	C	100	103	100	100	100	98	100	100	100
CH034	Gringo	Chillán	VIII	C	100	103	98	100	100	98	100	100	100
CH035	Mantequilla	Chillán	VIII	C	100	103	98	100	100	98	100	100	100
CH036	Juanita	Chillán	VIII	C	100	103	98	100	100	98	100	100	98
CH039	Manteca	Chillán	VIII	T	100	103	98	100	100	98	100	100	100
CH040	Cabrito chico	Chillán	VIII	C	100	103	98	100	100	98	100	100	100
CH041	Buey	Chillán	VIII	C	100	103	100	100	100	98	100	100	100
CH042	Oro	Chillán	VIII	C	100	103	98	100	100	98	100	100	100
CH043	Coscorrón americano	Chillán	VIII	C	100	103	100	100	100	98	100	100	98
CH044	Abalo	Chillán	VIII	C	103, 100	103	100	100	100	98	100	100	98
CH045	Burro	Cauquenes	VII	C	100	103	100	100	100	98	100	100	100
CH046	Burro argentino	Chillán	VIII	C	100	103	98	100	100	98	100	100	100
CH047	Presilla	Cauquenes	VII	S	100	103	98	100	100	98	100	100	100
CH070	Ch-60	Cañete	VIII	T	100	103	98	100	100	98	100	100	100
CH071	Ch-68	Cañete	VIII	T	103	103	98	100	100	100	95	100	98
CH072	Ch-77	Cañete	VIII	C	100	100	100	100	100	98	100	100	100
CH073	Araucano	Cañete	VIII	C	100	103	98	100	100	98	100	100	100
CH074	Ch-118	Mulchén	VIII	S	100	103	98	100	100	98	95	100	98
CH075	Señorita	Quilaco	VIII	C	100	103	98	100	100	98	100	100	98
CH076	Fideo	Quilaco	VIII	C	100	103	98	100	100	98	100	100	98
CH077	Ch-138	Loncopangue	VIII	S, T	100	103	98	100	100	100	95	100	98
CH080	Payar	Santa Bárbara	VIII	H	100	100	100	100	103	98, 100	100	100	98
CH081	Burro	Villucura	VIII	S	103	100	100	100	100	98, 100	100	null	98
CH082	Pajarito	Villucura	VIII	S	100	103	98	100	100	98	100	100	100
CH083	Ch-181	Racalhue	VIII	C	100	103	100	100	100	98	100	100	98
CH084	María	Racalhue	VIII	T	100	103	98	100	100	98	100	100	98
CH085	Borloto	Longaví	VII	T	100	103	98	100	100	98	100	100	98
CH086	Bayo	Los Niches	VII	T	100	103	98	100	100	98	100	100	100

CH087	Bayo argentino	Pelarco	VII	T	100	103	98	100	100	98	100	100	100
CH088	Ch-249	Cumpeo	VII	C	100	103	100	100	100	98	100	100	100
CH089	Gato	Tomé	VIII	C	100	103	100	100	100	100	100	100	100
CH091	Ch-283	Tomé	VIII	C	100, 103	100	100, 103	100	100	98	100	100	98
CH092	Ch-284	Tomé	VIII	T	103	103	98	100	100	98	95	100	98
CH093	Frutilla	Tomé	VIII	C	100	103	98	100	100	98	100	100	100
CH094	Ch-307	Coelému	VIII	C	100	103	98	100	100	98	100	100	98
CH095	Ch-336	Peumo	VI	C	100	103	98	100	100	98	100	100	100
CH096	Rancagüino	Guarilihue	VIII	T	100	103	100	100	100	100	100	100	100
CH097	Metro	Guarilihue	VIII	S	103	103	100	100	100	100	95	100	98
CH098	Ch-455	Ercilla	IX	C	100	103	98	100	100	98	95	null	98
CH099	7-semanas	Hualqui	VIII	T	100	100	98	100	100	98	95	100	98
CH100	Ch-479	Los Angeles	VIII	T	100	100	98	100	100	98, 100	100	100	100
CH101	Ch-498	Los Angeles	VIII	T	100	100	100	100	100	100	100	100	98
CH102	Ch-481	Yumbel	VIII	S	100	—	98	100	100	—	100	100	98
CH103	Correa	Curaco	VIII	C	103	103	98	100	100	—	100	100	98
CH104	Ch-520	Yumbel	VIII	C	100	103	100	100	100	98	100	100	100
CH105	Ch-533	Curanilahue	VIII	C	100	103	100	100	100	98	100	100	100
CH106	Ch-545	Curanilahue	VIII	C	100	103	100	10	100	98	100	100	100
CH107	Ch-550	Elicura	VIII	C, T	100	103	98	100	100	98	100	100	100
CH108	Ch-554	Elicura	VIII	T, C	100	103	98	100	100	98	100	100	100
CH109	Ch-564	Chanco	VIII	H	100	103	100	100	100	98	95	100	98
CH110	Ch-565	Chanco	VIII	C, S	100	103	98	100	199	98	100	100	98
CH111	Ch-566	Chanco	VIII	T	100	103	100	100	100	98	100	100	98
CH112	Ramillete	Arica	I	C	100	103	98	100	100	98	100	100	98
CH113	Cristal Bayo	Arica	I	C	100	103	98	100	100	98	100	100	98
CH114	Azufrados	Cerrillos de Rapel	IV	C	100	103	98	100	100	98	100	100	100
CH115	Sapo	Pangelillo	IV	C	100	103	98	100	100	98	100	100	98
CH116	Plomo	Pangelillo	IV	C	100	—	98	100	100	98	100	100	—
CH117	GB-94	La Capilla	IV	C	100	103	100	100	100	98	100	100	100
CH118	Tórtola	Higueras de Rapel	IV	C	100	—	98	100	100	—	100	100	—
CH119	Coscarrón	Quilimarí	IV	C	103	103	98	100	100	100	95	100	98
CH120	GB-93		IV	C	100	100	100	100	100	98	100	100	100
CH121	Vaquita	Concha Bajo	IV	H	100	103	100	100	100	98	100	100	100
CH122	NN-24	Valdivia	VI	C	100	103	100	100	100	98	100	100	100
CH124	PS-7	La Ligua	V	C	100	103	98	100	100	98	100	100	100
CH125	Borlotillo		V	C, H	100	103	98	100	100	98	100	100	100
CH126	Angelito		VI	C, H	100	103	98	100	100	98	100	100	100
CH127	GB-106	Quilimarí	IV	S	100	103	98	100	100	98	100	100	100
CH128	GB-32		VII	C	100	103	98	100	100	98	100	100	100
CH129	Colorado sin hilo	Mulchén	VIII	C	100	103	100	100	100	98	100	100	100
CH130	Buey	Angol	IX	C	100	103	98	100	100	98	100	100	100
CH131	Sapo	Nueva Imperial	IX	H	100	103	98	199	199	87	199	199	199
CH132	NN-18	Nehuentue	IX	S	103	100	100	100	100	100	95	100	98

Continued overleaf

Table 1—continued

ID	Genotype	Origin	Region	*Phs*	*Skdh*	*Lap-3*	*Rbcs*	*Mdh-1*	*Mdh-2*	*Me*	*Diap-1*	*Diap-2*	*Aco-2*
CH133	NN-165	Cajón	IX	C	100	103	98	100	100	98	100	100	100
CH134	NN-153	Las Hortensias	IX	T	103	100	98	100	100	100	95	100	98
CH135	NN-63	Galvarino	IX	C	100	103	98	100	100	98	100	100	100
CH136	Azufrados	Osorno	X	C	100	103	98	100	100	100	100	100	100
CH137	Chilotito	Chiloé	X	S	100	103	98	100	100	98	100	100	100
CH138	Intruso-1			C	100	100	98	100	100	100	100	100	100
CH139	Parrón			T	100	103	98	100	100	98	100	100	100
CH140	GB-26			C	100	103	98	100	100	98	100	100	100
CH141	FF-39			C, T	100	103	98	100	100	98	100	100	100

Table 2. Geographic distribution of phaseolin types among Chilean landraces

Geographic distribution	Sample size	Phaseolin type (%)[a]			
		"S"	"T"	"C"	"H"
Northern region (I, IV, V, VI)	20	5	0	65	5
Southern region (VII, VIII, IX, X)	75	13	22	55	3
Country-wide average	95	9	11	60	4
Previous data[b]	13	8	25	58	8

[a]Sums do not add up to 100% because only homogenous accessions were considered.
[b]Gepts et al. (1986).

within (Hs) populations indicated that most of the genetic variation was found between populations (0.156) and only little variation (0.004) was present within populations, which is consistent with the predominantly self-pollinated nature of the species (Koenig & Gepts, 1989b).

The analysis of genetic identity (Nei, 1973) indicated that the average genetic identity (I) of this population was 0.85, which is similar to the average genetic identity reported in *Lens culinaris*, a predominantly self-pollinated species, but lower than the identity value reported for *Zea mays* subsp. *mays* (I = 0.95), a cross-pollinated species (Doebley, 1989). As mentioned earlier this difference may be due to the fact that this analysis only included isozyme systems revealing polymorphisms, therefore estimates were biased downwards.

Eight of twelve loci analyzed showed polymorphism (*Diap-1*, *Diap-2*, *Lap-3*, *Mdh-1*, *Mdh-2*, *Me*, *Skdh*, *Rbcs*, and *Aco-2*) and four loci were monomorphic (*Gpi-c1*, *Gpi-c2*, *Lap-1*, *Lap-2*, and *Aco-1*). Except for a tight linkage between *Diap-1* and *Diap-2* and a loose linkage between *Rbcs* and *Me*, the polymorphic loci represent unlinked regions of the genome (reviewed in Gepts, 1993a; Gepts et al., 1993). In general, the level of isozyme polymorphism found in the samples analyzed was high compared with that reported previously by Hamrick & Godt (1989) and Koenig & Gepts (1989b) because only enzyme systems that showed polymorphism in earlier studies were selected. The absence of polymorphism for the *Gpi* loci

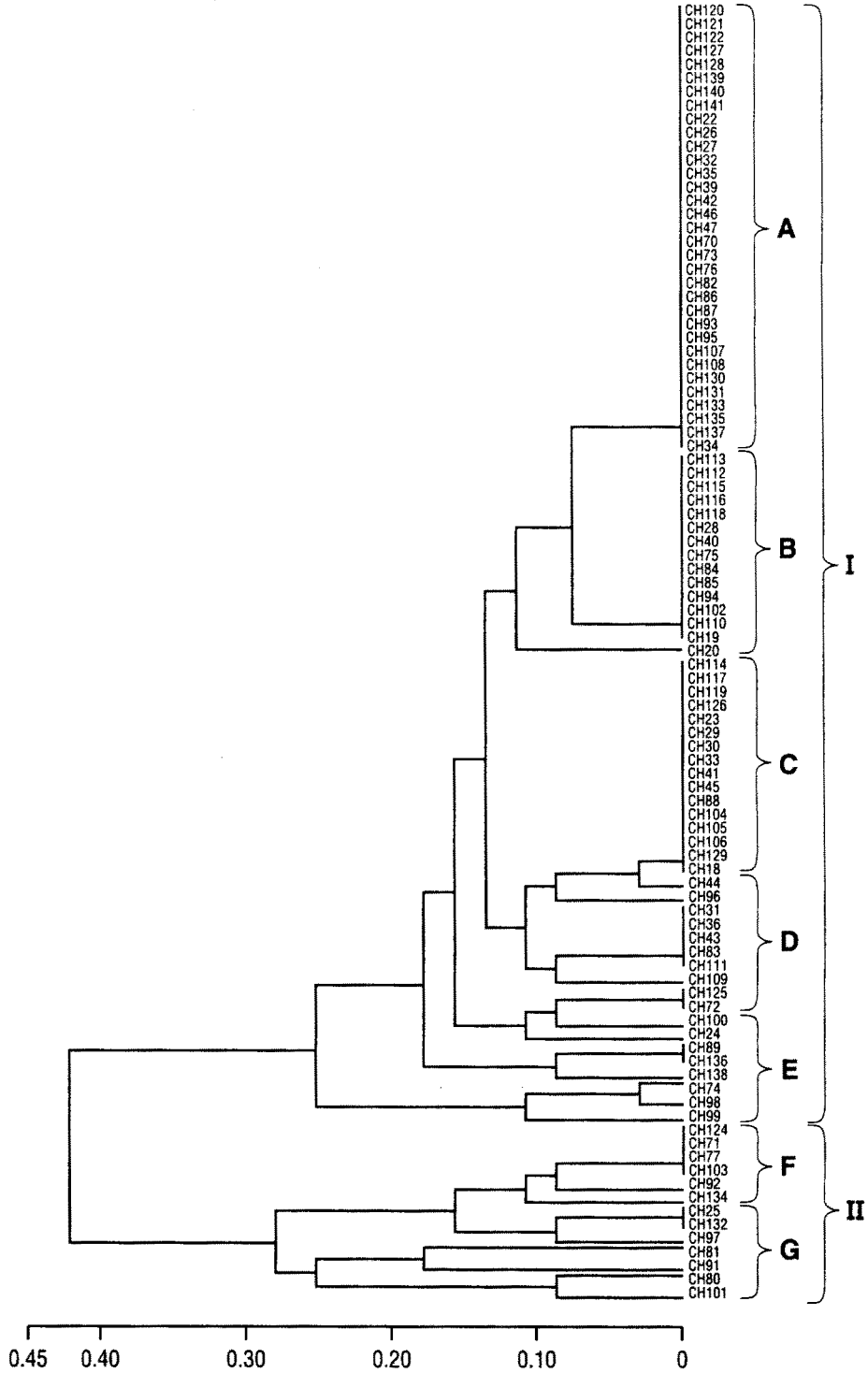

Fig. 1. Dendrogram of 95 Chilean common bean landraces based on isozyme frequency data. The abscissa represents Nei's distance.

agreed with results obtained by Sprecher (1988), even though a low level of polymorphism for the GPI system has been reported previously (Weeden, 1986; Koenig & Gepts, 1989b).

Cluster analysis based on Nei's genetic distance showed the presence of two major groups of genotypes (Fig. 1). None of the groups showed any clear pattern of geographical distribution that could be related to the isozyme and phaseolin constitution of the landraces. Most of the genotypes collected and produced preferentially in the Northern or the Southern part of the country are located together in the dendrogram (Fig. 1). However, group I contained 82 genotypes which constitutes 83.3% of the landraces studied. These genotypes were generally characterized by a medium seed size and mottled seed color and most of them are used for dry seed production. Genotypes of this group had different phaseolin patterns, but a high percentage of them had Andean phaseolin types. For instance, there were 51 genotypes that had a "C" phaseolin type, which represented 92.7% of all genotypes with a "C" phaseolin type. Twelve genotypes had a "T" phaseolin type (81.3%), 4 genotypes had an "H" phaseolin type (80.0%), and 7 genotypes had an "S" phaseolin type (63.6%).

The minor group (II) in the dendrogram (Fig. 1) included only 13 genotypes. These genotypes were characterized by having a larger seed size and lighter seed colors compared with those genotypes included in group I. Most of these genotypes are consumed as green pods.

These two main groups also exhibited a distinct isozyme pattern (Table 3). The larger group (I) had a higher frequency of the slow alleles for the *Skdh*, *Rbcs* and *Me* loci (*Skdh* ^{100}, *Rbcs* ^{98}, and *Me* ^{98}), and a higher frequency of the fast allele at the *Lap-3* and *Aco-2* loci (*Lap-3* ^{103} and *Aco-2* ^{100}). On the other hand, the minor group (II) was characterized by having a higher frequency of the fast allele of *Skdh* (*Skdh* ^{103}), slow allele for *Diap-1* and *Aco-2* (*Diap-1* ^{95} and *Aco-2* ^{98}), and intermediate allele of *Me* (*Me* ^{100}). The frequency of alleles at the *Mdh-1*, *Mdh-2*, and *Diap-2* loci were similar in both groups (Table 3).

This study also detected the presence at low frequency of the fast allele of *Mdh-1* (*Mdh-1* ^{103}) (0.02), the fast allele of *Mdh-2* (*Mdh-2* ^{102}) (0.08), and a null allele at the *Diap-2* locus (*Diap-2* ^{N}).

Table 3. Geographic frequency and national average of isozymes among Chilean landraces compared with previous data

Allozyme	Alleles	Group		Average	Race Chile[a]
		I	II		
Skdh	103	0.01	0.74	0.38	0.36
	100	0.99	0.26	0.62	0.64
Rbcs	100	0.32	0.49	0.41	0.50
	98	0.68	0.51	0.59	0.50
Lap-3	103	0.94	0.46	0.70	0.71
	100	0.06	0.54	0.30	0.29
Mdh-1	103	0.02	0.00	0.01	0.00
	100	0.98	1.00	0.99	1.00
Mdh-2	102	0.00	0.08	0.04	0.00
	100	1.00	0.92	0.96	1.00
Me	102	0.00	0.00	0.00	0.00
	100	0.05	0.77	0.41	0.29
	98	0.95	0.23	0.59	0.71
Diap-1	100	0.53	0.23	0.38	0.71
	95	0.47	0.77	0.62	0.29
Diap-2	100	0.00	0.00	0.00	0.07
	95	0.99	0.92	0.96	0.93
	null	0.01	0.08	0.04	0.00
Aco-2	100	0.73	0.00	0.37	—
	98	0.27	1.00	0.63	—

[a]Compiled from Singh et al. (1991c).

The *Mdh-1* ^{103} allele is characteristic of race Peru materials in the Andean gene pool (Singh et al., 1991a). Its presence may have resulted from hybridization with materials from race Peru or introduction of these materials into Chile. The *Mdh-2* ^{102} allele was observed previously only in a small group of cultivars belonging to race Jalisco in the Middle American gene pool and wild beans from the same region in Mexico. This allele may be indicative of gene flow between wild and cultivated beans in that region of Mexico (Singh et al., 1991a, 1991b, 1991c). The *Diap-2* ^{N} allele had been detected previously only in two Turkish genotypes of Andean origin (Sprecher, 1988). Furthermore, it was possible to identify polymorphisms at the *Aco-2* locus not yet described among Chilean bean landraces (Table 3).

The country-wide averages of most of the alleles agreed with previous studies that included a small sample (17) of Chilean landraces (Singh et al., 1991c). However, there were some differences in the frequency of alleles at the *Diap-1* and *Me* loci (Table 3).

Principal component analysis. In the principal component lysis, the first three principal components accounted for 44%, 23% and 13% of the total variation observed, respectively. Different allozyme alleles were responsible for the variation along the principal components. The Chilean landraces analyzed in this study were distributed in seven subgroups (A to G) in the principal component graph and in the dendrogram (Figs. 1 and 2). For instance, subgroup A included genotypes that were more closely related to typical Andean genotypes based on their isozyme and phaseolin data. The 33 genotypes of subgroup A differed from the Andean check only at the *Mdh-1* locus and represented the race Chile described by Singh et al. (1991a). On the other hand, some genotypes included in subgroup G differed from CDRK at the *Phs* (phaseolin) locus as well as at seven enzyme loci (data not shown).

Our observation confirmed the Andean origin of the Chilean landraces, but revealed that approximately 70% of the landraces had an isozyme constitution that diverged from the typical isozyme pattern of the Andean and Middle American gene pool (groups B, C, D, E, F and G). These landraces may have resulted from hybridization between the two gene pools (Fig. 2). Examination of the phaseolin type information indicated that most of the genotypes that clustered with the Middle American check (groups G and F) had an "S" phaseolin type. Although this suggested a Middle American origin, these genotypes had a large seed size, which is not characteristic of this gene pool. This observation also suggested the presence of some degree of hybridization in this population. Another observation supporting this hypothesis is the existence of genotypes with an "S" phaseolin type but with isozyme patterns that are typical of the Andean gene pool.

A comparison of the location of the genotypes in the dendrogram (Fig. 1) and the principal component analysis (Fig. 2) showed that the major

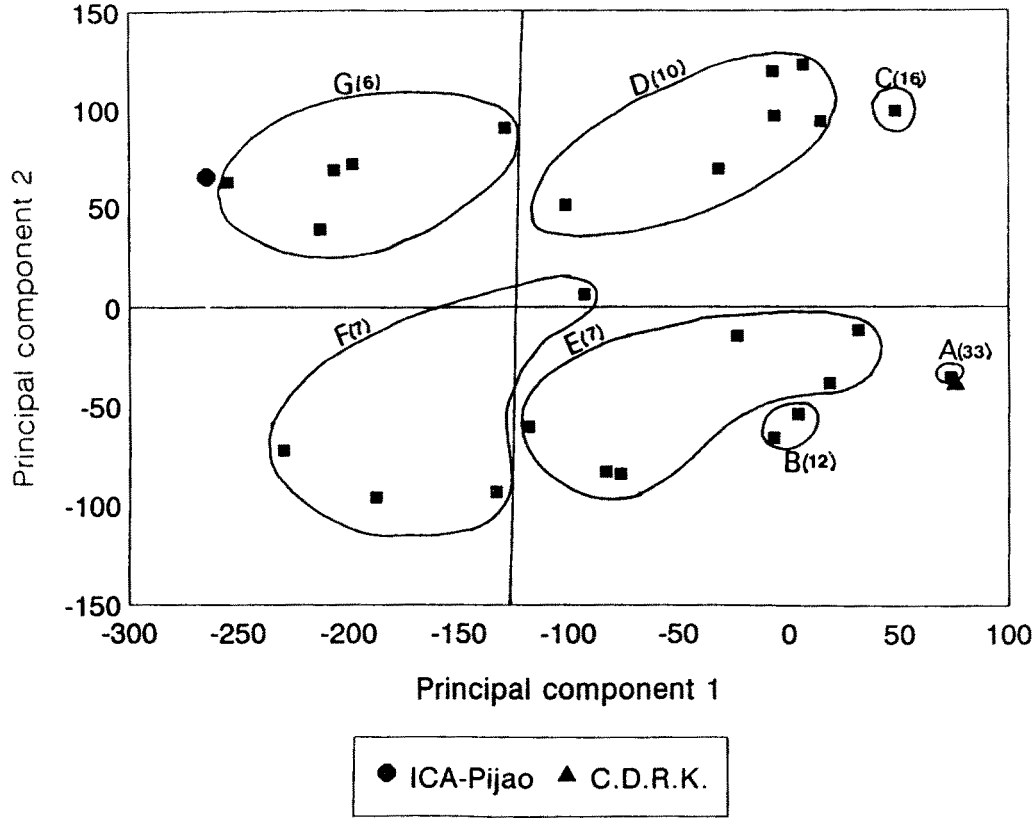

Fig. 2. Principal component analysis of allozyme frequency data in Chilean common bean landraces. Groups A to G correspond to groups identified in the dendrogram in *Fig.* 1. Numbers in parentheses are numbers of entries in each group.

branch in the dendrogram contained those genotypes that are more closely related to the Andean genotypes (subgroups A to E including genotypes CH120 to CH99), whereas group II included those genotypes (subgroup F and G including genotypes CH124 to CH101) that were least related to the Andean gene pool.

Discussion

The phaseolin analysis of 95 genotypes confirmed a previous report (Gepts et al., 1986) that there are four types of phaseolin among Chilean landraces: "C", "T", "S" and "H". The "C" type phaseolin was the most frequent type in all geographic regions whereas "T", "S" and "H" exhibited a more specific geographic distribution. Previous studies demonstrated that the Middle American and Andean gene pools have a characteristic isozyme pattern and seed size (Gepts & Bliss, 1986; Sprecher, 1988; Singh et al. 1991a). Although 92% of the Chilean landraces had a typical Andean phaseolin, only 30% of the genotypes had an isozyme constitution representative of the Andean gene pool. This observation suggests that part of the Chilean common bean germplasm may have resulted from hybridization between the Middle American and Andean gene pools in Chile although an alternative hypothesis is also possible (see below). The process of introgression between Middle American and Andean genotypes observed in this study can be supported by four lines of evidence: a) a high proportion of Andean landraces with an isozyme pattern characteristic of the Middle American gene pool, b) presence of large seed size characteristic of the Andean gene pool with genotypes with "S" phaseolin type and isozyme patterns that are typical of the Middle American gene pool, c) the presence of some rare alleles that were observed previously, although at a low frequency in the Middle American gene pool, and d) the absence of "S" phaseolin among wild beans of the Andean gene pool.

Although common bean is a predominantly self-pollinated species, its reproductive system is not inconsistent with hybridizations required to account for the introgression posited in the Chilean germplasm. Indeed, low to moderate levels of outcrossing have been reported for this species (Bliss, 1980; Brunner & Beaver, 1989; Wells et al., 1988). Occasional hybridizations over a long period of time would suffice to generate the hybrid pattern of the Chilean germplasm.

The higher incidence of Andean phaseolin types compared to that of isozyme variants may have resulted from a selection for larger seed size in the progeny of crosses involving Andean and Middle American genotypes. Indeed, Hartana (1986) showed that the Andean phaseolin types "T" and "C" were associated with larger seeds than the Middle American "S" phaseolin type. This process of introgression appears to have been occurring in the entire country as revealed by the isozyme constitution, phaseolin type, and seed size of the genotypes collected throughout Chile.

An alternative hypothesis that is not mutually exclusive with the introgression hypothesis is that the combination of what appear to be Middle American and Andean isozyme alleles actually results directly from domestication in the Southern part of the Andes. Isozyme data from wild beans from Argentina (Koenig & Gepts, 1989) show that some of these accessions carry isozyme alleles that are considered to be Middle American alleles (e.g. Me^{100}, $Rbcs^{100}$) in addition to Andean alleles. It is possible, however, that this mix of what has become known as Middle American and Andean alleles actually represents an ancestral state of the wild common bean gene pool. Isozyme analyses of wild common bean from northern Peru and Ecuador have shown that these accessions carry Middle American or Andean alleles depending on the isozyme locus (Debouck et al., 1993). Selection and genetic drift prior, during, or after domestication could have led to the current configuration of Middle American and Andean isozyme alleles in the cultivated gene pool (Singh et al., 1991c). This hypothesis, however, does not explain the presence of "S" phaseolin cultivars in the Chilean cultivars as no "S" phaseolin has been observed in wild common bean from the southern Andes (except in a weedy accession from Peru). It is therefore considered to be less likely in the current state of our knowledge. It could explain, however, the presence of "C" phaseolin cultivars as "C" phaseolin may have been identified in wild bean populations from southern Peru and Bolivia (Toro et al., 1990; Vargas et al., 1990). Further explorations are necessary in these areas to secure

additional wild bean germplasm, analyze its diversity at the biochemical level, and compare it to that of cultivars of the Andean gene pool.

Hybridization and recombination in progenies of crosses involving "S" phaseolin and "T" phaseolin genotypes could give rise to the raw material for the formation of the "C" type phaseolin. Brown et al. (1981) had previously suggested that the "C" phaseolin type could have resulted from recombination between "S" and "T" phaseolin types. It is not clear when this process of hybridization occurred. It could be an ancient event that took place between wild beans before domestication as suggested by the possible presence of "C" phaseolin among wild common bean (Koenig et al., 1990). It could also have occurred after domestication with the introduction of Middle American domesticates into the Andean region. Archaeological data suggest possible human contacts between Mesoamerica and South America as early as 3500–5000 BC (Zeidler, 1977–1978; Pearsall, 1977–1978). This process could have been accelerated more recently, since the Spanish conquerors introduced common bean into Chile approximately 500 years ago. On the other hand, the international market of common bean in Chile was developed only 50 or 60 years ago and the probability of introduction of new materials has increased only in the last few decades as a consequence of active bean breeding programs and favorable conditions for seed production to supply countries located in the Northern Hemisphere.

Hybrid weakness in the F_1 generation of some crosses between Middle American and Andean cultivated and wild types has been reported previously (Shii et al., 1980; Singh & Gutiérrez, 1984; Gepts & Bliss, 1985; Kelly, 1989; Koinange & Gepts, 1992). This phenomenon is controlled by two complementary semidominant genes *Dl-1* and *Dl-2* present in the Middle American and Andean gene pool, respectively. The presence of a high frequency of hybrids in the Chilean landraces could be explained by the presence and/or introduction of genotypes that are homozygous recessive at one or both loci. Hybridization of homozygous recessive genotypes would lead to viable progeny. Race Durango materials would be the most likely Middle American materials to be introduced in Chile because of similarities in growth habit (prostrate), seed types (medium-sized, light-colored) and ecological requirements (aridity). Both the Chile and Durango races are known to have low frequencies of the *Dl* genes (Singh et al., 1991a). On the other hand, mild temperatures in certain parts of the country could prevent or mitigate the expression of these genes because lethality is expressed more strongly at temperatures above 25°C (Shii et al., 1981). Alternatively, the ancestral materials involved in the introgression process were carriers of the *Dl-1* or *Dl-2* alleles and their progenies represent the fraction of the segregation that escaped the F_1 hybrid weakness because they lacked one or both of the *Dl-1* and *Dl-2* alleles. These various hypotheses can be tested in test crosses with genotypes that carry either the *Dl-1* or *Dl-2* alleles (e.g. Koinange & Gepts 1992). Such a test has been conducted in the common bean germplasm of Malawi. The low frequency of introgressants between the two major gene pools suggested by isozyme analyses (Sprecher, 1988) correlates well with the high frequency of *Dl* genes in that germplasm (W. C. Johnson & P. Gepts, unpubl. results).

The presence of a high frequency of genotypes that are apparently hybrids between the two gene pools suggests that hybridization between the two gene pools in breeding programs should lead to productive cultivars. It apparently contrasts with the difficulties faced by bean breeders in recovering useful germplasm from crosses between the two gene pools and suggests that the development of an adequate breeding methodology for this type of crosses ought to be pursued. Of particular importance would be the choice of parents and the mating and selection scheme in the progeny.

The lack of a clearer association between isozyme variation and geographic distribution of the landraces in the country could indicate several possibilities. Common bean was introduced into Chile to one specific area of the country from which it spread to other areas or the introduction of this species occurred simultaneously in different regions of the country. The morphological and seed characteristics used by the farmers to select their genotypes in different geographic areas are not related to isozyme variation. Isozyme variation is not associated with fitness values (adaptation) or the number of isozyme systems assayed was not large enough to discriminate among geographic

origins, because natural selection may act differently under diverse environmental conditions to produce local adaptation between landraces. At the subsistence level of bean production where landraces are cultivated, farmers interchange seeds with other farmers from closed related areas and/or occasionally they travel to the most important areas of production (Region VI to VIII) and exchange seeds, which could lead to a loss of a geographic identity. Chilean landraces could have a very wide geographic adaptation in the country, which could be explained by the absence of specific constraints associated with different bean production areas such as severe pathological problems, drastic climatic changes or moisture availability. Finally, most of the commercial bean production is carried out under irrigation. This could buffer some climatic (precipitation and temperature) and soil (texture and fertility) differences present between regions within the country and give rise to a more uniform bean production area.

In conclusion, the presence of high genetic variability in the Chilean common bean germplasm suggests that it is possible to find genetic variability at the phaseolin and isozyme level, making this germplasm very valuable from a genetic and breeding standpoint. The high levels of introgression observed in these materials from the Middle American gene pool suggest that these materials could be used as a potential genetic bridge between the Middle American and Andean gene pools.

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References

Bassiri, A. & M. W. Adams, 1978a. Evaluation of bean cultivar relationships by means of isozyme electrophoretic patterns. Euphytica 27: 707–720.

Bassiri, A. & M. W. Adams, 1978b. An electrophoretic survey of seedling isozymes in several *Phaseolus* species. Euphytica 27: 447–459.

Becerra Velasquez, V. L., 1992. Characterization of the genetic diversity in common beans (*Phaseolus vulgaris* L.) using RFLP markers. M.S. thesis, University of California, Davis.

Bliss, F. A., 1980. Common bean. In: W. R. Fehr & H. H. Hadley (Eds.), Hybridization of crop plants, pp. 273–284, Crop Science Society of America, Madison, WI.

Bliss, F. A., 1989. Utilization of genetic resources for crop improvement: the common bean. In: A. H. D. Brown, M. T. Clegg, A. L. Kahler & B. S. Weir (Eds.), Plant population genetics, breeding, and genetic resources, pp. 317–333, Sinauer, Sunderland, MA.

Bliss, F. A. & J. W. S. Brown, 1983. Breeding common bean for improved quantity and quality of seed protein. Plant Breed. Rev. 1: 59–102.

Brown, J. W. S., Y. Ma, F. A. Bliss & T. C. Hall, 1981. Genetic variation in the subunits of globulin-1 storage protein of French bean. Theor. Appl. Genet. 59: 83–88.

Brown, J. W. S., J. R. McFerson, F. A. Bliss & T, C. Hall, 1982. Genetic divergence among commercial classes of *Phaseolus vulgaris* in relation to phaseolin patterns. HortScience 17: 752–754.

Brunner, B. R. & J. S. Beaver, 1989. Estimation of outcrossing of the common bean in Puerto Rico. HortScience 24: 669–671.

Debouck, D. G., O. Toro, O. M. Paredes, W. C. Johnson & P. Gepts, 1993. Genetic diversity and ecological distribution of *Phaseolus vulgaris* in northwestern South America. Econ. Bot.: 408–423.

Doebley, J., 1989. Isozymic evidence and the evolution of crop plants. In: D. E. Soltis & P. S. Soltis (Eds.), Isozymes in plant biology, pp. 165–191, Dioscorides, Portland, OR.

Gepts, P., 1988. Phaseolin as an evolutionary marker. In: P. Gepts (Ed), Genetic resources of *Phaseolus* beans, pp. 215–241, Kluwer, Dordrecht, The Netherlands.

Gepts, P., 1989. Genetic diversity of seed storage proteins in plants. In: A. H. D. Brown, M. T. Clegg, A. L. Kahler & B. S. Weir (Eds.), Plant population genetics, breeding, and genetic resources, pp. 64–82, Sinauer, Sunderland, MA.

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

Gepts, P., 1993a. Linkage map of common bean (*Phaseolus vulgaris* L.). In: S. J. O"Brien (Ed), Genetic Maps, pp. 6.101–6.109, Cold SprigHbor Laboratory, Cold Spring Harbor, NY.

Gepts, P., 1993b. The use of molecular and biochemical markers in crop evolution studies. Evol. Biol. 27: 51–94.

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

Gepts, P. & F. A. Bliss, 1988. Dissemination pathways of common bean (*Phaseolus vulgaris*, Fabaceae) deduced from phaseolin electrophoretic variability. II. Europe. and Africa. Econ. Bot. 42: 86–104.

Gepts, P., T. C. Osborn, K. Rashka & F. A. Bliss, 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., K. Kmiecik, P, Pereira & F. A. Bliss, 1988. Dissemination pathways of common bean (*Phaseolus vulgaris*, Fabaceae) deduced from phaseolin electrophoretic variability. I. The Americas. Econ. Bot. 42: 73–85.

Hamrick, J. L. & M. J. W. Godt, 1989. Allozyme diversity in plant species. In: A. H. D. Borwn, M. T. Clegg, A. L. Kahler & B. S. Weir (Eds.), Plant population genetics, breeding, and genetic resources, pp. 43–63, Sinauer, Sunderland, MA.

Hattana, A., 1986. Components of variability for seed protein of common bean (*Phaseolus vulgaris* L.). Ph.D. thesis, University of Wisconsin, Madison.

INE, 1990/91. Estadísticas Agropecuarias, Cultivos Anuales Esenciales—Producción frutícola, Producción vitivinícola. Ministerio de Economía, Fomento y Reconstrucción. Santiago, Chile, pp. 11–12.

Kelly, J. D., 1989. The presence of dwarf lethal *Dl* genes in *Phaseolus* germplasm, lines, and cultivars. Annu. Rept. Bean Improv. Coop. 32: 74–74.

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

Koenig, R. & P. Gepts, 1989a. Segregation and linkage of genes for seed proteins, isozymes, and morphological traits in common bean (*Phaseolus vulgaris*). J. Hered. 80: 455–459.

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

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

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

Lioi, L., 1989a. Variation of the storage protein phaseolin in common bean (*Phaseolin vulgaris* L.) from the Mediterranean area. Euphytica 44: 151–155.

Lioi, L., 1989b. Geographical variation of phaseolin patterns in an Old World collection of *Phaseolus vulgaris*. Seed Sci. Technol. 17: 317–324.

Mutschler, M. A., F. A. Bliss & T. C. Hall, 1980. Variation in the accumulation of seed storage protein among genotypes of *Phaseolus vulgaris* L. Plant Physiol. 65: 627–630.

Nei, M., 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70: 3321–3323.

Pearsall, D. M., 1977–78. Early movement of maize between Mesoamerica and South America. J. Steward Anthrop. Soc. 9: 41–75.

SAS, 1988. SAS/STAT User's Guide, Release 6.03 Edition. SAS Institute, Cary, NC.

Schinkel, C. & P. Gepts, 1988. Phaseolin diversity in the tepary bean, *Phaseolus acutifolius* A. Gray. Plant Breed. 101: 292–301.

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

Shii, C. T., M. C. Mok & D. W. S. Mok, 1981. Developmental controls of morphological mutants of *Phaseolus vulgaris* L.: Differential expression of mutant loci in plant organs. Dev. Genet. 2: 279–290.

Singh, S. P., 1989. Patterns of variation in cultivated common bean (*Phaseolus vulgaris*, Fabaceae). Econ. Bot. 43: 39–57.

Singh, S. P. & A. J. Gutiérrez, 1984. Geographical distribution of the DL_1 and DL_2 genes causing hybrid dwarfism in *Phaseolus vulgaris* L., their association with seed size, and their significance to breeding. Euphytica 33: 337–345.

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

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

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

Smartt, J., 1990. Grain legumes: evolution and genetic resources. Cambridge Univ. Press, Cambridge.

Smith, J. S. C. & O. S. Smith, 1989. The description and assessment of distances between inbred lines of maize. II. The utility of morphological, biochemical, and genetic descriptors and a scheme for testing of distinctiveness between inbred lines. Maydica 34: 151–161.

Sneath, P. H. A. & R. Sokal, 1973. Numerical taxonomy. Freeman, San Francisco, CA.

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, MI.

Wall, J. R., 1968. Leucine aminopeptidase polymorphism in *Phaseolus* shows differential elimination of the donor parent genotype in interspecific backcrosses. Biochem. Genet. 2: 109–118.

Wall, J. R. & S. W. Wall, 1975. Isozyme polymorphisms in the study of evolution in the *Phaseolus vulgaris-P. coccineus* complex of Mexico. In: C. L. Markert (Ed), Isozymes IV, Academic Press, New York.

Weeden, N. F., 1984a. Linkage between the gene coding the small subunit of ribulose biphosphate carboxylase and the gene coding malic enzyme in *Phaseolus vulgaris*. Annu. Rept. Bean Improv. Coop. 27: 123–124.

Weeden, N. F., 1984b. Distinguishing among white-seeded bean cultivars by means of allozyme genotypes. Euphytica 33: 199–208.

Weeden, N. F., 1986. Genetic confirmation that the variation in the zymograms of 3 enzyme systems is produced by allelic polymorphism. Annu. Rept. Bean Improv. Coop. 29: 117–118.

Wells, W. C., W. H. Isom & J. G. Waines, 1988. Outcrossing rates of six common bean lines. Crop Sci. 28: 177–178.

West, N. B. & E. D. Garber, 1967. Genetic studies of variant enzymes. I. An electrophoretic survey of esterase and leucine aminopeptidase in the genus *Phaseolus*. Can. J. Genet. Cytol. 9: 640–645.

Zeidler, J., 1977–78. Primitive exchange, prehistoric trade and the problem of Mesoamerican-South American connection. J. Steward Anthrop. Soc. 9: 7–39.