Theor Appl Genet (1993) 87:506–516

Use of chloroplast DNA polymorphisms for the phylogenetic study of seven *Phaseolus* taxa including *P. vulgaris* and *P. coccineus*

V. Schmit[1,*], P. du Jardin[2], J. P. Baudoin[1], D. G. Debouck[3]

[1] Unité de Phytotechnie des Régions Chaudes, Faculté des Sciences Agronomiques de Gembloux, B-5030 Gembloux, Belgium
[2] Unité de Génétique et d'Amélioration des Plantes, Faculté des Sciences Agronomiques de Gembloux, B-5030 Gembloux, Belgium
[3] IBPGR Group for the Americas, C/o CIAT AA6713, Cali, Colombia

Received: 4 May 1993 / Accepted: 17 May 1993

Abstract. The genetic variability of seven *Phaseolus* taxa has been evaluated on the basis of molecular data and the results have used to clarify the phyletic relationships between several taxa of the *P. coccineus* L. complex. Chloroplast DNA (cpDNA) from 33 populations was digested with six restriction endonucleases, revealing some polymorphisms that made it possible to divide most of the taxa into two main groups: the subspecies of *P. coccineus* on the one hand, and *P. vulgaris* L., *P. polyanthus* Greenman and *P. costaricensis* (Freytag and Debouck) on the other hand. *P. polyanthus* is closer to *P. vulgaris* than the other taxa of the second group and should be considered as a separate species. The position of the wild species *P. costaricensis* is intermediate between *P. coccineus* and *P. polyanthus*. *P. glabellus* shows sufficient polymorphisms at the cpDNA level to be recognized as a separate species, as previously suggested from total seed-protein electrophoretic studies. These results favour the hypothesis of a common phylogeny for *P. vulgaris*, *P. polyanthus*, *P. costaricensis* and *P. coccineus* from a single wild ancestor. Although cpDNA is generally known to be uniform at the intraspecific level, some additional polymorphisms were also detected within *P. vulgaris*, *P. polyanthus* and *P. coccineus*. Further studies are required to understand the significance of the latter.

Key words: Chloroplast DNA polymorphisms – Phylogeny – Plant genetic resources – *Phaseolus* beans

Communicated by R. Hagemann
* *Present address:* Programme Haricot, Institut des Sciences Agronomiques du Burundi (ISABU), BP 795, Bujumbura, Burundi
Correspondence to: J. P. Baudoin

Introduction

Within the genus *Phaseolus* of neotropical origin, the common bean, *Phaseolus vulgaris* L., is the most important species. Its dry seeds are a major component in the diet of human populations in Latin America and in Central and East Africa (Londoño et al. 1981; Voyset 1983). However, other less-known *Phaseolus* species are also cultivated in small-scale farming in Latin America. This is the case for two food legumes adapted to the tropical highland areas: *P. coccineus* L., a perennial species found mainly in Central America (Mexico and Guatemala), and *P. polyanthus* Greenm., a pluri annual species present in the same geographical area as the former but extending also to the northern part of South America (Schmit and Baudoin 1987; Debouck 1991). Both species show interesting traits not, or only poorly, expressed in the primary gene pool of *P. vulgaris*, such as resistance to some diseases (*Ascochyta* leaf spot, bean golden mosaic virus), cold tolerance, long multinoded inflorescences, and thick stems (Schmit and Baudoin 1987). Furthermore, these two food legumes are closely related to the common bean: interspecific hybrids involving *P. vulgaris* × *P. coccineus* and *P. vulgaris* × *P. polyanthus* were obtained relatively easily and have been included in breeding programmes (Baudoin et al. 1992). Several wild *Phaseolus* species grouped together as early as 1926 by Piper under "*P. coccineus* and its allies" are indeed, at least morphologically, close to the two cultigens *P. coccineus* and *P. polyanthus*, and form a complex group of species, hereafter referred as the *P. coccineus* complex. The taxonomy and phylogeny of this complex, composed of predominantly allogamous taxa, are still poorly understood. In particular, *P. polyanthus* has been considered either as a subspecies of *P. coccineus*

(Maréchal et al. 1978; Delgado 1985, 1988) or as a distinct species in the genus (Smartt 1973). Although partial cpDNA restriction maps obtained by Llaca and Gepts (1991) were identical for *P. coccineus* and *P. polyanthus*, other data from morphological observations and interspecific hybridizations (Camarena and Baudoin 1987; Baudoin et al. 1992) and from biosystematic studies (Piñero and Eguiarte 1988; Schmit and Debouck 1991) favoured the attribution of a specific rank to *P. polyanthus*. Worth mentioning is the existence of a wild ancestral form for *P. polyanthus* discovered in Guatemala in 1978 (Schmit and Debouck 1991) and not considered in previous studies (Smartt 1973; Maréchal et al. 1978; Delgado 1985, 1988; Piñero and Eguiarte 1988). In Costa Rica, a wild form related to *P. coccineus* was recently collected and identified as *P. costaricensis* (Freytag and Debouck); natural hybrids were observed between that form and populations of *P. polyanthus* growing near the collection site (Debouck et al. 1989). The position of this new form within the *P. coccineus* complex is still unclear. The taxonomical status of another ecologically and morphologically distinct form, *P. glabellus* Piper, has also been questioned: it is considered either as a subspecies of *P. coccineus* by Maréchal et al. (1978) on morphological grounds or as a true species by Schmit and Debouck (1990) and Schmit et al. (1991) based on biochemical differences. On the other hand, no natural hybrids between this form and wild *P. coccineus* populations growing in the same environment (the eastern Sierra Madre in Mexico) have been observed by Debouck (1986) when collecting *P. glabellus* accessions.

Chloroplast DNA restriction patterns provide useful information with which to assess the phylogenetic relationships among plant species, due to the low rate of rearrangement, the relatively small size, and the predominantly maternal inheritance, of the cpDNA (Palmer 1987). This tool has been used mainy in several interspecific taxonomical works to study the genetic organization in centres of diversity or to establish the relationships between the cultigens and their related wild taxa; for instance, in the genera *Zea* (Thimothy et al. 1979; Doebley et al. 1987), *Oryza* (Dally and Second 1990), *Poa* (Soreng 1990), *Musa* (Gawel and Jarret 1991), *Nicotiana* (Kung et al. 1982), *Lycopersicon* (Palmer and Zamir 1982) and *Solanum* (Palmer and Zamir 1982; Hosaka et al. 1984; Buckner and Hyde 1985; Hosaka 1986; Hosaka and Hanneman 1988). At the intraspecific level, cpDNA analysis has so far been considered of limited interest as noted by Palmer (1987). Nevertheless, some studies have shown that intraspecific variants are more frequent than previously expected. Soltis et al. (1992) pointed out such variants in at least 60 species belonging to more than 15 families, while Milligan (1991) found some cpDNA polymorphisms even within a single population of *Trifolium pratense* L.

The present investigation was undertaken to analyze the chloroplast genome within a representative sample of *P. vulgaris* and of the *P. coccineus* complex, with a three-fold objective: (1) to establish the taxonomical position and the origin of *P. polyanthus*, (2) to determine the rank of *P. costaricensis* with respect to *P. coccineus* and *P. polyanthus* and (3) to obtain more information about the rank of *P. glabellus* vis-à-vis *P. coccineus*.

Materials and methods

Plant material

The plant material, as indicated in Table 1, involved 33 populations belonging to seven taxa covering various geographical origins and with a different biological status (wild, escaped and cultivated forms). Among the taxa, six are members of the *P. coccineus* complex and the seventh is a wild genotype of *P. lunatus* L. selected for its phyletic remoteness from the complex (Le Marchand and Maréchal 1977; Maréchal et al. 1978; Debouck 1991). The *Phaseolus* material also involves natural hybrids between *P. vulgaris* and *P. polyanthus* and between *P. coccineus* and *P. polyanthus*. All the populations come either from the Genetic Resources Unit of the "Centro Internacional de Agricultura Tropical" (CIAT, Cali, Colombia) or from the collection maintained in the "Faculté des Sciences Agronomiques de Gembloux" (FSAGx, Belgium).

Chloroplast isolation and DNA extraction

Chloroplast DNA was isolated following the method described by Hosaka (1986) but with some modifications. In the greenhouse, three-to-five-week-old plants were kept in darkness for 24 h just before harvesting the fresh leaflets. A total of 20–50 g of young leaflets were homogenized in a blender with four vol of ice-cold homogenization buffer (A buffer: 440 mM mannitol, 50 mM Tris-HCl, 15 mM EDTA, 1 mM β-mercaptoethanol and 0.1% bovine serum albumin, pH 8.0). The mixture was filtered through two layers of 1000-µm nylon mesh and four layers of 250-µm mesh. After centrifugation for 10 min at 2,000 g and 4 °C, the pellet was resuspended in 20 ml of cold wash buffer (B buffer: 440 mM mannitol, 10 mM Tris-HCl, pH 8.0) with a soft brush, and the chloroplasts were re-pelleted for 10 min at 2,000 g and 4 °C. Two additional washes were performed in the same way and the final pellet was resuspended in 4 ml of B bufer. The chloroplasts were purified on a sucrose step-gradient [15 ml 30% on 15 ml 60% sucrose (w/v) solution in 50 mM Tris-HCl, 3 mM EDTA, pH 8.0] and centrifuged at 53,800 g for 50 min at 4 °C. The chloroplasts were lysed with 0.5 ml of 10% lauryl sarcosinate and the suspension was rotated during 30 min with 2.5 ml of TE-buffered phenol (TE: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0). After centrifugation at 1,475 g for 10 min, the aqueous phase was re-extracted in the same way with phenol-chloroform (1 : 1, v/v) and precipitated by adding 1/10 vol of 3 M sodium acetate, pH 5.2, and 2 vol of cold ethanol (− 20 °C). The solution was kept overnight at − 20 °C and centrifuged for 30 min at 16,300 g and − 10 °C. The pellet was dried, solubilized in TE buffer and stored at 4 °C.

Restriction enzyme digestion and electrophoresis

Six endonucleases were used for digestion: *Eco*RI, *Bam*HI, *Ava*I, *Xho*I, *Eco*RV and *Hin*dIII. Chloroplast DNA extracts were digested with 5–10 units of restriction enzyme per microgram of

Table 1. Origin, identification and biological status of the material studied

Taxon	Identification	Origin[a]	Biological status[b]	Altitude (m)	Longitude	Latitude
P. lunatus	NI 1259	PER Junin	w	980	75.22 W	11.08 S
P. vulgaris	NI 1193	MEX Morelos	w	1350	–	–
	G 6040	GTA –	c	–	–	–
	DGD 1616	GTA Sacatepéquez	w	1550	90.50 W	14.32 N
	DGD 2423	GTA Chiquimula	w	1390	89.38 W	14.33 N
	DGD 2097	CRA San José	w	1750	84.07 W	9.49 N
	DGD 2769	ECD Chimborazo	w	1710	78.58 W	2.16 S
	DGD 2484	BLV Cochabamba	w	2270	65.10 W	17.47 S
	NI 637	BRZ –	c	–	–	–
	DGD 1716	ARG Salta	w	2600	65.00 W	22.15 S
P. polyanthus	G 35061	MEX Puebla	c	–	98.03 W	20.11 N
	G 35380	MEX Puebla	c	–	97.58 W	20.19 N
	G 35122	MEX Oaxaca	c	2103	96.24 W	17.00 N
	G 35337	MEX Oaxaca	c	1600	96.53 W	18.10 N
	DGD 1684	GTA Huehuetenango	c	2450	91.28 W	15.23 N
	DGD 1650	GTA San Marcos	c	2670	91.49 W	15.05 N
	DGD 1601	GTA Chimaltenango	c	2320	90.48 W	14.36 N
	DGD 1631	GTA Solola	w	1680	91.10 W	14.46 N
	DGD 2653	CLB Risaralda	e	2160	75.57 W	05.07 N
	G 35360	CLB Valle	e	2350	76.08 W	03.33 N
	DGD 602	CLB Cauca	e	1850	76.32 W	02.28 N
	G 35383	CLB Cauca	e	2470	76.32 W	02.27 N
	G 35625	CLB Cauca	c	2360	76.28 W	02.20 N
P. costaricensis	DGD 2132	CRA San José	w	1990	83.51 W	09.34 N
P. coccineus						
subsp. *coccineus*	NI 813	MEX Durango	w	2100	104.14 W	24.27 N
	NI 1204	MEX Morelos	w	2200	99.14 W	19.00 N
	NI 755	GTA –	c	2400	–	–
	NI 1108	MEX Morelos	w	2400	–	–
subsp. *purpurascens*	NI 722	MEX –	w	–	–	–
P. glabellus	DGD 2046	MEX San Luis Potosi	w	1430	99.06 W	21.11 N
Naturals hybrids						
between *P. vulgaris*	DGD 2975	CLB Tolima	c	2120	75.25 W	03.45 N
and *P. polyanthus*	DGD 2988	CLB Cundinamarca	c	1950	74.21 W	04.39 N
between *P. coccineus*	G 35841	CLB Nariño	c	2100	77.15 W	01.13 N
and *P. polyanthus*						

[a] PER, Peru; MEX, Mexico; GTA, Guatemala; CRA, Costa Rica; ECD, Ecuador; BLV, Bolivia; BRZ, Brazil; ARG, Argentina; CLB, Colombia

[b] w, wild; e, escaped; c, cultivated

DNA for approximately 5 h at 37 °C, as recommended by the supplier (BRL). In order to monitor the completeness of the digestions with an internal control, 5 ng of pUC 19 were added to aliquots of the digestion mixes and the plasmid digests were Southern-analyzed using a pUC derivative as probe. Agarose-gel electrophoresis and Southern blotting on nylon membranes (Hybond-N, Amersham) were performed using standard methods (Maniatis et al. 1982). Lambda DNA/*Hin*dIII and PhiX174RFDNA/*Hae*III restriction fragments (BRL) were used as molecular weight standards.

Statistical methods

In order to evaluate the genetic distances between the studied populations on the basis of the observed electrophoretic patterns, a classification was established using the complete linkage method of Jonhson (1967) via the SAS Cluster procedure (Department of Statistics and Informatics, Faculté des Sciences Agronomiques de Gembloux). The similarity matrix was calculated using the Jaccard coefficient (Sneath and Sokal 1973):

$$\text{Jaccard coefficient} = \frac{\text{Number of electrophoretic bands in common}}{\text{Number of different bands} + \text{number of bands in common}}$$

Results

Description of the restriction patterns

A typical electrophoretic separation of chloroplast DNA cut with the six restriction endonucleases used in the present study is shown in Fig. 1.

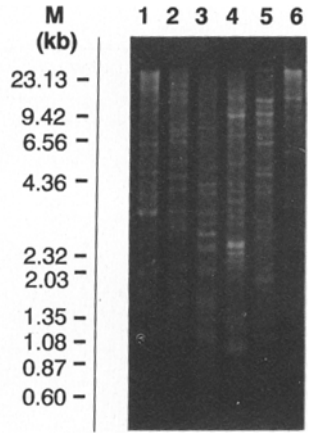

Fig. 1. Representative example of the electrophoretic patterns obtained (here in *Phaseolus polyanthus*) with the six restriction endonucleases used in the present study: 1, *Ava*I; 2, *Bam*HI; 3, *Eco*RI; 4, *Eco*RV; 5, *Hin*dIII; 6, *Xho*I. *M*, molecular weight standards. The gel is 0.8% agarose

The *Hin*dIII electrophoretic patterns were identical in all the tested material (data not shown). On the other hand, digestion by the other five enzymes revealed some polymorphic fragments enabling us to define distinct patterns. These are given in Table 2 together with the total number of polymorphic fragments for each restriction enzyme.

The *Phaseolus* populations were classified according to the pattern they presented for each enzyme. Table 3 indicates the patterns obtained for each population and Table 4 shows the pattern frequency for each enzyme within each taxon or natural hybrid. The results show that *P. lunatus* NI 1259 and *P. glabellus* DGD 2046 are isolated from the remaining taxa. For each of the five enzymes *Eco*RI, *Ava*I, *Bam*HI, *Xho*I and *Eco*RV the two genotypes are characterized by a distinct pattern, different from those observed in the other *Phaseolus* populations. For the latter, a common pattern is observed for the enzymes *Xho*I (pattern 1, Fig. 2A) and *Eco*RV (pattern 1, Fig. 2B). In *P. glabellus* DGD 2046, the pattern obtained with *Xho*I (pattern 2, Fig. 2A) differs from pattern 1 by the lack of a 3.9-kb fragment. For the same genotype, the pattern obtained with *Eco*RV (pattern 2, Fig. 2B) differs from pattern 1 by the lack of five fragments (17.0, 6.56, 4.25, 3.75 and 2.9 kb) and by the presence of an additional fragment of about 11 kb. In *P. lunatus* NI 1259, the *Xho*I pattern (pattern 3, Fig. 2A) differs from pattern 1 by two additional fragments of 6.2 and 4.36 kb and by one missing fragment of about 11 kb. For the same genotype, the pattern obtained with *Eco*RV (pattern 3, Fig. 2B) is characterized by the presence of five additional fragments of 12.0, 6.6, 4.6, 3.9 and 2.7 kb and by the lack of three fragments (17.0, 9.42 and 4.25 kb), as compared to pattern 1.

In the five remaining taxa, the digestion by three enzymes shows several cpDNA polymorphisms. The restriction patterns obtained with *Ava*I (Fig. 3) and *Eco*RI (Fig. 4) separate the populations into two groups. The first group includes *P. vulgaris*, *P. polyanthus*, *P. costaricensis* and the natural hybrids between *P. vulgaris* and *P. polyanthus* while the second group includes the subspecies of *P. coccineus* and the natural hybrid between *P. coccineus* and *P. polyanthus*. Table 2 and Figs. 3 and 4 show that the patterns of the first

Table 2. Restriction pattern numbering based on the polymorphic cpDNA fragments obtained using five endonucleases

Enzyme pattern no.	*Ava*I (kb)	*Bam*HI (kb)	*Eco*RI (kb)	*Eco*RV (kb)	*Xho*I (kb)
1	6.0+5.2+4.25	7.2+6.55+3.15+2.95	6.6+4.6+4.2+3.9+3.4+2.6	17.0+9.4+6.55+4.25+3.75+2.9	11.0+3.9
2	6.0+4.25	7.2+6.55+6.0+2.95	6.6+4.1+3.4+2.6	11.0+9.4	11.0
3	6.0+5.2+4.25+3.05	7.2+6.55+5.6+3.15+2.95	8.3+6.6+4.6+4.2+3.9+3.4+2.6	12.0+6.6+6.55+4.6+3.9+3.75+2.9+2.7	6.2+4.35+3.9
4	5.2+3.05+2.85	9.4+6.6+3.75	8.3+6.6+3.9+3.4+2.6		
5		8.3+7.2+6.55+3.6+3.15+2.95	6.6+4.2+3.9+3.4+2.6		
6			9.4+7.8+6.6+4.6+4.2+3.9+3.4+2.6		
7			12.0+7.2+4.6+4.2+3.9+3.4+2.6		
8			6.0+4.6+4.2+3.9		
9			6.6+6.0+4.6+4.35+4.2+3.9+3.4+2.6		
Number of polymorphic fragments	5	11	14	12	4

Table 3. Pattern repartition for each enzyme and *Phaseolus* population studied

Species	Genotype	*Eco*RI	*Ava*I	*Bam*HI	*Eco*RV	*Xho*I	*Hin*dIII
P. vulgaris	NI 1193	1	1	1	1	1	1
	G 6040	1	1	1	1	1	1
	DGD 1616	1	1	1	1	1	1
	DGD 2423	3	1	1	1	1	1
	DGD 2097	1	1	1	1	1	1
	DGD 2769	6	1	1	1	1	1
	DGD 2484	1	1	1	1	1	1
	NI 637	1	1	1	1	1	1
	DGD 1716	1	1	1	1	1	1
P. polyanthus	G 35061	1	1	1	1	1	1
	G 35380	1	1	1	1	1	1
	G 35122	1	1	1	1	1	1
	G 35337	1	1	1	1	1	1
	DGD 1684	7	1	1	1	1	1
	DGD 1650	7	1	1	1	1	1
	DGD 1601	1	1	1	1	1	1
	DGD 1631	6	1	1	1	1	1
	DGD 2653	1	1	1	1	1	1
	G 35360	1	1	1	1	1	1
	DGD 602	3	1	1	1	1	1
	G 35383	3	1	1	1	1	1
	G 35625	1	1	1	1	1	1
Natural hybrids between							
P. vulgaris and *P. polyanthus*	DGD 2975	1	1	1	1	1	1
	DGD 2988	1	1	1	1	1	1
P. cocc. and *P. polyanthus*	G 35841	5	2	1	1	1	1
P. costaricensis	DGD 2132	3	1	1	1	1	1
P. coccineus							
subsp. *coccineus*	NI 813	2	2	2	1	1	1
	NI 1204	5	2	1	1	1	1
	NI 755	4	2	3	1	1	1
	NI 1108	4	2	3	1	1	1
subsp. *purpurascens*	NI 722	5	2	1	1	1	1
P. glabellus	DGD 2046	8	3	4	2	2	1
P. lunatus	NI 1259	9	4	5	3	3	1

group for the enzymes *Ava*I (pattern 1, Fig. 3) and *Eco*RI (pattern 1, 3, 6 and 7, Fig. 4) present an additional fragment as compared to the patterns of the *P. coccineus* subspecies for the same two enzymes (pattern 2 for *Ava*I and patterns 2, 4 and 5 for *Eco*RI). The molecular weight of these additional fragments is 5.2 kb and 4.6 kb respectively for *Ava*I and *Eco*RI. Other variations are also observed in the *Eco*RI patterns within each group of taxa. Figure 4 shows that one or multiple fragments are identified between 12.0 and 6.56 kb. The *Bam*HI patterns indicate a high homogeneity. Nevertheless, some variations detected within *P. coccineus* reflect the high level of diversity in this species (Fig. 5). As compared to the other populations, the wild form NI 813 (pattern 2, Fig. 5) shows an additional fragment of 6.0 kb, while a 3.15-kb fragment is missing. On the other hand, both the cultivated *P. coccineus* form NI 755 and the wild population NI 1108 display an additional fragment of 5.6 kb (pattern 3, Fig. 5). As in the case of *Xho*I and *Eco*RV, the patterns obtained in *P. lunatus* and *P. glabellus* with the three enzymes *Ava*I, *Eco*RI and *Bam*HI are very different as compared to all other taxa (see Figs. 3, 4 and 5). For the enzyme *Ava*I, the pattern of *P. glabellus* (pattern 3, Fig. 3) is characterized by an additional fragment of 3.05 kb while that of *P. lunatus* (pattern 4, Fig. 3) shows more variations with two missing fragments (6.0 and 4.25 kb) and two new fragments (3.05 and 2.85 kb). For the enzyme *Eco*RI, the pattern of *P. glabellus* (pattern 8, Fig. 4) differs from the other patterns by three missing fragments (6.6, 3.4 and 2.6 kb) and an additional fragment of 6.0 kb. For the same enzyme, two additional fragments of 6.0 and 4.36 kb are observed in *P. lunatus* (pattern 9, Fig. 4). For the enzyme *Bam*HI, the pattern of *P. glabellus* (pattern 4, Fig. 5) is again different from the other patterns by three missing fragments (7.2, 6.56 and 2.95 kb) and three additional fragments (9.42, 6.6, 3.75 kb). On the other hand, the pattern of *P. lunatus* (pattern 5, Fig. 5) is less divergent with only two additional fragments of 8.3 and 3.6 kb.

Table 4. Chloroplast DNA pattern frequency within a taxon for each enzyme

Taxon	Biological status[a]	No. of populations studied	Pattern																												
			*Ava*I				*Bam*HI					*Eco*RI									*Eco*RV			*Xho*I							
			1	2	3	4	1	2	3	4	5	1	2	3	4	5	6	7	8	9	1	2	3	1	2	3					
P. vulgaris	c	2	2				2					2									2			2							
P. vulgaris	w	7	7				7					5	1				1				7			7							
P. polyanthus	c	8	8				8					6						2			8			8							
	e	4	4				4					2	2								4			4							
	w	1	1				1										1				1			1							
P. coccineus subsp. *coccineus*	c	1		1					1						1						1			1							
P. coccineus subsp. *coccineus*	w	3		3			1	1	1			1			1	1					3			3							
P. coccineus subsp. *purpurascens*	w	1		1			1									1					1			1							
P. costaricensis	w	1	1				1							1							1			1							
Natural hybrid between																															
P. vulgaris and *P. polyanthus*	c	2	2				2					2									2			2							
P. coccineus and *P. polyanthus*	c	1		1			1									1					1			1							
P. glabellus	w	1			1					1									1			1				1					
P. lunatus	w	1				1					1									1			1				1				
Total number of populations		33	25	6	1	1	28	1	2	1	1	17	1	4	2	3	2	2	1	1	31	1	1	31	1	1					

[a] c, cultivated; e, escaped; w, wild

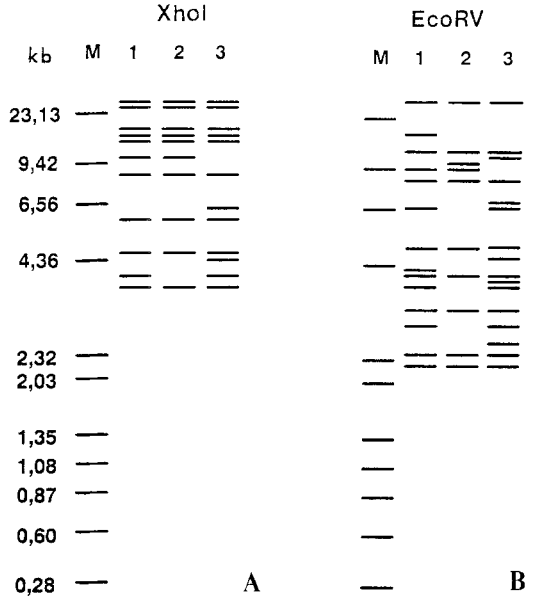

Fig. 2. **A** *Xho*I restriction patterns of: *1*, all the populations except DGD 2046 and NI 1259; *2*, *P. glabellus* DGD 2046 and *3*, *P. lunatus* NI 1259. **B** *Eco*RV restriction patterns of the same genotypes. *M*, molecular weight standards. The gel is 0.8% agarose

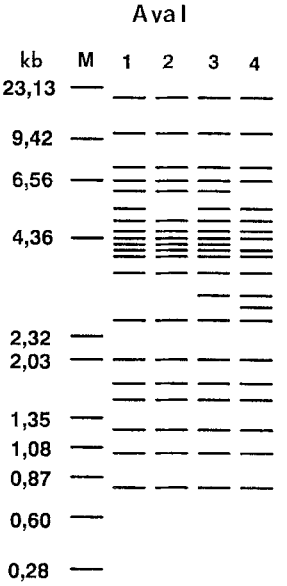

Fig. 3. *Ava*I restriction patterns of: *1*. *P. vulgaris*, *P. polyanthus*, *P. costaricensis* DGD 2132 and the two natural hybrids between *P. polyanthus* and *P. vulgaris*, DGD 2975 and DGD 2988; *2*, *P. coccineus* and the natural hybrid between *P. coccineus* and *P. polyanthus*, G 35841; *3*, *P. glabellus* DGD 2046 and *4*, *P. lunatus* NI 1259. *M*, molecular weight standards. The gel is 0.8% agarose

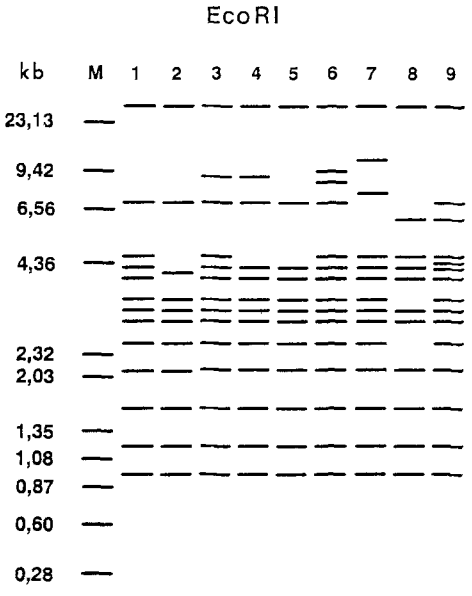

Fig. 4. *Eco*RI restriction patterns of: *1*, *P. vulgaris* NI 1193, DGD 1616, DGD 2097, DGD 2484, DGD 1716, NI 637, G 6040, *P. polyanthus* G 35061, G 35380, G 35122, G 35337, DGD 1601, DGD 2653, G 35360, G 35625 and natural hybrids between *P. polyanthus* and *P. vulgaris*, DGD 2975 and DGD 2988; *2*, wild *P. coccineus* NI 813; *3*, *P. vulgaris* DGD 2423, *P. polyanthus* DGD 602 and G 35383 and *P. costaricensis* DGD 2132; *4*, cultivated *P. coccineus* NI 755 and wild NI 1108; *5*, natural hybrid between *P. coccineus* and *P. polyanthus*, G 35841, wild *P. coccineus* NI 1204 and *P. coccineus* subsp. *purpurascens* NI 722; *6*, *P. vulgaris* DGD 2769 and wild *P. polyanthus* DGD 1631; *7*, *P. polyanthus* DGD 1684 and DGD 1650; *8*, *P. glabellus* DGD 2046 and *9*, *P. lunatus* NI 1259. *M*, molecular weight standards. The gel is 0.8% agarose

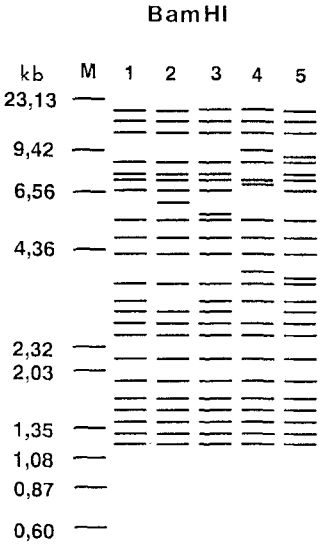

Fig. 5. *Bam*HI restriction patterns of: *1*, all populations except NI 813, NI 755, NI 1108, DGD 2046 and NI 1259; *2*, wild *P. coccineus* NI 813; *3*, cultivated *P. coccineus* NI 755 and wild *P. coccineus* NI 1108; *4*, *P. glabellus* DGD 2046 and *5*, *P. lunatus* NI 1259. *M*, molecular weight standards. The gel is 0.8% agarose

Intraspecific variation in P. vulgaris and P. polyanthus

Within the populations of *P. polyanthus*, pattern variations were observed only with *Eco*RI and concerned fragments above 6.0 kb (Fig. 4). Most of the populations studied show one 6.6-kb fragment. Only five genotypes differ: DGD 602 an G 35383 with an additional fragment of 8.3 kb, DGD 1631 with two additional fragments of 9.42 and 7.8 kb, and DGD 1650 and DGD 1684, both without the 6.6-kb fragment but with two new fragments of 12.0 and 7.2 kb.

The same variations were observed within *P. vulgaris*: DGD 2769 shows the same pattern as DGD 1631 while that of DGD 2423 is identical with G 35383 and DGD 602. The patterns obtained for the other populations are identical to those of most *P. polyanthus* genotypes.

Statistical analysis

A total of 46 polymorphic fragments have been identified for the five enzymes. Many populations show identical patterns and consequently identical values of the Jaccard coefficient. In order to reduce the dimensions of the similarity matrix, the populations were divided into nine groups, each of which includes 1 to 17 populations. The reduced matrix, as well as the description of each group, are indicated in Table 5. The dendrogram resulting from the complete linkage method is shown in Fig. 6. All genotypes of *P. vulgaris*, *P. polyanthus* and *P. costaricensis*, together with the natural hybrid *P. vulgaris* × *P. polyanthus*, form a large group divided into four smaller ones, with similarity coefficients ranging from 0.8 to 1.0. The *P. coccineus* populations and the natural hybrid between *P. coccineus* and *P. polyanthus* also form one group divided into three smaller ones, with similarity coefficients ranging from 0.727 to 1.0. The *P. coccineus* group is linked with the *P. vulgaris*, *P. polyanthus* and *P. costaricensis* group: the similarity coefficients between populations belonging to each of these two major groups range from 0.600 to 0.905. *P. glabellus* DGD 2046 and *P. lunatus* NI 1259 are isolated. The similarity coefficients reflect the particularity of their cpDNA. The coefficients between *P. glabellus* and the other taxa vary between 0.143 and 0.296, while the coefficients between *P. lunatus* and the other taxa vary between 0.250 and 0.400. The two species *P. glabellus* and *P. lunatus* are also distantly related with a very low similarity coefficient between them (0.171).

Table 5. Reduced similarity matrix[a, b]

Group	Group 1: DGD 2769	Group 2: DGD 1684	Group 3: DGD 2975	Group 4: DGD 2132	Group 5: NI 813	Group 6: NI 1204	Group 7: NI 1108	Group 8: DGD 2046	Group 9: NI 1259
Group 1	1.000								
Group 2	0.800	1.000							
Group 3	0.913	0.870	1.000						
Group 4	0.875	0.833	0.955	1.000					
Group 5	0.640	0.600	0.696	0.667	1.000				
Group 6	0.826	0.783	0.905	0.864	0.762	1.000			
Group 7	0.720	0.680	0.783	0.826	0.727	0.857	1.000		
Group 8	0.276	0.286	0.296	0.286	0.143	0.222	0.172	1.000	
Group 9	0.389	0.361	0.412	0.400	0.250	0.353	0.306	0.171	1.000

[a] Each group is represented by one of the populations that compose it. Composition of the groups: 1, DGD 2769, DGD 1631; 2, DGD 1684, DGD 1650; 3, DGD 2975, G 35360, DGD 1601, G 35122, G 35061, NI 637, DGD 2097, G 6040, NI 1193, DGD 1616, DGD 2484, DGD 1716, G 35380, G 35337, DGD 2653, G 35625 and DGD 2988; 4, DGD 2132, DGD 602, DGD 2423, G 35383; 5, NI 813; 6, NI 1204, G 35841 and NI 722; 7, NI 1108 and NI 755; 8, DGD 2046; 9, NI 1259

[b] Values plotted are Jaccard coefficients

Fig. 6. Dendrogram based on the complete linkage method. Group numbers are those of the reduced similarity matrix (see Table 5)

Discussion

Chloroplast DNA variation and phylogenetic relationships

As expected from previous phylogenetic studies (Maréchal et al. 1978; Sullivan and Freytag 1986), our results confirm the remoteness of *P. lunatus* from the other taxa investigated in this study. The results also show the isolation of *P. glabellus* from the other taxa studied and the cohesion of the *P. vulgaris-P. polyanthus-P. coccineus* group. Only three enzymes out of six revealed polymorphism between some taxa of that rather coherent group, and the distances between *P. lunatus* and/or *P. glabellus* and all the other taxa are similar, as shown in Fig. 6.

On the basis of two differences – the lack of presence of a specific DNA fragment in the patterns obtained by the digestion with *Ava*I and *Eco*RI – most of the taxa (except *P. glabellus* and *P. lunatus*) can be divided into two major groups: *P. vulgaris*, *P. polyanthus*, *P. costaricensis* and the natural hybrids between *P. vulgaris* and *P. polyanthus* on the one hand, and the sub-species of *P. coccineus* and the natural hybrid between *P. coccineus* and *P. polyanthus* on the other hand.

The affinity of the *P. coccineus* × *P. polyanthus* hybrid to the second group is correlated with the presence of several morphological traits closer to *P. coccineus* than to *P. polyanthus*, such as hypogeal germination, oval bracteoles, extrorse stigma, and red flowers (Schmit and Baudoin 1987). The greater proportion of traits from the *P. coccineus* parent could reflect a natural hybridization with the cytoplasm of that species. As cpDNA is usually transmitted maternally (Soltis et al. 1992) this would explain a restriction pattern of the hybrid identical to that of *P. coccineus* as reported here. Indeed, although paternal or biparental inheritance have been demonstrated in some taxa, as in *Medicago sativa* L. (Schuman and Hancock 1989; Masoud et al. 1990) and the genus *Pelargonium* (Metzlaff et al. 1981), there is no evidence for such a mode of cpDNA inheritance in *Phaseolus*.

The analysis of the DNA electrophoretic patterns reveals other interesting phyletic considerations. First, the position of *P. costaricensis* (a taxon with purple flowers) in the *P. vulgaris-P. polyanthus* group indicates that it is not a subspecies of *P. coccineus*, as for instance is *P. coccineus* subsp. *purpurascens*, a taxon which also has purple flowers (Schmit 1992). Our results confirm the hypothesis of an intermediate position of *P. costaricensis* between the *P. vulgaris-P. polyanthus* group on the one hand, and the *P. coccineus*

group on the other hand. This hypothesis has been based on both morphological and ecological data (Debouck et al. 1989; Schmit 1992) such as the epigeal germination (similar to *P. vulgaris* and *P. polyanthus*), the extrorse stigma (similar to *P. coccineus*), and the cloudy and humid habitat (similar to that of *P. polyanthus*). This hypothesis has also been suggested by Debouck et al. (1989) on the basis of the natural hybrids between *P. costaricensis* and *P. polyanthus* in the distribution area of these two species.

Second, all the *P. polyanthus* genotypes show a pattern different from those obtained with the *P. coccineus* genotypes, allowing the separation of these two taxa into two distinct botanical entities. These observations, together with results of hybridization (Smartt 1979; Hucl and Scoles 1985; Baudoin and Marechal 1991; Debouck 1991), lead to the hypothesis of a common phylogeny for the wild forms of *P. vulgaris*, *P. polyanthus*, *P. coccineus* and the other taxa of the *P. coccineus* complex. Is *P. costaricensis* involved in that common phylogeny? As inferred from the results for group 4 (Table 5 and Fig. 6), it is most likely involved, although it might be premature to consider it as the common ancestral branch leading to wild *P. vulgaris* and *P. polyanthus*. As a complement to a hypothesis presented elsewhere (Schmit and Debouck 1991), one could envision a dual evolutionary pathway, with hypogeal *P. coccineus*-like taxa on the one hand, and the epigeal taxa (*P. vulgaris*, *P. polyanthus* and *P. costaricensis*) on the other hand. *P. polyanthus* would thus be a distinct taxonomical entity being closer to *P. vulgaris* than to *P. coccineus*, and should therefore be maintained at the species level for the time being. On the basis of present results, and as discussed by Schmit and Debouck (1991), it seems that a hybrid origin of *P. polyanthus* between *P. coccineus* (as cytoplasm parent) and *P. vulgaris* can no longer be sustained.

Third, *P. glabellus* is well isolated, on the basis of cpDNA restriction patterns, from the two groups *P. vulgaris-P. polyanthus-P. costaricensis* and *P. coccineus*, as also indicated by Llaca and Gepts (1991). Despite some similarities with some *P. coccineus* wild forms (such as red flowers, stigma shape, perennial behaviour, and allogamous breeding system), *P. glabellus* should be maintained at the specific level for the time being on the basis of several other arguments. These include the particular ecology of the Mexican highland humid forest where the taxon has been found (Delgado 1985; Debouck 1986), the total glabrescence and the very tiny bracteoles of the plants (Maréchal et al. 1978; Delgado 1985, 1988), the strong incompatibility barriers which up to now have prevented successful hybridizations between *P. glabellus* and other related taxa (Debouck 1986 and unpublished observations), the distinct seed protein electrophoretic pattern (Schmit and Debouck 1990; Schmit et al. 1991, Schmit 1992) and the present results concerning cpDNA polymorphisms.

Intraspecific variation

Within *P. polyanthus*, our results confirm the identity between the wild and the cultivated forms and between the Mesoamerican and the Andean cultivated forms. No discriminant polymorphism is observed between forms with different biological status or geographical origin, pointing out the ancestral character of the wild form and the existence of only one gene pool. Some intraspecific variations are only obtained with *Eco*RI digestion and these concern five genotypes of *P. polyanthus* with different origins and biological status and two *P. vulgaris* genotypes. This polymorphism could not be related to other characteristics of the material. Among the five *P. polyanthus* genotypes, the distinct restriction pattern of the single wild form studied (DGD 1631) indicates that more wild accessions need to be analyzed. The two additional polymorphisms revealed by the wild *P. vulgaris* genotypes (DGD 2769 from Ecuador and DGD 2423 from Guatemala), out of the bulk of similar cpDNA patterns, are also interesting. These differences do not fit neatly into the concept of two major gene pools, of Mesoamerican and Andean origin, commonly recognized for that crop (Gepts 1988; Sprecher 1988; Khairallah et al. 1990). Instead, one can see a common cpDNA pattern across the range of biological materials of the common bean and across its gene pools, contrasting with the results presented elsewhere for mtDNA (Khairallah et al. 1992). Obviously, more material needs to be analyzed, even from neighbouring areas; the variation observed within Guatemala being illustrative in this regard (Table 3).

P. coccineus is the most polymorphic species, as also indicated by Llaca and Gepts (1991). Variations where detected with the two enzymes *Eco*RI and *Bam*HI, giving roughly twice the amount of polymorphism present in *P. polyanthus* and *P. vulgaris*, but no correlation between the biological status (wild and cultivated forms) or the subspecies classification (subspecies *coccineus* and subspecies *purpurascens*) and the cpDNA polymorphism was observed. A similar high level of variability was reported by Piñero and Eguiarte (1988) when analyzing eight enzymatic systems of eight taxa of the *P. vulgaris-P. coccineus* group.

Acknowledgements. The authors thank R. Maréchal and H. Bannerot for their helpful comments, M. Iwanaga and O. Toro from the CIAT Genetic Resources Unit for providing seeds for some of the populations studied, A. Deru, F. Kest and J. C. Martiat for their technical assistance and J. E. Muñoz for taking part to some electrophoretic analyses. This work was supported by the E. E. C. [contract number: TS2-0028-B (GDF)] and the

Belgian "Fonds de la Recherche Fondamentale Collective" (contract number: 2.45.15.22.).

References

Baudoin JP, Marechal R (1991) Wide crosses and taxonomy of pulse crops, with special emphasis on *Phaseolus* and *Vigna*. In: Ng NP, Perrino P, Attere F, Zedan H (eds) Proceedings of an African plant genetic resources Workshop, International Institute of Tropical Agriculture (Nigeria), 17–20 October 1988, pp 287–302

Baudoin JP, Camarena Mayta F, Schmit V (1992) Contribution à une meilleure connaissance de la position phylétique de la légumineuse alimentaire *Phaseolus polyanthus* Greenm. Bull Rech Agron Gembloux 27:167–198

Buckner B, Hyde BB (1985) Chloroplast DNA variation between the common cultivated potato (*Solanum tuberosum* ssp. *tuberosum*) and several South American relatives. Theor Appl Genet 71:527–531

Camarena F, Baudoin JP (1987) Obtention des premiers hybrides interspécifiques entre *Phaseolus vulgaris* et *Phaseolus polyanthus* avec le cytoplasme de cette dernière forme. Bull Rech Agron Gembloux 22:43–55

Dally AM, Second G (1990) Chloroplast DNA diversity in wild and cultivated species of rice (Genus *Oryza* section *Oryza*). Cladistic mutation and genetic-distance analysis. Theor Appl Genet 80:209–222

Debouck DG (1986) *Phaseolus* germplasm collection in the Huasteca and surrounding regions, Mexico. Trip Report, Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia

Debouck DG (1991). Systematics and morphology. In: van Schoonhoven A, Voysest O (eds) Common beans: research for crop improvement. Commonwealth Agricultural Bureaux International, Wallingford, United Kingdom, pp 55–118

Debouck DG, Araya VR, Ocampo S, Gonzalez UWG (1989) Collecting *Phaseolus* in Costa Rica. FAO/IBPGR Pl Genet Res Newslett 78/79:44–46

Delgado SA (1985) Systematics of the genus *Phaseolus* (*Leguminosae*) in North and Central America. PhD thesis, University of Texas, Austin

Delgado SA (1988) Variation, taxonomy, domestication and germplasm potentialities in *Phaseolus coccineus*. In: Gepts P (ed) Genetic resources of *Phaseolus* beans. Kluwer Academic Publishers, Dordrecht (The Netherlands), pp 441–463

Doebley J, Renfroe W, Blanton A (1987) Restriction site variation in the *Zea* chloroplast genome. Genetics 117:139–147

Gawel NJ, Jarret RL (1991) Chloroplast DNA restriction fragment length polymorphisms (RFLPs) in *Musa* species. Theor Appl Genet 81:783–786

Gepts P (1988) A middle American and an Andean common bean gene pool. In: Gepts P (ed) Genetic resources of *Phaseolus* beans. Kluwer Academic Publishers, Dordrecht (The Netherlands), pp 375–390

Hosaka K, Ogihara Y, Matsubayashi M, Tsunewaki (1984) Phylogenetic relationship between the tuberous *Solanum* species as revealed by restriction endonucleases analysis of chloroplast DNA. Jpn J Genet 59:349–369

Hosaka K (1986) Who is the mother of the potato? Restriction endonuclease analysis of chloroplast DNA of cultivated potatoes. Theor Appl Genet 72:606–618

Hosaka K, Hanneman RE Jr (1988) The origin of the cultivated tetraploid potato based on chloroplast DNA. Theor Appl Genet 76:172–176

Hucl P, Scoles GJ (1985) Interspecific hybridization in the common bean: a review. HortScience 20:352–357

Jonhson SC (1967) Hierarchic clustering schemes. Psychometrika 32:241–254

Khairallah MM, Adams MW, Sears BB (1990) Mitochondrial DNA polymorphisms of Malawian bean lines: further evidence for two major gene pools. Theor Appl Genet 80:753–761

Khairallah MM, Sears BB, Adams MW (1992) Mitochondrial restriction fragment length polymorphisms in wild *Phaseolus vulgaris* L.: insights on the domestication of the common bean. Theor Appl Genet 84:915–922

Kung SD, Zhu ZS, Chen GF (1982) *Nicotiana* chloroplast genome. III. Chloroplast DNA evolution. Theor Appl Genet 61:73–79

Le Marchand G, Maréchal R (1977) Chromosome pairing in interspecific hybrids reveals the value of pollen morphology for deducing phylogenic affinities in the genus *Phaseolus*. In: Interspecific hybridization in plant breeding. Proc 8th Congress Eucarpia, Madrid, pp 355–337

Llaca V, Gepts P (1991) Studies on *Phaseolus* inter- and intraspecific evolution using cpDNA as a molecular marker. Annu Rep Bean Improv Coop 34:145–146

Londoño NR, Gathee JW, Sanders JH (1981) Bean production trends in Africa 1966–1979. In: Proceedings Regional Workshop on Potential for Fields Beans in Eastern Africa, Lilongwe, Malawi, 1980. Centro Internacional de Agricultura Tropical, Cali, Colombia, pp 19–29

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Maréchal R, Masherpa JM, Stainier F (1978) Etude taxonomique d'un groupe complexe d'espèces des genres *Phaseolus* et *Vigna* (*Papilionaceae*) sur la base de données morphologiques et polliniques traitées par l'analyse informatique. Boissiera 28:1–273

Masoud SA, Jonhson LB, Sorensen EL (1990) High transmission of paternal plastid DNA in Alfalfa plants demonstrated by restriction fragment polymorphism analysis. Theor Appl Genet 79:49–55

Metzlaff M, Borner T, Hagemann R (1981) Variations of chloroplast DNAs in the genus *Pelargonium* and their biparental inheritance. Theor Appl Genet 60:37–41

Milligan BG (1991) Chloroplast DNA diversity within Land among populations of *Trifolium pratense*. Curr Genet 19:411–416

Palmer JD (1987) Chloroplast DNA evolution and biosystematic uses of chloroplast DNA variation. Am Nat 130 (Suppl):6–29

Palmer JD, Zamir D (1982) Chloroplast DNA evolution and phylogenetic relationships in *Lycopersicon*. Proc Natl Acad Sci USA 79:5006–5010

Piñero D, Eguiarte L (1988) The origin and biosystematic status of *Phaseolus coccineus* ssp. *polyanthus*: electrophoretic evidence. Euphytica 37:199–203

Piper CV (1926) Studies in American *Phaseolinae*. Contr US Nat Herb 22:663–701

Schmit V (1992) Etude de *Phaseolus polyanthus* Greenman et autres taxons du complexe *Phaseolus coccineus* L. Thèse de doctorat, Faculté des Sciences Agronomiques, Gembloux

Schmit V, Baudoin JP (1987) Multiplication et évaluation de *Phaseolus coccineus* L. et *Phaseolus polyanthus* Greenman, deux espèces intéressantes pour l'amélioration de la productivité des légumineuses vivrières. Bull Rech Agron Gembloux 22:235–253

Schmit V, Debouck DG (1990) *P. glabellus* Piper, a noteworthy variant of the *P. coccineus* complex? Annu Rep Bean Improv Coop 33:124–125

Schmit V, Debouck DG (1991) Observations on the origin of *Phaseolus polyanthus* Greenman. Econ Bot 45:345–364

Schmit V, Baudoin JP, Wathelet B (1991) Considerations about the taxonomical position of various taxa of the *P. coccineus* complex on basis of seeds protein electrophoresis and experimental hybridizations. Annu Rep Bean Improv Coop 34: 87–88

Schuman CM, Hancock JF (1989) Paternal inheritance of plastids in *Medicago sativa*. Theor Appl Genet 78:863–866

Smartt J (1973) The possible status of *P. coccineus* L. ssp. *darwinianus* Hdz X and Miranda C as a distinct species and cultigen of the genus *Phaseolus*. Euphytica 22:424–426

Smartt J (1979) Interspecific hybridization in the grain legumes – a review. Econ Bot 33:329–337

Sneath PHA, Sokal RR (1973) Numerical taxonomy. W. F. Freeman and Company, San Francisco

Soltis DE, Soltis PS, Milligan BG (1992) Intraspecific chloroplast DNA variation: systematic and phylogenetic implications. In: Soltis PS, Soltis DE, Doyle JJ (eds) Molecular Systematics of Plants. Chapman and Hall, New York, pp 117–150

Soreng RJ (1990) Chloroplast DNA phylogenetics and biogeography in a reticulating group: study in *Poa* (*Poaceae*). Am J Bot 77:1383–1400

Sprecher SL (1988) Allozyme differentiation between gene pools in common bean (*Phaseolus vulgaris* L.), with special reference to Malawian germplasm. PhD thesis, Michigan State University, EAst Lansing, Michigan, USA

Sullivan JG, Freytag G (1986) Predicting interspecific compatibilities in beans (*Phaseolus*) by seed protein electrophoresis. Euphytica 35:201–209

Thimothy DH, Levings CS III, Pring DR, Conde MF, Kermicle JL (1979) Organelle DNA variation and systematic relationships in the genus *Zea*: Teosinte. Proc Natl Acad Sci USA 76:4220–4224

Voyset O (1983) Variedades de frijol en America Latina y su origen. Centro Internacional de Agricultura Tropical, Cali, Colombia