Genetic Diversity and Origin of Slovene Common Bean (*Phaseolus vulgaris* L.) Germplasm as Revealed by AFLP Markers and Phaseolin Analysis

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ABSTRACT. There is a long tradition of common bean cultivation in Slovenia, which has resulted in the development of numerous landraces in addition to newly established cultivars. The genetic diversity of 100 accessions from the Genebank of the Agricultural Institute of Slovenia (AIS) were evaluated with amplified fragment length polymorphism (AFLP) markers and phaseolin seed protein. Twenty-seven standard accessions of known Mesoamerican and Andean origin, 10 wild *Phaseolus vulgaris* accessions and two related species, *P. coccineus* L. and *P. lunatus* L., were also included. Ten AFLP primer combinations produced 303 polymorphic bands, indicating a relatively high level of genetic diversity. Based on the marker data, unweighted pair group method with arithmetic mean (UPGMA) analysis and principal coordinate analysis (PCoA) all *P. vulgaris* accessions were separated into three well-defined groups. Two groups consisted of accessions of Mesoamerican and Andean origin, while the third was comprised of only four wild *P. vulgaris* accessions. A set of Slovene accessions formed a well-defined sub-group within the Andean cluster, showing their unique genetic structure. These data were supported by phaseolin analysis, which also revealed additional variants of “C” and “T” phaseolin types. The results are in agreement with previous findings concerning diversification of common bean germplasm introduced in Europe.

The genus *Phaseolus* L. includes numerous wild and cultivated species, originating in the New World. The exact number is still unknown (Debouck, 1999). Only five species are important as cultivated food crops, *P. vulgaris*, *P. polyanthus* Greenm., *P. coccineus*, *P. lunatus*, and *P. acutifolius* A. Gray. Because of its high nutritional value, the common bean (*P. vulgaris*) is by far the most important *Phaseolus* bean and is cultivated on all continents except Antarctica, under very diverse cultivation conditions (Singh, 1999).

Two major gene pools of common bean were first recognized in the wild form, Mesoamerican and Andean (Gepts, 1998). The evidence was based on morphological traits (Singh, 1989; Singh et al., 1991a), phaseolin seed protein (Gepts et al., 1986), isozymes (Koenig and Gepts, 1989), and molecular markers (Freyre et al., 1996; Tohme et al., 1996; Velasquez and Gepts, 1994). A third, genetically unique gene pool was later described in the northern Andes (Debouck, 1999; Tohme et al., 1996). The northern Andes gene pool is located in Ecuador and northern Peru and it is considered to be the nucleus of diversity, from where wild beans

dispersed both northward and southward (Broughton et al., 2003). A fourth gene pool in Colombia might also exist, but it is still poorly understood (Debouck, 1999; Tohme et al., 1996). After domestication, the common bean spread between Mesoamerica and South America and, after the European discovery of the Americas, to Europe and Africa, where it was cultivated in diverse environments and agricultural conditions (Chacon et al., 2005; Gepts et al., 1986). Post-domestication divergence gave rise to seven races, three in the Andean and four in the Mesoamerican gene pools (Chacon et al., 2005; Singh et al., 1991b).

The first documented appearance of common bean in Europe was as an ornamental plant in 1508 in France (Rodino and Drevon, 2004). Because this incident occurred before the exploration of Peru in 1528, it was presumably of Mesoamerican origin; later, a prevalence of Andean over Mesoamerican genotypes in Europe was generally observed (Gepts and Bliss, 1988; Rodino et al., 2003). The dissemination routes of common bean cultivars from their areas of domestication to Europe were studied by the analysis of phaseolin protein (Gepts and Bliss, 1988; Islam et al., 2002; Limongelli et al., 1996; Ocampo et al., 2005), but the exact origin of beans in Europe is still unclear and currently under discussion (Rodino and Drevon, 2004). There is no written evidence of the first introduction of common bean on Slovene territory; however, the naming of some accessions indicate that it could have come from France, Spain, and Italy through trade and the exchange of material. In the first historical review of Slovene territory by Valvasor (1689), the cultivation on a larger scale of two bean species, common bean and runner bean (*P. coccineus*), is already described. Common bean has therefore been an important crop for human consumption in Slovenia for

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centuries and is still cultivated in almost every home garden. Although new cultivars are displacing landraces, it is possible to find farmers and gardeners who grow bean landraces for their own consumption and for sale. There have been several collecting missions in Slovenia; 995 *Phaseolus* accessions (713 *P. vulgaris* var. *vulgaris* L., 203 *P. vulgaris* var. *nanus* G. Martens, 27 *P. vulgaris* accessions of intermediate growth habit, and 52 *P. coccineus*) were collected in the last decade and are stored at the Genebank of AIS, Ljubljana, Slovenia. The preliminary study of genetic diversity performed on a set of accessions using RAPD markers revealed that some accessions grouped within the Andean and Mesoamerican clusters, while the majority formed a separate cluster (Meglič et al., 1999).

The aim of this work was to evaluate, using AFLP and phaseolin seed protein analysis, the genetic variability and the relationship among 100 accessions of Slovene common bean landraces and cultivars, stored at the AIS Genebank, as well as to relate their origin to the Mesoamerican and Andean gene pools. The results will contribute new knowledge to improve germplasm bank management and conservation programs, and will improve the efficiency of the breeding process.

Materials and Methods

PLANT MATERIAL. A total of 139 *Phaseolus* accessions were evaluated for genetic diversity. These accessions included nine Slovene common bean cultivars, 91 landraces collected in various parts of Slovenia and stored at AIS (Table 1), 39 standard check accessions of which 27 were cultivars of known Andean and Mesoamerican origin, 10 wild accessions of *P. vulgaris*, and two accessions of *P. vulgaris* related species, *P. coccineus* and *P. lunatus* (Table 2). Standard accessions were obtained from the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany; Nationale Plantentuin van België, Meise, Belgium; Instituto Nacional de Investigación (INIA), Madrid, Spain; Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia (Table 2); and USDA, ARS-GRIN, Pullman, Wash. Twenty plants of each Slovene cultivar and landrace were grown in a field in 2003 at Jable, Trzin, Slovenia. At the end of the growing period, plants were harvested and dry seeds assessed for shape and weight (Table 1). These data are based on 1-year evaluation.

DNA EXTRACTION. Fresh leaf samples were taken from 10 field-grown plants of each Slovene accession, bulked, and DNA extracted using a modified CTAB method according to Kump and Javornik (1996). Standard accessions were grown in the greenhouse and DNA extraction was carried out as above, using five plants of each accession.

AFLP ASSAY. The AFLP technique was carried out as described by Vos et al. (1995) with some modifications (Bandelj et al., 2004). Total genomic DNA (500 ng) was restricted using two restriction enzymes, *EcoRI* and *MseI*, and ligated to *EcoRI* and *MseI* adaptors. Pre-amplifications were performed in 50- μ L volumes of 1 $\times$ PCR buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 75 ng *EcoRI* and *MseI* primers, 1.5 U Taq polymerase and 5 μ L of ligated DNA, using 20 cycles of 94 °C for 30 s, 56 °C for 60 s, and 72 °C for 60 s. Two-microliter aliquots of the diluted (1:10) pre-amplification product were used as templates for selective amplification with 10 primer combinations (E-AGC/M-CAA, E-AGC/M-CTC, E-AGC/M-CTT, E-AGG/M-CAG, E-AGG/M-CAA, E-ACC/M-CAT, E-ACT/M-CAT, E-ACT/M-CAA, E-ACT/M-CAC, E-ACG/M-CAG). Only *EcoRI* primers were labeled with Cy5 at the 5'

end for selective amplification, allowing fluorescence detection. The selective amplification was carried out in a 10- μ L volume using the following temperature profile: 13 cycles of 94 °C for 30 s, 65 °C for 30 s with a decrease of annealing temperature of 0.7 °C per cycle, and 72 °C for 60 s, followed by 23 cycles at the annealing temperature of 56 °C.

The PCR products were denatured by adding an equal volume of formamide loading dye (98% formamide, dextran blue 5 mg·mL⁻¹) and by heating at 94 °C for 4 min. The samples were separated on a 7.5% polyacrylamide denaturing gel in an automated ALFexpressII sequencer (Amersham Biosciences AB, Uppsala, Sweden). A fluorescence labeled size marker (Cy5 Sizer 50-500; Amersham Biosciences) was used as the molecular weight reference.

AFLP DATA ANALYSIS. AFLP profiles were scored for the presence (1) and absence (0) of each fragment. Only clear and reproducible fragments ranging in length from 50 to 500 bp were considered for data analysis. The binary data matrix was used to calculate Jaccard's similarity coefficients (Jaccard, 1908); $GS_{ij} = a/(a + b + c)$, where GS_{ij} is the similarity between two individuals i and j , a is the number of bands present in both i and j , b is the number of bands present in i and absent in j and c is the number of bands present in j and absent in i . Associations among the 139 accessions were displayed by cluster analysis using the UPGMA algorithm in the FreeTree computer package (Pavlicek et al., 1999). A cophenetic matrix was derived from the similarity matrix to test the goodness of fit of the cluster analysis, by comparing the two matrices using the Mantel matrix correspondence test in the NTSYS-pc package (Rohlf, 1998). In order to estimate the degree of independence between AFLP profiles generated from individual primer-pairs, similarity matrices were computed and subjected to Mantel tests (Mantel, 1967). FreeTree was used also to calculate bootstrap values for 1000 replicates to evaluate branch support for the obtained tree (Felsenstein, 1985). Based on the genetic distance matrix, genetic relationships among accessions were additionally analyzed by PCoA using NTSYS-pc (Rohlf, 1998).

PHASEOLIN ANALYSIS. Five seeds of each of the 123 accessions analyzed for phaseolin type were ground into fine powder. Initially, the flour sample was suspended for 0.5 h in a 0.5 M NaCl solution at room temperature. The suspension was then centrifuged and the supernatant mixed with an equal volume of cracking buffer [0.5 M Tris-HCl; pH 6.8; 2 mM EDTA; 4% (w/v) SDS; 20% (w/v) glycerol; 3% (v/v) 2-mercaptoethanol; and 0.02% (w/v) bromophenol blue marker dye]. The extract was heat-treated at 100 °C for 5 min. After centrifugation, the supernatant was separated by one-dimensional SDS/PAGE according to Ma and Bliss (1978), using 1-mm-thick slab gels with 10% (w/v) acrylamide in the running gel, and 4% (w/v) acrylamide in the stacking gel. The electrophoresis was carried out at 40 mA for 1 h and 80 mA for another 2.5 h. At the end of the electrophoresis, the gels were stained with Coomassie Brilliant Blue. 'Cornell 49242', G24404, 'Tendergreen', 'Contender', and 'Sanilac' were chosen as standards for "B," "H," "T," "C," and "S" phaseolin types, respectively.

Results

AFLP ANALYSIS. All primer pair combinations produced well-defined and scorable amplification products and showed polymorphisms among the 139 analyzed *Phaseolus* accessions (Table 3). The 10 AFLP primer combinations used in this study generated a

Table 1. Seed shape, 100-seed weight, and phaseolin type of 100 Slovene *Phaseolus vulgaris* accessions included in AFLP analysis.

Serial no.	Accession name/no.	Seed shape	100 seed wt (g)	Phaseolin type	Status ^z	Serial no.	Accession name/no.	Seed shape	100 seed wt (g)	Phaseolin type	Status ^z
1	Jeruzalemčan	Elliptical	45.9	C ^s	C	50	PHA315	Elliptical	21.0	C	L
2	Cipro	Elliptical	55.5	C ^s	C	51	PHA316	Elliptical	40.3	S	L
3	Ptujski maslenec	Elliptical	60.6	C ^s	C	52	PHA318	Kidney shaped	47.2	T ^s	L
4	Jabelski pisanec	Elliptical	65.2	C ^s	C	53	PHA327_I	Kidney shaped	33.7	T ^s	L
5	Klemen	Elliptical	45.6	C	C	54	PHA327_II	Kidney shaped	45.6	T ^s	L
6	Jabelski stročnik	Elliptical	55.8	C ^s	C	55	PHA336	Elliptical	38.8	T	L
7	Zorin	Narrowly elliptical	52.1	T ^s	C	56	PHA346	Elliptical	28.8	T	L
8	Češnjevec	Elliptical	50.8	C ^s	C	57	PHA347	Elliptical	24.9	T	L
9	Starozagorski	Elliptical	49.7	T	C	58	PHA358	Kidney shaped	33.8	T	L
10	PHA6	Narrowly elliptical	47.8	C ^s	L	59	PHA363	Elliptical	39.2	T ^s	L
11	PHA7	Kidney shaped	35.8	S	L	60	PHA368	Elliptical	31.3	S	L
12	PHA11	Elliptical	42.0	C ^s	L	61	PHA371	Elliptical	26.0	S	L
13	PHA29	Elliptical	48.5	C ^s	L	62	PHA374	Elliptical	42.5		L
14	PHA32	Globose	44.4	C ^s	L	63	PHA377	Elliptical	41.7	T ^s	L
15	PHA39	Elliptical	36.0	C ^s	L	64	PHA386	Elliptical	37.8	T	L
16	PHA41	Elliptical	36.3	T ^s	L	65	PHA388	Kidney shaped	56.7	T ^s	L
17	PHA54	Elliptical	41.5	C ^s	L	66	PHA390	Narrowly elliptical	38.5	C ^s	L
18	PHA55	Kidney shaped	46.6	S	L	67	PHA396	Elliptical	41.5	T ^s	L
19	PHA59	Kidney shaped	34.6	C ^s	L	68	PHA397	Elliptical	36.2	T ^s	L
20	PHA77	Elliptical	30.6	C ^s	L	69	PHA418	Kidney shaped	55.9	T	L
21	PHA79	Elliptical	41.4	C ^s	L	70	PHA419	Kidney shaped	42.8	S	L
22	PHA90	Elliptical	62.1	T ^s	L	71	PHA423	Elliptical	33.8	T	L
23	PHA91	Elliptical	35.2	C ^s	L	72	PHA438	Elliptical	41.9	C ^s	L
24	PHA93	Kidney shaped	49.8	T ^s	L	73	PHA446	Kidney shaped	43.9		L
25	PHA95	Globose	32.8	C ^s	L	74	PHA452	Kidney shaped	23.8	C	L
26	PHA97	Narrowly elliptical	23.9	S	L	75	PHA491	Narrowly elliptical	43.9		L
27	PHA99	Narrowly elliptical	43.3	C ^s	L	76	PHA498	Elliptical	48.6	T ^s	L
28	PHA121	Elliptical	57.4	C ^s	L	77	PHA499	Elliptical	44.3	C ^s	L
29	PHA124	Elliptical	43.6	C ^s	L	78	PHA627	Elliptical	52.5	C ^s	L
30	PHA131	Globose	44.7	T ^s	L	79	PHA637	Elliptical	53.1	C ^s	L
31	PHA132	Elliptical	45.5	C ^s	L	80	PHA639	Elliptical	50.3	C ^s	L
32	PHA137	Elliptical	21.8	C ^s	L	81	PHA642	Elliptical	52.2	C	L
33	PHA147	Kidney shaped	61.9	C	L	82	PHA681	Elliptical	39.7	C ^s	L
34	PHA150	Elliptical	42.2	C ^s	L	83	PHA691	Elliptical	35.5	C ^s	L
35	PHA153	Elliptical	42.0	C ^s	L	84	PHA717	Elliptical	46.9	S	L
36	PHA181	Kidney shaped	36.5	C ^s	L	85	PHA777	Elliptical	50.6	C ^s	L
37	PHA207	Elliptical	30.2	C ^s	L	86	PHA860	Kidney shaped	23.9	S	L
38	PHA214	Narrowly elliptical	37.6	T ^s	L	87	PHA903	Elliptical	38.8	C ^s	L
39	PHA218	Kidney shaped	30.8	S	L	88	PHA909	Elliptical	41.2	C ^s	L
40	PHA222	Globose	39.5	C ^s	L	89	PHA914	Kidney shaped	33.4	C ^s	L
41	PHA225	Elliptical	62.7	C ^s	L	90	PHA915	Elliptical	20.3	S	L
42	PHA235	Elliptical	46.1	C ^s	L	91	PHA938	Elliptical	68.3	C ^s	L
43	PHA245	Elliptical	39.3	S	L	92	PHA941	Elliptical	29.2	S	L
44	PHA259	Elliptical	37.5	C ^s	L	93	PHA942	Elliptical	59.8	S	L
45	PHA260	Elliptical	32.2	C ^s	L	94	PHA950	Elliptical	42.6	C ^s	L
46	PHA264	Elliptical	52.0	C ^s	L	95	PHA954	Elliptical	46.1	C	L
47	PHA304	Narrowly elliptical	23.7	T	L	96	PHA967	Globose	62.6	C ^s	L
48	PHA306	Elliptical	57.7	T	L	97	PHA971	Elliptical	55.6	T	L
49	PHA307	Narrowly elliptical	37.2	T	L	98	PHA973	Elliptical	53.9	T	L
						99	PHA1073	Narrowly elliptical	43.4	T	L
						100	PHA1074	Elliptical	49.2	C ^s	L

^zC = Slovene cultivar; L = Landrace. "T^s" and "C^s" are the variants of "T" and "C" phaseolin type, respectively.

Table 2. *Phaseolus* accessions chosen as standards representing common bean gene pools. Phaseolin types taken from Tohme et al. (1996) are indicated with an asterisk. “T^s” and “C^s” are the variants of “T” and “C” phaseolin types, respectively.

Serial no.	Species	Accession name	Phaseolin type	Gene pool ^z	Status ^u	Source of material (Accession no.)
1	<i>P. vulgaris</i>	Cornell 49242	B	MA ^y	C	CIAT
2	<i>P. vulgaris</i>	Widusa	T ^s	MA ^y	C	CIAT
3	<i>P. vulgaris</i>	G 2333	B	MA ^y	C	CIAT
4	<i>P. vulgaris</i>	Tu	S	MA ^y	C	CIAT
5	<i>P. vulgaris</i>	Ab 136	S	MA ^y	C	CIAT
6	<i>P. vulgaris</i>	Michelite	S	MA ^y	C	CIAT
7	<i>P. vulgaris</i>	Gloriabamba (Apetito)	S	MA ^x	C	IPK (IPK-PHA 5542)
8	<i>P. vulgaris</i>	Rosa de castilla	S	MA ^x	C	IPK (IPK-PHA 5078)
9	<i>P. vulgaris</i>	Mexico 222	T ^s	MA ^x	C	CIAT
10	<i>P. vulgaris</i>	To	S	MA ^y	C	CIAT
11	<i>P. vulgaris</i>	Bayos	S	MA ^x	C	IPK (IPK-N 1131)
12	<i>P. vulgaris</i>	Tamaulipas 9-3	S	MA ^x	C	IPK (IPK-PHA 5597)
13	<i>P. vulgaris</i>	PI 207262	S	MA ^y	C	USDA, ARS-GRIN
14	<i>P. vulgaris</i>	Aurora	T	MA ^v	C	IPK (IPK-PHA 604)
15	<i>P. vulgaris</i>	Azufrados	T	MA ^x	C	IPK (IPK-PHA 967)
16	<i>P. vulgaris</i>	Coscorron blanco	S	A ^w	C	IPK (IPK-N 388)
17	<i>P. vulgaris</i>	Caballeros	S	A ^w	C	IPK (IPK-N 1137)
18	<i>P. vulgaris</i>	Xan159		A ^v	C	IPK (IPK-PHA 5628)
19	<i>P. vulgaris</i>	Sangretero	T ^s	A ^x	C	IPK (IPK-N 684)
20	<i>P. vulgaris</i>	Mortino	C ^s	A ^x	C	Nationale Plantentuin van België (HBBRMAI-618)
21	<i>P. vulgaris</i>	Canario	T ^s	A ^x	C	INIA (CRF-BG-019198)
22	<i>P. vulgaris</i>	Kaboon	T	A ^y	C	CIAT
23	<i>P. vulgaris</i>	Uribe redondo	T ^s	A ^x	C	IPK (IPK-PHA 1390)
24	<i>P. vulgaris</i>	Frijol bola roja	C	A ^w	C	IPK (IPK-PHA 5088)
25	<i>P. vulgaris</i>	Perry Marrow	T	A ^v	C	CIAT
26	<i>P. vulgaris</i>	Michigan Dark Red Kidney	T	A ^v	C	CIAT
27	<i>P. vulgaris</i>	Frutilla	C ^s	A ^w	C	IPK (IPK-N 1141)
28	<i>P. vulgaris</i>	G24757 (OT-443)	T*		W	CIAT (G24757)
29	<i>P. vulgaris</i>	G23580A	I*		Wd	CIAT (G23580A)
30	<i>P. vulgaris</i>	G24404 (OT-113)	H*		Wd	CIAT (G24404)
31	<i>P. vulgaris</i>	G24423 (OT-140)	CH*		W	CIAT (G24423)
32	<i>P. vulgaris</i>	G24408 (OT-118)	H*		W	CIAT (G24408)
33	<i>P. vulgaris</i>	G24605	M24*		W	CIAT (G24605)
34	<i>P. vulgaris</i>	G21245	I*		W	CIAT (G21245)
35	<i>P. vulgaris</i>	G23583	I*		W	CIAT (G23583)
36	<i>P. vulgaris</i>	G23579	I*		W	CIAT (G23579)
37	<i>P. vulgaris</i>	G23723	I*		W	CIAT (G23723)
38	<i>P. coccineus</i>					
39	<i>P. lunatus</i>					

^zMA = Mesoamerican gene pool; A = Andean gene pool.

^yData from Balardin and Kelly (1998).

^xData from Velasquez and Gepts (1994).

^wData from Singh et al. (1991b).

^vData from Johns et al. (1997).

^uC = cultivated; W = wild; Wd = weedy.

total of 424 scorable amplification products. The number of bands varied from 24 (E-ACT/M-CAC) to 56 (E-ACT/M-CAA), with an average of 42.4 per primer pair. The percentage of polymorphism ranged from 58% to 83%, with an average of 71%. The combined patterns obtained by the 10 primer-pairs were adequate to discriminate all genotypes except two, PHA971 and PHA973. The largest Jaccard's similarity coefficient (1.000) was calculated for these two accessions, while the lowest (0.462) belonged to the pair *P. lunatus* and 'Coscorron blanco'. The largest similarity coefficient involving *P. lunatus* was 0.506, while the similarity coefficients of the representative of *P. coccineus* did not exceed 0.666. The lowest similarity coefficient (0.699) among 137 *P.*

vulgaris accessions was identified between G23723 and PHA717 and the smallest (0.728) among 100 Slovene accessions between PHA132 and PHA7. Similarity coefficients of 0.889 and more were observed among nine Slovene cultivars. A comparison of the similarity matrices based on data from individual primer-pairs revealed a relatively high correspondence ($0.45 < r < 0.78$) between each pair, indicating that different primer-pairs generated similar banding profiles.

UPGMA cluster analysis divided the studied accessions into three groups (Fig. 1). The cophenetic correlation between the dendrogram and the similarity matrix revealed high degree of fit ($r = 0.96$). High bootstrap values, indicated on the dendrogram,

Table 3. Total number of AFLP fragments, number of polymorphic fragments, and percentage of polymorphisms observed among 139 *Phaseolus* accessions using 10 AFLP primer pair combinations.

Primer pair combination	Amplified fragments (no.)	Polymorphic fragments (no.)	Polymorphisms (%)
E-AGC/M-CAA	42	32	76
E-AGC/M-CTC	47	37	79
E-AGC/M-CTT	50	30	60
E-AGG/M-CAG	29	24	83
E-AGG/M-CAA	40	28	70
E-ACC/M-CAT	43	32	74
E-ACT/M-CAT	53	41	77
E-ACT/M-CAC	24	14	58
E-ACT/M-CAA	56	41	73
E-ACG/M-CAG	40	24	60
Total	424	303	71
Mean	42.4	30.3	71

showed that all group-defining branches are well supported. The majority of accessions clustered into Group 1 and Group 2. The latter was formed by 13 Mesoamerican standard cultivars, 15 Slovene landraces and two Andean standard cultivars, while Group 1 comprised one small and two large sub-groups. Large Sub-group A contained 51 Slovene landraces, one Andean standard cultivar and six Slovene cultivars, while Sub-group B included five Andean standard accessions, one Mesoamerican standard accession, 23 Slovene landraces, and the remaining three Slovene cultivars. Small Sub-group C had only four wild *P. vulgaris* genotypes. A set of accessions, one Mesoamerican and four Andean standard cultivars, two Slovene landraces, and two wild *P. vulgaris* accessions, from Group 1, did not cluster within these three sub-groups. Another four wild *P. vulgaris* genotypes formed minor Group 3, while the two representatives of *P. lunatus* and *P. coccineus* are only distantly related to all other studied accessions.

The majority of the standard cultivars clustered according to the gene pool of origin determined in previous studies (Balardin and Kelly, 1998; Johns et al., 1997; Singh et al., 1991b; Velasquez and Gepts, 1994). Ten out of 12 standard Andean cultivars clustered together into Group 1, while 13 out of 15 Mesoamerican cultivars belonged to Group 2. These two groups clearly represent two common bean gene pools of origin, Andean and Mesoamerican. Two cultivars, 'Aurora' and 'Azufrados', from the Mesoamerican gene pool and two Andean cultivars, 'Caballeros' and 'Coscorron blanco', did not group as described by Johns et al. (1997), Singh et al. (1991b), and Velasquez and Gepts (1994). In order to obtain additional information on the grouping of the 139 analyzed accessions, PCoA was carried out.

The PCoA plot (Fig. 2) clearly reflected the relationships among the studied accessions that had also been obtained from the dendrogram. The groups indicated in the PCoA plot are analogous to the clusters in the UPGMA dendrogram. The first three principal coordinates accounted for 46% of the total variation. The first axis in Fig. 2 clearly classified Mesoamerican accessions apart from the Andean as well as the Andean among themselves. The second axis clearly separated two large Andean sub-groups, Sub-group A and Sub-group B.

PHASEOLIN ANALYSIS. Electrophoretic analysis of the phaseolin showed that Slovene common bean landraces and cultivars possess the three phaseolin patterns typical of the Mediterranean basin: "C," "T," and "S" (Gepts and Bliss, 1988). The "C" pattern was observed in 47 landraces and seven cultivars, while the "T" pattern

was common to 27 landraces and two cultivars. Other than the 14 landraces, no cultivar with an "S" pattern was identified.

Among the 83 "C" and "T" accessions, 63 showed electrophoretic profiles that were different in one band from the standard accessions for "C" phaseolin type ('Contender') and "T" type ('Tendergreen'). This new band, indicated by an arrow in Fig. 3, was found in both "T" and "C" phaseolin types. We designated these types of phaseolin as "Ts" and "Cs," respectively. These two variants were also found in the standards and are therefore not unique to the Slovene common bean germplasm.

As shown in the cluster analysis of the AFLP data, there is a tendency for accessions to distribute according to their phaseolin type. The majority of the accessions included in Group 2 have "S" and "B" phaseolin patterns and a low mean seed weight (Tables 1 and 2, Fig. 1), characteristics of Mesoamerican genotypes. The same findings have already been reported by many authors (Gepts and Bliss, 1988; Johns et al., 1997; Rodino et al., 1991; Singh et al., 1991b). The accessions from Group 1 have "C" and "T" phaseolin patterns with their variants "Cs," "Ts," and a high seed weight, typical of Andean genotypes. Phaseolin analysis supports the differentiation of Group 1 into sub-groups. Namely, 48 accessions from Sub-group A exhibited "C," whereas "T" phaseolin type was detected in only nine accessions. On the other hand, "T" phaseolin prevailed in Sub-group B, where "T" type was identified in 25 accessions and "C" type in only five accessions.

Discussion

In the present study, AFLP markers were used to evaluate the structure of genetic diversity among common bean accessions cultivated in Slovenia. Ten primer pairs produced 303 polymorphic bands among the 139 accessions, which indicates a relatively high level of genetic diversity within cultivated common bean germplasm, even within a restricted geographic region such as Slovenia. The high number of polymorphic bands per primer pair confirms that AFLP is capable of detecting substantial numbers of polymorphisms with a relatively small number of primer pairs (Maciel et al., 2003; Tohme et al., 1996), compared with other marker systems applied to *Phaseolus* germplasm analysis, such as RAPD (Haley et al., 1994) and RFLP (Sonnante et al., 1994; Velasquez and Gepts, 1994).

The results of UPGMA clustering and PCoA analysis showed that *P. vulgaris* genotypes of both Andean and Mesoamerican origin are grown in Slovenia and that these two gene pools are distinct from each other. In the dendrogram, 15 Slovene landraces clustered with 13 standard accessions representing the Mesoamerican gene pool (Group 2), while the majority of landraces (76) grouped with 10 standard accessions of Andean origin (Group 1). The robustness of clusters was also indicated by high bootstrap indices. The prevalence of Andean genotypes might be due to better adaptation to the cooler and shorter summer in this region, as well as a preference for growing large seed types. The same assumptions were proposed by Rodino et al. (2003). As observed in the dendrogram, all nine studied Slovene cultivars clustered with standard accessions of Andean origin. The relatively close relationships among cultivars suggest that only a small portion of the existing variability of *P. vulgaris* has been used in breeding programs.

Molecular data have shown that wild and cultivated common bean accessions of the same geographic origin have a similar genetic base, as also observed by Ocampo et al. (2005) and Velasquez and Gepts (1994). This was reflected in the cluster

Fig. 2. Principal coordinate analysis of 139 bean accessions analyzed by AFLP markers.

analysis, where three wild and one weedy bean genotypes from Sub-group C clustered near the cultivated genotypes of Andean origin from Sub-group A and Sub-group B. The latter contained the most Andean standard accessions, while Sub-group A comprised more than half of all the landraces studied and only one Andean standard accession. This is an evidence of separate Slovene bean accessions with unique genetic diversity and confirmation of our preliminary results (Meglič et al., 1999). As suggested by Negri and Tosti (2002), Santalla et al. (2002), and Zeven (1997) new introductions and gene exchange between Andean and Mesoamerican germplasm could have played a major role in the evolution of additional diversification centers for this species. Due to the long tradition of common bean cultivation in Slovenia, it could be hypothesized that a set of original genotypes of Andean origin has undergone an adaptive evolutionary process, resulting in today's new variations in this region.

While historical information provides little evidence about the origin and introduction of common bean in Europe, the narrow variation of the phaseolin protein pattern has allowed studying the dissemination pathways of each type from the domestication areas to the rest of the world (Gepts et al., 1986). It has been shown that landraces and cultivars of various geographic origin

display different frequencies of the phaseolin types. Four patterns, designated as "T," "C," "S" and "B," have been identified among the cultivars of common bean in the Mediterranean area (Gepts and Bliss, 1988; Lioi, 1989). In this study, we identified two variants of "T" and "C" phaseolin patterns, denominated "Ts" and "Cs." This is the first report of such phaseolin variants but for their characterization we need to screen a larger number of different phaseolin types.

Gepts and Bliss (1988) found that 40% of Iberian cultivars had "C" pattern, indicating that most of the Iberian cultivars may have been introduced from Chile, where a high frequency of the "C" phaseolin pattern has also been observed. However, recent studies have revealed a high proportion of the "T" phaseolin pattern among Iberian cultivars (Escribano et al., 1998; Rodino et al., 2003). According to Santalla et al. (2002), the Iberian Peninsula landraces may have been disseminated to other parts of Europe, such as Greece, Cyprus, and Italy, where similar frequencies of "T" and "C" types have been observed (Limongelli et al., 1996; Lioi, 1989). In our study we found a very high proportion of "C" phaseolin

type and its variant "Cs," which place Slovene accessions among accessions typical of the Mediterranean area.

Slovene landraces clustered together in the dendrogram according to their phaseolin type; "C" and "T" types were characteristic of landraces from two sub-groups of Andean Group 1, while "S" type predominated in Mesoamerican Group 2. Similar findings were also reported by Maciel et al. (2003, 2001), who studied the genetic diversity of Brazilian common bean germplasm using biochemical and molecular markers. As suggested by Singh et al. (1991b), the predominance of "C"-type cultivars in the Mediterranean area could be the result of deliberate selection for photoperiod adaptation and a specific consumption category; cultivars grown for their green pods from Europe are mainly coupled to "T" and "C" types. The phaseolin analysis provided new information about the identity of four standard cultivars, which did not cluster in the dendrogram as described in previous studies; 'Aurora' and 'Azufrados', considered to be Mesoamerican standard accessions, clustered with Andean genotypes and had Andean "T" phaseolin, while 'Caballeros' and 'Coscarron Blanco', considered to be Andean standard accessions, grouped with Mesoamerican genotypes and showed Mesoamerican "S" phaseolin type.

Fig. 3. One-dimensional SDS/PAGE of seed proteins of some analysed *Phaseolus* accessions. The phaseolin pattern extends between the two horizontal lines. Type "H" (lane 2: G24404); type "C" (lanes 3 and 11: 'Contender', lanes 15-17: PHA315, PHA642, 'Klemen'); type "Cs" (lanes 12-14: PHA627, PHA639, PHA390); type "T" (lanes 4 and 10: 'Tendergreen', lane 5: PHA418); type "Ts" (lanes 6-9: PHA377, PHA396, PHA363, PHA93); molecular weight marker (lanes 1 and 18: SMO661, Fermentas). An arrow indicates the band that distinguishes type "Ts" from type "T" and type "Cs" from type "C."

In conclusion, the results presented in this study have provided the most detailed picture of genetic variation of Slovene common bean germplasm to date. The knowledge of the genetic structure of Slovene common bean genetic resources provided by this study will have a significant impact on further decisions regarding germplasm bank management, conservation programs and breeding activities at AIS.

Literature Cited

- Balardin, R.S. and J.D. Kelly. 1998. Interaction between *Colletotrichum lindemuthianum* races and gene pool diversity in *Phaseolus vulgaris*. J. Amer. Soc. Hort. Sci. 123:1038–1047.
- Bandelj, D., J. Jakše, and B. Javornik. 2004. Assessment of genetic variability of olive varieties by microsatellite and AFLP markers. Euphytica 136:93–102.
- Broughton, W.J., G. Hernandez, M. Blair, S. Beebe, P. Gepts, and J. Vanderleyden. 2003. Beans (*Phaseolus* spp.)—Model food legumes. Plant Soil 252:55–128.
- Chacon, M.I., S.B. Pickersgill, and D.G. Debouck. 2005. Domestication patterns in common bean (*Phaseolus vulgaris* L.) and the origin of the Mesoamerican and Andean cultivated races. Theor. Appl. Genet. 110:432–444.
- Debouck, D.G. 1999. Diversity in *Phaseolus* species in relation to the common bean, p. 25–52. In: S.P. Singh (ed.). Common bean improvement in the twenty-first century. Kluwer Academic Publ., Dordrecht, The Netherlands.
- Escribano, M.R., M. Santalla, P.A. Casquero, and A.M. de Ron. 1998. Patterns of genetic diversity in landraces of common bean (*Phaseolus vulgaris* L.) from Galicia. Plant Breeding 117:49–56.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783–791.
- Freyre, R., R. Rios, L. Guzman, D.G. Debouck, and P. Gepts. 1996. Ecogeographic distribution of *Phaseolus* spp (Fabaceae) in Bolivia. Econ. Bot. 50:195–215.
- Gepts, P. and F.A. Bliss. 1988. Dissemination paths 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, and 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. 1998. Origin and evolution of common bean: Past events and recent trends. HortScience 33:1124–1130.
- Haley, S.D., P.N. Miklas, L. Afanador, and J.D. Kelly. 1994. Random amplified polymorphic DNA (RAPD) marker variability between and within gene pools of common bean. J. Amer. Soc. Hort. Sci. 119:122–125.
- Islam, F.M.A., K.E. Basford, C. Jara, R.J. Redden, and S. Beebe. 2002. Seed compositional and disease resistance differences among gene pools in cultivated common bean. Genet. Resources Crop Evol. 49:285–293.
- Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. Bul. Société Vaudoise des Sciences Naturelle 44:223–270.
- Johns, M.A., P.W. Skroch, J. Nienhuis, P. Hinrichsen, G. Bascur, and C. Munoz-Schick. 1997. Gene pool classification of common bean landraces from Chile based on RAPD and morphological data. Crop Sci. 37:605–613.
- Koenig, R. and P. Gepts. 1989. Allozyme diversity in wild *Phaseolus vulgaris*—Further evidence for two major centers of genetic diversity. Theor. Appl. Genet. 78:809–817.
- Kump, B. and B. Javornik. 1996. Evaluation of genetic variability among common buckwheat (*Fagopyrum esculentum* Moench) populations by RAPD markers. Plant Sci. 114:149–158.
- Limongelli, G., G. Laghetti, P. Perrino, and A.R. Piergiovanni. 1996. Variation of seed storage proteins in landraces of common bean (*Phaseolus vulgaris* L.) from Basilicata, southern Italy. Euphytica 92:393–399.
- Lioi, L. 1989. Geographical variation of phaseolin patterns in an old world collection of *Phaseolus vulgaris*. Seed Sci. Technol. 17:317–324.
- Ma, Y. and F.A. Bliss. 1978. Seed proteins of common bean. Crop Sci. 18:431–437.
- Maciel, F.L., L.T.S. Gerald, and S. Echeverrigaray. 2001. Random amplified polymorphic DNA (RAPD) markers variability among cultivars and landraces of common beans (*Phaseolus vulgaris* L.) of south-Brazil. Euphytica 120:257–263.
- Maciel, F.L., S. Echeverrigaray, L.T.S. Gerald, and F.G. Graziotin. 2003. Genetic relationships and diversity among Brazilian cultivars and landraces of common beans (*Phaseolus vulgaris* L.) revealed by AFLP markers. Genet. Resour. Crop Evol. 50:887–893.
- Mantel, N.A. 1967. The detection of disease clustering and a generalized regression approach. Cancer Res. 27: 209–220.
- Meglić, V., M.A. Johns, and J. Sustar-Vozlic. 1999. Genetic diversity in a Slovenian bean germplasm collection as related to the Andean *Phaseolus* gene pool, p. 28. In: World Seed Conf. 1999: 6–8 Sept. 1999, Cambridge, U.K.: ISTA 75th anniversary: A unique event: Programme and abstracts. Zürich.
- Negri, V. and N. Tosti. 2002. *Phaseolus* genetic diversity maintained on-farm in central Italy. Genet. Resources Crop Evol. 49:511–520.
- Ocampo, C.H., J.P. Martin, M.D. Sanchez-Yelamo, J.M. Ortiz, and O. Toro. 2005. Tracing the origin of Spanish common bean cultivars using biochemical and molecular markers. Genet. Resources Crop Evol. 52:33–40.
- Pavlicek, B.A., S. Hrdá, and J. Flegr. 1999. FreeTree—Freeware program for construction of phylogenetic trees on the basis of distance data and bootstrap/jackknife analysis of the tree robustness, Application in the RAPD analysis of the genus *Frenkelia*. Folia Biologica 45:97–99.
- Rodino, A.P. and J.J. Drevon. 2004. Migration of a grain legume, *Phaseolus vulgaris*, in Europe, p. 61–71. In: D. Werner, (ed.). Biological resources and migration. Springer-Verlag, Berlin.
- Rodino, A.P., M. Santalla, A.M. De Ron, and S.P. Singh. 2003. A core collection of common bean from the Iberian peninsula. Euphytica 131:165–175.
- Rohlf, F.J. 1998. NTSYS-pc. Numerical taxonomy and multivariate analysis system. Appl. Biostat., New York.
- Santalla, M., A.P. Rodino, and A.M. De Ron. 2002. Allozyme evidence supporting southwestern Europe as a secondary center of genetic diversity for the common bean. Theor. Appl. Genet. 104:934–944.
- Singh, S.P. 1989. Patterns of variation in cultivated common bean (*Phaseolus vulgaris*, Fabaceae). Econ. Bot. 43:39–57.
- Singh, S.P. 1999. Production and utilization, p. 1–24. In: S.P. Singh (ed.). Common bean improvement in the twenty-first century, Kluwer Academic Publ., Dordrecht, The Netherlands.
- Singh, S.P., P. Gepts, and D.G. Debouck. 1991b. Races of common bean (*Phaseolus vulgaris*, Fabaceae). Econ. Bot. 45:379–396.
- Singh, S.P., J.A. Gutierrez, A. Molina, C. Urrea, and P. Gepts. 1991a. Genetic diversity in cultivated common bean: II. Marker-based analysis of morphological and agronomic traits. Crop Sci. 31:23–29.
- Sonnante, G., T. Stockton, R.O. Nodari, V.L.B. Velasquez, and P. Gepts. 1994. Evolution of genetic diversity during the domestication of common bean (*Phaseolus vulgaris* L.). Theor. Appl. Genet. 89:629–635.
- Tohme, J., D.O. Gonzalez, S. Beebe, and M.C. Duque. 1996. AFLP analysis of gene pools of a wild bean core collection. Crop Sci. 36:1375–1384.
- Valvasor, J.V. 1689. Die Ehre des Hertzogthums Crain. Reprinted in 1978, p. 65. Mladinska knjiga, Ljubljana, Slovenija.
- Velasquez, V.L.B. and P. Gepts. 1994. RFLP diversity of common bean (*Phaseolus vulgaris*) in its centers of origin. Genome 37:256–263.
- Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. Vandelee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, and M. Zabeau. 1995. AFLP—A new technique for DNA-fingerprinting. Nucleic Acid Res. 23:4407–4414.
- Zeven, A.C. 1997. The introduction of the common bean (*Phaseolus vulgaris* L.) into western Europe and the phenotypic variation of dry beans collected in the Netherlands in 1946. Euphytica 94:319–328.