

## CHARACTERIZATION AND GENETIC DIVERSITY CHANGES IN THE SLOVENIAN COMMON BEAN, ČEŠNJEVEC LANDRACE

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The common bean has been cultivated in Slovenia for centuries, resulting in the development of numerous landraces that are still grown today. The objectives of this study were to define the genetic background and to estimate genetic diversity changes in the traditional Češnjevec landrace of the Slovenian common bean over the last 50 years of cultivation. Fourteen microsatellite loci were analyzed for the presence, number and size distribution of alleles in 231 individuals, representing 67 common bean accessions, including 19 new and five old accessions of landrace Češnjevec collected in the 1950's and stored at the Agricultural Institute of Slovenia (AIS). In factorial correspondence analysis and UPGMA cluster analysis, Češnjevec clustered apart from both Mesoamerican and Andean gene pools. It is suggested that occasional outcrossing, adaptation to particular environmental conditions and strong selection for consumer preferences for seed types could have played a significant role in the evolution of the additional variation in the common bean in this region. Three alleles present in old Češnjevec accessions were undetected in new Češnjevec accessions. The results presented here provide a firm basis for important and informed decisions concerning further conservation strategy and the breeding program in Slovenia.

**Key words:** Common bean, Češnjevec, gene pool, genetic diversity, microsatellites.

### INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) cultivation has a long tradition in Slovenia; its cultivation was already described in the 17th century (Valvasor, 1689). This resulted in the development of many landraces, which are still cultivated at the present time. Češnjevec, first described in the beginning of the last century as an indeterminate climbing genotype grown for green pods (Zaplotnik, 1952), is one of the most abundant and popular landraces grown in Slovenia. Two types of medium-size seeds have been described for Češnjevec: elliptic round dark red seeds, and round dark red seeds that are yellow-variegated. However, there is great confusion about the identity of the Češnjevec landrace in Slovenia today. Growers often name their varieties Češnjevec although they do not correspond to the original Češnjevec in plant type or in other morphological characters (e.g., size and color of seeds and pods). Numerous true and false Češnjevec accessions are

stored at the Agricultural Institute of Slovenia (AIS), which houses an ex situ collection of 943 common bean and 52 runner bean accessions collected from various parts of Slovenia during the last decade. A set of 50 nonviable bean accessions from the 1950s has been preserved as well. There is a lack of information on the genetic diversity of Slovenian common bean germplasm. Slovenian landraces need to be characterized before effective genetic resource management is begun. Until then it will not be known how narrow the genetic base of common bean germplasm is, and what changes in genetic diversity have occurred in the last 50 years. That period saw changes in cropping systems, unsustainable land use, deforestation and agroecosystem deterioration, leading to the loss of traditional cultivars and landraces (Gao, 2003).

DNA markers provide the most precise tool for measuring genetic relationships, because they are potentially unlimited in number and are not affected by the environment (Maciel et al., 2003). In the com-

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TABLE 1. Common bean accessions used in determining allelic variation at 14 microsatellite loci. 'Češnjevec' accessions were selected from old and new collections according to Zaplotnik (1952)

Name of group	Accessions investigated	Seed shape	Seed ground color	Seed secondary color	Seed pattern	No. of individuals analyzed
New Češnjevec	PHA152	Elliptic round	Dark red	Yellow	Marbled	5
New Češnjevec	PHA153	Elliptic round	Dark red	Yellow	Marbled	5
New Češnjevec	PHA263	Elliptic round	Dark red	Yellow	Marbled	5
New Češnjevec	PHA283	Elliptic round	Dark red	Yellow	Marbled	5
New Češnjevec	PHA430	Elliptic round	Dark red	Yellow	Marbled	5
New Češnjevec	PHA609	Elliptic round	Dark red			5
New Češnjevec	PHA669	Elliptic round	Dark red			5
New Češnjevec	PHA729	Elliptic round	Dark red			5
New Češnjevec	PHA731	Elliptic round	Dark red			5
New Češnjevec	PHA732	Elliptic round	Dark red	Yellow	Marbled	5
New Češnjevec	PHA735	Elliptic round	Dark red	Yellow	Marbled	5
New Češnjevec	PHA739	Elliptic round	Dark red			5
New Češnjevec	PHA740	Elliptic round	Dark red	Yellow	Marbled	5
New Češnjevec	PHA741	Elliptic round	Dark red	Yellow	Marbled	5
New Češnjevec	PHA742	Elliptic round	Dark red	Yellow	Marbled	5
New Češnjevec	PHA750	Elliptic round	Dark red	Yellow	Marbled	5
New Češnjevec	PHA759	Elliptic round	Dark red	Yellow	Marbled	5
New Češnjevec	PHA764	Elliptic round	Dark red	Yellow	Marbled	5
New Češnjevec	PHA954	Elliptic round	Dark red	Yellow	Marbled	5
Old Češnjevec	S17	Elliptical	Dark purple			5
Old Češnjevec	S30	Elliptical	Dark purple			5
Old Češnjevec	S36	Elliptical	Dark purple			5
Old Češnjevec	S47	Elliptical	Dark purple			5
Old Češnjevec	S48	Elliptical	Dark purple			5
New Slovenian bean	PHA15	Elliptical	Grey			5
New Slovenian bean	PHA28	Elliptical	Black			1
New Slovenian bean	PHA29	Elliptical	White	Brown	Spotted	5
New Slovenian bean	PHA65	Elliptical	Brown	Dark brown	Striped	1
New Slovenian bean	PHA192	Elliptical	Brown	Black	Punctate	1
New Slovenian bean	PHA280	Globose	Black			1
New Slovenian bean	PHA301	Elliptical	Brown	Dark brown	Striped	1
New Slovenian bean	PHA363	Elliptical	Brown	Dark brown	Striped	5
New Slovenian bean	PHA374	Globose	Brown			1
New Slovenian bean	PHA413	Globose	Brown	Black	Striped	1
New Slovenian bean	PHA432	Narrowly elliptical	White	Brown	Spotted	1
New Slovenian bean	PHA438	Elliptical	Brown	Dark brown	Striped	5
New Slovenian bean	PHA505	Elliptical	Brown	Dark brown	Striped	1
New Slovenian bean	PHA642	Elliptical	White	Brown	Spotted	1
New Slovenian bean	PHA643	Elliptical	White			1
New Slovenian bean	PHA802	Elliptical	Brown	Dark brown	Striped	1
New Slovenian bean	PHA819	Elliptical	Brown	Dark brown	Striped	1
New Slovenian bean	PHA834	Elliptical	Brown	Dark brown	Striped	1
New Slovenian bean	PHA913	Elliptical	White	Violet	Striped	1
New Slovenian bean	PHA1011	Globose	Brown	Dark brown	Striped	1

TABLE 1 (continued)

Name of group	Accessions investigated	Seed shape	Seed ground color	Seed secondary color	Seed pattern	No. of individuals analyzed
Old Slovenian bean	S1	Elliptical	Brown			5
Old Slovenian bean	S3	Globose	White			1
Old Slovenian bean	S5	Globose	Brown			1
Old Slovenian bean	S6	Narrowly elliptical	White			1
Old Slovenian bean	S7	Narrowly elliptical	White			1
Old Slovenian bean	S10	Elliptical	White			5
Old Slovenian bean	S13	Narrowly elliptical	White			1
Old Slovenian bean	S19	Kidney shaped	Brown			1
Old Slovenian bean	S21	Globose	Grey			1
Old Slovenian bean	S25	Globose	White	Dark brown	Spotted	1
Old Slovenian bean	S26	Elliptical	White	Black	Spotted	5
Old Slovenian bean	S35	Elliptical	Dark red	Dark brown	Striped	1
Old Slovenian bean	S41	Elliptical	White	Black	Spotted	5
Old Slovenian bean	S43	Elliptical	Brown	Black	Striped	5
Old Slovenian bean	S44	Elliptical	Dark red	Dark brown	Striped	5
Old Slovenian bean	S49	Globose	Dark red			1
Mesoamerican outgroup	Michelite					5
Mesoamerican outgroup	PHA7	Elliptical	White			5
Mesoamerican outgroup	PHA316	Elliptical	Brown			5
Andean outgroup	XAN159					5
Andean outgroup	Michigan Dark Red Kidney (MDRK)					5
Andean outgroup	PHA309	Narrowly elliptical	Dark red	White	Marbled	5
Andean outgroup	PHA358	Kidney shaped	Brown			5

mon bean (*Phaseolus vulgaris*), restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs) and amplified fragment length polymorphisms (AFLPs) have been used to identify two gene pools, Mesoamerican and Andean (Velasquez and Gepts, 1994; Haley et al., 1994; Maciel et al., 2001; Tohme et al., 1996). More recently, simple sequence repeats (SSRs) were shown to be a very useful tool in common bean germplasm characterization (Metais et al., 2002; Gaitan-Solis et al., 2002). Due to their high to moderate polymorphism rates, codominance and predominantly single-copy nature, genomic and gene-derived microsatellites have been used effectively in genetic mapping of the common bean (Blair et al., 2003).

In this work we investigated microsatellite polymorphism at 14 loci of 24 old and new Češnjevec accessions stored at AIS. The objectives were to define the genetic background of the Slovenian common bean landrace Češnjevec and to estimate genetic diversity changes within this landrace over the last

50 years. The study should clarify the confusion about the identity of landrace Češnjevec, which has been a constant subject of argument.

## MATERIALS AND METHODS

### PLANT MATERIAL

Accessions were selected on the basis of morphological characteristics and multicrop passport data. A total of 231 individuals representing 67 different common bean accessions were studied (Tab. 1). These accessions included 43 new common bean accessions from the gene bank at AIS; 19 of them were named Češnjevec and corresponded phenotypically to the Češnjevec landrace (New Češnjevec group); 20 accessions were phenotypically diverse and included 14 accessions designated Češnjevec (New Slovenian bean). From the old AIS common bean collection accumulated in the 1950s, 21 accessions were included in the analyses; 5 defined as the

TABLE 2. Microsatellite markers, repeat type, size range and number of alleles for the analyzed microsatellite loci of six studied common bean groups

Designation	Repeat type	Range of allele sizes (bp)						Number of alleles						Total allele number
		N-C	O-C	N-SLO	O-SLO	MA	A	N-C	O-C	N-SLO	O-SLO	MA	A	
ATA2 <sup>1</sup>	(TAA) <sub>20</sub> (GAA) <sub>10</sub>	108–123	108–108	108–126	108–129	117–123	120–126	3 (0)	1 (0)	5 (0)	6 (1)	3 (0)	3 (0)	6
ATA3 <sup>1</sup>	(TAA) <sub>9</sub>	118–130	124–130	121–136	118–130	124–136	130–133	5 (0)	3 (0)	6 (0)	4 (0)	5 (0)	2 (0)	7
ATA4 <sup>1</sup>	(TTA) <sub>8</sub>	128–140	134–137	131–140	131–140	134–143	131–134	5 (1)	2 (0)	4 (0)	4 (0)	3 (1)	2 (0)	6
ATA5 <sup>1</sup>	(TTN) <sub>42</sub> , N=A,G,T	188–191	188–191	188–191	182–191	188–188	188–191	2 (0)	2 (0)	2 (0)	3 (1)	1 (0)	2 (0)	3
ATA6 <sup>1</sup>	(TAT) <sub>19</sub>	134–155	128–137	125–143	128–143	125–143	131–143	5 (1)	3 (0)	5 (0)	5 (0)	6 (0)	4 (0)	8
ATA7 <sup>1</sup>	(ATA) <sub>11</sub>	124–133	127–130	127–133	124–133	127–136	127–133	4 (0)	2 (0)	3 (0)	4 (0)	4 (1)	3 (0)	5
ATA9 <sup>1</sup>	(AAT) <sub>12</sub>	185–194	188–194	182–194	188–194	182–191	185–197	4 (0)	3 (0)	5 (0)	3 (0)	3 (0)	5 (1)	6
ATA10 <sup>1</sup>	(ATT) <sub>11</sub>	103–127	103–106	103–127	100–130	103–127	103–106	3 (0)	2 (0)	4 (1)	5 (3)	2 (0)	2 (0)	7
ATA13 <sup>1</sup>	(TTA) <sub>68</sub>	236–272	248–287	245–281	245–287	263–266	263–272	9 (1)	6 (0)	8 (1)	8 (0)	2 (0)	3 (0)	13
ATA16 <sup>1</sup>	(TAA) <sub>14</sub>	136–139	139–139	139–154	139–157	139–142	139–154	2 (1)	1 (0)	4 (1)	3 (1)	2 (0)	3 (1)	7
GATS9 <sup>1</sup>	(GA) <sub>17</sub>	213–247	223–225	213–253	213–249	213–225	213–225	5 (1)	2 (0)	9 (3)	8 (1)	4 (0)	4 (0)	12
BM170 <sup>2</sup>	(CT) <sub>5</sub> CCTT (CT) <sub>12</sub>	151–177	151–165	153–169	153–177	151–155	163–167	7 (1)	5 (0)	6 (0)	6 (0)	2 (0)	3 (0)	9
BM183 <sup>2</sup>	(TC) <sub>14</sub>	145–169	145–149	147–159	141–167	141–143	130–149	5 (1)	3 (0)	3 (1)	6 (3)	2 (1)	4 (2)	13
BM210 <sup>2</sup>	(CT) <sub>15</sub>	166–170	166–166	166–182	166–182	170–184	166–172	3 (0)	1 (0)	5 (0)	3 (0)	4 (1)	3 (0)	6
Total								62 (7)	36 (0)	69 (7)	68 (10)	43 (4)	43 (4)	108
Number of alleles per primer pair								4.4	2.6	4.9	4.9	3.1	3.1	7.7

Microsatellite marker designations are according to Metais et al. (2002)<sup>1</sup> and Gaitan-Solis et al. (2002)<sup>2</sup>. N-C – New Češnjevec; O-C – Old Češnjevec; N-SLO – New Slovenian bean; O-SLO – Old Slovenian bean; MA – Mesoamerican outgroup; A – Andean outgroup. Numbers in brackets are the numbers of group-specific alleles.

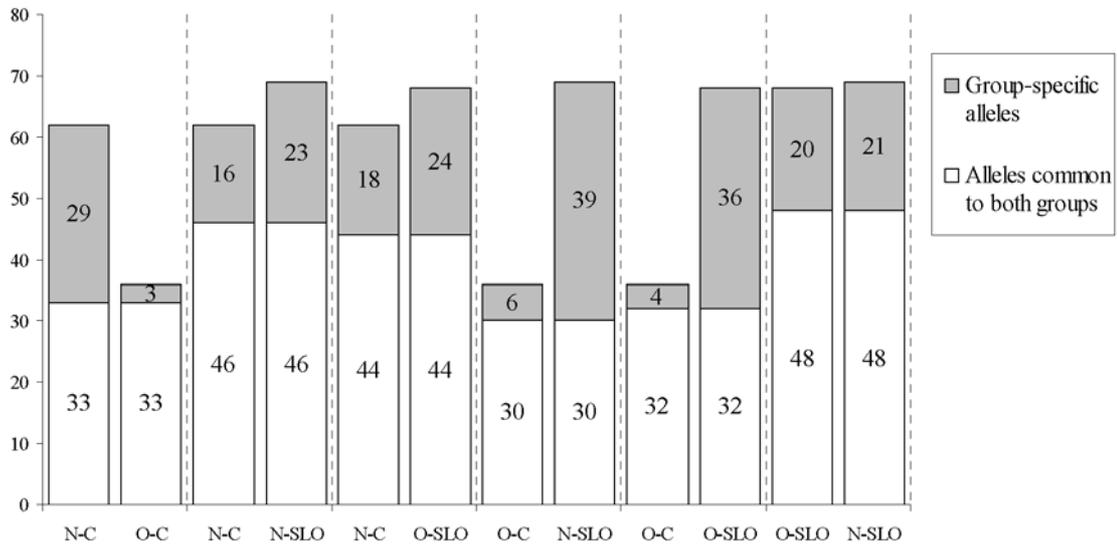
Češnjevec landrace, with dark purple elliptical seeds (Old Češnjevec), and 16 phenotypically diverse accessions (Old Slovenian bean). To investigate the genetic structure of the Češnjevec landrace more comprehensively, each Češnjevec accession was represented by 5 individuals; the majority of the New Slovenian bean and Old Slovenian bean accessions were represented by a single individual.

Three other accessions (Michelite, PHA7, PHA316) were included as outgroups for the Mesoamerican (Mesoamerican outgroup), and four (XAN159, Michigan Dark Red Kidney – MDRK, PHA309, PHA358) for the Andean gene pool (Andean outgroup). Michelite, MDRK and XAN159 were obtained from the Genebank at Gatersleben. The origin of four AIS accessions (PHA7, PHA309,

PHA316, PHA358) was determined in a previous study (Meglič et al., 1999).

#### DNA EXTRACTION

Since the seeds of the accessions from our old collection are nonviable and few, total DNA from these samples was extracted from single seeds with the GenElute Plant Genomic DNA Miniprep Kit (Sigma). Embryos from single seeds were isolated and ground to fine powder in liquid nitrogen. DNA was extracted following the manufacturer's instructions and stored at -20°C. DNA integrity and quality was evaluated by electrophoresis on 0.8% agarose gels. DNA concentration was determined with a Hoefer TKO-100 DNA fluorometer.



**Fig. 1.** Number of group-specific alleles and alleles shared by both groups in some pairwise comparisons. N-C – New Češnjevec; O-C – Old Češnjevec; N-SLO – New Slovenian bean; O-SLO – Old Slovenian bean.

#### MOLECULAR ANALYSIS

Fourteen nuclear microsatellite loci identified by Metais et al. (2002) and Gaitan-Solis et al. (2002) were analyzed (Tab. 2). The forward primer of each primer pair was fluorescence-labelled, and 10  $\mu$ l total volume of the PCR reaction mix contained 0.25  $\mu$ M of each primer, 200  $\mu$ M dNTP, 2 mM MgCl<sub>2</sub>, 1  $\times$  PCR buffer, 0.5 U Taq polymerase (PE Applied Biosystems) and 20 ng genomic DNA. Loci were amplified using the polymerase chain reaction, with a profile of initial denaturation (95°C, 3 min), followed by 30 cycles of strand denaturation (94°C, 30 s), primer annealing (47°C–62°C, 30 s) and DNA extension (72°C, 30 s), in a GeneAmp PCR System 9700 programmable thermocycler. Aliquots of fluorescence-labelled amplified DNA were mixed with formamide and GeneScan 350 Rox Size Standard (Perkin-Elmer) and genotyped on an ABI Prism 310 Genetic Analyzer using GeneScan™ Analysis Software 2.1.

#### DATA ANALYSIS

Microsatellite genetic diversity was quantified as allele frequency and number of alleles per locus, using GENETIX 4.02 software (Belkhir et al., 2001). Genetic differentiation between populations was tested by permutating individuals between samples and by computing  $F_{st}$  for each matrix. In most cases, estimation of  $F_{st}$  is inappropriate for defining where a population or accession limit occurs. Therefore, the extent of population differentiation based on microsatellite allelic frequency and at the same time the population structure were additionally assessed

by factorial correspondence analysis (FCA) using GENETIX 4.02 (Belkhir et al., 2001). The principle of FCA is based on transformation of genetic data into a contingency table (samples  $\times$  alleles), in which each sample is described by the presence/absence of the allele. The  $\chi^2$  distance is used to measure the relatedness between any two samples in the multidimensional space. The resulting factorial axes that optimize the differences between the analyzed individuals are ordered according to their values. The first axis corresponds to the largest value and explains the most general pattern or structure contained in the data set (Guinand, 1996). A UPGMA algorithm in the PHYLIP (Felsenstein, 1993) package, with Nei's standard distance as implemented in MICROSAT (Minch et al., 1997), was used to construct a dendrogram representing the relationships between accessions. Statistical support estimates for major nodes in the dendrogram were obtained with 1000 bootstrap iterations across loci and individuals and are presented as percentages.

#### RESULTS

All microsatellite markers yielded polymorphic fragments in the 67 accessions studied, and 108 alleles (average 7.7 per locus) were identified (Tab. 2). The number of alleles per locus ranged from three (ATA5) to 13 (BM183 and ATA13).

Of the total data set, the New Slovenian bean and Old Slovenian bean groups were the most variable, with an average 4.9 alleles per primer pair

TABLE 3.  $F_{st}$  values, indicating the level of similarity between six analyzed groups of common bean

	N-C	O-C	MA	A	N-SLO	O-SLO
N-C		0.0483**	0.3772***	0.3456***	0.0941***	0.2389***
O-C			0.4981***	0.4691***	0.1805***	0.3337***
MA				0.3466***	0.2766***	0.2495***
A					0.1984***	0.0577**
N-SLO						0.1134***
O-SLO						

N-C – New Češnjevec; O-C – Old Češnjevec; N-SLO – New Slovenian bean; O-SLO – Old Slovenian bean; MA – Mesoamerican outgroup; A – Andean outgroup; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ .

(Tab. 2). In comparing the two Češnjevec groups, 29 alleles were identified in the New Češnjevec that were absent in the Old Češnjevec group (Fig. 1). Three alleles present in the Old Češnjevec at low frequencies (ATA6–128, 0.04; ATA13–284, 0.16; ATA13–287, 0.04) were absent from the New Češnjevec group.

Alleles with high frequencies in Old Češnjevec were present at high frequencies in the New Češnjevec group as well. The difference in  $F_{st}$  values between these two groups was significant (Tab. 3), and was the smallest of all the possible pair-wise comparisons ( $F_{st} = 0.0483$ ;\*\*). The level of similarity between the Old Slovenian bean and Andean accessions was very similar ( $F_{st} = 0.0577$ ;\*\*). On

the other hand, the highest dissimilarity values found, between the Old Češnjevec group and the outgroups, exceeded even those identified between the two outgroups.

An FCA graph (Fig. 2) and a UPGMA dendrogram (Fig. 3) were constructed to describe the relationships between individuals at the primary level and between groups at the secondary level. It is evident that all the accessions clustered in three groups common to both the FCA graph and UPGMA dendrogram. Three major clusters observed in the UPGMA dendrogram were well supported, as indicated by the high bootstrap values. Among the three groups identified in the FCA graph, Group 1 was the smallest and included all three control Mesoamerican accessions, three Old Slovenian bean accessions, and one accession of New Slovenian bean. Group 2 comprised four Andean control accessions, one New Češnjevec accession, eight Old Slovenian bean and six New Slovenian bean accessions. Group 3 was the largest, consisting of 5 Old Češnjevec, 18 New Češnjevec, 13 New Slovenian bean and 5 Old Slovenian bean accessions. All accessions except two (S6, S7) clustered in the same groups in the UPGMA dendrogram and the FCA graph. Of the 24 Češnjevec accessions, 23 clustered together in Group 3 (Fig. 4), indicating high similarity between the old and new Češnjevec accessions; one of them (PHA430) clustered within the Andean gene pool (Group 2).

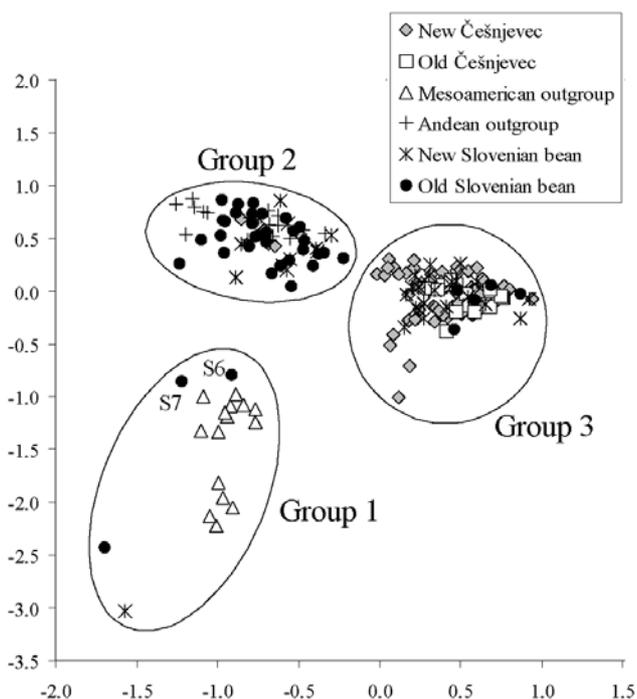
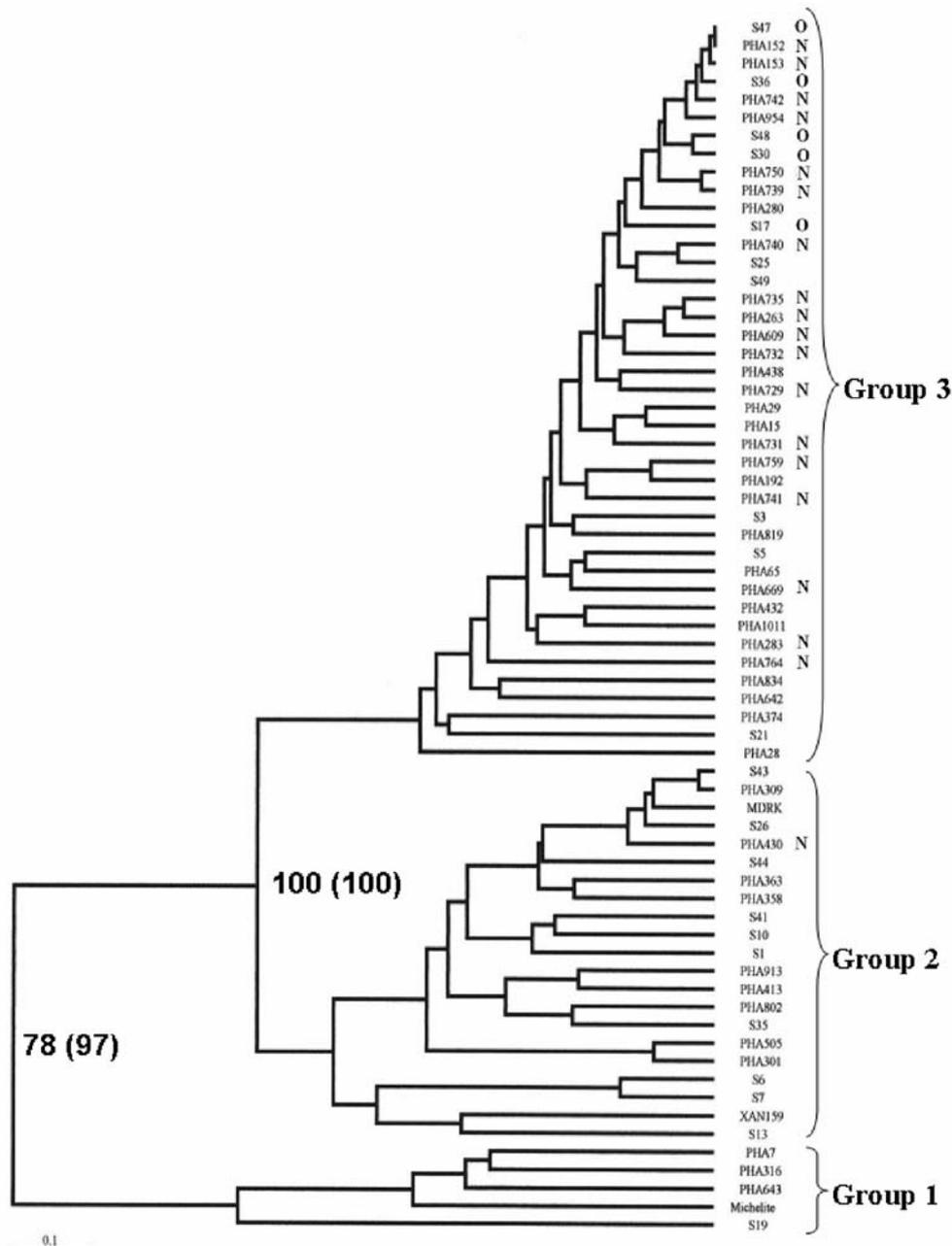


Fig. 2. FCA graph representing relationships between 231 individuals (67 accessions).

## DISCUSSION

The polymorphism of microsatellite loci observed in this study is consistent with previous studies on common bean by Hamann et al. (1995), Yu et al. (1999), Gaitan-Solis et al. (2002) and Metais et al. (2002), who found microsatellite markers to be a useful tool for characterizing common bean genotypes.

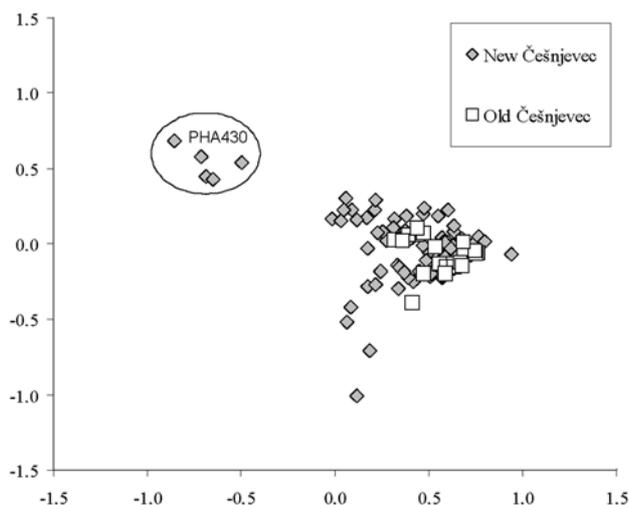
Factorial correspondence analysis and UPGMA cluster analysis clearly discriminated all the studied common bean accessions on the gene pool level.



**Fig. 3.** UPGMA dendrogram describing genetic relationships among 67 accessions of common bean based on analyses of 14 microsatellite loci. N and O designate New Češnjevec and Old Češnjevec accessions, respectively. Numbers at major nodes represent statistical support obtained with 1000 bootstrap iterations across loci and individuals (in parentheses).

This is in agreement with Galvan et al. (2003), who demonstrated the usefulness of microsatellite markers in discriminating Andean and Mesoamerican common bean gene pools. With one exception, (PHA430), all new and old Češnjevec accessions clustered in Group 3, distant from the two major common bean gene pools comprising Group 1 and

Group 2. PHA430, designated Češnjevec, has seed characteristics corresponding to the original description of Češnjevec, but according to growth habit (bush type) and molecular data, it could not be considered one of the Češnjevec genotypes. Landrace Češnjevec raises an interesting questions about the nature of the additional variation described in this



**Fig. 4.** FCA graph describing relationships among 19 (95 individuals) new and 5 (25 individuals) old accessions of landrace Češnjevec.

paper. In a study of genetic diversity of *Phaseolus vulgaris* landraces from central Italy using SSR and ISSR markers, Sicard et al. (2005) found that the most polymorphic locus encodes a pathogenesis-related protein. This suggests that adaptation to heterogeneous environments and interactions with other species, such as parasites, are involved in maintaining the diversity of local bean varieties. Zeven (1997), Santalla et al. (2002), Negri and Tosti (2002) and Sicard et al. (2005) have suggested that occasional outcrossing, different cropping systems and strong selection for consumer preferences for seed types may have played a significant role in the evolution of the additional variation in the common bean introduced into Europe. Thus, Češnjevec could constitute a new germplasm that emerged from recombination between the Mesoamerican and Andean gene pools and/or an evolutionary process that proceeded through centuries of common bean cultivation under specific environmental conditions.

The majority of Old Slovenian bean accessions clustered near the Andean control accessions (Group 2), and only a few near the Mesoamerican control accessions (Group 1). Though phenotypically not corresponding to landrace Češnjevec, about a third of the Old Slovenian bean accessions clustered near Češnjevec accessions into the unique Group 3, indicating that the additional genetic variation observed in this study has already been present for decades in this area and comprises many different phenotypic variants. The New Slovenian bean accessions, including 14 accessions designated Češnjevec though not corresponding to the original description of landrace Češnjevec, clustered similarly. This indi-

cates that the classification of these 14 accessions is incorrect.

In terms of the allelic diversity of the Old Češnjevec and New Češnjevec groups, we identified 33 alleles shared between them, as well as 3 alleles specific to the Old Češnjevec group and 29 alleles to New Češnjevec. The difference in the number of alleles detected was due primarily to the discrepancy in the number of accessions studied in these two groups. Nevertheless, 5 Old Češnjevec accessions (25 individuals) generated more than half of the microsatellite variations generated by 19 New Češnjevec accessions (95 individuals). Presumably this represents only a portion of the genetic diversity of Češnjevec from the 1950s, so the number of different Old Češnjevec alleles is probably underestimated in the present study.

Genetic resources such as the old Češnjevec accessions preserved in the old AIS common bean collection are not suitable for breeding purposes and cultivation, due to the loss of seed viability. This underlines the need to establish an appropriate strategy of genetic resource management, since the range of genetic diversity within a species is critical to its survival and adaptation to changing environments. The key parameter for gene conservation, either in situ or ex situ, is the allelic richness of the population or sample (Gao, 2003). Studies of genetic diversity using molecular marker and DNA sequencing techniques are necessary if we are to understand a population's genetic structure and phylogeography, identify the center of genetic diversity of a species, and develop effective conservation strategies (Gao, 2003). In the present work we detected additional genetic variation in Slovenian bean accessions, and also gathered and processed information about changes in the genetic diversity of traditional Slovenian common bean landrace, information that should prove useful in formulating new strategies for conservation of the genetic resources of native common bean germplasm. Local populations need to be collected, described and efficiently managed, and as much of their original variability as possible needs to be conserved, in order to maintain the adaptive potential of the landraces on which the breeding of new cultivars will be based.

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