UDC 575.630
DOI: 10.2298/GENSR1602729M
Orginal scientific paper

GENETIC DIVERSITY OF COMMON BEAN ACCESSIONS FROM FORMER YUGOSLAV REPUBLIC OF MACEDONIA AS REVEALED BY MOLECULAR AND MORPHOLOGICAL MARKERS

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Maras M., A. Ibusoska, S. Kratovalieva, R. Agić, J. Šuštar-Vozlič, V. Meglič (2016): *Genetic diversity of common bean accessions from Former Yugoslav Republic of Macedonia as revealed by molecular and morphological markers*- Genetika, Vol 48, No. 2, 729-742.

Cultivation of common bean has a long tradition in the Former Yugoslav Republic of Macedonia (FYROM) and is still nowadays important part of the human diet. In a study reported here 71 accessions from the FYROM were assessed for genetic diversity with the aim to provide information on genetic structure of Macedonian common bean germplasm and to depict its peculiarities. A total of 71 accessions were assessed using 13 microsatellite and 16 morphological markers. The average number of alleles per microsatellite was 5.8, and ranged from three to 16 alleles. High capacity of selected markers for distinguishing genotypes was identified by the calculation of a very low value of probability of identity. The relationship among 71 studied accessions was assessed by hierarchical cluster analysis. A very clear separation of accessions into two groups was observed in the UPGMA dendrogram. The larger represented Andean gene pool and contained 40 accessions (56% of total), while the other 31 accessions (44% of total) composed Mesoamerican gene pool. The two groups were successfully discriminated by eight morphological traits. Within the larger Andean cluster in the UPGMA dendrogram a sub-group of 16 climbing accessions was separated from 24 bush accessions. The absence of the string in the pods of the climbers suggests that this sub-group comprises snap beans grown primarily for their fresh pods. There were eight morphological traits in total that distinguished the two Andean sub-groups. Assessment

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of genetic relationship among accessions, their classification into respective gene pool and identification of morphological peculiarities provided valuable information for the management of plant gene bank and Macedonian bean breeding program.

Key words: *Phaseolus vulgaris* L., landraces, diversity, microsatellite markers, morphological traits

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most important edible food legume in the world, representing 50% of grain legumes for direct human consumption. Fresh green beans represent excellent source of cellulose fiber in the diet (BROUGHTON *et al.,* 2003). Common bean is an excellent pre-culture in intensive agricultural production because of the symbiotic relationship with nitrogen-fixing bacteria of the genus *Rhizobium* (SADOWSKY *et al*., 1988).
Separate domestications in the Andes and in Middle America (Mesoamerica) led to two distinct cultivated gene pools in this species (GEPTS and BLISS, 1988). Soon after the discovery of American continent common bean expanded in all parts of Europe and the Mediterranean area where many landraces and varieties evolved that were grown to provide dry seed or fresh pods. Wide variability as revealed by diverse phenotypes and polymorphisms of DNA markers reside in European collections of common bean (ANGIOI *et al*., 2010; LOGOZZO *et al*., 2007; PEREZ-VEGA *et al*., 2009; ŠUŠTAR-VOZLIČ *et al*., 2006).

In the last few decades hundreds of common bean landraces were collected in Balkan Peninsula region and stored in national genebanks. Using microsatellite markers MARAS *et al.* (2015) found extensive genetic variability which was evenly distributed within the territory of former Yugoslavia. Andean genotypes were shown to prevail in collections from Slovenia, Croatia, Bosnia and Herzegovina and Serbia. On the other hand, almost half of the studied accessions from the Former Yugoslav Republic of Macedonia (FYROM) were assigned into Mesoamerican gene pool. Cultivation of common bean has a long tradition in the FYROM and is still nowadays important part of the diet of local communities. Farmers in the rural areas have been growing various landraces for decades (IVANOVSKA and POPSIMONOVA, 2006) which have developed special features due to the action of specific natural conditions and traditional way of cropping system where beans are sown in combination with maize. The FYROM is classified among the countries with higher average seed yield due to favourable conditions for the cultivation of this crop. The most important areas for bean cultivation are: Strumicki, Kumanovski, Prilepski, Poloshki and area of Strushko-Ohridski. Common bean types with different seed shapes grown in FYROM occupy an important segment of the diversity of agricultural crops (IVANOVSKA *et al*., 2013). The most common colour of the bean grain is white and the most popular variety is “Tetovski grav” with white coloured kidney shape seeds and low percentage of the shell. Cultivated on the area of 5000 hectares this cultivar gives an average yield of 1,500 to 2,000 kg/ha, and up to 2,500 kg per hectare in optimal conditions.

As shown by MARAS *et al.* (2015) genetic distinctness of Macedonian common bean might be ascribed to the gene flow with surrounding countries, i.e. Bulgaria and Greece, where high ratio of Mesoamerican genotypes in cultivation of common bean has been reported (LIOI, 1989; SVETLEVA *et al*., 2006). In a complementary study reported here 43 additional accessions from the FYROM were assessed for genetic diversity with the aim to provide additional information on genetic structure of Macedonian common bean germplasm and to depict its peculiarities. A total of 71 accessions were assessed using microsatellite or SSR markers which

are known for their polymorphism, wide distribution in plant genomes, co-dominant nature (POWELL *et al*., 1996; YU *et al*., 1999; PIPAN *et al.*, 2013) and were shown to be efficient in (1) distinguishing bean genotypes and (2) their classification according to the gene pool of origin (BLAIR *et al*., 2006, 2009; MARAS *et al.*, 2006, 2013, 2015). In addition, all accessions were screened for 16 morphological traits in order to identify morphotypes that are efficient in discerning among Macedonian common bean landraces.

MATERIALS AND METHODS

Plant material

A total of 71 common bean accessions from the national gene bank of the Former Yugoslav Republic of Macedonia were used in this study (Table 1). Out of these 71 accessions, 28 have already been assessed for genetic diversity in our previous study (MARAS *et al.,* 2015) and were used here as a reference material for determination of gene pool of origin of the remaining 43 accessions (passport data of the accessions are available upon request).

Table 1. List of 71 accessions of P. vulgaris from the FYROM used in the current study. A set of 43 accessions assessed for SSR polymorphism for the first time are written out in bold fonts. Gene pool of origin of 28 accessions as determined by MARAS et al. (2015) is reported in parentheses. MA = Mesoamerican gene pool; A = Andean gene pool

S.No.	Accession	S.No.	Accession	S.No.	Accession	S.No.	Accession
1	GN-1 (A)	21	**GN-32**	41	**GV-17**	61	**GV-43**
2	GN-2 (A)	22	GN-34 (A)	42	**GV-18**	62	**GV-44**
3	GN-3 (A)	23	GN-35 (MA)	43	GV-19 (MA)	63	**GV-45**
4	**GN-5**	24	**GN-36**	44	GV-20 (MA)	64	**GV-46**
5	GN-6 (A)	25	**GN-37**	45	GV-21 (A)	65	**GV-47**
6	**GN-8**	26	**GN-38**	46	GV-22 (MA)	66	**GV-48**
7	GN-9 (A)	27	GN-41 (MA)	47	**GV-23**	67	**GV-49**
8	**GN-11**	28	GV-1 (A)	48	**GV-24**	68	GV-50 (MA)
9	**GN-14**	29	**GV-2**	49	**GV-25**	69	**GV-51**
10	**GN-16**	30	GV-4 (MA)	50	GV-27 (MA)	70	**GV-52**
11	GN-17 (A)	31	GV-5 (MA)	51	GV-28 (MA)	71	**GV-54**
12	**GN-18**	32	**GV-6**	52	**GV-29**		
13	GN-19 (MA)	33	GV-7 (MA)	53	GV-31 (MA)		
14	**GN-20**	34	**GV-8**	54	**GV-33**		
15	GN-22 (A)	35	GV-10 (MA)	55	GV-34 (MA)		
16	**GN-23**	36	**GV-12**	56	**GV-35**		
17	GN-26 (A)	37	GV-13 (A)	57	**GV-36**		
18	GN-28 (A)	38	**GV-14**	58	**GV-37**		
19	**GN-29**	39	**GV-15**	59	**GV-38**		
20	**GN-31**	40	**GV-16**	60	**GV-42**		

Molecular analyses

DNA extraction

The total DNA was extracted from bulked leaf material of 10 plants of each accession using BioSprint15 DNA Plant Kit (Qiagen, Germantown, MD) and MagMax Express Magnetic Particle Processor (Life Technologies, Grand Island, NY) following manufacturer's instructions. Integrity and quality of DNA was evaluated by electrophoresis on 1.0 % agarose gels. Concentrations of DNA samples were determined with a DyNA Quant 200 Fluorometer (Hoefer, Holliston, MA).

SSR analysis

Thirteen SSR loci developed by METAIS *et al.* (2002) and GAITAN-SOLIS *et al.* (2002) were used in this study (Table 2). Amplification reactions were performed with Veriti Thermal Cycler (Life Technologies) in a 10-µl reaction mixture. Each reaction contained 1 x PCR buffer, 2 mM $MgCl_2$, 200 µM dNTPs, 0.25 µM unlabeled right primer, 0.25 µM labeled left primer, 0.5 U of *Taq* DNA Polymerase (Biotools, Madrid, Spain) and 20 ng of genomic DNA. Loci were amplified using a profile of initial denaturation at 95 °C for 3 min, followed by 30 cycles of strand denaturation at 94 °C for 30 s, primer annealing at 47 °C to 62 °C for 30 s, DNA extension at 72 °C for 30 s, and final extension at 72 °C for 4 min. Fluorescently labeled PCR products were mixed with formamide and internal size standard GeneScan350 ROX (Life Technologies) and genotyped on the 3130xL Genetic Analyzer (Life Technologies).

Data analyses

Alleles of different sizes were scored for each SSR marker. Observed number of alleles (Na), expected heterozygosity (He), polymorphic information content (PIC), and probability of identity (PI) were calculated in Identity 1.0 (WAGNER and SEFC, 1999) and Microsatellite-Toolkit (PARK, 2001). GenAlEx 6.1 (PEAKALL and SMOUSE, 2006) was used in estimation of Shannon's information index (I).

Populations 1.2.28 software (LANGELLA, 2002) was used in computation of Nei's standard genetic distance–Ds (NEI, 1972) from allele frequencies and construction of UPGMA (unweighted pair group method with arithmetic mean) dendrogram in cluster analysis.

Structure 2.3.3 software (PRITCHARD *et al.*, 2009) was employed for inferring population structure using Bayesian approach. Ten independent runs for each *K* (from 1 to 9) in the case of no admixture model were performed and burning period of 10,000 followed by 100,000 Markov Chain Monte Carlo (MCMC) repeats were used. The ideal *K*-value was selected based on the increases in likelihood ratios between runs using Evanno's delta *K* statistic (EVANNO *et al*., 2005) implemented in Structure Harvester (EARL and VON HOLDT, 2011).

Morphological characterization

All 71 accessions were screened for 16 morphological traits. These included 10 qualitative and six quantitative traits (Table 3) from different descriptor lists for *P. vulgaris*. For the purposes of morphological characterization a field trial was carried out on experimental plots of Institute of Agriculture in Skopje by random complete block design during 2008.

Following hierarchical cluster analysis based on SSR data the established groups in the dendrogram were compared for differentiation for every single trait using Chi-square statistics (qualitative traits) or ANOVA (quantitative traits).

Table 2. List of SSR markers assessed in the present study with size and number of alleles (Na) and calculations of expected heterozygosity (He), polymorphic information content (PIC), Shannon's information index (I), and probability of identity (PI)

Locus	Amplification range	Na	He	PIC	I	PI
ATA2[z]	108-120	5	0.62	0.55	1.15	0.208
ATA3[z]	121-130	3	0.51	0.45	0.87	0.300
ATA4[z]	131-143	5	0.70	0.64	1.32	0.148
ATA5[z]	185-194	3	0.43	0.35	0.66	0.410
ATA6[z]	122-143	7	0.80	0.77	1.72	0.070
ATA7[z]	127-136	4	0.68	0.63	1.26	0.152
ATA9[z]	182-200	4	0.65	0.59	1.19	0.179
ATA10[z]	91-130	6	0.70	0.65	1.38	0.139
ATA16[z]	142-172	5	0.58	0.49	1.00	0.270
GATS91[y]	215-275	16	0.91	0.90	2.54	0.016
BM170[y]	154-179	6	0.57	0.48	1.01	0.277
BM183[y]	143-163	5	0.61	0.55	1.16	0.208
BM210[y]	166-182	6	0.73	0.68	1.42	0.125
Total		75				3.0×10^{-11}
Average		5.77	0.65	0.59	1.28	0.192

[z]SSR markers developed by METAIS *et al*. (2002). [y]SSR markers developed by GAITAN-SOLIS *et al*. (2002).

Table 3. Morphological attributes recorded on 71 common bean genotypes. [1]characteristic of Mesoamerican genotypes (SINGH et al., 1991); [2]characteristic of Andean genotypes (SINGH et al., 1991)

No.	Plant organ	Trait	Variants of the trait
1.	Stem	Growth type	1-determinate bush, 2-indeterminate upright bush, 3-indeterminate prostrate non-climbing and semi-climbing bush, 4-indeterminate climbing
2.	Leaf	Shape	1-cordate[1], 2-ovate[1], 3-rhombohedric[2], 4-hastate[2]
3.	Leaf	Width	Average width of three center trifoliate leaves
4.	Leaf	Length	Average length of three center trifoliate leaves
5.	Leaf	Leaf hairs	1-sparse, short[1], 2-dense, long[2]
6.	Flower	Bracteole size	1-small, 2-medium, 3-large
7.	Pod	Colour of fresh pod	1-green, 2-yellow, 3-green with purple stripes, 4-yellow with purple stripes
8.	Pod	Presence of string	1-absent, 5-present
9.	Pod	Length	Average of 10 pods
10.	Seed	Length	Average of 10 seeds
11.	Seed	Width	Average of 10 seeds
12.	Seed	Shape	1-globularis/round, 2-elliptical, 3-cuboid, 4-kidney shaped, 5-truncated
13.	Seed	Mass	Weight of 100 seeds
14.	Seed	Character of seed pattern	1-marbled, 2-punctate, 3-striped, 4-spotted 5-no pattern
15.	Seed	Seed ground colour	1-white, 2-grey, 3-yellow, 4-brown-yellow, 5-brown, 6-red, 7-violet, 8-black
16.	Seed	Seed secondary colour	1-white, 2-grey, 3-yellow, 4-brown-yellow, 5-brown, 6-red, 7-violet, 8-black

RESULTS

A total of 75 alleles were scored across the full set of accessions. The average number of alleles per SSR was 5.8, and ranged from three alleles for ATA3 and ATA5 to 16 alleles for GATS91 (Table 2). The latter also generated the highest values of expected heterozygosity (0.91), polymorphic information content (0.90) and Shannon's information index (2.54). High capacity of GATS91 for distinguishing genotypes was further evidenced by a very low value of probability of identity (0.016) (Table 2).

Based on collected microsatellite data, the relationship among 71 studied accessions was assessed by hierarchical cluster analysis (Figure 1). A very clear separation of accessions into two large groups could be observed in the UPGMA dendrogram. Based on the classification of 28 Macedonian accessions which served as reference genotypes (see Table 1) we were able to assign other 43 accessions into the respective gene pool of origin, Andean or Mesoamerican.

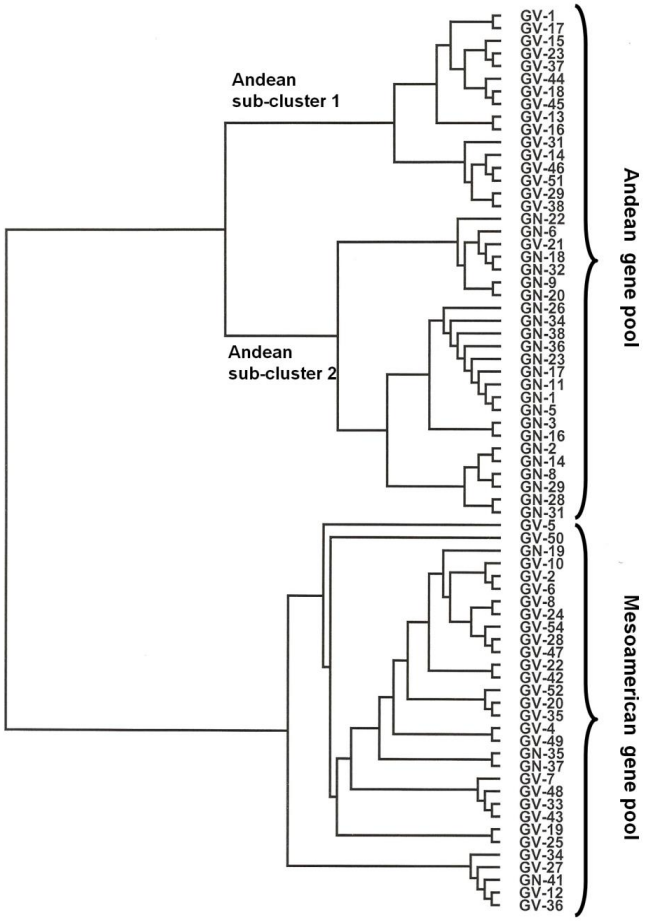

Figure 1. UPGMA dendrogram depicting genetic relationship among 71 Macedonian common bean accessions based on Nei's genetic distance

Andean cluster was the largest and contained 40 accessions (56% of total), including all 14 Macedonian accessions representing Andean references (Figure 1). Mesoamerican cluster consisted of 31 accessions (44% of total) and comprised also 14 Macedonian accessions with known Mesoamerican gene pool of origin.

Table 4. Morphological traits with variants recorded on 71 common bean genotypes classified into two groups, Andean and Mesoamerican gene pools

No.	**Plant organ**	**Trait**	**Most common variant of the trait / range (R) and average (A)**		**Statistical significance of the difference**
			Andean gene pool (N=40)	**Mesoamerican gene pool (N=31)**	
1.	Stem	Growth type	1-determinate bush (58%)	4-indeterminate climbing (87%)	0.0001 ***
2.	Leaf	Shape	1-cordate (38%)	1-cordate (65%)	0.025 *
3.	Leaf	Width	R: 4.8-9.1 cm A: 6.2 cm	R: 5.6-9.5 cm A: 6.8 cm	0.005 **
4.	Leaf	Length	R: 6.6-11.1 cm A: 8.9 cm	R: 7.1-10.3 cm A: 9.0 cm	0.645
5.	Leaf	Leaf hairs	1-sparse, short (90%)	1-sparse, short (87%)	0.722
6.	Flower	Bracteole size	2-medium (63%)	3-large (45%)	0.089
7.	Pod	Colour of fresh pod	1-green (85%)	1-green (90%)	0.620
8.	Pod	Presence of string	5-present (65%)	5-present (81%)	0.188
9.	Pod	Length	R: 7.4-14.3 cm A: 11.0 cm	R: 8.5-17.8 cm A: 11.5 cm	0.204
10.	Seed	Length	R: 1.0-1.9 cm A: 1.4 cm	R: 1.0-2.0 cm A: 1.5 cm	0.680
11.	Seed	Width	R: 0.5-1.0 cm A: 0.8 cm	R: 0.7-1.0 cm A: 0.8 cm	0.029 *
12.	Seed	Shape	4-kidney shaped (50%)	3-cuboid (35%)	0.009 **
13.	Seed	Mass	R: 28.6-73.4 g A: 50.6 g	R: 30.0-62.0 g A: 48.1 g	0.361
14.	Seed	Character of seed pattern	1-marbled (40%)	1-marbled (71%)	0.013 *
15.	Seed	Seed ground colour	1-white (25%) and 5-brown (25%)	1-white (90%)	0.000 ***
16.	Seed	Seed secondary colour	4-brown-yellow (23%)	1-white (55%)	0.000 ***

**Significant at $P < 0.05$; **Significant at $P < 0.01$; ***Significant at $P < 0.001$.

Majority of the accessions (58%) from Andean gene pool were found to form determinate bush growth type (Table 4). On contrary, 87% accessions of Mesoamerican gene pool had indeterminate climbing growth habit. The difference between the two groups as estimated by chi-square statistic was strongly significant for this morphological trait ($P<0.001$) (Table 4). The other qualitative traits that significantly distinguished the two groups were leaf shape and four seed related traits: shape, pattern, ground colour and secondary colour. In general, majority of Mesoamerican genotypes had bright colored seeds while half of the Andean genotypes showed colorful seeds. Length and density of hairs on leaf surface was found inefficient to classify genotypes in the corresponding gene pool, which is not consistent with the observation of SINGH *et al.* (1991) who found this trait to distinguish between the landraces from Andean and Mesoamerican regions. On the other hand, statistically significant difference in the distribution of leaf shape variants was detected between the two gene pools in the present study which is consistent with the reports of SINGH *et al.* (1991). However, the same variants of this trait were present in both groups of genotypes indicating that the trait is as inefficient as leaf hairiness in determination of the origin of the genotypes. Beside qualitative traits, there were also two quantitative traits that discriminated the two gene pools: leaf width and seed width (Table 4).

Within the larger Andean cluster in the UPGMA dendrogram a smaller group of 16 accessions (40% of Andean and 23% of total) was distinguished based on the classification of three Macedonian accessions (GV-1, GV-13, and GV-31) previously found to cluster within genetically distinctive Andean sub-cluster (MARAS *et al.*, 2015). As shown in Table 5 all accessions from the smaller group (Andean sub-cluster 1) were climbers, while the larger group (Andean sub-cluster 2) contained 23 bush accessions and only one climbing accession. Another striking difference between these two groups was presence/absence of the string in the pods (Table 5). The absence of the string in the pods of the majority of accessions from Andean sub-cluster 1 suggests that this group comprises beans especially suitable for growing fresh pods. On the other hand, the accessions from Andean sub-cluster 2 with string in their pods represent beans grown primarily for their dried seeds. The accessions from the two sub-clusters differentiated also in the seed color. The majority of the accessions (71%) from sub-cluster 1 had brighter ground colours ranging from white to brown-yellow, whereas majority of the accessions (81%) from sub-cluster 2 showed darker ground colours ranging from brown to black. Other than these, statistically significant difference between the two Andean sub-clusters was detected for colour of fresh pod, character of seed pattern, and width of both leaf and seed.

Bayesian clustering was employed in Structure software to identify distinct genetic populations and assign accessions to these populations (Figure 2). Delta *K*, an ad hoc statistic that has been recommended to help identify the best-fitting number of populations within a sample (EVANNO *et al.*, 2005), was highest at $K = 3$. The division of Andean and Mesoamerican genotypes was observed at $K = 2$. Compared to hierarchical cluster analysis Structure analysis assigned accessions into identical gene pool with one exception, GV-5, which was classified into different gene pools by the two cluster analyses (Figure 1 and 2).

Table 5. Morphological traits with variants recorded on 40 common bean genotypes classified into two Andean sub-groups

No.	Plant organ	Trait	Most common variant of the trait / range (R) and average (A)		Statistical significance of the difference
			Andean sub-cluster 1 (N=16)	**Andean sub-cluster 2 (N=24)**	
1.	Stem	Growth type	4-indeterminate climbing (100%)	1-determinate bush (96%)	0.0001 ***
2.	Leaf	Shape	3-rhombohedric (38%)	1-cordate (50%)	0.056
3.	Leaf	Width	R: 4.8-7.0 cm A: 5.8 cm	R: 5.3-9.1 cm A: 6.5 cm	0.009 **
4.	Leaf	Length	R: 7.5-10.5 cm A: 9.0 cm	R: 6.6-11.1 cm A: 8.9 cm	0.720
5.	Leaf	Leaf hairs	1-sparse, short (88%)	1-sparse, short (92%)	0.667
6.	Flower	Bracteole size	2-medium (56%)	2-medium (67%)	0.796
7.	Pod	Colour of fresh pod	1-green (63%)	1-green (100%)	0.005**
8.	Pod	Presence of string	1-absent (63%)	5-present (83%)	0.003**
9.	Pod	Length	R: 7.4-14.3 cm A: 11.3 cm	R: 8.4-13.2 cm A: 10.7 cm	0.263
10.	Seed	Length	R: 1.0-1.9 cm A: 1.3 cm	R: 1.0-1.8 cm A: 1.5 cm	0.057
11.	Seed	Width	R: 0.7-0.9 cm A: 0.8 cm	R: 0.5-1.0 cm A: 0.8 cm	0.034 *
12.	Seed	Shape	3-cuboid (31%) and 4-kidney shaped (31%)	4-kidney shaped (63%)	0.131
13.	Seed	Mass	R: 30.2-73.4 g A: 47.2 g	R: 28.6-71.8 g A: 52.8 g	0.164
14.	Seed	Character of seed pattern	2-punctate (31%)	1-marbled (54%)	0.038 *
15.	Seed	Seed ground colour	5-brown (44%)	1-white (29%)	0.007 **
16.	Seed	Seed secondary colour	8-black (50%)	4-brown-yellow (38%)	0.000 ***

**Significant at $P < 0.05$; **Significant at $P < 0.01$; ***Significant at $P < 0.001$.

The existence of two Andean sub-clusters in Macedonian germplasm was identified at $K = 3$ (Figure 2). The results of both cluster analyses were in congruence considering sub-group level with only one discrepancy; accession GN-22 which clustered within Andean sub-cluster 2 in UPGMA dendrogram was assigned in Structure analysis within Andean sub-cluster 1.

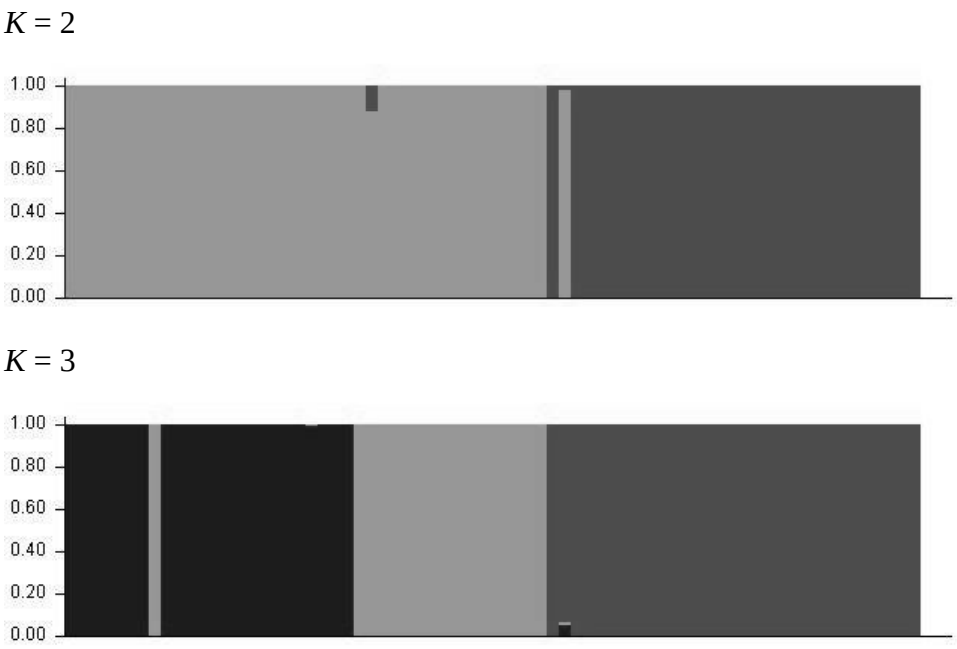

Figure 2. Population structure and membership fraction at $K = 2$ and $K = 3$ for 71 Macedonian common bean accessions based on STRUCTURE analysis. Each accession is represented by a vertical histogram with two colour segments at $K = 2$ (dark gray = Mesoamerican gene pool; pale gray = Andean gene pool) or three colour segments at $K = 3$ (dark gray = Mesoamerican gene pool; pale gray = Andean sub-cluster 1; black = Andean sub-cluster 2) that represent the accession's membership fraction in three clusters

DISCUSSION

In the study reporter here 71 accessions from Former Yugoslav Republic of Macedonia have been assessed for genetic diversity using 13 SSR markers. This set of markers has proven highly efficient in previous studies to discern common bean genotypes and classify them into respective gene pool of origin, Andean or Mesoamerican (MARAS *et al*., 2006, 2013). The investigation reported here is complementary to the previous genetic diversity assessments of common bean from the Western Balkans (MARAS *et al*., 2015; SAVIĆ *et al.*, 2014), which showed that extensive genetic diversity resides in the region and that the majority of the cultivated genotypes originate from Andean domestication centre. A total of 75 SSR alleles or 5.8 per marker were detected in this study which is less than reported earlier by MARAS *et al*. (2013, 2015), who found 10.5 alleles on average in 167 bean accessions from Slovenia and Austria and 9.1 alleles on average in 119 bean accessions from the Western Balkans, respectively. This is not unexpected since fewer accessions covering narrower geographic area (FYROM) were included herein.

MARAS *et al*. (2015) who analysed 28 Macedonian accessions reported that genotypes from Andean and Mesoamerican domestication centres are evenly distributed in FYROM. In the

present study with 71 Macedonian accessions included hierarchical cluster analysis and UPGMA dendrogram revealed a mild prevalence of Andean genotypes (56%) over the Mesoamerican (44%). The occurrence of the former is still lower than in Bosnia and Herzegovina (60%), Croatia (67%), Serbia (63%) and Slovenia (67%) (MARAS *et al*., 2015), while the ratio of the Mesoamerican genotypes is identical to the one observed in Austria (Maras et al., 2013) but still much lower than in Bulgaria (79%) (SVETLEVA *et al.*, 2006). These results suggest that expansion routes leading through the territory of former Yugoslavia were crucial in shaping up the genetic structure of the Macedonian germplasm, but the gene flow from the adjacent Bulgaria might have also played an important role by introducing larger amount of Mesoamerican alleles. Confirmation for the classification of the accessions based on UPGMA dendrogram came from Bayesian cluster analysis. Using no admixture model in Structure software we found all accessions but one (GV-5) in exactly the same cluster at $K = 2$ as in the UPGMA dendrogram. The results for $K = 3$ were also in strong congruence with UPGMA dendrogram showing clear sub-grouping of Andean genotypes into two clusters. Only one accession (GN-22) was observed to deviate between the two cluster analyses at $K = 3$.

With regards to morphological characterization studied accessions showed clear differentiation on the level of gene pools as well as on sub-group level. While Mesoamerican accessions exhibited predominantly climbing growth habit, the Andean group included both bush (N=23) and climbing genotypes (N=17). Interestingly, accessions within the Andean gene pool strictly differentiated according to the type of the growth habit. The larger Andean sub-cluster included 23 bush and only one climbing genotype. The smaller Andean sub-cluster comprised 16 climbing genotypes including three accessions which clustered within Andean sub-cluster in a study reported by MARAS *et al.* (2015). These climbing beans lack strings in their pods making them especially suitable for production of green pods. On the other hand, we have not observed differentiation of accessions based upon the 100 seed mass which is beside phaseolin type one of the paramount markers that discern between Andean and Mesoamerican genotypes (SINGH *et al.*, 1991). It is typical for European genotypes of Mesoamerican origin to show a larger seed size in comparison to Mesoamerican genotypes cultivated in Latin America (LOGOZZO *et al.*, 2007; RODINO *et al*., 2003). It is possible that Mesoamerican races Durango and Jalisco with larger seeds were introduced into Europe or selection for larger seeds might have favoured the introgression of Andean large seed beans into Mesoamerican types (LOGOZZO *et al.*, 2007). Two traits, leaf hairiness and leaf shape, shown by SINGH *et al.* (1991) to discriminate between the two gene pools failed to do so in the present study. Similar weakness of informative morphological markers depicted by SINGH *et al.* (1991) has been reported also elsewhere (BLAIR *et al*., 2007, 2009; ASFAW *et al*., 2009).

The diversity pattern of Macedonian germplasm revealed in this paper largely resembles the structure reported by RAGGI *et al.* (2013) for Italian collection of common bean. Examination of 146 landraces cultivated in different parts of the Apennine Peninsula by morphological, biochemical and SSR markers showed that landrace diversity is structured in three clusters. One cluster included phaseolin S genotypes with prevailing climbing growth habit which corresponds to Mesoamerican cluster identified in this study. The other two clusters contained phaseolin C genotypes with predominantly climbing growth habit mixed together with phaseolin T genotypes with bushy type of growth which resemble to the two Andean sub-clusters identified here. This similarity provided additional evidence that the expansion of

common bean on the territory of the Western Balkans occurred in the past through the western Mediterranean routes.

To sum up, the results of molecular characterization revealed that genotypes of both gene pools, Andean and Mesoamerican, are cultivated in Macedonia but not at exactly the same ratio as indicated in the previous study (MARAS *et al*., 2015). Additionally, morphological characterization showed that Mesoamerican gene pool comprises climbing genotypes with large and bright colored seeds, whereas the Andean gene pool consists of bush genotypes grown for their large and bright colored seeds as well as climbing genotypes grown for their green pods with colourful seeds. The data gathered in this study provided relevant and much needed information for optimal identification of valuable accessions and for the design of appropriate approaches to ex-situ and in situ on-farm management of plant genetic resources.

ACKNOWLEDGEMENTS

This work was financially supported by FP7 Project CropSustaIn, grant agreement FP7-REGPOT-CT2012-316205, by grant No. 168/01 from the SEE-ERA.NET.PLUS FP7 Regional Programme and by grant P4-0072 from the Slovenian Research Agency.

Received August 28th, 2015
Accepted February 16th, 2016

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ISPITIVANJE GENETSKE RAZNOLIKOSTI OBIČNOG GRAHA S PODRUČJA BIVŠE JUGOSLAVENSKE REPUBLIKE MAKEDONIJE POMOČU MOLEKULARNIH I MORFOLOŠKIH MARKERA

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Izvod

Gajenje običnog graha u Bivšoj Jugoslovenskoj Republici Makedoniji ima dugu tradiciju pa tako i danas predstavlja važan dio u humanoj ishrani. U ispitivanje genetske raznolikosti pomoču mikrosatelitnih i morfoloških markera uključeno je 71 akcesija sa područja Makedonije. U molekularnoj analizi utvrđena je visoka kapaciteta 13 mikrosatelita za razdvajanje genotipova graha. Na osnovu sakupljenih molekularnih podataka izrađen je UPGMA dendrogram, gdje su se akcesije razdvojile u dvije grupe. Veča grupa sadržavala je 40 akcesija andskog porjekla, a preostalih 31 akcesija formirala je grupu srednjeameričkog porjekla. Na razini fenotipa ove je dvije grupe razdvojilo osam od ukupno 16 istraženih morfoloških svojstava. Na UPGMA dendrogramu unutar andske grupe uočili smo dvije podgrupe sa 16 odnosno 24 akcesija. Manju grupu obuhvačala je visoke genotipove graha, a veča grupa niske. Odsustvo niti u mahunima kod visokih genotipova prave ih vrlo pogodnim u privredi mahuna. Istraživanje odnosa između akcesija, određivanje njihovog porijekla i identifikacija morfolških osobina pridonijelo je brojne informacije bitne za daljnjeg upravljanje genske banke i programe oplemenjivanja.

Primljeno 28. VIII 2015.
Odobreno 16. II. 2016.