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Original scientific paper

GENETIC DIVERSITY OF COMMON BEAN (*Phaseolus vulgaris* L.) BREEDING COLLECTION IN SERBIA

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The characterization of 41 common bean cultivars and landraces from breeding collection of Institute of Field and Vegetable Crops, Novi Sad, Serbia, was done based on phenotypic traits and microsatellite markers. Phenotypic traits were chosen from Bioversity International descriptor list. In addition, main yield components were investigated. Analysis of phaseolin type revealed affiliation of cultivars and landraces to Mesoamerican or Andean gene pool. Cultivars and landraces demonstrated significant diversity level with regard to studied phenotypic traits. Identified variation showed high potential for developing new cultivars with desirable combination of traits. Principal component analysis based on phenotypic traits separated bean cultivars and landraces in two groups, which corresponded to Mesoamerican and Andean determined according to phaseolin type. Putative hybrids, with combination of traits between gene pools were also identified. Analysis of microsatellite data, using twenty-two SSR primer pairs, showed medium gene diversity in studied material. Microsatellite-based cluster analysis separated genotypes in two discrete clusters and several subclusters. No clear separation according to gene pool was found between the clusters, however grouping according to gene pool and patterns of phenotypic variation, following these gene pools, were observed within subclusters. Knowledge on detailed relationships of cultivars and landraces based on phenotypic and molecular data would facilitate identification of candidates for future breeding.

Keywords: phaseolin, *Phaseolus vulgaris*, phenotypic diversity, molecular diversity

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.), due to its good nutritional value and the possibility for using as both fresh and processed food, is among the most significant legumes in the world (CAROVIĆ-STANKO *et al*., 2017). In the first half of the twentieth century, in former Yugoslavia common bean was usually intercropped with maize. In the last five decades, the production methods changed. Nowadays, common bean production in Serbia is in pure stands. Therefore, the need emerged to expand domestic assortment of bush bean cultivars that would have characteristics of favorite local populations in terms of grain color and size, however with improved traits, especially those concerning seed yield and quality. In the last fifty years, Serbian institutes have bred around 35 common bean cultivars representing several recognized market classes. These cultivars were developed from crosses or by selection of plants with desired characteristics from locally adapted material (VASIĆ, 2004). In order to define future breeding strategy, it is essential to estimate the genetic relationships among the existing cultivars and landraces and to determine the genetic variability in the collection.

Phenotypic characterization of breeding material is the first step in determination of differences among genotypes (KUMAR *et al*., 2009). Both qualitative and quantitative traits are used in common bean germplasm screening, including important agronomical traits. Phenotypic characterization is usually followed by biochemical methods that rely on protein and isozyme electrophoretic patterns, with special interest in main storage protein, phaseolin. Besides assessing genetic diversity, phaseolin represents important protein marker in studies of common bean evolution and geographic origin (PAPA *et al*., 2006). Molecular markers are effective tools for determining genetic variation in cultivated and wild species (LIOI and PIERGIOVANNI, 2013). Simple Sequence Repeats (SSRs) are especially suitable for genetic diversity studies due to their abundance in genome, high reproducibility and codominant nature (RAGGI *et al*., 2013). LIOI *et al*. (2012) and MARAS *et al*. (2015) have used these types of markers in common bean germplasm characterization.

There are two major common bean gene pools, Mesoamerican (from Central America) and Andean (from South America). Cultivars and landraces belonging to each of these two gene pools distinguish by their phenotypic traits, phaseolin type, isozymes, as well as on molecular level (SINGH, 1989; BLAIR *et al*., 2010). There are several phaseolin types, of which the most frequent are S type, representing Mesoamerican gene pool; T and C types representing Andean gene pool (PAPA *et al*., 2006; RAGGI *et al*., 2013). SINGH (1989) and BLAIR *et al*. (2010) observed patterns of phenotypic variation in relation to common bean gene pool affiliation and phaseolin type. ANGIOI *et al.* (2010) implied partial reproductive barrier between bean genotypes belonging to these two differentiated gene pools. Even though viable and fertile progenies could be achieved through inter-gene pool crosses, difficulties may arise in transfer of traits that are quantitatively inherited (GIOIA *et al*., 2013).

The objective of this study was to assess the variability of domestic and foreign common bean cultivars and landraces as well as their genetic relationships based on phenotypic traits, phaseolin type and SSR markers. Knowledge on existing variability should provide information concerning potential value of the tested material for breeding.

MATERIALS AND METHODS

Plant material. The material consisted of 38 common bean cultivars (18 domestic and 20 foreign) and three landraces (Table 1). Tested material was cultivated during two growing seasons 2014 and 2015, at the experimental field of Institute of Field and Vegetable Crops, Novi Sad, Serbia. Trail was set as randomized complete block design. Main plot consisted of three rows, 2 m long, with distance between rows of 60 cm and 5 cm in the row. Phenotypic traits scored according to Key access and utilization descriptors for bean genetic resources, developed by Bioversity International (2009) were: plant growth habit (Type) – I (determinate bush), II (indeterminate bush), III (indeterminate prostrate of vining but not climbing); color of flower standard (BLV) – 1 (white), 3 (lilac), 8 (carmine red), 9 (purple); color of flower wings (BLW) – 1 (white), 3 (lilac), 8 (purple); pod color (PDC) – 7 (normal green), 8 (shiny green), 9 (dull green); seed coat pattern (SPC) – 0 (absent), 1 (mottled), 2 (white with spot around hilum); seed coat color (SCL) – 1 (white), 2 (cream), 4 (brown), 5 (pink), 6 (red), 7 (purple), 8 (black), 9 (golden-yellow), 10 (greenish-yellow); seed brilliance (BRL) – 3 (dull), 5 (medium), 7 (shiny); seed shape (SHP) – 1 (round), 2 (oval), 3 (cuboid), 4 (kidney), 5 (markedly truncated); days to blossom (DM), days to maturity (MAT) and 1000-seed weight (SHKM). In addition, number of pods per plant (NPP), number of seeds per plant (NSP) and seed yield per plant (SYP) were analyzed.

Phaseolin. Isolation and determination of phaseolin was done according to method proposed by GEPTS *et al*. (1986) with SDS-PAGE electrophoresis.

Molecular analyses. Genomic DNA was isolated from bulk of ten plants grown in greenhouse, using CTAB method (DOYLE and DOYLE, 1990). Twenty-two fluorescently labeled microsatellite loci (SSR) from different linkage groups were used for the analysis (YU *et al*., 2000; GAITAN-SOLIS *et al*., 2002; BLAIR *et al*.; 2003; 2008), Table 3. Final PCR (polymerase chain reaction) mix consisted of 30 ng genomic DNA, 0.2 mM dNTP, 2 mM $MgCl_2$, 1x Taq buffer with KCl, 0.1 µg/µl BSA (Bovin Serum Albumin), 1.25 U Taq polymerase and 0.2 µM of each primer pair. PCR amplification was done on GeneAmp PCR System 9700, 96-Well Gold-Plated (Applied Biosystems). Fragment analysis was done in mixture of 2 µl amplified fragments, 7.8 µl Hi-Di formamide (Applied Biosystems) and 0.2 µl GeneScan 500-LIZ Size Standard. Separation of PCR products was done by capillary electrophoresis on Genetic Analyzer Prism 3130, and their sizes were identified with GeneMapper software, version 4.0 (Applied Biosystems).

Data analysis. Shannon's diversity index (SHANNON and WEAVER, 1949) was used in order to determine diversity and discriminative power of qualitative phenotypic traits. Shannon's diversity index was calculated for each trait according to formula:

$$H' = \sum_{i-1}^{n} - P_i \log_2 P_i$$

where Pi is the frequency of the total number of entries (n) in i^{th} class.

For tested quantitative data, main statistical parameters were analyzed: mean, minimum, maximum and coefficient of variation (%). Differences between genotypes were analyzed by employing Fisher's LSD test at the 95% probability level using statistical package XlStat (trial version) (Addinsof, New York, USA; http://www.xlstat.com). Same package was used to perform principal component analysis. Two components were used to visualize distribution of the genotypes in a biplot graph.

Number of alleles (Na), number of effective alleles (Ne), Shannon's information index (I), expected heterozygosity (He) and polymorphic information content (PIC) were calculated for each SSR marker using GenAlEx 6.1 (PEAKALL and SMOUSE, 2006). Nei's standard genetic distance (NEI, 1972) was calculated from allele frequencies using software Populations 1.2.28 (LANGELLA, 2002). The same program was used to construct unweighted pair group method with arithmetic mean (UPGMA) dendrogram in cluster analysis. The UPGMA dendrogram was visualized in TreeView (PAGE, 1996).

RESULTS AND DISCUSSION

The putative origin of tested cultivars and landraces, and their separation in two major gene pools based on phaseolin type was determined (Table 1). Phaseolin S type was observed in 61% of tested cultivars, indicating Mesoamerican origin, while 39% of cultivars and landraces were of Andean origin. SINGH (1989) and TOMLEKOVA (2012) observed higher, more stable yields, better tolerance to abiotic stress (including drought) in Mesoamerican beans comparing to their Andean counterpart, which makes Mesoamerican cultivars more economical for production. Therefore it is not surprising that majority of domestic, as well as foreign cultivars analyzed in this paper were characterized by phaseolin type S. According to RAGGI *et al.* (2013) phaseolin S type prevails among white-seeded genotypes, thus the dominance of Mesoamerican beans among tested material could also be explained with commercial advantage of white-seeded cultivars in Serbia.

Table 1. List of cultivars and landraces, origin, phaseolin type and qualitative phenotypic traits observed in growing seasons 2014 and 2015

Cultivar	Country of origin	Phaseolin type	Type	BLV	BLW	PDC	SPC	SCL	BRL	SHP
Sataja 425	USA	S	II	9	8	8	0	8	5	2
A55	COL	S	II	9	8	8	0	8	5	2
Naya nayahit	USA	S	II	9	8	8	0	8	5	2
Red kidney dark	USA	T	I	3	3	8	0	6	5	4
Rozalija	SRB	T	I	3	3	7	0	5	5	3
Creola	SVK	T	I	3	3	7	1	5	7	3
Žutotrban	SRB	T	I	1	3	7	2	1	7	3
Butmirski trešnjo*	BIH	T	I	3	3	7	1	5	5	2
Jovandeka*	SRB	T	I	3	3	9	1	7	7	3
Vukovarski zlatnožuti*	HRV	T	I	3	3	8	0	9	7	3
Royal Dutch	NLD	T	I	8	3	9	0	2	5	2
Magna	CZE	T	I	1	1	7	0	1	3	2
Vulkan	BGR	S	III	1	1	7	0	1	5	5
Astor	BGR	S	III	1	1	7	0	1	5	2
Prelom	BGR	S	I	1	1	8	0	1	7	2
Dobrudžanski 7	BGR	C	III	1	1	7	0	1	7	2
Laker	USA	S	I	1	1	7	0	1	5	2
Sremac	SRB	T	I	3	3	8	0	10	3	3

Balkan	SRB	S	I	3	3	7	0	1	7	2
Slavonski zeleni	SRB	T	I	3	3	7	0	10	5	3
P-1	SRB	S	II	1	1	7	0	1	7	2
Biser	SRB	S	I	1	1	9	0	1	7	1
Medijana	SRB	S	II	1	1	9	0	1	7	2
C-20	USA	S	II	1	1	8	0	1	3	2
Kutjevački rani	HRV	S	II	1	1	7	0	1	5	2
Seawave	USA	S	I	1	1	7	0	1	5	2
Spinel	USA	S	II	1	1	7	0	1	5	2
Oplenac	SRB	T	I	1	1	7	0	1	5	3
Panonski gradištanac	SRB	C	I	1	1	7	0	1	5	5
Panonski tetovac	SRB	C	I	1	1	7	0	1	7	5
Aster	SRB	S	I	1	1	9	0	1	5	4
Poboljšani gradištanac	SRB	S	III	1	1	8	0	1	5	4
Gerle	BGR	S	I	1	1	7	1	4	5	5
Galeb	SRB	S	I	1	1	7	0	1	5	2
Zlatko	SRB	T	I	3	3	7	0	9	5	3
Dvadesetica	SRB	S	I	1	1	7	0	1	7	4
Belko	SRB	S	I	1	1	7	0	1	5	2
Maksa	SRB	S	I	1	1	7	0	1	5	3
Ludogorje	BGR	S	II	1	1	7	0	1	5	3
Harwood	CAN	S	I	1	1	7	0	1	7	2
Julia	SVK	S	I	1	1	7	0	1	5	3
H’			0.74	0.63	0.73	0.79	0.39	0.56	0.77	0.77
Average			0.67							

* landrace; Type- plant growth habit, BLV- color of flower standard, BLW- color of flower wings, PDC- pod color, SPC- seed coat pattern, SCL- seed coat color, BRL- seed brilliance, SHP – seed shape

Generally, studied cultivars and landraces showed medium phenotypic variation in qualitative traits with average estimated diversity (H’) of 0.67 (Table 1). Majority of the studied genotypes had plant growth Type I, white color of flower standard and flower wings, normal green pod color, oval seed shape and predominantly white seed coat. Plant growth Types II and III were also present, mostly in foreign cultivars of Mesoamerican origin. Pink, golden-yellow and greenish-yellow seed types were observed among domestic cultivars, while black, red, brown and purple seed coat colors were found among foreign cultivars. LIOI *et al.* (2012) and BLAIR *et al.* (2010) recorded heterogeneity in terms of seed color in bean germplasm from Italy and Central Africa, which was in accordance with results obtained in this paper.

Prevalence of white seeded cultivars in Serbian farms could be due to the producers and consumers preference of this seed type (VASIĆ, 2004). On the other hand, presence of cultivars with various seed coat colors indicates changes in farmer’s habits and cultivation of beans for different production purposes. According to GARCIA *et al.* (1997) color of flower standard and flower wings, together with other qualitative phenotypic traits are important for plant breeders since they represent criterion for varietal purity and are easy to assess. RANA *et al.* (2015) stated that dominance of one growth habit type is mainly associated with cropping system and agro-ecological adaptations of the growing region. STOILOVA and BEROVA (2009) and ZEVEN *et al.* (1999) investigating Bulgarian and Dutch bean germplasm found plant growth Type I as most prevalent since bean producers in their countries prefer bush determinate beans, which is also the situation in Serbia. Bean producers consider indeterminate plant growth Types II and III, together with Type I, as bush bean suitable for mechanized harvest, which could explain the presence of these types of cultivars in Serbian bean production and their introduction from other collection for breeding purposes.

Results for the quantitative phenotypic traits are presented in Table 2. Majority of cultivars were medium late. The earliest cultivars with short blossom and maturity stages were Creola and Julia, while Laker distinguished as late. The lowest levels of variation were observed for days to blossom and days to maturity with coefficients of variation of 7.16% and 4.3%, respectively, which was in accordance to the results of CASQUERO *et al.* (2006) and STOILOVA *et al.* (2013). The low recorded variation of these two characters indicates adaptation of studied material to growing conditions in Serbia. Majority of cultivars and one landrace had medium seed weight (250-400 g 1000 grains^{-1}). The second most numerous group were large-seeded cultivars and landraces (>400 g 1000 grains^{-1}), while only ten cultivars had small seeds (<250 g 1000 grains^{-1}). Both domestic and foreign cultivars were dispersed in all three groups. Seed weight, along with seed color and shape, determine commercial class and acceptability of cultivar by farmers (RANA *et al.,* 2015). In previous studies on Serbian common bean germplasm, genotypes belonging to all three groups of seed weight were present, with dominance of medium-seeded white beans, which is in accordance with the results obtained in this paper (VASIĆ, 2004; SAVIĆ *et al*., 2014).

Foreign cultivars Sataja 425, A55 and Naya Nayahit that were of Mesoamerican origin with plant growth Type II scored the highest values for number of pods per plant and number of seeds per plant. On the contrary, the lowest value for these traits was observed in domestic cultivars Žutotrban and Rozalija, which have growth Type I and originate from Andean gene pool. Another domestic cultivar, Pasuljica P-1, which is one of the oldest cultivars, made for grower's preferences at the time, distinguished as the lowest yielding, while the highest yield per plant had Bulgarian cultivars Astor and Dobrudžanski 7, with plant growth Type III. It was observed that cultivars with plant growth habit Types II and III mostly had higher number of pods per plant, number of seeds per plant and seed yield per plant. These findings support the work of SINGH (1989), who stated that cultivars with indeterminate growth habit Types II and III yield better than determinate bush Type I cultivars. Thus, foreign cultivars with bush indeterminate growth plant types could be good candidates for yield enhancing. High levels of variability were observed among bean cultivars and landraces for seed weight, number of pods per plant, number of seeds per plant and seed yield per plant, with coefficients of variation of 32.7%, 41.5%, 50.6% and 36.1%, respectively (Table 2). BITOCHI *et al.* (2012) and RANA *et al.* (2015) found similar diversity levels for analyzed traits for their common bean germplasms. These findings indicate significant phenotypic diversity among tested cultivars and landraces, showing a great potential for developing cultivars with combinations of traits suitable for various market requirements.

Table 2. Quantitative traits measured in two growing seasons 2014 and 2015

Accession name	DB	MAT	SHKM (g)	NPP	NSP	SYP (g)
Sataja 425	53	101	224.1	19.6	61.8	12.8
A55	53	102	225.9	17.3	68.8	14.2
Naya Nayahit	55	100	222.2	20.3	72.1	13.2
Red Kidney Dark	45	99	384.6	6.2	16.7	7.1
Rozalija	43	99	545.2	5.1	12.1	5.7
Creola	43	87	307.5	9.3	35.2	8.2
Žutotrban	45	99	315.7	4.1	12.9	4.7
Butmirski trešnjo	43	94	356.5	5.3	14.7	4.7
Jovandeka	47	99	501.8	7.1	17.4	7.9
Vukovarski zlatnožuti	44	97	576.5	4.9	13.4	7.4
Royal Dutch	45	102	183.6	7.5	18.6	7.4
Magna	44	92	401.3	8.8	25.3	9.2
Vulkan	46	99	586.4	7.1	23.8	11.9
Astor	45	92	303.5	14.6	62.8	15.2
Prelom	53	98	319.1	13.1	49.4	14.2
Dobrudžanski 7	46	98	283.6	14.7	62.9	15.2
Laker	51	107	240.2	12.9	35.8	7.3
Sremac	47	97	482.7	6.1	17.9	8.8
Balkan	46	97	306.1	8.5	26.9	8.1
Slavonski zeleni	46	99	425.9	6.6	18.1	5.9
P-1	48	98	218.5	7.8	25.6	5.4
Biser	45	94	358.4	12.4	37.7	11.2
Medijana	44	99	263.6	10.3	33.3	7.1
C-20	48	103	244.1	18.8	49.5	8.7
Kutjevački rani	46	100	262.3	19	49.9	8.7
Seawave	46	94	208.4	9.5	32.2	6.3
Spinel	52	101	265.5	8.8	22.8	8.8
Oplenac	44	90	422.8	9.2	24	8.9
Panonski gradištanac	45	92	451.1	9.8	28.9	13.2
Panonski tetovac	44	95	533.1	9.9	27.4	13.1
Aster	47	102	455.5	6.8	15.8	6.5
Poboljšani gradištanac	50	99	329.8	10.6	57.8	10.8
Gerle	44	96	407.9	14.2	44.2	17.4
Galeb	45	95	474.1	8.1	23.5	9.2
Zlatko	45	102	483.1	6.1	16.3	7.4
Dvadesetica	43	101	359.8	11.7	37.7	13.4
Belko	43	97	222.7	13.4	47.7	3.3
Maksa	42	95	395.8	6.6	18.9	6.8
Ludogorje	52	99	162.8	11.9	36.9	7.5
Harwood	46	99	333.1	8.7	31.1	7.5
Julia	46	87	286.1	11.5	39.9	10.9
LSD (0.05)	0.9	0.9	41.7	3.7	10.3	3.2
min	42	87	162.8	4.1	12.0	3.3
max	55	107	586.4	20.3	72.1	17.4
$\bar{X}$	46.5	97.5	349.5	10.3	33.4	9.3
CV (%)	7.16	4.3	32.7	41.5	50.6	36.1

DB- days to blossom, MAT- days to maturity, SHKM- 1000 seed weight, NPP- pod per plant, NSP- seeds per plant, SYP- seed yield per plant

The relationships among cultivars and landraces based on 14 qualitative and quantitative phenotypic traits and phaseolin type are presented on PCA biplot (Figure 1). PC1 and PC2 accounted for 30.04% and 20.09% of variation, respectively, explaining together

50.13% of total variation. Traits such as growth habit type, seed weight, phaseolin type, number of pods per plant and number of seeds per plant contributed mostly to PC1, while days to blossom, color of flower standard and flower wings, pod color, days to maturity and seed color contributed to PC2. In addition, PCA biplot showed positive correlations between seed color, color of flower standard and flower wings, phaseolin type, seed weight and seed shape. Positive correlations were also observed between days to blossom, days to maturity, plant growth habit type, number of pods per plant, seeds per plant and seed yield per plant. On the other hand, these two groups of variables were negatively correlated to each other. ZEVEN *et al.* (1999) obtained similar results in studies of Dutch common bean germplasm applying PC analysis. Therefore selection directed towards PC1 would generate cultivars with smaller seeds but with higher values of other yield components and vice versa.

The first coordinate separated bean cultivars and landraces in two groups, which corresponds to the affiliation to gene pool, Mesoamerican or Andean, implying that phenotypic traits chosen for PC analysis were discriminatory enough. In addition, it was possible to identify cultivars and landraces with higher values of number of pods and seeds per plant and seed yield per plant (Mesoamerican) opposed to group with lower values of these traits, but with greater seed weight (Andean). RANA *et al.* (2015) used PC analysis with large number of genotypes from Indian bean collection, in order to determine contribution of analyzed traits to divergence of accessions and germplasm diversity. DURAN *et al.* (2005), in studies of bean landraces from Caribbean distinguished two major groups of accessions, Mesoamerican and Andean, which was in accordance with our results.

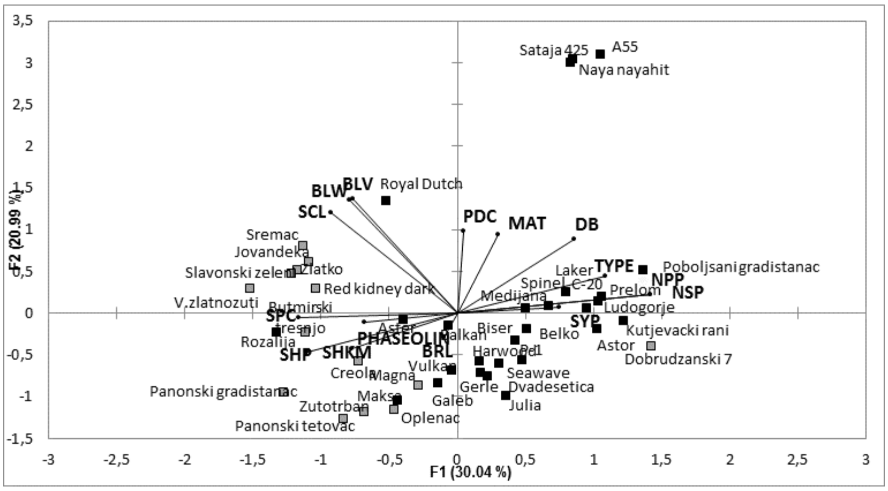

Fig. 1. Principal component analysis (PCA) of 41 studied cultivars and landraces based of phenotypic traits; black – Mesoamerican, grey – Andean gene pool; Type- plant growth habit, BLV- color of flower standard, BLW- color of flower wings, PDC- pod color, SPC- seed coat pattern, SCL- seed coat color, BRL- seed brilliance, SHP – seed shape, DB- days to blossom, MAT- days to maturity, SHKM- 1000 seed weight, NPP- pod per plant, NSP- seeds per plant, SYP- seed yield per plant

Although there was a clear separation of genotypes between gene pools on PCA biplot, cultivars having combinations of traits of both gene pools were identified and had intermediate position on biplot graph. Bulgarian cultivar Dobrudžanski 7 grouped with Mesoamerican beans due to smaller seed weight, higher yield per plant and growth habit Type III. Domestic cultivars Balkan, Galeb, Maksa and Bulgarian cultivar Vulkan clustered with Andean beans due to higher seed weight and intermediate values for other yield components. It could be suggested that these cultivars represent putative hybrids between gene pools, based on phenotypic traits. SANTALLA *et al.* (2002), RODINO *et al.* (2006) and LEITÃO *et al.* (2017) found putative hybrids between Andean and Mesoamerican gene pools in bean accessions from Iberian Peninsula, whereas it is believed that large percentage of European samples could be spontaneous hybrids between the two gene pools (ANGIOI *et al*., 2010). These cultivars, identified as putative hybrids, could be potential parents for future crosses between genotypes originating from different gene pools (ZEVEN *et al.*, 1999). In addition, through gene-pool introgression new combinations of traits may have emerged, such as higher tolerance to biotic and abiotic stress and better nutritional quality (BLAIR *et al*., 2010). GONZALEZ *et al.* (2009) observed signs of introgression between gene pools mostly in cultivars of white seeds, which was the case in this study. In Europe, including Serbia, consumers prefer cultivars with larger seeds (VASIĆ *et al.*, 2001), thus the goal of many breeding programs is to maintain or increase seed size, and many breeders seek to recombine higher yields and other traits of Mesoamerican beans with larger seeds of Andean genotypes (GONZALEZ *et al.*, 2009).

Using 22 polymorphic SSR primers for 41 common bean cultivars and landraces, total number of 93 alleles was identified (Table 3). The number of detected alleles in this paper was higher comparing to analysis of 87 bean landraces from Calabria (57 alleles), MERCATI *et al.*, (2013), but slightly lower than those found in 128 bean accessions from Slovenia (130 alleles), MARAS *et al*., (2006). The highest values of expected heterozygosity (0.79), Shannon's information index (1.72) and PIC (0.77) were scored for locus BM141, which proved to be the most efficient in determining genetic diversity for the tested material. Average value of expected heterozygosity (He) for all analyzed loci was 0.54 with average PIC value of 0.48 (Tab. 4), indicating medium gene diversity in studied material. COELHO *et al*. (2009) recorded similar level of gene diversity in preliminary study of 20 Portuguese accessions (0.57), whereas greater allelic diversity was found in analysis of 87 Calabrian landraces (MERCATI *et al*., 2013). Differences in allelic frequency and gene diversity found in this paper comparing to other studies can be contributed to the choice and number of polymorphic SSR markers used as well as nature of studied material.

The relationships among 41 cultivars and landraces based on SSR data were assessed by cluster analysis. UPGMA dendrogram showed two discrete clusters with subdivision of second cluster in several groups (Figure 2). Group I comprised of mostly Mesoamerican small-seeded cultivars along with one domestic cultivar of Andean origin, Rozalija. Majority of domestic cultivars of Andean origin with colored seed coats and medium to large seeds clustered in group IIa, together with two foreign cultivars. Exceptions of this grouping pattern were domestic white large-seeded cultivar Panonski gradištanac and white medium-seeded cultivar Julia, for which was determined to have Mesoamerican origin. Group IIb, again, included mostly Mesoamerican cultivars, along with one landrace, Jovandeka and cultivar Dobrudžanski 7, which were of

Andean origin. Cultivars in this group were mainly with higher values of yield components and plant growth Types II and III. It was previously determined in PC analysis that Dobrudzanski 7 and Vulkan had a combination of traits of two gene pools and it was expected that they clustered together. Group IIc comprised of only two Bulgarian cultivars, Astor and Gerle.

Table 3. SSR loci used for the analyses of common bean genotypes. Na - number of alleles, Ne -effective number of alleles, I - Shannon's information index, He - expected heterozygosity, PIC - polymorphic information content

Locus	Na	Ne	I	He	PIC
Pvctt001[b]	5	1.83	0.88	0.45	0.41
BMd16[a]	**2**	1.22	0.33	0.18	0.16
BMd33[a]	3	1.31	0.46	0.24	0.22
BM114[c]	**9**	3.95	1.65	0.75	0.72
BMd53[a]	**2**	1.99	0.69	0.49	0.37
BM172[c]	5	4.06	1.45	0.75	0.71
BM199[c]	5	2.17	0.95	0.54	0.46
BM141[c]	7	4.88	1.72	0.79	0.77
BM210[c]	4	1.85	0.86	0.46	0.42
BM154[c]	4	2.31	1.05	0.57	0.52
BM184[c]	3	2.51	1.00	0.60	0.53
BM181[c]	4	2.64	1.10	0.62	0.55
BM205[c]	3	2.86	1.07	0.65	0.58
BMd45[a]	**2**	1.99	0.69	0.50	0.38
BMd37[a]	3	1.54	0.60	0.35	0.31
BM165[c]	8	4.57	1.73	0.78	0.75
ATA2[d]	2	1.75	0.62	0.43	0.34
ATA4[d]	3	1.41	0.54	0.29	0.26
ATA5[d]	4	2.95	1.16	0.66	0.59
ATA6[d]	6	3.34	1.44	0.70	0.66
ATA9[d]	4	3.49	1.30	0.71	0.66
ATA16[d]	5	1.60	0.71	0.37	0.34
Total	93	-	-	-	-
Average	4.23	2.55	1.00	0.54	0.48

References: a- BLAIR *et al*. (2003); b- YU et al. (2000); c- GAITAN-SOLIS *et al*. (2002); d- BLAIR *et al*. (2008)

Four domestic white-seeded cultivars and one landrace, Vukovarski zlatnožuti, clustered in group IId. Characteristics of this group are medium to large seeds and mainly plant growth type I, with both Andean and Mesoamerican genotypes. Group IIe comprised of one domestic, one foreign cultivar and one landrace, without patterns observed on the level of phenotypic traits. Five domestic and four foreign cultivars of mostly Mesoamerican origin were included in Group IIe. Even though there was apparent phenotypic variability in studied traits among cultivars in group IIe, it was shown that they were more similar on DNA level. Distribution of domestic and foreign common bean cultivars in different groups indicated certain level of heterogeneity among cultivars of the same geographic origin. Even though there was no clear division of genotypes in two main groups, according to two recognized gene pools, Mesoamerican and Andean, it can still be observed that cultivars and landraces clustered in smaller groups in relation to gene pool affiliation and familiar patterns of phenotypic variation following these gene pools.

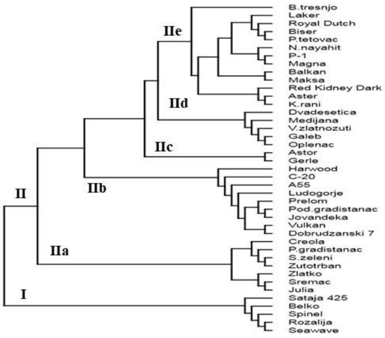

Fig. 2. UPGMA dendrogram explaining genetic relationship among 41 common bean cultivars and landraces

Cultivars from the same cluster are less divergent comparing to cultivars belonging to different groups and their crossing would not provide progeny with enhanced agronomical traits (KUMAR *et al.*, 2009). Quantifying level of genetic diversity of studied cultivars and landraces, and grouping them into smaller homogenous cluster could help breeders to choose suitable candidates for future breeding program (RANA *et al.*, 2015).

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GENETIČKI DIVERZITET KOLEKCIJE PASULJA (*Phaseolus vulgaris* L.) NAMENJENE OPLEMENJIVANJU U SRBIJI

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Izvod

Fenotipska i molekularna procena 41 genotipa (sorte i loklane populacije) oplemenjivačke kolekcije pasulja Instituta za ratarstvo i povrtarstvo iz Novog Sada, urađena je u ovom radu. Ispitivanje fenotipa je vršeno na osnovu osobina odabranih prema međunarodnom Bioversity International deskriptoru za pasulj, uključujući osnovne komponente prinosa. Analizom fazeolina utvđena je pripadnost sorti i lokalnih populacija srednjeameričkom ili andskom centru porekla. Značajan nivo diverziteta u pogledu proučavanih osobina je zabeležen među ispitivanim sortama i lokalnim populacijama. Identifikovana fenotipska varijacija ukazuje na značajan potencijal ispitivanog materijala za oplemenjivanje novih sorti sa poželjnim kombinacijama osobina. Primenom PC analize, genotipovi pasulja su podeljeni u dve grupe, koje su odgovarale srednjeameričkom ili andskom centru porekla, na osnovu fenotipa i tipa fazeolina. Identifikovani su potencijalni hibridi sa kombinacijom osobina karakterističnim za svaki centar porekla pojedinačno. Analizom mikrosatelitnih podataka, primenom 22 SSR para prajmera, u ispitivanom materijalu je utvrđen srednji nivo diverziteta gena. Klaster analizom, na osnovu rezultata dobijenih mikrosatelitnim markerima, ispitivani genotipovi su podeljeni u dve osnovne grupe i nekoliko podgrupa. Jasna podela prema centru porekla nije primećena između grupa, ali je grupisanje prema centru porekla i poznatim obrascima fenotipske varijacije primećeno unutar podgrupa. Poznavanje detaljnih veza između sorti i lokalnih populacija, na fenotipskom i molekularnom nivou, trebalo bi da olakša odabir roditeljskih komponenti za buduće programe oplemenjivanja.

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