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MORPHO-CHEMICAL CHARACTERIZATION OF DRY AND SNAP BEAN (*Phaseolus vulgaris* L.) LANDRACES COLLECTED ON FRUŠKA GORA MT.

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Disappearance of old cultivars, including dry and snap bean (*Phaseolus vulgaris* L.) has been accelerated in last five to six decades, which mainly led to great genetic impoverishment. For all the humanity and its future, particularly is important the maintenance and evaluation of old cultivar's seeds. The research presented in this paper has been conducted on the territory of southwestern Fruška gora Mt. Of the collected samples of field and vegetables crops, as well as wild plants on the mountain, 13 accessions of snap bean and 21 accessions of dry bean have been analyzed in this paper. Seed color, seed shape, 1000-seed mass and phaseolin type was determined for all the accessions. Seeds of collected bean and snap bean accessions were predominantly white and cylindrical in shape. Mass of 1000 seeds ranged between 104,90 g and 634,96 g. T phaseolin type dominated, while S type of phaseolin was present in six bean and in two snap bean accessions.

Key words: landraces, collection, main traits, phaseolin type, *Phaseolus vulgaris*

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INTRODUCTION

Traditional cultivars are important source of genetic variability and adaptability (HAMMER *et al,* 2003), and broad genetic diversity is basis for successful breeding process and adaptation to environmental conditions (VASIĆ *et al*., 2009), as well as pathogens. Disappearance of old cultivars has been dramatically accelerated in last five to six decades (MILOŠEVIĆ *et al.,* 2010). Large areas have been planted with single genotype of newly created cultivars (BOROJEVIĆ, 1981), especially of most cultivated species. Among species that have not been

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done much on their breeding, old cultivars and landraces have been preserved. Collecting them should not be neglected and omitted (VASIĆ *et al.,* 2009; GVOZDANOVIĆ-VARGA *et al*., 2013).

Due to its rich nutritional value dry and snap bean (*Phaseolus vulgaris* L.) take important place in human diet. They are suitable for organic farming system and their cultivation areas are in increase in organic production (VASIĆ *et al*., 2011). In the process of plant breeding the emphasis is on the increment of the cultivated species yield, including dry and snap bean, while it haven't been paid much attention on preserving the nutritional quality of food (KELLY *et al.,* 1998). With both dry and snap bean, there was a cessation of growing old landraces, especially by introducing into production cultivars made by modern principles of genetics (VASIĆ, 2004).

Due to the position of Balkan Peninsula, presence of different cultures, soil and climatic conditions, as well as the roads intersections, there have been a significant divergence of *Phaseolus vulgaris* L. in this region (VASIĆ, 2004). Of particular interest are domestic dry and snap bean landraces, which could be found in home gardens and on smaller farms (TODOROVIĆ *et al*., 2012). These populations have continued its evolution in their traditional surroundings, where they have been grown in traditional way, without selection pressure of man. Thus they represent exquisite source of genetic variability. This is especially important from the aspect of biodiversity preservation. They also represent valuable genetic potential for breeding for desirable culinary properties, as well as for creating varieties of high nutritive quality (MILOŠEVIĆ *et al.,* 2012). From the reason of biodiversity preservation, prevention of genetic erosion, food security and providing resources for plant breeding, including organic breeding (ZDRAVKOVIĆ *et al*., 2010) collection, preservation and investigation of local landraces and cultivars have been organized (TODOROVIĆ *et al*, 2011).

Two main centers of origin of species *Phaseolus vulgaris* L. are Central America and Andes. Belonging to one of these centers indicates the landraces suitability to certain climatic conditions (GEPTS *et al.,* 1986). *Phaseolus vulgaris* L. was brought to Europe form America. It arrived on Balkan Peninsula from two directions: from south – from Turkey and north – from France and Italy (VASIĆ, 2004). Reserve proteins, like phaseolin, are reliable markers in studies of domestication and dispersion of bean varieties, and in analysis of phytogene relationship between species inside *Phaseolus* genus (NIKOLIĆ *et al.,* 2007).

The aim of this work was morpho-chemical characterization of collected dry and snap bean landraces from several localities from Fruška gora Mt. Collected accessions present important genetic potential for future challenges breeders are going to face, including climate changes and drought.

MATERIALS AND METHODS

Landraces of dry and snap bean, collected from several villages on southwestern part of Fruška gora Mt., were investigated. In total, 30 dry bean (Table 1, from 5 to 22) and snap bean (Table 1, from 23 to 34) landraces were collected. For each collected accession, full documentation on where the seed is from, who was the donor, how to plant and use it, as well as short description and photographs were made (MILOŠEVIĆ *et al*., 2012). Accessions were compared with four dry bean cultivars, Dvadesetica, Balkan, Zlatko and Sremac, created at the Institute of Filed and Vegetable crops in Novi Sad (IFVCNS) (Table 1).

Table 1. Collection number, common name, origin and habitus of collected dry bean (white area) and snap bean (shaded area) accessions

	Accession name	Common name	Origin	Habitus
1	Dvadesetica		Institute of Field	d
2	Balkan	Control Cultivars	and Vegetable	d
3	Zlatko		Crops, Novi Sad	d
4	Sremac		(IFVCNS)	d
5	NK1/12	yellow bean	Pavlovci	d
6	NK10/12	domestic white bean	Mali Radinci	d-i
7	NK11/12	domestic mottled bean	Mali Radinci	d
8	NK12/12	lemonade	Mali Radinci	d-i
9	NK14/12	greenish bean	Stejanovci	d
10	NK29/12	mottled bean	Stejanovci	d
11	NK65/12	white cylindrical	Erdevik	d-i
12	NK66/12	white subcomprssus	Erdevik	d-i
13	NK67/12	greenish bean	Erdevik	d
14	NK75/12	greenish-yellow bean	Erdevik	d
15	NK76/12	soybean-like bean	Erdevik	d
16	NK83/12	white bean	Ljuba	d
17	NK103/12	American bush bean	Ljuba	d
18	NK108/12	mottled bean	Sremska Mitrovica	d
19	NK109/12	round lemonade bean	Sremska Mitrovica	d
20	NK114/12A	domestic bean	Deronje	d
21	NK114/12B	domestic bean	Deronje	d
22	NK34/12	mottled bean	Stejanovci	d
23	NK2/12	butter snap bean	Pavlovci	i
24	NK4/12	short pencil snap bean	Pavlovci	d
25	NK25/12	spotted snap bean	Stejanovci	i
26	NK38/12	patterned snap bean	Stejanovci	d-i
27	NK39/12	pencil snap bean	Stejanovci	d
28	NK40/12	yellow spotted snap bean	Stejanovci	i
29	NK52/12	bush green snap bean	Stejanovci	d
30	NK53/12	bush patterned snap bean	Stejanovci	d
31	NK54/12	bush yellow snap bean	Stejanovci	d
32	NK62/12	yellow pencil snap bean	Erdevik	d
33	NK71/12	green pencil snap bean	Erdevik	d
34	NK116/12	patterned snap bean	Deronje	d

Main market characteristics of seed were assessed – 1000-seed mass, seed color, seed shape and phaseolin type.

Seed color was determined visually based on FAO/IPGRI Multi-Crop Descriptors (ALERCIA *et al*., 2001). Seed weight of dry and snap bean was determined based on 1000-seed

mass or absolute mass (ISTA RULES, 2013). Seed shape was calculated from the ratio of: (i) seed length and seed width, and (ii) seed thickness and seed width, according to the method of DEKAPLEREVIĆ (after KOJNOV, 1973). Sample for measuring seed dimensions consisted of 40 seeds of each accession. 1D-SDS PAGE electrophoresis of phaseolin was done according to RODINO *et al* (2001).

Values for each of three seed dimensions (length, width, thickness) for each accession were statistically processed with statistical package Statistica ver. 12 using analysis of variance (ANOVA) and Student Newman Keuls (S-N-K) test for the significance level of 5%.

RESULTS AND DISCUSSION

Local dry and snap bean landraces suggest eating habits of local population. Traits that indicate these habits are main market characteristics, which include 1000-seed mass, seed color and shape. Our work was based on investigating these traits.

Many authors impart (ROSIĆ, 1970; MITRANOV, 1981) that most common in the region of Balkan Peninsula is dry bean of white seed, but greenish-yellow and greenish beans are also present in high number. Our results are in accordance with these findings, even though mottled dry and snap beans are present among our collected accessions. Presence of dry and snap beans of different colors and shapes in Fruška gora Mt., especially mottled ones is determined by migrations of populations that came here from different part of former Yugoslavia (VASIĆ *et al*., 2009). During expeditions in villages of Fruška gora Mt. it was observed that each family, samples were obtained from; grow all three types of beans – white, green and mottled, which do not differ much between different villages. It was also observed that there exists exchange of seeds between the villages, especially mottled ones, then greenish-yellow and greenish beans. As for the white dry and snap beans collected on these localities, there are many types which are different in shape, habit, weight and height, much more than green and mottled beans. The most numerous collected snap bean accessions are ones with white seed, but brown and mottled accessions could also been found.

Seed weight is important quality trait, as well as main yield component and market characteristic (VASIĆ *et al.,* 2010; KELLY *et al.,* 1998). Among our accessions, it ranged from around 100 g to over 630 g, which suggest that there is important difference between them (Table 2). Averaged 1000-seed mass of bean accessions was 379, 44 g. Snap bean had smaller seed with 247, 98 g of 1000-seed mass in average (Table 2). Species *Phaseolus vulgaris* L. based on the seed weight can be divided into three groups: (1) small-seeded –with 1000 seed mass below 200 g, (2) medium-seeded – up to 450 g and (3) large-seeded –over 451 g (TODOROVIĆ *et al.*, 2008). Within accessions all three groups are present: small (7), medium (21), including all four cultivars, and large-seeded beans (8). Farmers choose seeds of specific size and shape for the sowing (PAPA and GEPTS, 2003) and in that way indirectly affect the selection and phenotype of local landraces. No matter what the objectives of bean breeding are, the main goal is to create high yielding cultivars with different seed weights (VASIĆ *et al.,* 2010). It is important to collect local landraces and accessions, which are source of significant genetic variability.

Table 2. Seed dimensions of collected bean and snap bean populations

	Accession name	1000 seed mass	Seed length (cm)	Seed width (cm)	Seed thickness (cm)
1	Dvadesetica	405,03	1,39^{cde}	0,80^{cde}	0,58^{efg}
2	Balkan	284,88	1,07 ^{no}	0,70^{ghi}	0,54^{ghij}
3	Zlatko	395,96	1,38^{cdef}	0,72^{ghi}	0,58^{efg}
4	Sremac	331,36	1,24^{ijklm}	0,68^{hi}	0,57^{efg}
5	NK1/12	284,27	1,17^{lm}	0,63^{j}	0,57^{efgh}
6	NK10/12	604,70	1,73^{a}	0,78^{de}	0,70^{c}
7	NK11/12	578,90	1,48^{c}	0,90^{a}	0,78^{a}
8	NK12/12	597,16	1,61^{b}	0,86^{b}	0,70^{c}
9	NK14/12	342,30	1,26^{hijkl}	0,69^{ghi}	0,61^{e}
10	NK29/12	488,83	1,41^{cde}	0,82^{cd}	0,73^{bc}
11	NK65/12	389,60	1,57^{b}	0,74^{fg}	0,61^{e}
12	NK66/12	311,70	1,35^{efgh}	0,76^{ef}	0,51^{jkl}
13	NK67/12	368,00	1,23^{jklm}	0,74^{fg}	0,65^{d}
14	NK75/12	255,10	1,23^{jklm}	0,61^{j}	0,49^{klm}
15	NK76/12	104,90	0,79^{q}	0,48^{l}	0,44^{o}
16	NK83/12	238,00	1,29^{fghij}	0,62^{j}	0,48^{lmn}
17	NK103/12	191,06	1,05^{no}	0,62^{j}	0,46^{mno}
18	NK108/12	634,96	1,47^{c}	0,89^{a}	0,75^{b}
19	NK109/12	535,53	1,28^{ghijk}	0,81^{cd}	0,70^{c}
20	NK114/12 A	413,86	1,34^{efghi}	0,71^{ghi}	0,59^{ef}
21	NK114/12 B	212,10	0,92^{p}	0,63^{j}	0,53^{hijk}
22	NK34/12	395,80	1,36^{defgh}	0,83^{c}	0,65^{d}
23	NK2/12	278,97	1,37^{defg}	0,73^{fgh}	0,52^{ijk}
24	NK4/12	283,43	1,41^{cde}	0,70^{ghi}	0,48^{lmn}
25	NK25/12	137,07	0,99^{o}	0,61^{j}	0,44^{no}
26	NK38/12	202,00	1,25^{ijklm}	0,68^{hi}	0,57^{efg}
27	NK39/12	161,26	1,18^{klm}	0,56^{k}	0,39^{p}
28	NK40/12	529,00	1,46^{cd}	0,77^{ef}	0,52^{ijkl}
29	NK52/12	119,90	1,03^{no}	0,46^{l}	0,38^{p}
30	NK53/12	213,33	1,16^{m}	0,70^{ghi}	0,46^{mno}
31	NK54/12	267,60	1,28^{ghijk}	0,61^{j}	0,45^{mno}
32	NK62/12	193,97	1,26^{hijkl}	0,61^{j}	0,43^{o}
33	NK71/12	213,26	1,23^{jklm}	0,59^{j}	0,55^{fghij}
34	NK116/12	228,27	1,09^{n}	0,67^{i}	0,49^{klm}
min		104,90	0,79	0,46	0,38
max		634,96	1,73	0,90	0,78

Seed length, width and thickness are main characteristics of the cultivar whose ratio doesn't vary much under different environmental conditions (VASIĆ, 1986). Tested accessions differed on these properties (table 2). Seed length ranged from 0, 79 cm, with landrace NK76/12

which is very small bean, to 1, 73 cm with landrace NK10/12, that has white large cylindrical grain. Other two seed dimensions, width and thickness, demonstrated a minor variation than the length. The lowest variability of the three seed dimensions among collected accessions had seed width (Table 2).

Table 3. Seed color and shape, and phaseolin type of collected bean and snap bean populations

	Collection number	Seed shape	Seed color	Phaseolin type
1	Dvadesetica	*Oblongus*	white	S
2	Balkan	*Ellipticus*	white	S
3	Zlatko	*Oblongus*	golden-yellow	T
4	Sremac	*Oblongus*	greenish	T
5	NK1/12	*Oblongus*	yellow	T
6	NK10/12	*Oblongus*	white	T
7	NK11/12	*Ellipticus*	seed coat pattern	T
8	NK12/12	*Oblongus*	yellow	T
9	NK14/12	*Oblongus*	greenish-yellow	T
10	NK29/12	*Oblongus*	seed coat pattern	T
11	NK65/12	*Oblongus*	white	T
12	NK66/12	*Compressus*	white	S
13	NK67/12	*Ellipticus*	greenish	T
14	NK75/12	*Oblongus*	greenish-yellow	T
15	NK76/12	*Ellipticus*	white	S
16	NK83/12	*Oblongus*	white	T
17	NK103/12	*Ellipticus*	seed coat pattern	S
18	NK108/12	*Ellipticus*	seed coat pattern	T
19	NK109/12	*Ellipticus*	yellow	T
20	NK114/12 A	*Oblongus*	brownish-green	T
21	NK114/12 B	*Ellipticus*	white	S
22	NK34/12	*Ellipticus*	seed coat pattern	T
23	NK2/12	*Oblongus*	brown	S
24	NK4/12	*Compressus*	white	T
25	NK25/12	*Ellipticus*	white	T
26	NK38/12	*Oblongus*	seed coat pattern	T
27	NK39/12	*Compressus*	white	T
28	NK40/12	*Compressus*	white	S
29	NK52/12	*Oblongus*	brown	T
30	NK53/12	*Subcompressus*	seed coat pattern	T
31	NK54/12	*Oblongus*	white	T
32	NK62/12	*Compressus*	white	T
33	NK71/12	*Oblongus*	seed coat pattern	T
34	NK116/12	*Ellipticus*	various colors	T

Cylindrical=ssp. *Oblongus*; Ellipsoid= ssp. *Ellipticus*; Kidney-shaped= ssp. *Compressus*; Semi-flat= ssp. *Subcompressus*

Based on the ratios of: (i) seed length and seed width, and (ii) seed thickness and seed width, collected seed samples were classified into five basic groups of seed shape: round, ellipsoid, cylindrical, semi-flat and kidney-shaped. Shape and color of the seed are main market characteristics based on which genotypes are divided into types or trade classes (VOYSEST *et al.,* 1994). Seeds of collected bean accessions have three, while snap bean seeds have four different shapes, and none of them was round (Table 3). The largest number of accessions has cylindrical seed shape, including cultivars Dvadesetica, Zlatko and Sremac. Seed coats of bean accessions were of five different colors, while in the snap bean group seed coats were of three different colors. Seeds of collected bean and snap bean accessions were predominantly white and cylindrical in shape (Table 3).

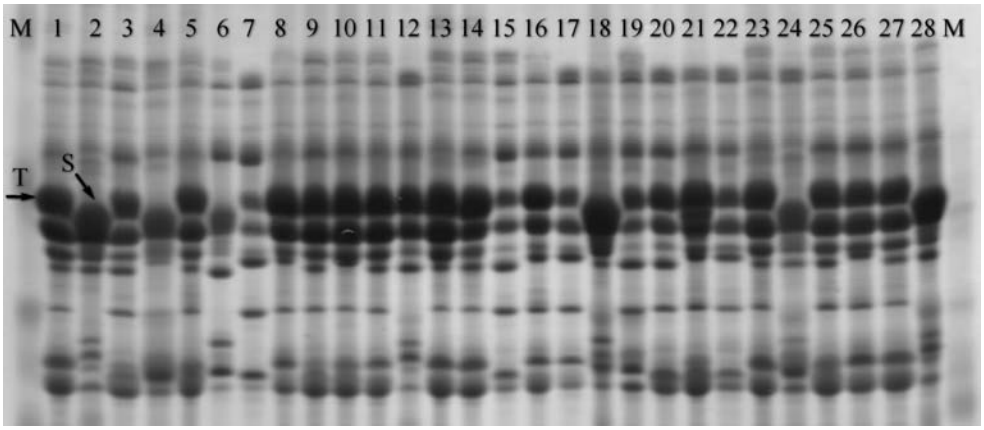

Figure 1. SDS-PAGE-generated phaseolin patterns among dry and snap bean (*Phaseolus vulgaris* L.) landraces collected on Fruska gora Mt M–protein marker (120-20 kDa); 1,3-control T type phaseolin (Zlatko, Sremac); 2,4–control S type phaseolin (Dvadesetica, Balkan); 5–NK 1/12; 6–NK 66/12; 7–NK 10/12; 8–NK 11/15; 9–NK 12/12; 10–NK 14/12; 11-NK 29/12; 12–NK65/12; 13–NK 67/12; 14–NK 75/12; 15-NK 83/12; 16–NK 108/12; 17–NK 114/12 A; 18–NK 103/12; 19-NK 109/12; 20-NK 34/12; 21-NK 4/12; 22-NK25/12; 23-NK 38/12; 24-NK 2/12; 25-NK 52/12; 26- NK 39/12; 27-NK116/12; 28-NK 40/12.

In studying the germplasm of *Phaseolus vulgaris* L. important role has phaseolin as protein marker (LIOI, 2005, NIKOLIĆ *et al*., 2007). It allows determining evolutionary and geographic distribution of the bean (ŠUŠTAR-VOZLIČ *et al.,* 2006). Presence of different phaseolin types indicates the belonging of bean germplasm to certain center of origin and its suitability to specific climatic conditions. Well is known the existence of two centers of origin of species *Phaseolus vulgaris* L., Mesoamerican and Andean (DEBOUCK and TOHME, 1989). S phaseolin type indicates the belonging to Mesoamerican, and T type to Andean center of origin (KOENING, SINGH and GEPTS, 1990).

Among our accessions T type dominates, while S phaseolin type is present with six dry and two snap bean accessions (Table 3, Figure 1). It was noticed in this paper that T phaseolin

type occurs mainly with white seeded snap bean landraces, while among dry bean landraces it was not observed significant relationship between seed color and phaseolin type. Among dry bean cultivars, including those taken as a control in this paper, it was found that S phaseolin type occurs more commonly with cultivars of white grain, and T type among those with colored grain (NIKOLIĆ *et al*., 2007). It is believed that this difference in distribution of various phaseolin types shows the farmers preference in choosing accessions with certain traits. TOMLEKOVA (2012) noted that domination of T phaseolin type among accessions from Bulgarian germplasm occurs because of its relationship with white seed color, which is primer choice in the dishes of local population. S type of phaseolin is associated with diseases resistance (KOENIG *et al*., 1989), which explains its presence in local germplasm of this region. Research with Bulgarian cultivars also confirmed that varieties with S phaseolin type were better adapted to high temperatures and rainfall shortage comparing to varieties with T phaseolin type (GENČEV *et al.,* 2002). *Phaseolus vulgaris* L. with S phaseolin type originated from Central America and thus is better suited to arid conditions characteristic for that region. On the contrary, dry and snap beans with T phaseolin type are better adapted to humid climates like Andes, where they came from (GEPTS *et al*., 1986).

CONCLUSION

The research confirms existence of large number and significant breeding potential of dry and snap bean landraces on the area of Fruška gora Mt. It was observed the existence of significant variability and diversity in terms of tested traits among these landraces, as well in relation to domestic newly created cultivars. The variety of shapes, colors and seed weights of dry bean cultivars made in IFVCNS are the reason from which they are selected to be compared with accessions collected on Fruška gora Mt. By examining the shape and seed color, it was found that landraces with white and cylindrical shape dominated. Seed weight varied. Most numerous accessions in term of seed weight were medium-seeded, but small and large-seeded accessions were also present. T phaseolin type dominated among landraces, while S phaseolin type was present only with six bean and two snap bean accessions. Cultivars made at IFVCNS with white seed have S phaseolin type, while cultivars with colored seed have T phaseolin type. The diversity of studied landraces characteristics indicates presence of different eating habits of population living in this area. All this shows that landraces were brought from different places as the inhabitants were settling in this region, thus they are adapted to different climatic conditions. Due to all said above, there is reason to include collected landraces in breeding programs.

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MORFO-HEMIJSKA KARAKTERIZACIJA LOKALNIH POPULACIJA PASULJA I BORANIJE (*Phaseolus vulgaris* L.) SAKUPLJENIH NA FRUŠKOJ GORI

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Izvod

Nestanak starih sorata, uključujući pasulj i boraniju (*Phaseolus vulgaris* L.) je ubrzan u poslednjih pet do šest decenija, što je dovelo do velikog genetičkog osiromašenja. Za celo čovečanstvo i njegovu budućnost posebno je važno očuvanje i ispitivanje semena starih sorata. Ispitivanje predstavljeno u ovom radu je vršeno na teritoriji jugozapadne Fruške gore. Od sakupljenih uzoraka ratarskog, povrtarskog i samoniklog bilja, 13 akcesija boranije i 21 akcesija pasulja je analizirana u ovom radu. Boja zrna, oblik zrna, masa 1000 zrna i tip fazeolina su idređeni za svaku akcesiju. Seme sakupljenih akcesija pasulja i boranije je bilo većinom belo i valjkastog oblika. Masa 1000 zena se kretala od 104,90 g do 634,96 g. Dominirao je T tip fazeolina, dok se S tip nalazio kod šest akcesija pasulja i dve akcesije boranije.

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