STUDIA UBB CHEMIA, LXIX, 2, 2024 (p. 135-150)
(RECOMMENDED CITATION)
DOI:10.24193/subbchem.2024.2.10

NUTRITIONAL COMPOSITION AND ANTIOXIDANT CAPACITY OF COMMON BEAN (*PHASEOLUS VULGARIS* L.) CORE COLLECTION

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ABSTRACT. Variation of common bean (*Phaseolus vulgaris* L.) core collection was assessed based on the main nutritive and bioactive components. Nutritional profile was described for each cultivar and landrace. Protein content was in the familiar range for common bean (19.6-31.6%). Detected variability for potassium, sulphur, iron and zinc was 7.78, 16.7, 14.99, and 40.17%, respectively. Total phenolic content ranged from 1.8 to 14.1 mg GAE /g DW, with high variation (CV = 41.3%). Likewise, antioxidant tests DPPH, ABTS and FRAP had high, genotype-based, CV in range 29-46%. With the application of PCA and cluster analysis, better insight in underlying germplasm structure was acknowledged, as well accession's grouping based on the studied traits. Cultivars Vulkan and Panonski tetovac, breeding line HR45, landraces L24, L92, L119, L120, and L125 had larger amounts of iron, nitrogen, and proteins. Elevated phenolic content was observed in cultivars Balkan and Spinel, as well as landraces L19, L29, L41 and L60. In addition, cultivar Royal Dutch was recognized for higher levels of zinc, and higher antioxidant capacity revealed by DPPH, ABTS, and FRAP assays. Therefore, these tests could be used in the selection of the accessions for breeding for nutritive quality enhancing.

Keywords: *nutritive value, bioactive compounds, variability, landraces, cultivars*

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INTRODUCTION

Due to its high nutritional value, common bean (*Phaseolus vulgaris* L.) is one of the most valuable legumes for human consumption worldwide. Moreover, it is recognized as a very diverse crop in terms of different types of cultivation methods, phenotypic and genotypic variability, as well as a wide range of environments to which it is adapted [1]. All these traits have established this species as a significant component in the traditional diet and life of many nations, keeping in mind that it is usually the only source of proteins and other nutrients in developing countries and people living in rural and marginal areas. It is also getting higher in demand as a source of vegetable proteins in the nutrition of urban population due to the rise in the adoption of flexitarian, vegetarian and vegan diets [2].

The species *Phaseolus vulgaris* is also recognized for the existence of Mesoamerican and Andean gene pools which derived from two independent domestication events in Middle and South America [3]. Accessions belonging to these two gene pools distinguish between themselves according to their phenotypic, biochemical, nutritional, adaptive and genotypic differences [4,5]. A great diversity of the common bean is identified in Europe, mainly as a result of beans' evolution under diverse cropping systems, agro-ecological conditions and farmer's preference in term of types of seeds (market classes) and use [6]. Genetic collections of the species *Phaseolus vulgaris* in Serbia are maintained within breeding institutes. These collections accommodate the seeds of traditional and modern domestic and foreign cultivars, breeding lines and landraces from the territory of Serbia and neighbouring countries. These genetic collections have been characterized for their phenotypic, agronomic and genotypic variability; however, information on nutraceutical value is missing. Nutritional value is an important component in breeding, both from the aspect of increasing the content of certain nutrients, but also from selection of high yielding genotypes of good nutritive and bioactive properties.

Common bean is rich in proteins, carbohydrates, fibres, vitamins, and minerals, as well as a variety of bioactive compounds [7]. Even though common beans are considered vegetables due to the high fibre and mineral content, it is also a protein crop. Dry seed of the beans contains 20-35% of crude proteins, which is more than any other plant-based food. These are high-quality proteins, with almost all essential amino acids in higher quantities, except for methionine and tryptophan. As for carbohydrates, they make up to 70% of seeds' dry matter, with starch being the major component of complex sugars which degrades slowly, making beans low glycaemic index food. Dietary fibres (18-20%) are another component of beans' seed, important for the health of the human digestive tract and cardiovascular system. In addition to this, most of the fat

found in bean seeds are unsaturated fats, essential for the prevention of coronary heart disease, high blood pressure and stroke [8]. Common bean is also recognized for high amount of minerals which are required for proper function of human body system, including macronutrients (sulphur, potassium, phosphorus, calcium, magnesium) and micronutrients (iron, zinc, copper, manganese, iodine). Moreover, beans are rich in vitamins, especially B group vitamins (folates), vitamins E and K [9].

Beans are good sources of bioactive compounds with antioxidant properties, including plant phenolics, tannins, flavonoids, anthocyanins, among others [10]. These phenolic compounds play an important role in antioxidative response through free radicals scavenging activity against harmful effect of ROS [11]. Moreover, the antioxidative activity of the common bean secondary metabolites could protect human cells against damages caused by oxidative stress [12]. Other health effects of beans include prevention of diabetes type 2, peripheral vascular diseases, hypertension, heart attack and in combat of Alzheimer's, Parkinson's disease and various type of cancers [13]. Many products are developed from common bean, including gluten-free flour, biscuits with lower antinutrient contents, high-nutritional bread and other [14]. Therefore, the main objective of this study was to assess nutritional value of a selected set of common bean accessions comprising *Phaseolus vulgaris* core collection with greatest phenotypic and genetic variation.

RESULTS AND DISCUSSION

Variability of common bean accessions based on studied nutritive traits was presented in Table 1. For all the studied samples, mean values of protein, nitrogen, phosphorus, and sulphur contents were 23.22%, 3.71%, 0.40% and 0.26%, respectively. Cultivar Balkan had the lowest protein (19.62%) and nitrogen (3.14%) content, while the least amount of phosphorus was found among the breeding line BAT477 and landrace L9 (0.33%). On the other hand, landrace L92 distinguished with the largest amount of proteins (31.61%), nitrogen (5.06%) and phosphorus (0.51%) in its seeds. The largest potassium content was found among the cultivar Poboljšani gradištanac and landrace L120 (1.33%), while for the sulphur it was in landrace L29 (0.47%). On the contrary, breeding line BAT477 had the lowest seed amount of both potassium (0.93%) and sulphur (0.20%). Iron content ranged from 45.93 mg/kg in cultivar Dobrudžanski 7 to 104.70 mg/kg in landrace L125, with mean value of 64.18 mg/kg for all studied samples. Other genotypes with increased iron content were landrace L24 (85.63 mg/kg) and breeding line HR45 (83.78 mg/kg). Landrace L120 had the largest zinc content (101.60 mg/kg), as opposed to landrace L10 with the

smallest value (24.17%). Moreover, cultivars Royal Dutch and Vulkan were recognized for elevated zinc content (84.22 mg/kg and 90.02 mg/kg, respectively) compared to average found for all the samples (38.16 mg/kg). The highest variability was observed for zinc (CV = 40.17%); moderate variation was recorded for sulphur (CV = 16.70%) and iron (CV = 14.99%) contents while the lowest variability was found for the contents of protein (CV = 10.33%), nitrogen (CV = 10.33%), phosphorus (CV = 8.49%) and potassium (CV = 7.78%).

Table 1. Variability of studied nutritive traits in IFVCNS common bean core collection

	Proteins	**N**	**P**	**K**	**S**	**Fe**	**Zn**
	%					**mg/kg**	
Mean	23.22	3.71	0.40	1.12	0.26	64.18	38.16
SE	0.32	0.05	0.005	0.001	0.001	1.27	2.03
CV%	10.33	10.33	8.49	7.78	16.70	14.99	40.17
Range	19.62	3.14	0.33	0.93	0.20	45.93	24.17
	31.61	5.06	0.51	1.33	0.47	104.70	101.60

Observed amounts of both nitrogen and protein contents were in the variation range recorded for common bean worldwide [14,8]. However, bean accessions in our study had slightly more nitrogen and protein contents, with greater variability, when compared to the results of Celmeli et al. [15] and de Lima et al. [16]. This could be due to the nature of the studied material, conferring greatest captured phenotypic and genotypic diversity of larger collection, but can also be related to more samples analysed in our study. Plant based proteins make up to 65% of the world's total supply of protein for human consumption, of which 45-50% come from legumes and cereals [17]. This indicates the importance of investigation of new sources of this type of proteins, but also making attempts in increasing protein contents through breeding. Results of this research showed that some landraces had more proteins compared to commercial cultivars, which makes them valuable components in breeding with aforementioned purpose. On the other hand, even though the accessions in the core collection were chosen according to their variability, not significant differences were observed for phosphorus and potassium contents. Recorded values of these two macronutrients were similar to those found in Paredes et al. [18]. Obtained results argue that investigated core collection is not a suitable source of variability for increasing phosphorus and potassium contents via breeding.

Iron and zinc contents recorded in this study were in the same range as observed in research of Islam et al. [19] and Guzman-Maldonado et al. [20]. In addition, the average zinc value corresponded to that found in research of 1000 common bean genotypes from the CIAT's common bean collection [21], while iron content was even higher. As for newer research, iron and zinc contents

were higher compared to the results of Ramirez-Ojeda et al. [22] and de Lima et al. [16]. Therefore, results of this study demonstrated great variability and accumulation of iron and zinc in evaluated bean germplasm, which, in turn, gives the possibility of the selection of cultivars with large quantities of these nutrients. It is estimated that 17% of the population worldwide suffer from zinc deficiency, while for the iron that number reaches 30%. A large percentage of child deaths are associated with zinc insufficiency, while anaemia often occurs due to low iron bioavailability, especially in food of plant origin [23]. By providing sufficient amounts of these micronutrients into the human diet, normal pregnancies may be ensured, as well as adequate child growth and development, immune system function and neurobehavioral development [24].

The total phenolic content ranged from 1.80 mg GAE/g DW (cultivar Biser) to 14.1 mg GAE/g DW (landrace L29), with mean value of 6.46 mg GAE/g DW and relatively large variation (CV = 41.3%). The highest amount of total tannins content was observed in landrace L48 (5.70 mg GAE/g DW and the lowest in landrace L73 (2.70 mg GAE/g DW, with moderate variability, CV = 18.6% (Table 3). Carbas *et al*. [14] observed similar values for total phenolic contents, while de Lima *et al.* [16] found similar total tannin values in bean landraces from Brazil. On contrary, Sahaskul *et al*. [25] recorded smaller variation of total phenolic content, (0.72 to 3.12 mg GAE/g DW, in range), in the study of more than one legume species (2 Phaseolus, 4 Vigna and one Glycine species). Moreover, reported concentrations of these compounds are higher in common beans compared to lentils, chickpea, soybeans, which is related to the better antioxidant and nutraceutical properties of beans and implication on human health [10]. Total phenolic and tannins content, alone or in combination with other constituents, are a potential candidate as a selection criterion for antioxidant activity in beans.

Results of seven non-enzymatic antioxidant assays (DPPH, ABTS, FRAP, NBT, TAA, TRC, and NO inhibitory), involving the measurement of the ability of compound to act as free radical scavengers, and one enzymatic assay LP (lipid peroxidation) are represented in Table 2. Landrace L29 was with highest DPPH and ABTS values (59.20 and 94.00 μmol TE/g DW, while the highest FRAP level was observed in landrace L40 (94.80 μmol TE/g DW. On the contrary, cultivar Biser had the lowest values for DPPH, ABTS and FRAP, with 9.60, 6.80 and 18.00 μmol TE/g DW, respectively. In comparison to the results of Carbas et al. [16] twice as high mean DPPH and FRAP values were observed in our study, and therefore better antioxidant potential. Good free radical scavenging properties with protective roles in cellular oxidative stress caused by dietary habits, inflammation, microbial interactions and other were detected among investigated common bean germplasm. NBT levels ranged from 0.03 U/g DW in breeding line BAT 477 to 0.26 U/mg DW in landrace L120, with mean value of 0.23 U/mg DW.

Table 2. Variability of bioactive compounds in IFVCNS common bean core collection

	TPC mg GAE	TTC mg GAE	DPPH µmol TE	ABTS µmol TE	FRAP µmol TE	NBT U	TAA µmol TE	TRC µmol TE	NO %	LP nmol MDA/ mg protein
	g DW					mg DW	g DW			
Mean	6.46	4.14	35.27	51.48	51.94	0.23	210.1	113.6	0.02	0.12
SE	0.4	0.1	1.2	0.4	0.4	0.01	0.4	1.2	0.01	0.01
CV %	41.3	18.6	29.2	46.1	32.5	13.4	10.9	8.3	12.1	5.2
Range	1.80	2.70	9.60	6.80	18.00	0.03	262.8	134.8	0.01	0.11
	14.1	5.70	59.20	94.00	94.80	0.26	144.8	85.2	0.03	0.13

TPC – total phenolic content, TTC – total tannin content, NBT – nitroblue tetrazolium test, TAA – total antioxidant activity, TRC – total redox capacity, NO – nitric oxide test, LP – lipid peroxidation

Total antioxidant activity ranged from 144.8 µmol TE/g DW in cultivar Vulkan to 262.8 µmol TE /g DW in cultivar Naya Nayahit, with mean value of 210.1 µmol TE/g DW and variability of CV = 10.9%. Landrace L18 exhibited the lowest total redox capacity (85.2 µmol TE/g DW) compared to landrace L5 with the highest value (134.8 µmol TE/g DW). On the contrary, the same landrace L5 displayed the lowest potential for nitric oxide inhibition (0.01%), while the landrace L120 (0.03%) was distinguished as best for the value of this bioactive compound. Nitric oxide acts in plant–microbe interactions, responses to abiotic stress, stomatal regulation and a range of developmental processes [26]. Additionally, nitric oxide plays an important role in symbiotic organisms, particularly between legumes and *Sinorhizobium* [27]. In beans, NO is involved in lipid and photosynthesis recovery under Mn stress conditions, it is assumed that NO beneficial effects are attributable to NO/Mn cross-talk [28]. According to only enzymatic assay performed in this study, lipid peroxidation ranged from 0.11 nmol MDA/mg protein in landrace L121 to 0.13 nmol MDA/mg protein in landrace L124, with variability of only CV = 5.2% (Table 3). Low variability (CV below 10%) in TRC and LP indicates that differences for these parameters among tested genotypes could be obtained only under stress conditions.

Different nutritional and bioactive profiles were observed between groups generated according to the seed coat traits (market classes). In general, accessions from the Albus group had the lowest level of radical scavenging compounds revealed by ABTS and FRAP assays (36.04 µmol TE/g DW and 41.36 µmol TE/g DW respectively), but relatively large amounts of zinc (42.12 mg/kg). On the other hand, Roseus group was recognized for the large levels of total phenolics (8.50 mg GAE/g DW), DPPH (39.0 µmol TE/g DW), ABTS (83.0 µmol TE/g DW), NBT (0.24 U/g DW) and TRC (124.4 µmol TE/g DW), but the smallest amounts of both iron (58.21 mg/kg) and zinc (29.21 mg/kg). The least amounts of proteins (21.61%), nitrogen (3.46%) and potassium (1.02%)

were found in the Griseus group. Conversely, accessions from Aureus seed form displayed largest amounts of nitrogen (4.09%) and proteins (25.59%), with elevated levels of sulphur (0.30%) and iron (70.72 mg/kg). Highest amounts of iron (83.52 mg/kg) and TAA (242.6 µmol TE/g DW), but lowest amounts of zinc (29.22 mg/kg) were observed in the Niger group. Highest value for zinc content (45.75 mg/kg), total phenolic content (10.46 mg GAE/g DW), DPPH (48.72 µmolTE/ g DW) and FRAP (77.60 µmol TE/g DW) was recorded for Crepito accessions (Table 3). Differences between common bean seed forms (market classes) in terms of their nutritive value were observed in other studies [29,14]. Even though it was suggested that dark-coloured beans have overall better antioxidant properties, that was not completely the case in our study.

Table 3. Nutritional and bioactive profile of different bean groups generated according to seed coat traits

	Albus	Roseus	Versicolor	Griseus	Aureus	Niger	Crepito	Vinosus	Brunneus
N %	3.71	3.69	3.76	3.46	4.09	3.64	3.66	3.91	3.53
P %	0.41	0.40	0.40	0.37	0.43	0.39	0.42	0.40	0.40
K %	1.13	1.13	1.15	1.02	1.18	1.12	1.12	1.12	1.05
S %	0.26	0.23	0.25	0.23	0.30	0.25	0.34	0.29	0.30
Fe mg/kg	63.27	58.21	62.13	65.46	70.72	83.52	59.86	60.89	64.28
Zn mg/kg	42.12	29.21	36.33	33.08	31.42	29.22	45.75	40.06	35.39
Proteins %	23.17	23.06	23.53	21.61	25.59	22.74	22.90	24.46	22.05
TPC mg GAE/g DW	5.44	8.50	5.39	7.13	6.64	5.30	10.46	7.30	8.85
TTC mg GAE/ g DW	4.47	4.25	3.73	4.21	3.72	3.30	3.92	4.30	4.40
DPPH µmol TE/g DW	32.27	39.0	34.52	36.74	31.92	32.0	48.72	37.60	38.80
ABTS µmol TE/g DW	36.04	83.0	52.12	61.14	61.52	43.6	78.64	58.8	60.4
FRAP µmol TE/g DW	41.36	67.6	54.4	55.54	47.20	61.20	77.60	66.40	51.40
NBT U/mg DW	0.22	0.24	0.23	0.20	0.23	0.23	0.23	0.23	0.23
TAA µmol TE /g DW	205.5	216.6	207.9	214.6	207.6	242.6	206.9	218.4	222.6
TRC µmol TE g/DW	112.4	124.4	111.8	115.1	117.2	117.4	111.5	115.4	110.2
NO %	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
LP nmol MDA/mg protein	0.12	0.13	0.12	0.12	0.12	0.11	0.12	0.12	0.12

TPC – total phenolic content, TTC – total tannin content, NBT – nitroblue tetrazolium test, TAA – total antioxidant activity, TRC – total redox capacity, NO – nitric oxide test, LP – lipid peroxidation

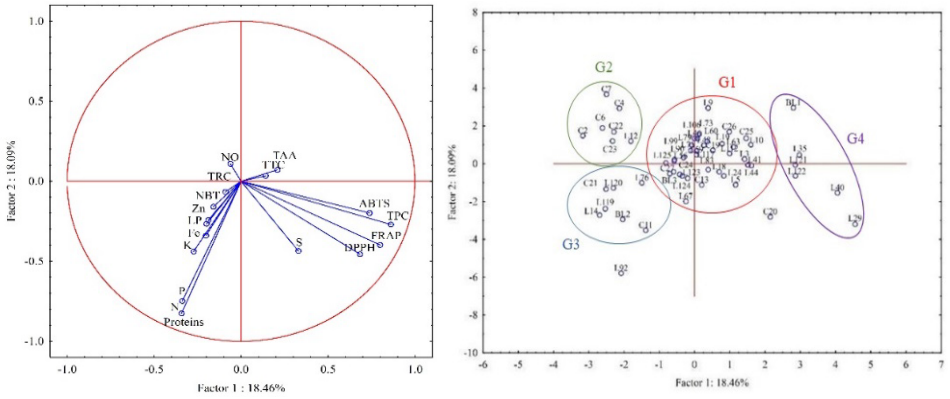
Principal component analysis reduced the initial number of studied variables (traits) from 16 to 6 with eigenvalues (λ) larger than 1, which explained 69.3% of variability of the examined dataset, in total (Table 4). First two principal components explained 18.46% and 18.09% total variability, respectively, and were separated for graphical representation of relationships among studied traits and accessions. The first principal component was associated with traits such as total phenolics, DPPH, ABST and FRAP. Nitrogen, phosphorus and protein content defined second principal component. The third main component was related to the total antioxidant activity, sulphur and zinc contents. NBT and LP defined forth, while total tannins and iron content defined fifth and sixth principal component, respectively.

Table 4. Principal component analysis of studied common bean core collection: eigenvalues, total variance, and cumulative variance

Principal component	Eigenvalue	Total variance %	Cumulative variance %
1	3.14	18.46	18.46
2	3.07	18.09	36.55
3	1.69	9.95	46.51
4	1.46	8.57	55.08
5	1.29	7.60	62.69
6	1.12	6.61	69.29

Vector projections of studied traits and position of accessions on biplot graph enable identification of their relationships according to investigated nutritive value. Therefore, positive correlations could be observed between the traits that defined first PC (TPC, ABTS, FRAP, DPPH) which were in negative association with traits of the second PC (proteins, nitrogen, phosphorus). Majority of accessions grouped in the middle of the biplot chart (G1), comprising of 28 landraces, one breeding line and six cultivars (Naya Nayahit, Spinel, Alubia, Poboljšani gradištanac, Gerle and Harwood). Both nutritive and bioactive compounds levels found in these accessions were around the average for the entire core collection. Second group (G2) consisted of six cultivars (Žutotrban, Dobrudžanski 7, Balkan, Pasuljica P1, Biser, C-20) and one landrace (L12). Main features of these cultivars and landrace were the lowest observed values for total phenolics content (2.80 mg GAE/g DW), DPPH (14.97 μmol TE/g DW), ABTS (25.14 μmol TE (g/DW) and FRAP (24.34 μmol TE/g DW) in average. Two cultivars (Vulkan and Panonski tetovac), one breeding line (HR45) and four landraces (L14, L76, L119 and L120) comprised third group (G3) and were characterized with largest mean content of TRC (120.80 μmol TE/g DW), nitrogen (4.27%), phosphorus (0.44%), zinc (52.21 mg kg-1) and protein (26.73%) contents. The largest average amount of total phenolics content (11 mg GAE/gDW), DPPH (47.00 μmol TE/g DW), ABTS (78.37 μmol TE/g DW) and FRAP (77.93 μmol

TE/g DW) were recorded in accessions of the fourth group (G4) that clustered around the same vectors on biplot graph. This group included one breeding line (BAT 477) and five landraces (L29, L35, L40, L121, and L122). Two accessions had separate position, cultivar Royal Dutch which distinguished according to higher amounts of DPPH (50 μmol TE/g DW), ABTS (86.4 μmol TE/g DW), FRAP (83.2 μmol TE/g DW) and zinc content (84.22 mg/kg) and landrace L92 with elevated levels of nitrogen (5.06%), phosphorus (0.51%), potassium (1.26%) and proteins (31.61%) contents (Fig. 1).



TPC – total phenolic content, TTC – total tannin content, NBT – nitroblue tetrazolium test, TAA – total antioxidative activity

Figure 1. Principal component analysis biplot representing distribution of nutritional and biochemical characteristics and common bean accessions in the first and second dimension

Hierarchical cluster analysis based on squared Euclidean distance was applied in order to assess the relationships among accessions in more detail (Figure 2). The dendrogram divided landraces and cultivars in three main clusters (A, B, C), with additional subdivision within the third cluster (C1, C2, C3). Cluster A included only two cultivars of Bulgarian origin, Vulkan and Dobrudzanski 7 and one landrace (L120). These genotypes are recognized for the largest amount of zinc content on average (91.95 mg/kg). Four accessions were organized within cluster B, one breeding line, HR45, and three landraces (L24, L92 and L125). Largest amount of iron (88.36 mg/kg), nitrogen (4.29%) and protein content (26.84%) was observed in this group. Accessions comprising cluster C accounted for 87.7% of the studied core collection. Most of these accessions had values of studied traits around or below the average, with certain deviation. Subcluster C1 included cultivars Balkan and Spinel, and landraces L19, L29, L41, L60. Main features of these accessions were higher values of total phenolics content (7.85 mg GAE/g DW). Largest group was subcluster C2, which, together with

subcluster C3 had better total antioxidant activity (213.53 and 212.34 μmol TE/g DW), respectively), while C3 subcluster also had in average more iron (73.07 mg/kg) and total phenolics (7.27 mg GAE/ g DW) in their seeds.

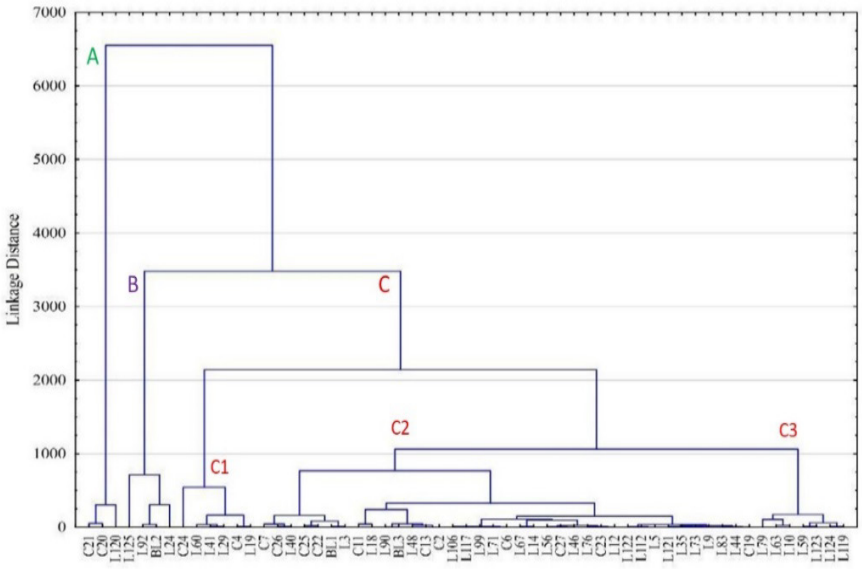


Figure 2. Hierarchical cluster analysis based on squared Euclidean distance of common bean core collection

After describing each accession according to its nutritional profile, application of PCA and cluster analysis revealed underlying structure of studied common bean germplasm. With the deployment of cultivars and landraces in several small homogenous groups, accessions with specific combinations of nutritional compounds were acknowledged. It was possible to distinguish accessions with superior composition in terms of specific nutritional and bioactive compounds. This information is important for the selection of preferable accessions for nutritive quality enhancing through breeding. Moreover, results of this research will enable accessions of already known good agronomic performance associated with elevated nutritional value to be advanced in production as functional food.

CONCLUSIONS

Bean accessions proved to have high nitrogen and protein contents, with greater variability. However, some landraces (L92, L14, L67, and L119) had more proteins compared to commercial cultivars, which makes them valuable

components in breeding with afore mentioned purpose. Results of this study demonstrated great variability and accumulation of iron and zinc in evaluated bean germplasm, which, in turn, gives the possibility of the selection of cultivars with large quantities of these nutrients. Among different antioxidant tests applied DPPH, ABTS and FRAP had high, genotype-based, CV in range 30-40%.

Therefore, these tests could be used in the selection of accessions according to non-enzymatic antioxidant capacity. With the application of PCA and cluster analysis, nutritive profile was determined for each accession, while underlying structure and grouping patterns of studied germplasm were revealed. All of these results could help in the choice of new genotypes with desirable traits in further nutritive quality breeding.

EXPERIMENTAL SECTION

A total of 57 accessions from the common bean core collection maintained at the institute of Field and Vegetable Crops, Novi Sad (IFVCNS), Serbia was analysed in this paper.

The material was selected based on the greatest phenotypic and genetic variability detected in previous studies. This included 33 landraces collected from various locations in Serbia, 5 landraces of foreign origin and 1 landrace of unknown origin, 6 domestic cultivars, 9 foreign cultivars and 3 foreign breeding lines. Landraces, cultivars and breeding lines were also classified according to the seed traits (seed coat colour, seed coat patterns) in several seed forms, most commonly grown in Serbia: Roseus (pink seed colour), Versicolor (seed coat pattern), Griseus (greenish-yellow seed colour), Aureus (yellow and golden-yellow seed colour), Albus (white seed colour), Niger (black seed colour), Crepito (cream seed colour), Vinosus (red seed colour) and Brunneus (brown seed colour). In addition, gene pool for each accession was determined based on phaseolin types in previous studies [30] and accessions were designated as Mesoamerican or Andean (Table 5; Figure 3).

Bean accessions were grown in a one-year trial under field conditions, since the primary goal was to make comparison between the accessions grown under the same environmental conditions. Field trial was set as a randomised complete block design with three replications at the experimental field of IFVCNS. The plot was arranged in three rows, 2 m long, with a distance between the rows of 60 cm and 5 cm in the row. The beans have been sown in the beginning of May and harvested in September. After harvest, air-dried 100 g seeds of each bean accession were grounded using a hand mill for the following analyses. The analyses were carried out by the IFVCNS and Faculty of Agriculture, University of Novi Sad.

Table 5. List of the accessions from IFVCNS common bean core collection chosen for the study

No	Accession / origin	Seed form	Gene pool	No	Accession / origin	Seed form	Gene pool
1	L3 (SRB[1])	*Roseus*	A*	30	L106 (SRB)	*Albus*	A
2	L5 (SRB)	*Roseus*	A	31	L112 (SRB)	*Albus*	A
3	L9 (SRB)	*Griseus*	A	32	L117 (SRB)	*Albus*	A
4	L10 (SRB)	*Griseus*	A	33	L119 (SRB)	*Aureus*	M
5	L12 (SRB)	*Albus*	M	34	L120 (KAZ)	*Albus*	A
6	L14 (SRB)	*Versicolor*	A	35	L121 (BIH)	*Versicolor*	A
7	L18 (SRB)	*Versicolor*	A	36	L122 (BIH)	*Crepito*	A
8	L19 (SRB)	*Versicolor*	A	37	L123 (HRV)	*Aureus*	A
9	L24 (SRB)	*Griseus*	A	38	L124 (BIH)	*Versicolor*	A
10	L29 (SRB)	*Crepito*	A	39	L125 (N/A)	*Niger*	M
11	L35 (SRB)	*Brunneus*	A	40	C2 (**Žutotrban**, SRB)	*Versicolor*	A
12	L40 (SRB)	*Crepito*	A	41	C4 (**Balkan**, SRB)	*Albus*	M
13	L41 (SRB)	*Vinous*	A	42	C6 (**Pasuljica P-1**, SRB)	*Albus*	M
14	L44 (SRB)	*Aureus*	A	43	C7 (**Biser**, SRB)	*Albus*	M
15	L46 (SRB)	*Aureus*	A	44	C11 (**Panonski tetovac**, SRB)	*Albus*	A
16	L48 (SRB)	*Albus*	A	45	C13 (**Poboljšani gradištanac**, SRB)	*Albus*	M
17	L56 (SRB)	*Versicolor*	A	46	C19 (**Naya Nayahit**, USA)	*Niger*	M
18	L59 (SRB)	*Albus*	A	47	C20 (**Royal Dutch**, NLD)	*Crepito*	A
19	L60 (SRB)	*Griseus*	A	48	C21 (**Vulkan**, BGR)	*Albus*	M
20	L63 (SRB)	*Griseus*	A	49	C22 (**Dobrudžanski 7**, BGR)	*Albus*	A
21	L67 (SRB)	*Vinosus*	A	50	C23 (**C-20**, USA)	*Albus*	M
22	L71 (SRB)	*Crepito*	A	51	C24 (**Spinel**, USA)	*Albus*	M
23	L73 (SRB)	*Griseus*	A	52	C25 (**Alubia**, BRA)	*Albus*	A
24	L76 (SRB)	*Versicolor*	A	53	C26 (**Gerle**, BGR)	*Versicolor*	M
25	L79 (SRB)	*Albus*	M	54	C27 (**Harwood**, CAN)	*Albus*	M
26	L83 (SRB)	*Albus*	M	55	BL1 (**BAT 477**, BGR)	*Griseus*	M
27	L90 (SRB)	*Brunneus*	M	56	BL2 (**HR45**, CAN)	*Albus*	M
28	L92 (SRB)	*Aureus*	M	57	BL3 (**Oreol L-xan**, BGR)	*Albus*	A
29	L99 (SRB)	*Versicolor*	A				

* A – Andean, M - Mesoamerican

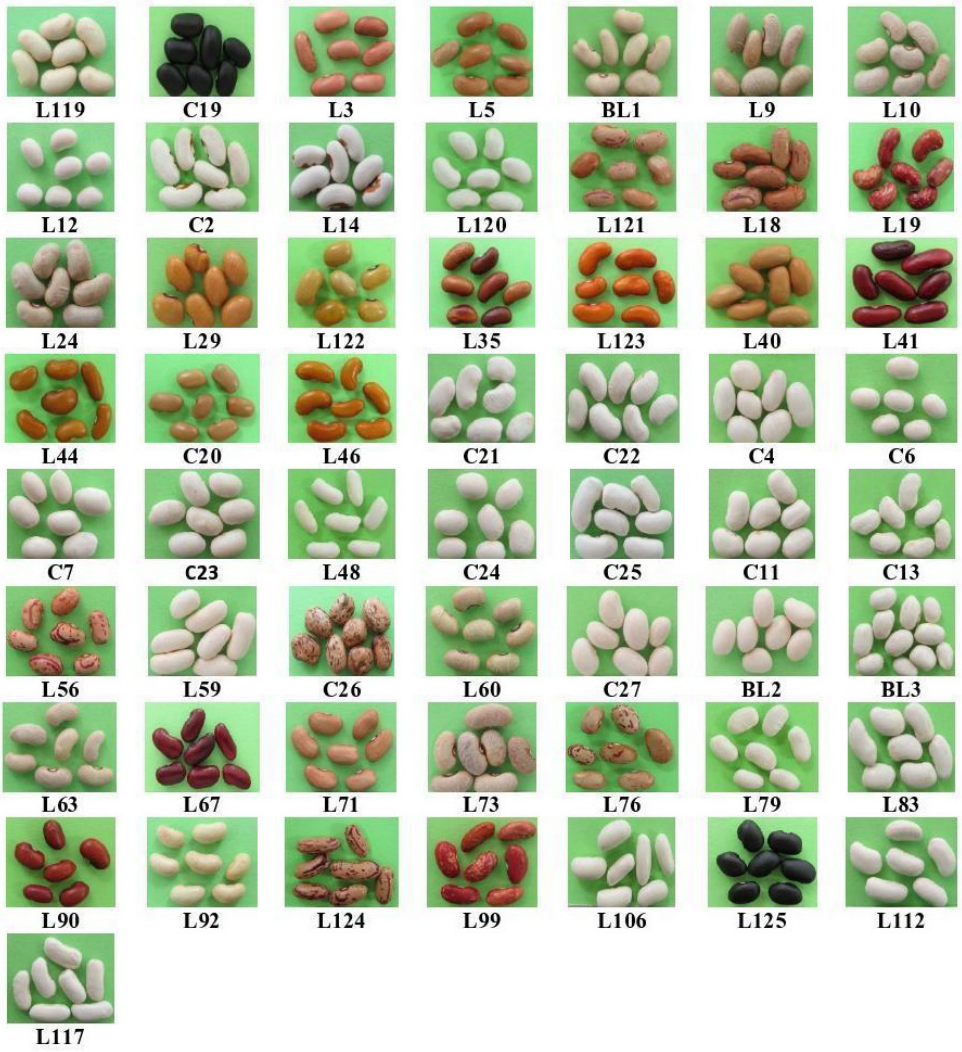


Figure 3. Studied accessions from the IFVCNS, Serbia, common bean core collection

The main common bean nutritive quality parameters assessed in this study included content of proteins (%), nitrogen (%), phosphorus (%), potassium (%), sulphur (%), iron (mg/kg^{-1}) and zinc (mg/kg^{-1}), with the application of the following methods. Nitrogen and sulphur content were determined in the elemental analysis with the application of CHNS elemental analyser Elementar III Vario El; protein content was determined with the conversion factor (N × 6.25), where

nitrogen content was multiplied by 6.25. Potassium, phosphorus, iron and zinc contents were determined with the method of inductively coupled plasma on the apparatus ICP-OES Varian. Content of the metals was assessed after the destruction of organic matter by mineral acids (10 ml HNO_3 and 2 ml H_2O_2) in the process of microwave decomposition at 180°C, in the apparatus Ethos1-MILESTONE.

Analysis of biochemical parameters was performed in the Laboratory for Biochemistry, Faculty of Agriculture Novi Sad (Serbia). Grounded seed material of each accession (500 mg) was extracted with methanol solution (50 ml) by sonication for 20 minutes in an ultrasonic bath at ambient temperature. The extracts were rapidly vacuum-filtered through a sintered glass funnel and kept refrigerated until assayed. The total phenolic content (TPC) was determined using a Folin-Ciocalteu colorimetric method [31]. Total tannins content (TTC) was determined by the Folin-Ciocalteu procedure, after removal of tannins by their adsorption on insoluble matrices [32]. Calculated values were subtracted from TPC and TTC and expressed as mg of gallic acid equivalent per g of seed dry weight (mg GAE/g DW). The scavenging efficiency of free radicals was tested in a DPPH (2,2-diphenyl-1-picrylhydrazyl) methanol solution [33]. Ferric-reducing antioxidant power (FRAP) assay was carried out according to Valentao *et al*. [34].

The ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) assay was based on a method developed by Miller *et al*. [35]. The total antioxidant activity (TAA) and NBT (nitroblue tetrazolium test) were evaluated by method of Kalaskar and Surana [36]. A reducing power assay (total reduction capacity) (TRC) was performed by the method of Saha *et al* [37]. A methanol solution of known trolox concentrations was used for calibration and formation of standard curve. The DPPH, FRAP, ABTS, TAA and TRC are expressed in μmol trolox equivalent antioxidant capacity per g seed dry weight (μmol TE/g DW). NBT is expressed as U per mg of seed dry weight (U/mg DW). Protein content in homogenates was determined using bovine serum albumin as a protein standard test [39]. Lipid peroxidation (LP) was measured at 532 nm using the thiobarbituric acid (TBA) test [39] and expressed as nmol malondialdehyde equivalents per mg protein (nmol MDA (mg/protein). NO-nitric oxide test was performed according to Shirinova *et al* [40]. Percentage was calculated based on the ability of extracts to inhibit NO formation.

Statistical analysis was performed using software Statistica, version 13.2 (Dell Inc., USA). Descriptive statistics, including mean, standard error (SE) of mean, range and coefficient of variation was estimated for all studied traits. Principal component analysis (PCA) was performed based on correlation variances to identify significant traits responsible for overall variability, as well

as to identify underlying structure of studied collection. In addition, for better assessment of genotypes grouping cluster analysis based on complete linkage and squared Euclidean distance was performed.

ACKNOWLEDGMENTS

This research was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant No. 451-03-47/2023-01/200032.

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