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ORIGINAL PAPER

MULTI-ELEMENTAL COMPOSITION, NUTRIENTS AND TOTAL PHENOLICS IN SEEDS OF *PHASEOLUS VULGARIS* L. BREEDING MATERIAL*

Barbara Pipan, Lovro Sinkovič

Crop Science Department
Agricultural Institute of Slovenia, Ljubljana, Slovenia

Abstract

Diversity of common bean (*Phaseolus vulgaris* L.) lines can disclose accessions of interest for breeding and enables breeders to examine different traits of interest at the morphological and nutritional level. This study aimed to determine variation in macro- and microelements, selected nutrients and total phenolics in seeds of the most prospective common bean breeding material. Seeds of eleven common bean breeding lines from different filial generations (F5 – F7) were produced under an open-field experiment during the growing season 2020. Characterisation of seeds comprised a multi-elemental analysis, determination of the dry matter, moisture, proteins, crude proteins, crude fats and total phenolics (TPC). The macro- (N, K, Mg, P, Ca, S) and microelement (Na, Cr, Mn, Fe, Co, Cu, Zn, Mo) content varied considerably among the studied common bean breeding lines. The highest coefficient of variation among the macroelements was determined for N and among the microelements - for Na. Proteins ranged from 17.8 to 27.9% and crude fats varied from 1.0 to 1.3%. The protein content is one of the most important common bean quality parameters and non-destructive protein determination using a whole seed analyser Infratec NOVA showed to be a reliable solution despite dealing with small samples of 35-40 middle-sized seeds. TPC varied from 1.5 to 4.1 mg GAE g^{-1}. Correlation between crude protein determined using the Kjeldahl method and protein determined non-destructively was very high. Higher variance was noticed for mineral content and TPC, which can potentially serve as a basis for further breeding. The common bean breeding material of different filial generations examined in this study showed great potential for registration of new varieties in the next few years.

Keywords: breeding line, common bean, elements, protein, crude fats, ICP-MS.

Barbara Pipan, PhD, Research associate, Crop Science Department, Agricultural Institute of Slovenia, Hacquetova ulica 17, SI-1000 Ljubljana, Slovenia, EU; phone: +386 (0)1 280 52 73, e-mail: barbara.pipan@kis.si

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INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is both nutritionally and economically the most important food legume in many countries worldwide (Singh et al. 2020). The main products of common bean for food production and culinary use rare dry beans harvested at the complete plant maturity stage, shell beans harvested at the physiological maturity, and snap beans that are tender pods with reduced fibre harvested before the seed development phase (Singh, Singh 2015).

Common bean is an inexpensive and important source of essential nutrients such as proteins, complex carbohydrates, vitamins and minerals as well as phenolic compounds, which exhibit high antioxidant capacity and promote health benefits by reducing oxidative stress (Mojica et al. 2015, Faria et al. 2018). Around twenty of the known elements are defined as essential ones because they act as important phytochemicals and have a significant role in the maintenance of human health (Dias et al. 2016, Zoroddu et al. 2019). Essential elements are classified into macroelements, namely Na, K, Mg, Ca, Cl, P and S, and microelements or trace minerals, eg. Fe, Cu, Mn, Zn, Co, Mo (Zoroddu et al. 2019). Mineral malnutrition is still among the most serious global challenges to humankind (Weber 2017). Beans are nutritionally valuable vegetable, providing proteins (22-45%) and complex carbohydrates (~60%), but are generally low in fat, with less than 5% energy from fat (Maphosa, Jideani 2017). Phenolic compounds in beans are present more in seed coats, with lower amounts accumulated in the cotyledons. Their concentration is highly influenced by growing conditions and genetic factors (García-Díaz et al. 2018). Bean breeding aims to sustainably increase grain production under challenging environmental conditions. The important breeding objectives are the resistance to biotic/abiotic stresses such as drought tolerance, pest and disease resistance (Keller et al. 2020), and high mineral content, especially for Fe and Zn (Mukankusi et al. 2019). Breeding genotypes capable of producing high-quality seeds are still scarce and should be a priority for breeders to ensure viable, well-adapted crops with high nutritional quality for future generations (Mecha et al. 2021).

In Slovenia, the long tradition of common bean production is evidenced by many local populations or traditional varieties. These are mainly grown at small-scale farms and gardens through low input production systems. In 2019, Slovenian dry beans production was carried out on 634 ha, producing a yield of 1499 t, while snap bean production covered 356 ha and yielded 1975 t (SI-STAT 2021). Breeding of the common bean at the Agricultural Institute of Slovenia was held by individual selection from autochthonous populations or directed selection of the desired genotypes in population breeding in the past. From 2016 onwards, the targeted hand-pollination of intermedius and determinate beans has been successfully applied through a national breeding program. Gene entry for different traits took place through crossings of parents' candidates and further selection based on observations and evaluations during the growing seasons (e.g. plant growth and development, resistance to pathogens and pests, tolerance to abiotic stress) and technological maturity (e.g. morphological characteristics, yield, seed quality). To accelerate an efficient breeding process, MAS (Marker Asssisted Selection) for selected agro-ecologic and nutritional quality characteristics using trait-related DNA markers was implemented (Pipan, Meglič 2019, Sedlar et al. 2020). The present study aimed to investigate the variation in multi-elemental composition, selected nutrients (moisture, dry matter, proteins, fats) and total phenolics in seeds of the most prospective common bean breeding materials.

MATERIAL AND METHODS

The most promising 11 breeding lines of common bean (*Phaseolus vulgaris* L.) from the Slovenian national common bean breeding programme were studied. The list of these lines and their basic characteristics are summarised in Table 1. An open-field experiment was performed at the Agricultural Institute of Slovenia (46°03′N, 14°31′E; 297 m a.s.l.) during the growing season 2020 according to the established cultivation technology on polyethylene (PE) black foil with a drip irrigation system. The experiment was carried out in twin rows with 10 cm distance in the row and 0.60 m between rows, thus as 450 seeds per breeding line to assure representativeness of each breeding line. For intermedius growth types, climbing support using beanpoles was arranged during the growing season. Soil type on the trial site is classified as Umbric Planosols (Plu) with the prevailing silt loam texture. Bulk density, pH value and the mean content of P, K and Mg in the upper 25 cm of soil are 1.61 g cm^{-3}, 6.3, 256 mg kg^{-1}, 200 mg kg^{-1} and 145 mg kg^{-1}, respectively. Growing conditions in the experimental field enable growth under natively present *Rhizobium phaseolus* without any additional fertilizers. After harvest, the pods were air-dried, and seeds manually removed. A climate-controlled drying chamber (25°C, 15% of relative humidity) was used to reduce water content in the seeds below 12%. The moisture (%) and protein (%) content of representative seed samples were determined non-destructively with a Infratec™ NOVA (Denmark) grain analyser using sample transport module (STM29; 75 mL) for small samples and FOSS global calibration curve for beans and pulses. All samples were then homogenised using a laboratory ball mill (Retsch MM 400) at a frequency of 30 Hz for 3 min.

For multi-elemental analysis, inductively coupled plasma mass spectrometry (ICP-MS) was used. Before the determination on an Agilent 7900 ICP-MS, microwave digestion and dilution of each sample was performed. The calibration curve was prepared using IV-STOCK-50 standard solution and single

Table 1

List of the common bean (*Phaseolus vulgaris* L.) breeding lines and their seed characteristics

Sample name	Breeding background	Filial generation	Germplasm origin	Plant growth type
KIS20_Pv_BL1	425 × 417	F7	Slovenian	determinate
KIS20_Pv_BL2	491 × 498	F6	Slovenian	determinate
KIS20_Pv_BL3	452 × dif_1(a)	F5	European	intermedius
KIS20_Pv_BL4	452 × dif_1(b)	F5	European	intermedius
KIS20_Pv_BL5	452 × dif_1(c)	F5	European	intermedius
KIS20_Pv_BL6	428 × češ	F5	Slovenian	determinate
KIS20_Pv_BL7	425 × 301	F6	Slovenian	determinate
KIS20_Pv_BL8	131 × 867	F6	Slovenian	determinate
KIS20_Pv_BL9	306 × 452	F6	European	determinate
KIS20_Pv_BL10	309 × 425	F6	Slovenian	determinate
KIS20_Pv_BL11	417 × 316	F6	Slovenian	determinate
Sample name	culinary use (snap beans, dry beans)	seed coat characteristics		
		number of colours	main colour (largest area)	predominant secondary colour
KIS20_Pv_BL1	dual use	one	brown	/
KIS20_Pv_BL2	dry beans	more than two	brown; violet; red	brown
KIS20_Pv_BL3	dual use	one	grey	/
KIS20_Pv_BL4	dual use	more than two	beige	violet; brown; grey
KIS20_Pv_BL5	dual use	one	white	/
KIS20_Pv_BL6	dry beans	two	beige; brown	red
KIS20_Pv_BL7	dry beans	two	beige; brown	red
KIS20_Pv_BL8	dry beans	more than two	beige; brown	red; brown
KIS20_Pv_BL9	dry beans	two	beige; brown	red; violet
KIS20_Pv_BL10	dual use	more than two	brown	brown; grey
KIS20_Pv_BL11	dry beans	two	brown; red	/

standard solutions of P and S (Inorganic Ventures, USA) were added separately to the mixture. The accuracy of the results was checked with two certified reference materials NIST SRM 1573a Tomato leaves and NIST SRM 1547 Peach leaves (Gaithersburg, MD, USA). All results are quoted on a dry weight basis and expressed as g kg^{-1} for macroelements or mg kg^{-1} for microelements. To compare results from the non-destructive method obtained with a grain analyser, crude protein (and total N content) was analysed according to the Kjeldahl method (ISO 5983:2) using factor 6.25. Crude fat was analysed with petroleum ether extraction (152/2009 App. III H). Total phenolics (TPC) were determined using the Folin-Ciocalteu (FC) assay, as first described by Singleton and Rossi (1965). Samples were first extracted with 70%

ethanol using an ultrasonic bath and thoroughly mix on a vortex several times. After 1 h extraction at room temperature, sample solutions were centrifuged (15.000×g; 5 min) and filtered through 0.45 µm PTFE syringe filters. A reagent mixture was prepared with the mixing diluted FC reagent and sample extract, followed by the addition of 20% Na_2CO_3. Total phenolics were determined in triplicates and expressed as mg gallic acid equivalent (GAE) g^{-1}.

The differences among the breeding lines were analysed through least-squares mean tests (Statgraphics Centurion XVI 2009). Statistics included mean, minimum (Min), maximum (Max), standard deviation (SD) and coefficient of variation (CV). Principal component analysis (PCA) was performed to reveal the most influential variables. A dendrogram was drawn to combine individual variables into larger clusters using the Ward's method and Squared Euclidian distance.

RESULTS AND DISCUSSION

This manuscript describes the nutritional seed characteristics of the most perspective and diverse Slovenian common bean (*Phaseolus vulgaris* L.) breading material from different filial generations (F5 - F7) for the first time. A total of twenty parameters were determined in the seeds of several common bean breeding lines and the results showed high variability among them. Multi-elemental composition and summary statistics for seeds of the studied breeding lines are shown in Table 2. A total of fourteen elements were determined and divided into macroelements (g kg^{-1}) N, Mg, P, S, K and Ca, and microelements (mg kg^{-1}) Mn, Fe, Cu, Na, Cr, Co, Zn and Mo (Table 2). These elements can be ordered from the most to the least abundant according to the data as follows: N (29-45 g kg^{-1}), K (15-19 g kg^{-1}), P (5-7 g kg^{-1}), S (2-3 g kg^{-1}), Ca (1-2 g kg^{-1}), Fe (61-190 mg kg^{-1}), Zn (27-42 mg kg^{-1}), Mn (13-16 mg kg^{-1}), Na (6-68 mg kg^{-1}), Cu (7-13 mg kg^{-1}), Mo (0.3-3.9 mg kg^{-1}), Cr (0.2-2.3 mg kg^{-1}) and Co (0.05-0.14 mg kg^{-1}). The highest coefficient of variation among macroelements was calculated for N (14.20%) and the lowest for K (6.87%), and among the microelements for Na (133.84%) and Mn (8.31%), respectively. The highest level for the most abundant element N was seen for KIS20_Pv_BL3 (44.6 g kg^{-1}). Palčić et al. (2018) reported similar contents of macroelements in Croatian common bean landraces. Beebe et al. (2000) reported a lover range for the microelement Fe (34-89 mg kg^{-1}) and a similar range for Zn (21-54 mg kg^{-1}) among 2000 analysed accessions at CIAT. Delfini et al. (2020) analysed 1512 accessions from Brasilian common bean germplasm to evaluate the diversity of the mineral composition. Similarly to our study, the highest variability in nutrients was observed for Na.

Nutritional characteristics and summary statistics for seeds of 11 breeding lines are presented in Table 3. Dry matter in seeds ranged from 86.32%

Table 2

Multi-elemental composition and summary statistics for seeds of 11 common bean (*Phaseolus vulgaris* L.) breeding lines

Sample name	Macroelement (g kg^{-1} DM)					
	N	K	P	S	Mg	Ca
KIS20_Pv_BL1	33.7	17.7	5.47	2.83	1.85	1.43
KIS20_Pv_BL2	36.9	16.3	5.90	2.72	1.64	1.08
KIS20_Pv_BL3	44.6	17.3	6.81	3.08	1.73	1.38
KIS20_Pv_BL4	38.5	18.0	6.16	2.77	1.64	1.62
KIS20_Pv_BL5	42.7	18.5	7.15	3.30	1.90	1.90
KIS20_Pv_BL6	28.5	15.2	4.73	2.13	1.81	1.52
KIS20_Pv_BL7	31.4	17.5	5.21	2.39	1.82	1.41
KIS20_Pv_BL8	31.9	17.0	5.29	2.53	1.98	1.70
KIS20_Pv_BL9	35.1	18.2	6.01	3.01	1.82	1.18
KIS20_Pv_BL10	31.9	18.4	5.67	2.53	1.93	1.33
KIS20_Pv_BL11	106/8	15.2	5.16	2.41	1.56	1.84
Mean ±SD	35.2±5.0	17.2±1.2	5.77±0.73	2.70±0.34	1.79±0.13	1.49±0.26
Min - Max	28.5-44.6	15.2-18.5	4.73-7.15	2.13-3.30	1.56-1.98	1.08-1.90
CV (%)	14.20	6.87	12.61	12.75	7.40	17.30

Sample name	microelement (mg kg^{-1} DM)							
	Fe	Zn	Mn	Na	Cu	Mo	Cr	Co
KIS20_Pv_BL1	72.9	32.9	14.7	67.7	7.6	0.40	0.40	0.06
KIS20_Pv_BL2	70.6	33.0	12.8	9.2	9.0	1.84	0.31	0.07
KIS20_Pv_BL3	83.7	36.6	13.1	6.3	11.8	2.71	1.57	0.09
KIS20_Pv_BL4	193.8	38.6	15.0	10.9	12.7	3.94	2.29	0.14
KIS20_Pv_BL5	86.5	42.4	15.3	9.0	11.6	2.92	0.28	0.12
KIS20_Pv_BL6	67.5	27.0	13.2	8.3	7.2	1.14	0.23	0.07
KIS20_Pv_BL7	63.0	31.1	13.5	7.4	7.4	0.32	0.22	0.07
KIS20_Pv_BL8	63.8	29.2	15.3	10.1	8.6	0.61	0.37	0.07
KIS20_Pv_BL9	61.6	30.8	12.8	6.1	7.1	1.08	0.20	0.05
KIS20_Pv_BL10	60.8	30.2	14.9	5.6	7.3	0.53	0.22	0.05
KIS20_Pv_BL11	61.3	29.5	16.1	7.8	7.2	0.27	0.26	0.07
Mean ±SD	80.5 ±38.6	32.9 ±4.6	14.3 ±1.2	13.5 ±18.1	8.9 ±2.1	1.43 ±1.25	0.58 ±0.69	0.08 ±0.03
Min - Max	60.8-193.8	27.0-42.4	12.8-16.1	5.6-67.7	7.1-12.7	0.27-3.94	0.20-2.29	0.05-0.14
CV (%)	47.96	14.00	8.31	133.84	24.13	87.33	119.67	36.10

SD – standard deviation, CV – coefficient of variation, DM – dry matter

Table 3

Nutritional characteristics and summary ststistics for seeds of 11 common bean (*Phaseolus vulgaris* L.) breeding lines

Sample name	Dry matter (%)	Moisture (%)	Protein (%)	Crude protein (%)	Crude fat (%)	TPC (mg GAE g^{-1})
KIS20_Pv_BL1	88.90	11.10	23.53	21.09	1.20	2.49
KIS20_Pv_BL2	89.25	10.75	24.08	23.09	1.00	2.84
KIS20_Pv_BL3	91.16	8.84	24.81	27.85	1.20	2.64
KIS20_Pv_BL4	86.32	13.68	24.22	24.06	1.10	2.57
KIS20_Pv_BL5	91.37	8.63	25.13	26.71	1.10	1.48
KIS20_Pv_BL6	89.08	10.92	22.93	17.80	1.20	2.86
KIS20_Pv_BL7	88.47	11.53	23.65	19.62	1.10	2.93
KIS20_Pv_BL8	89.70	10.30	23.24	19.91	1.30	3.03
KIS20_Pv_BL9	89.10	10.90	23.92	21.95	1.10	4.09
KIS20_Pv_BL10	89.28	10.72	23.44	19.91	1.20	2.40
KIS20_Pv_BL11	89.67	10.33	23.54	20.21	1.30	3.27
Mean ±SD	89.30±1.34	10.70±1.34	23.86±0.66	22.02±3.13	1.16±0.09	2.78±0.64
Min - Max	86.32-91.37	8.63-13.68	22.93-25.13	17.80-27.85	1.00-1.30	1.48-4.09
CV (%)	1.50	12.49	2.77	14.21	7.94	22.83

TPC – total phenolics, SD – standard deviation, CV – coefficient of variation

to 91.37%, and moisture content was from 8.63% to 13.68%. The protein content determined non-destructively using a grain analyser varied from 22.93 to 25.13%, while crude protein varied over a larger range, from 17.80 to 27.85%. However, the calculated correlation between these two parameters was very high (0.96), which means that the use of a grain analyser Infratec NOVA enabled us to determine the protein content in whole grains reliably despite having small samples, approximately 35-40 middle-sized seeds. The protein content is an important seed quality parameter and reliable non-destructive protein determination on a small seed sample is very useful for a breeder. The crude fat content in seeds of breeding lines varied over a smaller range of 1.00% to 1.30%. The protein and fat contents are in accordance with previous reports on dry beans (Maphosa, Jideani 2017, Carbas et al. 2018, Celmeli et al. 2018). The TPC ranged considerably from 1.48 mg GAE kg^{-1} to 4.09 mg GAE kg^{-1}. The highest TPC was determined for KIS20_Pv_BL9 and the lowest for KIS20_Pv_BL5. A similar range for TPC was reported by Yang et al. (2020) in their study on common beans from China. The highest coefficient of variation was calculated for the parameter TPC (22.83%), and the lowest for dry matter (1.50%). Low coefficients of variation were observed also for protein (2.77%) and crude fat (7.94%). Variance in the protein content was relatively low, which is a positive characteristic since proteins provide high energy to the nutritional value of the beans.

The protein concentrations were comparable with those from the study on 1512 accessions from Brasilian common bean germplasm, where the protein content ranged from 16.9 to 24.8% (Delfini et al. 2020). Although it was slightly lower than in our study, the variance was higher. The coefficient of variation in the mentioned research was 5.66, but it was still the lowest among nutrients, same as in our study.

Based on the cluster analyses (the Ward's method) performed on the studied parameters, common bean breeding lines were grouped into clusters. A dendrogram for 11 studied breeding lines along with representative seed photos is presented in Figure 1, where the combination of twenty variables established three main clusters. The highest number of breeding lines was ranked in cluster 3 (six), followed by cluster 1 (three) and cluster 2 (two). A PCA plot of component weights for twenty variables is presented in Figure 2.

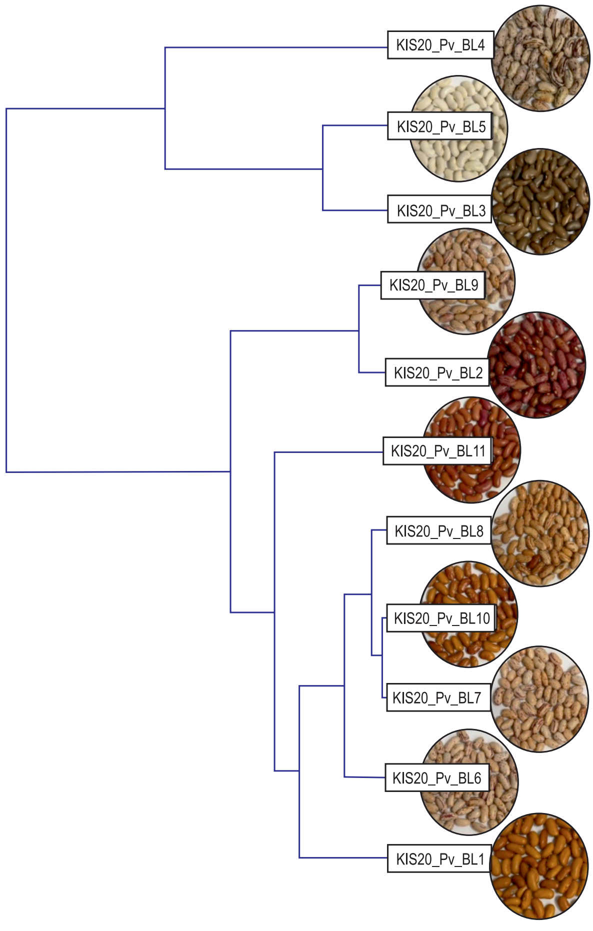

Fig. 1. Dendrogram and representative photo of the seeds of the studied common bean breeading material

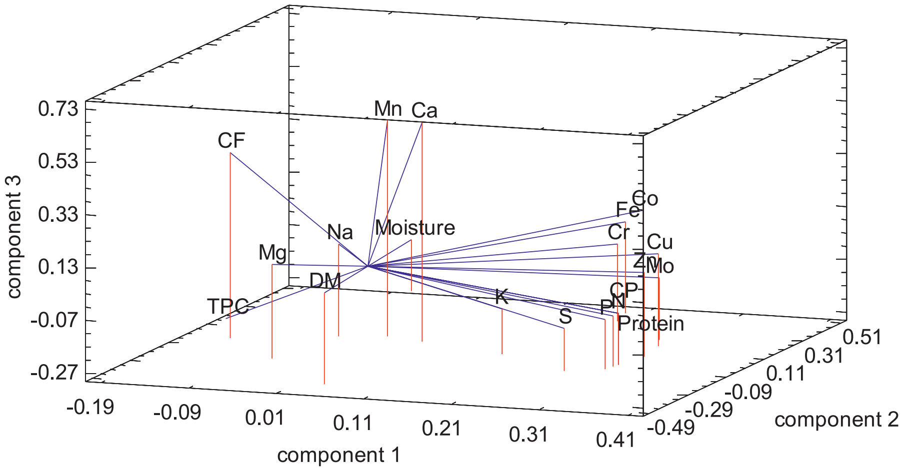

Fig. 2. PCA plot of component weights

The content of Zn, N, Cu, protein and P were the major contributors to component 1, moisture, the content of Fe and Cr to component 2, and content of Ca, Mn and crude fat to component 3.

CONCLUSIONS

This research represents the first overview of multi-elemental composition and nutritional characteristics of the most prospective common bean breeding lines from the national Slovenian common bean breeding programme. Correlation between crude protein determined using the Kjeldahl method and protein determined non-destructively on whole grains using an analyser Infratec NOVA was very high (0.96). The higher variance was noticed for mineral content and total phenolics which can potentially serve as a basis for further breeding steps. Among microminerals, N achieved the highest variance, while among microelements the highest variance was observed for Na. Analysis of nutrients showed the highest variance for crude protein and the lowest for dry matter. In general, breeding material of different filial generations (F5 - F7) shows great potential for possible registration of new common bean varieties with different traits of interest shortly.

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