

ARTICLE

Chemical composition as related to seed color of common bean

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Abstract - This study aimed to quantify the levels of protein and minerals in common bean lines from the Germplasm Bank of Universidade Federal de Lavras and to investigate the relationship between color and the mineral and protein contents of the beans. One hundred common bean lines with carioca, black and other grain color patterns were assessed for the protein levels and the mineral contents of phosphorus, potassium, calcium, magnesium, copper, manganese, zinc, and iron. Genetic variability was detected among the different groups and also between lines within each group. In general, the protein, iron and zinc contents were highest in the black bean lines. The carioca grain was outstanding for manganese and magnesium and the other grain colors for calcium. Positive correlations between most nutrients were observed. This indicates the possibility of obtaining lines with higher nutritional value by selection.

Key words: *Phaseolus vulgaris* L., nutrients, correlation.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is a nutrient-rich food that contains nutrients essential for humans, such as proteins, minerals, vitamins (especially B-complex), carbohydrates, and fiber. In Brazil, common bean consumption supplies on average 28% of the daily protein and 5-11% of the daily calorie demand (Silva 2010).

The protein quality in common bean is high and many cultivars available for cultivation in Brazil have sufficiently high levels of essential and nonessential amino acids to meet the daily nutritional needs (Ribeiro et al. 2007), according to standards of the Food and Agriculture Organization - FAO - (FAO 1998). The protein content of common beans is about half of the content in soybean, while the protein digestibility is higher (78.70%) (Pires et al. 2006).

For minerals, the main bioavailable sources are foods of animal origin (Costa and Liberato 2003). However, in view of the high cost, they are inaccessible to many people. In view of the high mineral content in the relatively cheap seeds, the consumption of common beans is beneficial to health (Mesquita et al. 2007, Ribeiro et al. 2008, Jost et al. 2009) and can be a means of prevention of a number of mineral deficiencies. The high nutritional quality of common beans and their importance in human nutrition has aroused

the interest of national and international breeding programs to verify the existence of variability for these nutrients in their genebanks (Mesquita et al. 2007, Ribeiro et al. 2008, Bassinello et al. 2010). As lines with higher nutritional quality are identified, improvement programs that combine a high nutritional quality with agronomic traits of interest can be initiated.

The type of common beans consumed in different regions differs, especially with regard to grain color. In the Zona da Mata of Minas Gerais, e.g. black or red beans are preferred. In other regions the carioca bean is predominant. This preference is often explained by some factor associated with the chemical composition. However, no studies have been published comparing the chemical composition of representative samples of the different bean color patterns.

In the over 40 years since the beginning of the common bean breeding program of the Federal University of Lavras (UFLA) in 1970, many common bean lines have been collected, selected and stored in the germplasm bank. As there is no information about the chemical composition of the grains of these lines, this study was conducted with the objective of quantifying the protein and mineral levels from a representative sample of these lines and to investigate the relations between seed color and protein and mineral contents.

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MATERIAL AND METHODS

One hundred lines of the Federal University of Lavras (UFLA) Common Bean Germplasm Bank (51 with carioca, 13 with black and 36 with other grain colors) were sown in an experimental field of UFLA, in the southern region of the state of Minas Gerais (lat 21° 14' S, long 45° 59' W, 919 m asl). The seeds were sown in plots with two 2-m rows, spaced 0.50 m apart, in the dry season (sowing in February 2009). Fertilization consisted of 400 kg ha⁻¹ NPK fertilizer (8- N; 28- P₂O₅; 16 -K₂O) at sowing and 150 kg ha⁻¹ of ammonium sulfate were top dressed, 25 days after sowing. In this way, 62 kg ha⁻¹ N was applied to the areas. Sprinkler irrigation was applied when necessary.

After harvest, three 50-g seed samples per plot were analyzed in a completely randomized design for the protein and mineral contents (P, K, Ca, Mg, Cu, Mn, Zn, and Fe). For this purpose, the seeds were air-dried to a moisture content of approximately 13%. Each sample was ground in a micro-mill (particle size < 1 mm), stored in completely sealed plastic bags, labeled, and refrigerated until laboratory tests. The chemical analyses were performed in the leaf analysis laboratory of the UFLA Chemistry Department.

The protein content was quantified based on the total N content, by the Kjeldahl method, which consists in heating the N substance in concentrated sulfuric acid in the presence of a catalyst, so that N and H present are converted into ammonium salt. Nitrogen in the form of ammonia is removed in the distillation stage. The distillate is then titrated and the N content of the sample determined, which is converted to crude protein by the factor 6.25 (AOAC 2005).

For the mineral analysis, nitroperchloric digestion was carried out with 0.5 g of each sample. At the end of the digestion, the extract volume was completed with deionized water to 15 mL. This digestion removes the elements from the organic compounds of the sample or adsorbed to them. Ca, Mg, Cu, Mg, Fe, and Zn contents were determined by atomic absorption spectroscopy, using a SpectrAA 110 model (Varian Inc.), calibrated for each element under specific conditions of wavelength, slit width and gas mixture. P and S were analyzed by UV spectrophotometry in a Perkin Elmer Lambda 25 UV/Vis and K by flame emission photometry in a Micronal B262 unit. For all analyses, the procedures described by Malavolta et al. (1997) were used. After the analysis, the data were corrected to a dry basis by the method of moisture content, measured by the water loss from the sample at a drying temperature of 100-105 °C (AOAC 2005).

The data of the chemical analyses of the 100 lines were subjected to analysis of variance, according to the following statistical model, considering all effects random, except the mean: $Y_{ij} = \mu + I_i + e_{ij}$, where: Y_{ij} : value observed in the line, within the replication j ($j=1, 2, 3$); μ : general mean of the experiment; I_i : effect of line i , where $i = 1, 2, \dots, 100$; e_{ij} : experimental error associated with observation Y_{ij} , assuming that errors are independent and normally distributed with mean 0 and variance σ_e^2 . For the statistical analyses the softwares Genes (Cruz 2006) and SAS Institute (2008) were used.

The selection accuracy was estimated by the methodology proposed by Resende and Duarte (2007). Means were compared by the Scott-Knott (1974) grouping test. The heritability was also estimated (Bernardo 2002), considering that the selection would be based on the means of the lines and confidence intervals, according to Knapp et al. (1985). The correlation coefficients between nutrients were estimated by the methodologies of Vencovsky and Barriga (1992).

To obtain a selection index involving the data of all nutrients, the sum of the standardized variables (Z) was estimated (Mendes et al. 2009). As variable Z assumes negative and positive values, the value three was added to make all values positive. In this case, the mean of the lines would come to be three instead of zero. Analysis of variance of $\sum Z$ was performed and the coefficient of variation of Z (CVZ) per line estimated.

RESULTS AND DISCUSSION

Initially it was found that all accuracy estimates were high (> 90%) (Table 1), indicating good experimental precision (Resende and Duarte 2007). The source of variation of lines and of the lines of each color group differed significantly from each other ($P \leq 0.01$) (Table 1). This result indicates the existence of genetic variability between the different groups and also between the lines of each group.

The existence of a difference between the lines for all nutrients may also be detected by the estimates of the mean amplitude of variation of line means. The difference was especially significant for Mn, Zn and Fe. Several studies in the literature quantified the nutritional contents of common bean (Osborn 1988, Maldonado and Sarmám 2000, Araújo et al. 2003, Mesquita et al. 2007, Ribeiro et al. 2007). The averages for the minerals P, Cu and Mn found in this study were higher than previously reported (Barampama and Simard 1993), whereas the average contents of protein and the other minerals were similar to those reported in other studies (Osborn 1988, Mesquita et al. 2007). This variation can be ascribed to the genotype and environmental conditions (Ribeiro et al. 2007).

Table 1. Summary of the analysis of variance, heritability estimates with their lower (LL) and upper limits (UL), accuracy (\hat{r}_{gg}) and general mean for protein and mineral (P, K, Ca, Mg, Cu, Mn, Zn, and Fe) contents of the 100 tested common bean lines of the Common Bean Germplasm Bank

SV	df	MS								
		Protein	Phosphorus	Potassium	Calcium	Magnesium	Copper	Manganese	Zinc	Iron
Lines	99	15.0400**	0.0100**	0.0500**	0.0600**	0.0010**	16.4900**	98.6400**	152.2600**	1061.8100**
Between groups	2	45.1200**	0.0800**	0.1700**	0.3400**	0.0100**	12.4500**	27.6400**	307.0000**	865.1900**
between carioca grain lines	52	14.1200**	0.0100**	0.0500**	0.0300**	0.0007**	15.5500**	90.3200**	153.4700**	1228.8000**
between black grain lines	12	8.1000**	0.0030**	0.0300**	0.0600**	0.0010**	15.9300**	97.2200**	141.9600**	1203.6300**
between colored grain lines	33	17.1600**	0.0080**	0.0500**	0.0900**	0.0010**	18.4500**	116.5700**	144.7200**	758.9900**
Error	200	0.7800	0.0002	0.0015	0.0019	0.0001	0.2643	1.2762	3.2617	27.5801
h ² (%)		94.78	98.29	97.01	96.82	94.41	98.40	98.71	97.86	97.40
LL		92.59	97.56	95.75	95.35	92.00	97.72	98.16	96.95	96.31
UL		96.26	98.77	97.86	97.66	95.96	98.85	99.07	98.46	98.14
\hat{r}_{gg} (%)		97.36	99.14	98.49	98.40	97.15	99.20	99.35	98.92	98.69
Mean of carioca lines		24.54 c ¹	0.53 a	1.81 a	1.40 b	0.26 a	11.03 b	22.98 a	48.62 b	89.00 b
Mean of black lines		26.08 a	0.53 a	1.83 a	1.40 b	0.24 b	11.70 a	21.65 b	52.94 a	92.21 a
Mean of colored lines		25.34 b	0.50 b	1.76 b	1.48 a	0.23 b	11.55 a	22.70 a	48.80 b	85.10 c
General mean		25.00	0.52	1.80	1.43	0.25	11.30	22.71	49.24	88.14
Range (%)		21.6	23.0	19.4	15.4	12.0	31.9	48.4	34.0	69.7

** Significant by the F test at 1% probability.

¹ Means followed by the same letter in a column belong to the same group, by Scott Knott (1974) analysis, at 5% probability.

The heritability estimates, all non-zero and above 94%, allow the conclusion that the chance of success with selection for lines with outstanding nutritional quality is high (Table 1). The high heritability estimate obtained in this study must be attributed to the fact that the evaluated lines are from a genebank involving lines with differentiated expression of several traits. Many of these phenotypes are not necessarily related to the acceptance or not of a line by farmers. However, the heritability value indicates that there is a possibility of success in identifying lines with improved nutritional value.

As the sample contained beans of different skin colors, associations were sought between color and nutrient content. The lines were grouped into carioca, black and other color patterns. As already mentioned, significant differences ($P \leq 0.01$) between groups were found (Table 1). In general, black bean lines stood out for protein, Fe and Zn. For Ca, the average levels were higher in the lines of the colored group. Carioca beans, which are the most consumed in Brazil, stood out with highest average

Mg and Mn contents. Although the protein content of the black group lines was on average higher (26.08%), line CIAT-A-257 of the red bean (color) group had the highest protein percentage (30.4%) (Table 2). It is noteworthy that, as this line is not adapted to the regional growing conditions, it could be used in the hybridization program, in particular with the carioca grain lines, with a view to increase its protein content.

Unfortunately, there is little information in the literature relating nutrient contents with the skin color. Moraghan et al. (2002) observed that the amount of iron found in beans can vary according to the color; they attributed this difference to the tannin content, which is higher in black grain cultivars, since tannins can complex iron. Lombardi-Boccia et al. (1998) reported a higher Ca content in colored beans, especially in white beans. In this study, where a representative sample of the different colors was used, it can be inferred that a considerable proportion of nutrients must be located in the skin, as reported elsewhere (Leleji et al. 1972, Moraghan and Etchevers 2006, Jost et al. 2009).

Table 2. Classification of 10 common bean lines with the highest and 10 lines with the lowest levels for each nutrient (protein, P, K, Ca, Mg, Cu, Mn, Zn, and Fe)

Rank	Line	Protein (%)	Line	P (g 100g ⁻¹)	Line	K (g 100g ⁻¹)
1	Ciat-A-257	30.40	CI-128	0.69	BRS Campeiro	2.06
2	Bolinha	30.19	CI-164	0.69	CI-128	2.05
3	Iapar 81	29.25	R-29	0.64	CNF 05	2.04
4	Linea 29	29.10	Pérola	0.63	G 2333	2.02
5	Roxo PV	28.95	BRSMG Pioneiro	0.62	ICA Pijão	2.00
...
96	Small White	20.60	Batatinha	0.40	Bolinha	1.60
97	RC-1.8	20.44	Ouro	0.40	Mineiro Precoce	1.52
98	P-1. 103	20.34	Esal 627	0.40	Esal 543	1.48
99	FP-5.9	20.30	Esal 512	0.40	Flor de Mayo	1.46
100	BRSMG Majestoso	19.60	Small White	0.40	Esal 521	1.45

to be continued...

Rank	Line	Ca (g 100g ⁻¹)	Line	Mg (g 100g ⁻¹)	Line	Cu (mg kg ⁻¹)
1	Safira	1.80	Carioca	0.29	CI-128	15.99
2	Baetão	1.76	Franguinho	0.28	CNF 10	15.60
3	Fortuna 1895	1.76	AN 910522	0.28	Esal 515	15.45
4	CNF 05	1.75	RP-1	0.28	Fortuna 1895	15.45
5	Ouro	1.73	BRSMG Pioneiro	0.28	Milionário	15.10
...
96	BRSMG Majestoso	1.24	AN 910523	0.20	Paraná	7.40
97	IAC Maravilha	1.24	Batatinha	0.20	Esal 518	7.02
98	IAC CAR. Pyata	1.23	Roxo PV	0.20	Esal 521	6.68
99	MAI-8.9	1.21	Mineiro Precoce	0.20	Flor de Mayo	5.78
100	BRSMG Majestoso	19.60	Small White	0.40	Esal 516	5.76

to be continued...

Rank	Line	Mn (mg kg ⁻¹)	Line	Zn (mg kg ⁻¹)	Line	Fe (mg kg ⁻¹)
1	VR-3	36.78	R-1	65.50	P-180	161.50
2	AN 910523	36.13	CI-128	64.61	R-1	146.80
3	AN 910522	33.50	Milionário	63.50	Linea 29	135.30
4	Dor 95	33.44	AN 910523	60.83	Small White	133.20
5	Milionário	33.40	ICA Pijão	60.00	AN 910523	125.10
...
96	Esal 521	13.40	Mineiro Precoce	38.81	BP -28	58.95
97	Esal 543	13.11	BP -28	37.22	Esal 521	58.12
98	Esal 516	12.45	Esal 521	35.41	Esal 518	56.46
99	Iapar 81	10.58	Flor de Mayo	32.51	Batatinha	55.71
100	Mineiro Precoce	9.19	Esal 516	29.33	Flor de Mayo	54.20

The correlation estimates (Table 3) show a positive association among most nutrients. The same was observed by Beebe et al. (2000). This situation is favorable for improving nutritional quality, indicating the possibility of obtaining lines with higher nutritional value, since selection may be effective for two or more minerals.

To identify lines with superior chemical composition, the selection index based on the sum Z ($\sum Z$) was used. The

line with the highest $\sum Z$ estimate associated with a small coefficient of variation (CV) is ideal, indicating the line with highest levels of most nutrients and a small variation in the chemical composition as a whole. The $\sum Z$ and CV estimates were plotted on a graph (Figure 1). It was observed that most of the lines associated high $\sum Z$ with a low CV estimate, indicating that the chemical composition of the lines being improved and/or planted in the state is good. Nevertheless,

Table 3. Phenotypic genetic and environmental correlation coefficients between nutrients levels assessed in 100 common bean lines from the Common Bean Germplasm Bank

	Protein	Phosphorus	Potassium	Calcium	Magnesium	Copper	Manganese	Zinc	Iron
Protein	1	0.35 ± 0.09 ¹ (0.34) **	-0.12 ± 0.10 (-0.11)	0.10 ± 0.10 (0.10)	0.09 ± 0.11 (0.09)	0.29 ± 0.10 (0.28) **	-0.15 ± 0.10 (-0.14)	0.24 ± 0.10 (0.24) *	-0.17 ± 0.10 (-0.16)
Phosphorus	0.14	1	0.37 ± 0.09 (0.37) **	-0.14 ± 0.10 (-0.14)	0.32 ± 0.10 (0.32) **	0.27 ± 0.02 (0.27) **	0.13 ± 0.10 (0.13)	0.53 ± 0.07 (0.53) **	0.05 ± 0.10 (0.05)
Potassium	0.02	0.49	1	0.10 ± 0.10 (0.10)	0.17 ± 0.10 (0.18)	0.44 ± 0.08 (0.44) **	0.26 ± 0.10 (0.26) **	0.49 ± 0.08 (0.49) **	0.31 ± 0.03 (0.31) **
Calcium	0.10	0.11	0.20	1	-0.22 ± 0.10 (-0.20)	0.60 ± 0.07 (0.58) **	0.24 ± 0.10 (0.24) *	0.08 ± 0.10 (0.08)	0.13 ± 0.10 (0.08)
Magnesium	0.14	0.39	0.39	0.27	1	0.08 ± 0.71 (0.08)	0.35 ± 0.09 (0.35) **	0.19 ± 0.08 (0.20) *	0.03 ± 0.10 (0.03)
Copper	0.01	0.30	0.47	0.12	0.25	1	0.52 ± 0.07 (0.52) **	0.63 ± 0.06 (0.62) **	0.41 ± 0.09 (0.41) **
Manganese	0.11	0.29	0.44	0.17	0.28	0.48	1	0.53 ± 0.07 (0.52) **	0.37 ± 0.09 (0.37) **
Zinc	0.14	0.53	0.48	0.28	0.50	0.46	0.44	1	0.55 ± 0.07 (0.54) **
Iron	0.26	0.03	0.09	0.14	0.08	0.14	0.11	0.08	1

** , * Significant at 1 and 5% probability by the t test, respectively. ¹ Standard errors of genetic correlations (Holland 2006). In brackets: phenotypic correlation; upper diagonal: genetic correlation and lower diagonal: environmental correlation.

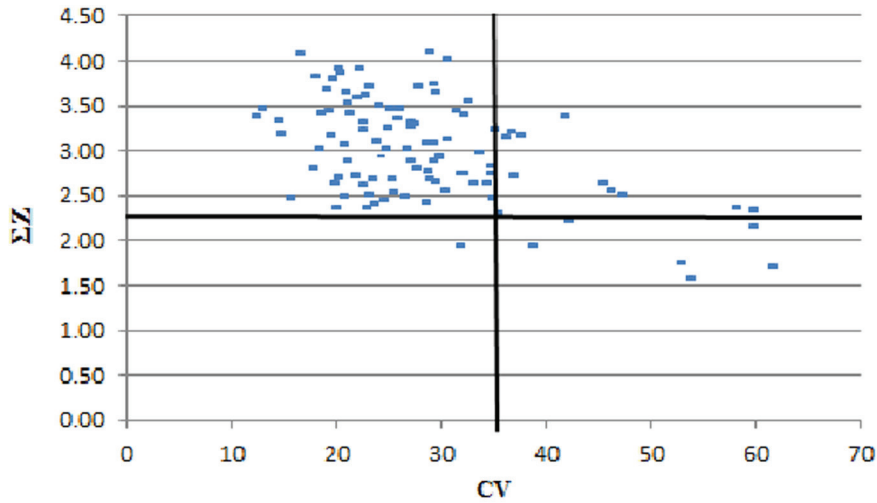


Figure 1. Graphic dispersion of the 100 lines evaluated for the sum of standardized variables Z (ΣZ) and the coefficient of variation of each line (CV).

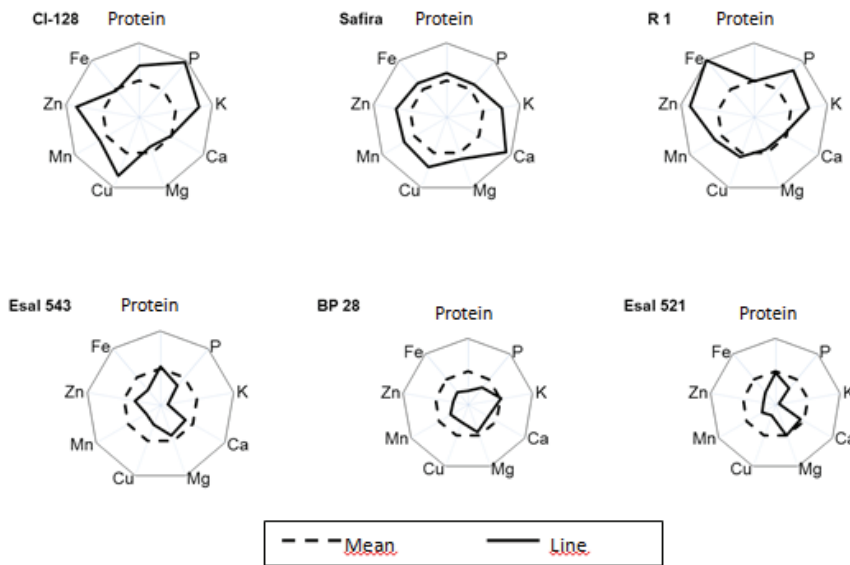


Figure 2. Graphical representation of the standardized values for protein and minerals (P, K, Ca, Mg, Cu, Mn, Zn, and Fe) of the three lines with highest and three lines with lowest ΣZ estimates.

the possibility of a successful selection for each particular nutrient or for the chemical composition as a whole is evident. In this context, the lines CI 128, Safira and RP-1 had the highest ΣZ estimates and lowest CV (Figure 2). The lines CI-128 and RP-1 belong to the Carioca group and Safira, to the colored group (red coat). On the other hand, the worst lines were ESAL 543 (red skin), BP-28 (Carioca) and ESAL 521 (Carioca).

It is worth noting that although some currently recommended lines had undergone selection for mineral and protein contents, the levels of all nutrients were high, e.g., in the case of the recently recommended BRS Cometa and BRSMG Pioneiro (Moreira et al. 2006, Faria et al. 2007) and RP-1 which is about to be officially recommended (Table 2). These were always ranked among the best 15 lines.

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Composição química e sua associação com a cor das sementes de feijão comum

Resumo – Visando a quantificar os teores de proteína e minerais de linhagens de feijão pertencentes ao Banco de Germoplasma da Universidade Federal de Lavras e verificar se existe relação entre a cor do grão e o teor de nutrientes foi realizado o presente trabalho. Foram avaliadas 100 linhagens de feijão dos tipos carioca, preto e cores, quanto aos teores de proteína e dos minerais fósforo, potássio, cálcio, magnésio, cobre, manganês, zinco e ferro. Observou-se variabilidade genética entre os diferentes grupos avaliados e, também, entre as linhagens dentro de cada grupo. Em média, as linhagens de grãos pretos se destacaram para os teores de proteína, ferro e zinco. Já os grãos tipo carioca, para manganês e magnésio e os do tipo cores, para cálcio. Detectou-se correlação positiva entre a maioria dos nutrientes, condição essa favorável para a seleção.

Palavras-chave: *Phaseolus vulgaris L.*, nutrientes, correlação.

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