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Research article

Chemical and nutritional characterization of bean genotypes (*Phaseolus vulgaris* L.)

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Abstract: Beans (*Phaseolus vulgaris* L.) are the most important legume for human consumption, and have essential nutrients for physiological processes. As examples, we can mention Fe and Zn. As a strategy to increase these nutrients in the population's diet, beans stand out as a potential candidate, as it already has high levels of these minerals compared to other foods. The objective of this study was to evaluate 14 bean genotypes from the EPAGRI Bean Genetic Improvement Program, Brazil, regarding Fe and Zn content, availability of Fe and Zn in vitro, amount of phytic acid, tannins, proteins, and moisture. The results showed a high positive correlation between the total amount of Zn with bioavailable Zn; the total amount of Fe, and the total amount of Zn; and the total amount of bioavailable Fe with Zn. Furthermore, there were statistically significant differences for all characteristics evaluated, showing an interesting variability that can be considered for future crosses aiming at nutritional quality.

Keywords: Fe; mineral availability; nutrition content; Zn; breeding

1. Introduction

Brazil is the third-largest producer of beans in the world. The production of 2019/2020 was about 3.02 million tons, an increase of 1.5% compared with the 2018 harvest [1,2].

In Brazil, the consumption of beans per capita is about14 kg per year [3]. It is estimated that, on

average, more than 200 million people are totally dependent on beans in their daily consumption [4]. In African countries, where consumption is higher, it reaches 66 kg per person per year, being the second most important item in the supply of total daily calories. In addition, beans are a central element in the diet of more than 400 million people in the tropics.

Beans significantly strengthen food and nutritional security among low-income consumers, reducing the risk of cardiovascular disease and diabetes. The main reason for this is the large amount of minerals, macronutrients and micronutrients. In 100 g of cooked beans, on average, the following values are found: 9.0 g of protein, 2.0 mg of Fe, and 0.9 mg of Zn [5]. It is estimated that, worldwide, 60 to 80% of people have some nutritional deficiency. Of these, about 17% suffer from Zn deficiency and 4.4% of child deaths are associated with Zn deficiency [6,7]. According to WHO, 30% of the population suffers from Fe deficiency, which leads to the appearance of anemias and other various complications [8]. Anemia is due to the low bioavailability of Fe in foods, especially those of plant origin.

According to [9], variations among bean grains can occur when referring to their proximate composition, minerals, lignin, polyphenols, peroxidase activity, polyphenol oxidase activity, in vitro protein digestibility, and water absorption capacity since there is wide genetic variability among species. Data from the CIAT (International Center for Tropical Agriculture) revealed an amount of 37,987 accessions of beans in its germplasm bank [10]. In this way, the possibilities for crossbreeding and obtaining new genetic materials are encouraging.

According to data from scientific publications, the search for new bean genotypes that can meet the population's nutritional needs is fundamental. However, the work of selection and development of new genotypes with higher nutritional quality is relatively time-consuming [11]. The characterization of genotypes in terms of their chemical and proximate composition may help select new cultivars in breeding programs that can meet the demand for agronomic and nutritional characteristics.

Thus, the objective of this work was to evaluate the concentration and availability of Fe and Zn, amount of phytic acid, tannins, ash, moisture, and proteins in grains of 14 bean genotypes from a genetic improvement program (EPAGRI).

2. Materials and methods

Seeds from 14 common bean genotypes (*Phaseolus vulgaris* L.) of the carioca and black varieties were selected: CHP 01-238-80; CHP 01-182-12; CHP 05-282-04; CHC 05-262-100; CHP 01-238-10; CHP 01-182-48; CHP 01-231-10; CHP 04-239-01; CHC 98-42-215; RC3 PUMA (PUMA X EXPLENDOR); CHP 04-239-52; CHC 01-175-1; CHC 98-42-160; CHC 05-262-102, from the Bean Genetic Improvement Program (PMGF) - Agricultural Research and Rural Extension Company of Santa Catarina (EPAGRI), provided by the Family Agriculture Research Centre (CEPAF) in Chapecó. The seeds were breeding lines, at the stage of Cultivation Value Testing (VCU) in the 2018 and 2019 harvests.

These grains were harvested in the 2018 "water period". The exact geographic location was: Latitude: −23,7641, Longitude: −53.3184. This region has a subtropical climate (Köppen climate classification-Geiger: Cfa).

For the analyses, the bean grains were previously hydrated in distilled water in the proportion of 1:3 (grain: water) for 24 hours and cooked in the proportion of 1:2 (grain: water) for 10 minutes at

121 ℃ in an autoclave according to methodology suggested by [12]. After cooking, the samples were placed in aluminum trays and dried in an oven with forced air circulation at a temperature of 50 ℃ (± 2 ℃) until constant weight (approximately 24 hours). After drying, the samples were crushed in a knife mill, and sieved in a 30 "mesh" with the dried broth and kept in a freezer at 4 ℃ until the time of analysis.

2.1. Concentration of moisture and protein

The moisture content of the samples was established using a gravimetric method at 105 ℃ [13]; the fixed mineral residue was determined by calcining the samples in a muffle at 550 ℃ according to the method described by [14]; protein content was performed using the Kjeldahl method [15] with a total nitrogen conversion factor of 6.25.

2.2. Determination of Nitrogen

Nitrogen determination was performed using the Kjeldahl method [15], in which 0.5 g of the ground sample, in triplicate, was dried at 65 ℃ to constant weight and then stored in parchment paper. After preparing the digestor/sulfuric acid (H_2SO4), boric acid (H_3BO_3) indicator, hydrochloric acid (HCl), and sodium hydroxide (NaOH) solution, the digestion of the sample was carried out in a Marconi MA 4025 digester block, adding 5 mL of Aldrich® sulfuric acid (PA) to the glass tubes, which were heated gradually, over 3 hours, until reaching 450 ℃. The digested sample was distilled in a Marconi MA 036 distiller, adding 25 mL of hydroxide 50% sodium. With the distillation steam at 100 ℃, the distilled sample was collected in an Erlenmeyer flask containing 25 mL of 4% indicator boric acid. The distilled sample was titrated with 0.1 M HCl until turning from green to pink. Afterward, the calculation of the percentage of nitrogen was carried out.

2.3. Determination of phytic acid and tannin

The determination of phytic acid was made according to the methodology [16], with adaptations. The measurement of concentrations was performed at 515 nm in the SPECTRAMAX PLUS 384 microplate reader (ELISA). The tannin values shown in Table 1 are expressed in mg of catechin kg^{-1} of beans. The results were expressed in mg kg^{-1} of phytic acid equivalents per g^{-1} of the sample using the standard catechin curve. The determination of the tannin content followed the protocol described by [17], with adaptations. The sample was analysed in a spectrophotometer at 500 nm on the ELISA device. A Standard Curve was built to obtain the equation of the genotype.

2.4. Fe and Zn Concentration

The analyses of Fe and Zn concentration were determined by the official method of analysis [14] with adaptations, where 1:12 HNO_3 1 M was added at 32 ℃ for 48 h, heated to 100 ℃ until evaporation. After, were added 1:6 of H_2O_2, filtered and the volume adjusted to 10 mL. The measurement occured in a Flame Atomic Absorption Spectrophotometer (brand GBC model 932).

2.5. In vitro availability of Fe and Zn

The in vitro availability of Fe and Zn followed the protocols described by [14] with adaptations, where 250 mg of the sample were homogenized in 15 ml of ultrapure water, in an orbital shaker-type at 37 ℃/200 rpm for 1 h. After removal from the shaker, 5 M HCl pH 2 and 0.75 mL pepsin (previously prepared with 0.1 M HCl) was added to the sample. For hydrolysis, the pH was adjusted to 6 with 1 M sodium bicarbonate, followed by the addition of the pancreatin mixture and centrifuged at 250 rpm at 4 ℃. Subsequently, 5 mL of NaCl + 5 mL 5 M KCl at 37 ℃ were added and stirred at 200 rpm for 1 h. Afterward, the precipitate and the supernatant were dried in an air oven at 60 ℃ until constant mass. After drying, 1:12 nitric acid (HNO_3) 1M was added at 32 ℃ for 48 h, followed by heating at 100 ℃ until the complete evaporation of the solution. After, 1:6 of hydrogen peroxide (H_2O_2) was added, filtered, and the volume was adjusted to 10 mL. Values were obtained from a Flame Atomic Absorption Spectrophotometer (GBC model 932).

2.6. Statistical analysis

For the analysis, all samples were processed in triplicate. The results were subjected to analysis of variance ($p \leq 0.0001$). The variables that did not obtain normality through the Shapiro-Wilk test were subjected to transformation by the box cox method. A comparison analysis of averages was performed based on the Tukey analysis, at 5% probability, using an statistical program [18].

The results were also submitted to Pearson's correlation coefficient estimate (r), which allowed to quantify the degree of association between the two variables. In this analysis, the results range from −1 to 1, with the highest correlations occurring when the results approach −1 (high negative correlation) and 1 (high positive correlation). From this, it was possible to evaluate the models of simple genotype regression. The genotypes were grouped according to the similarity found from the evaluated characteristics, using the Ward method, and a Euclidean distance matrix was generated. These data were generated from the Statistic 7 program [19].

3. Results

3.1. Concentration of moisture, protein, nitrogen, phytic acid, tannin

The protein content of beans (Table 1) ranged from 14.50 g per 100 g of beans (CHC 98-42-215) to 22.64 g per 100 g of beans (CHC 98-42-160). As shown in Table 1, the nitrogen content ranged from 3.19 mg per kg^{-1} (CHC 05-262-102) to 4.96 mg per kg^{-1} (RC3). For both characteristics, there was a difference of about 35% from the highest to the lowest value.

The tannin values are shown in Table 1. It was found that the bean genotype CHC 01-175-1 had the lowest concentration (4.67 mg catechin per kg^{-1}) while the genotype CHC 98-42-160 obtained the highest value (6.14 mg of catechin per kg^{-1}) of beans. For the other genotypes, catechin values were around the average. It was observed in Table 1 that the bean genotypes showed different concentrations of phytic acid. The genotype CHP 01-231-10 has 39 mg per kg^{-1} of phytic acid, while the genotype CHC 98-42-160 had 63 mg per kg^{-1}. The moisture values ranged from 7.8 g per 100g (CHP 04-239-52) to 5.14 g per 100 g (CHC 05-262-100), a 65% difference between the highest and the lowest value.

Table 1. Statistical analysis regarding the characteristics of phytic acid, tannin, moisture, protein, and nitrogen in 14 bean genotypes.

Genotype	Phytic acid (mg per kg^{-1})	Tannin (mg catechin per kg^{-1})	Moisture (g per 100g)	Protein (g per 100g)	Nitrogen (%)
CHP 01-238-80	63 a	5.91 ab	7.36 a	21.61 ab	3.71 de
CHP 01-182-12	58 de	5.65 abc	6.52 b	20.46 ab	4.44 ab
CHP 05-282-04	54 f	5.32 bcd	5.43 d	21.85 ab	4.44 abc
CHC 05-262-100	58 d	5.45 bcd	5.14 d	19.2 abc	4.4 abcd
CHP 01-238-10	57 e	5.87 abc	6.45 b	19.29 ab	3.91 bcde
CHP 01-182-48	61 b	6.02 ab	7.43 a	21.27 ab	3.89 bcde
CHP 01-231-10	39 i	5.71 abc	7.21 a	19.29 abc	4.22 abcd
CHP 04-239-01	62 b	6.04 ab	7.69 a	19.32 ab	3.78 cde
CHC 98-42-215	53 g	5.23 cd	5.79 cd	14.5 c	4.21 abcd
RC3 PUMA	58 de	5.55 bc	6.4 bc	16.87 bc	4.96 a
CHP 04-239-52	60 c	5.85 abc	7.8 a	19.09 abc	4.83 a
CHC 01-175-1	48 h	4.67 d	6.53 b	17.88 bc	4.43 abc
CHC 98-42-160	63 a	6.14 a	6.36 bc	22.64 a	4.26 abcd
CHC 05-262-102	55 f	5.77 abc	7.37 a	22.15 a	3.19 e
Average	58	5.74	6.52	19.30	4.24
CV %	0.67	4.21	3.25	7.25	5.80

3.2. Fe and Zn Concentration

The Fe concentration of the bean genotypes varied from 14.43 to 27.33 mg per kg^{-1} (Figure 1). The analysis of variance ($p \leq 0.0001$) showed that the genotypes have significantly different Fe concentrations in the grains (Figure 1). The highest Fe concentration was observed in genotype CHP 05-282-04 (Figure 1). CHP 05-282-04 showed 47% more Fe than the genotype with the lowest value, CHC 98-42-215.

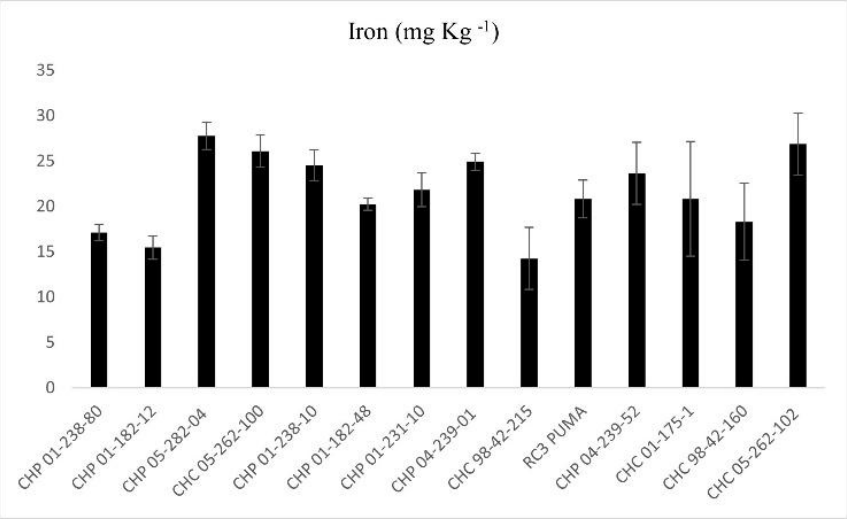

Figure 1. Amount of Fe (mg per kg^{-1}) in 14 common bean genotypes.

The Zn concentration of the bean genotypes varied from 12.11 to 26.69 mg per kg $^{-1}$ (Figure 2). The analysis of variance ($p \leq 0.0001$) showed that the genotypes have significantly different values in relation to the concentration of Zn in the grains (Figure 2). The highest values were for the CHC 05-262-102 (Figure 2). The CHC 05-262-102 had 54% more Zn than CHC 98-42-215, which was the genotype with the lowest observed value.

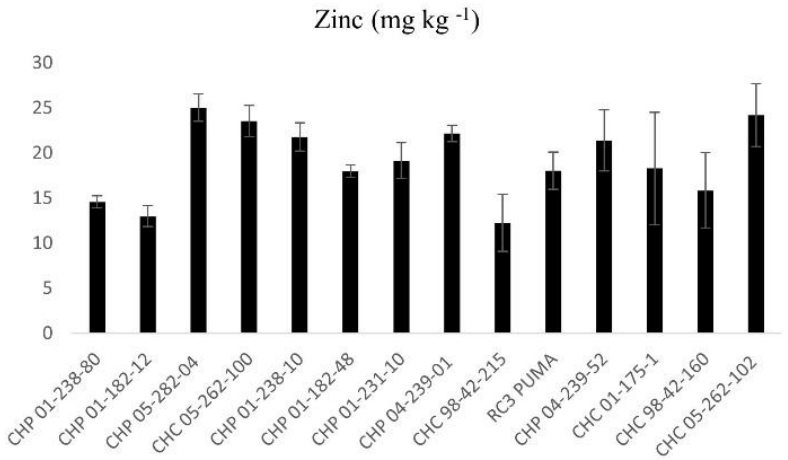

Figure 2. Amount of Zn (mg per kg^{-1}) in 14 common bean genotypes.

3.3. In vitro availability of Fe and Zn

The concentration of bioavailable Zn in the bean genotypes varied from 1.18 to 1.99 mg per kg $^{-1}$. The analysis of variance ($p \leq 0.0001$) identified significant variation in the evaluated samples (Figure 3). The highest values for the concentration of Zn solubilized in beans were for the genotype CHC 05-262-100 (Figure 3). The CHC 05-262-100 presented 40% more Zn than the genotype with the lowest value, CHP 01-182-12.

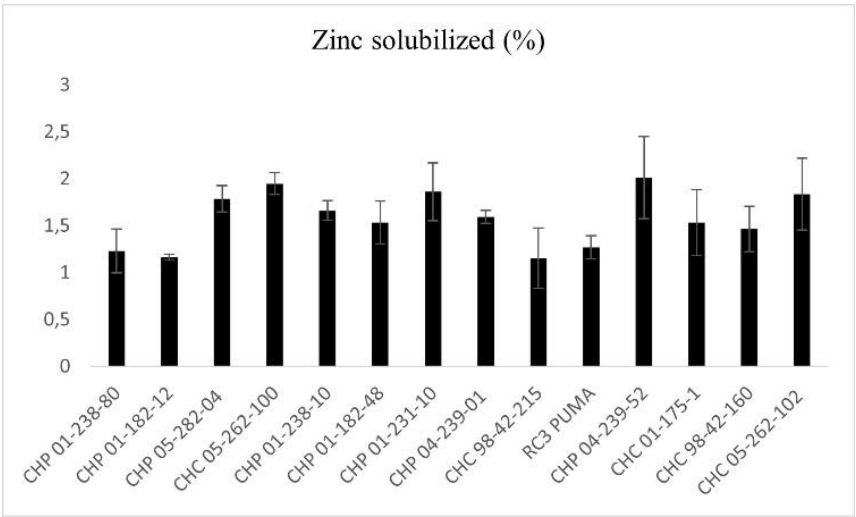

Figure 3. Amount of Zn solubilized (%) in 14 common bean genotypes.

The bioavailable Fe concentration of the bean genotypes ranged from 4.27 to 22.73% (Figure 4). The analysis of variance ($p \leq 0.0001$) showed that the genotypes significantly differed regarding the concentration of Fe in the grains (Figure 4). The highest values for the concentration of Fe solubilized in beans were for the genotype CHP 01-238-80 (Figure 4). The genotype CHP 01-238-80 showed 81% more Fe solubilized than the genotype CHC 05-562-102.

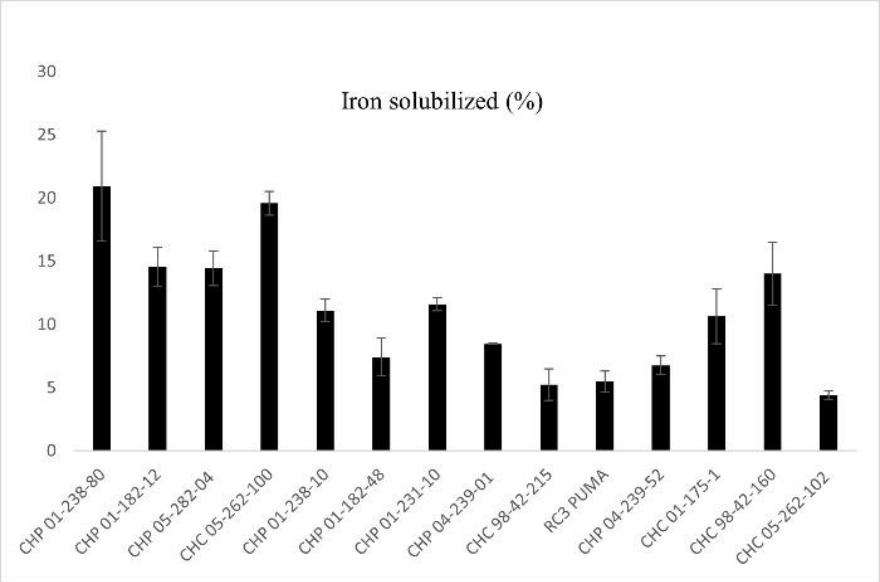

Figure 4. Amount of bioavailable Fe (%) in 14 common bean genotypes.

3.4. Pearson correlation analysis

Pearson's correlation analysis between the variables evaluated in the bean genotypes (Figure 5) indicates that there was a high positive correlation between the total amount of Zn with bioavailable Zn; the total amount of Fe, and the total amount of Zn; and for the total amount of bioavailable Fe with Zn. In addition, there was a medium positive correlation between tannin and phytate; and tannin and protein.

	Total Iron	Total Zinc	Iron Availability	Zinc Availability	Moisture	Nitrogen	Tannin	Phytate	Protein
Total Iron	1,00								
Total Zinc	1,00	1,00							
Iron Availability	-0,01	-0,02	1,00						
Zinc Availability	0,84	0,86	0,03	1,00					
Moisture	0,02	0,02	-0,30	0,16	1,00				
Nitrogen	-0,20	-0,20	-0,02	-0,11	-0,40	1,00			
Tannin	-0,02	-0,03	-0,01	0,00	0,45	-0,29	1,00		
Phytate	-0,11	-0,11	0,12	-0,29	0,07	-0,06	0,58	1,00	
Protein	0,22	0,21	0,35	0,16	0,13	-0,35	0,55	0,37	1,00

Figure 5. Pearson's R correlation analysis considering all variables evaluated: Total Fe, Total Zn, Fe availability, Zn availability, Moisture, Nitrogen, Tannin, Phytate, and Protein.

3.5. Cluster analysis

According to the cluster analysis (Figure 6), the data were divided into 5 distinct groups from the cutoff point set at 4. The first group was composed by the genotypes CHP 04-239-52, CHC 05-262-102, CHP 01-182-48, CHP 04-239-01 and CHP 01-238-10. The second group was composed by the genotypes CHC 05-262-100 and CHP 05-282-04. The third group was composed by RC3 PUMA and CHC 98-42-215 genotypes. The fourth group consisted of the genotypes CHC 01-175-1 and CHP 01-231-10. Finally, the fifth group was composed by the genotypes CHC 98-42-160, CHP 01-182-12, and CHP 01-238-80. Of these results, the genotypes CHP 04-239-01 and CHP 01-182-48, belonging to group 1 were the ones with the shortest distance, with 1.81, while the genotypes CHC 98-42-215 (group 3) and CHC 05-262-102 (group 1) had the longest distances, with 6.69.

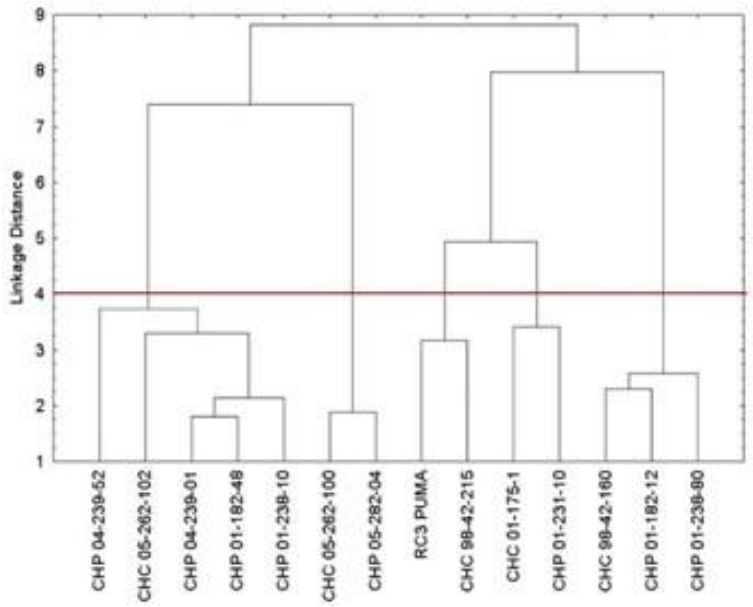

Figure 6. Cluster analysis of 14 bean genotypes, using Ward's clustering method, through Euclidean distance.

4. Discussion

4.1. Concentration of moisture, protein, phytic acid, and tannin

The protein values found in the evaluated bean genotypes had a similar variability when compared to [20,21]. Beans are an important source of vegetable protein. In these genotypes, the amount of protein has a considerable value, which is, in 100 g of beans, it is possible to obtain ⅓ of the average daily protein recommendations for an adult.

Phytate has long been considered an antagonist of nutrient absorption in legumes because it is responsible for the chelation of cationic metals. However, many antitumor and antioxidant properties have been attributed to phytate, and in this way, it can be used for different industries [22]. On the other hand, several studies show that the decrease in phytate in legumes allows for an increase in the absorption of elements such as Fe in human food [23].

In addition, phytic acid has important biological functions, such as the supply of phosphorus, essential for plant growth and development, in addition to assisting metabolic pathways involved in protection against pathogens. Thus, it is very important to know the genetic basis of phytic acid, so that the best genetic gains can be achieved. The literature tells us that phytates and proteins are closely related [24]. In our study, phytate and proteins had a medium correlation. These results can be attributed to several factors, such as, in part, by the variation in the number of proteins and in part by the moderate amount of phytic acid found in these samples, since the number of phytates directly influences the protein accumulation.

4.2. Amount of Fe and Zn

The results showed that the genotypes evaluated have the lowest amount of Fe compared to [25], who found amounts 5 times greater in the genotypes evaluated by them at the time (but with raw beans), and the same amount of Zn; while comparing the results with those obtained by [26], it is possible to observe similar values to those found in our study for Fe and smaller amounts for Zn.

When comparing the results with a study obtained by [27], it is possible to note that the Fe and Zn content of the genotypes obtained by our study are higher. Our results show that the amount of Fe and Zn is consistent with the Brazilian food composition table [28], which ranges from 11 to 19 mg per kg^{-1} for Fe and 7 to 13 mg per kg^{-1} for Zn.

These results demonstrate a great variability for the accumulation of the minerals Fe and Zn within the evaluated common bean genotypes. This is an important result because it reflects the possibility of genetic combinations that can favor the development of genotypes that have these nutrients in large quantities. This is a fundamental point that can contribute to the supply of nutrients to the population.

The results obtained in our study correspond to those found by several other researchers who report great variability of these nutrients both in cultivated and wild genotypes [20,29]. Genetic improvement programs seek genotypes that satisfy the producer's need in terms of high productivity. Some studies report that the amount of Fe and Zn is negatively correlated with productivity [30]. However, it is possible to control these characteristics with a selection index for the 3 characteristics [31].

In our study, it was possible to observe a large positive correlation between the amount of Fe and Zn. This result is explained by [32,33], who found a large number of QTLs for these characteristics, closely linked at the same intervals and in several linkage groups. This suggests that them can be pleiotropic or genetically linked, being controlled at the same time. However, other authors have also identified genes associated only with Zn accumulation [34], showing the diversity of genetic control for this trait.

As the genotypes evaluated in this study came from a bean genetic improvement program, them can be used in the future for crosses aimed at cultivars with high Fe contents. For that, the literature shows some strategies that can be adapted to maximize the gains of this characteristic. The study carried out by [35] found that inbred lines from the cross between 2 genotypes where the mother had higher levels of Fe, showed a progeny with also high levels of Fe. This work indicates that maternal effects may be involved in the concentration of Fe. This same study suggests that for the future selection of both traits, it is interesting to select genotypes with higher concentrations of Zn and then evaluate them for the concentration of Fe since the heritability of Zn in relation to Fe is higher. Thus,

the genotypes with the highest concentrations of Zn, in decreasing order, are: CHP 04-239-52, CHP 01-238-10, CHP 04-239-01, CHC 05-262-100, CHC 05-262- 102 and CHP 05-282-04.

4.3. Availability of Fe and Zn

In a study carried out by [36], using the same method as presented in this research, similar values for the bioavailability of Fe were obtained, ranging between 6% and 40%. These are similar values and values higher than those found in our study. This fact can be explained by the difference in the genotypes used. [37] obtained bioavailability values similar to those found here.

For Zn, the results were similar to those found by [38,39]. However, when we examine the correlation between the bioavailability of Fe and Zn; and the correlation between Fe concentration and Zn bioavailability, we verified that these results are in agreement with the literature [35].

Our results were different from those found in [40], which used Caco-2 type cells and these cells correlate very well with the actual functioning of Fe and Zn absorption in the human digestive tract. Thus, we emphasize the importance of confirming the data obtained in this study with Caco-2 cells.

The availability of Fe and Zn is influenced by antinutritional factors, such as phytates and tannins, and the composition of other nutrients, such as calcium [39]. The food process also greatly influences availability since cooking directly influences these antinutritional compounds.

4.4. Cluster analysis

Cluster analysis allows the identification of distinct genotypes that may be important for crosses that aim to increase the evaluated traits. With cluster analysis, it is possible to identify groups of more similar and more distinct genotypes and, in this way, evaluate possible genotypes for future crosses. However, we emphasize the importance of evaluating additional genetic components in contrasting genotypes in order to understand better the genetics associated with these characteristics, since most of the analyses evaluated in this study have multigenic loci for the characteristics studied and, thus, only the biochemical evaluation it is not enough to infer possible and future crosses.

5. Conclusions

We conclude that there was a statistically significant difference for all traits evaluated in the 14 bean genotypes from the EPAGRI PMGF. The publication of studies such as this one are important because increases the availability of genotype information so that breeders can access it from the most diverse research institutions. The obtained results in this study are important because shows the variability of the evaluated traits within the genotypes used and, thus, these genotypes may become important sources for crosses in a breeding program that have the objective of increasing the evaluated traits.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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