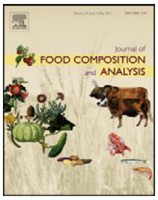
Journal of Food Composition and Analysis 26 (2012) 89–95

Contents lists available at SciVerse ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca



Original Research Article

Mineral concentrations in the embryo and seed coat of common bean cultivars

Nerinéia Dalfollo Ribeiro [a,*], Sandra Maria Maziero [a], Marina Prigol [b], Cristina Wayne Nogueira [b], Daniele Piano Rosa [a], Micheli Thaise Della Flora Possobom [a]

[a] *Crop Breeding Sector, Rural Sciences Institute, Federal University of Santa Maria, Santa Maria, 97105-900, Rio Grande do Sul, Brazil*
[b] *Laboratory of Synthesis, Reactivity, Pharmacological and Toxicological Evaluation of Organochalcogens, Natural and Exact Sciences Institute, Federal University of Santa Maria, Santa Maria, 97105-900, Rio Grande do Sul, Brazil*

ARTICLE INFO

Article history:
Received 10 November 2011
Received in revised form 24 February 2012
Accepted 6 March 2012

Keywords:
Phaseolus vulgaris
Grain legumes
Common bean
Gene pool
Mineral accumulation
Genetic variability
Genotype × environment interaction
Nutritional quality
Food analysis
Food composition

ABSTRACT

The distribution of calcium, potassium, iron, zinc and copper in the embryo and seed coat fractions of 16 common bean cultivars of the Middle American and Andean gene pools, obtained in two crop cycles, was investigated. Genetic factors affected the accumulation of minerals in the embryo and seed coat. Common bean seeds contained over 94.5% calcium in their seed coat and from 76.0 to 89.7% potassium in their embryo. Iron, zinc and copper concentrations varied widely between the embryo and seed coat fractions in different cultivars. The BRS Supremo cultivar has a high concentration of calcium (1044 mg 100 g^{-1} dry matter [DM]) and iron (24.88 mg 100 g^{-1} DM) in its seeds, whereas the Iraí cultivar stands out for its potassium (1720 mg 100 g^{-1} DM), zinc (6.51 mg 100 g^{-1} DM) and copper (0.47 mg 100 g^{-1} DM) concentrations. The BRS Supremo and the Iraí cultivars have high nutritional value, and their dietary use is therefore recommended.

1. Introduction

The common bean (*Phaseolus vulgaris* L.) represents 50% of the grain legumes consumed worldwide (Talukder et al., 2010). It is the main source of protein in the diet of countries such as Brazil, Mexico, Rwanda, Uganda, and other parts of East Africa and Latin America (Broughton et al., 2003). Moreover, it is an excellent source of complex carbohydrates, polyunsaturated free fatty acids (linoleic and linolenic), fiber, vitamins and minerals (calcium, potassium, phosphorus, magnesium, iron, zinc and copper) (Sathe et al., 1984). Thus, the common bean is one of the world's most important food sources, especially in developing countries, in terms of caloric and nutrient intake (Reyes-Moreno et al., 1993).

The mineral concentrations in common bean seeds show genetic variability. Values ranging from 0.30 to 2.80 g calcium kg^{-1} dry matter (DM), 15.10–24.80 g of potassium kg^{-1} DM, 71.37–126.90 mg iron kg^{-1} DM, 36.93–63.90 mg zinc kg^{-1} DM and 14.93–28.90 mg copper kg^{-1} DM were observed in 21 common bean genotypes grown in Brazil (Mesquita et al., 2007).

Common bean genotypes of Middle American origin have superior mineral content in their seeds compared to genotypes of Andean origin. Navy bean seeds (small seeds with a mass of less than 16.9 g per 100 seeds), which belong to the Middle American gene pool, contain, on average, 90% more calcium than kidney bean seeds (large seeds with a mass of more than 47.8 g per 100 seeds) and cranberry bean seeds (large seeds with a mass of more than 43.1 g per 100 seeds) (Moraghan and Grafton, 1997). Talukder et al. (2010) assessed the zinc and iron contents of the seeds of 29 common bean genotypes and observed that cultivars with Middle American genotypes have 16.1 and 11.3% more zinc and iron, respectively, than cultivars with Andean genotypes.

Seeds are composed of the following two fractions: the embryo, including cotyledons (tissue fertilization), and the seed coat (maternal tissue), and there is no vascular connections between these two tissues (Wolswinkel, 1992). In common bean seeds, the embryo (cotyledons and embryonic axis) represents approximately 90.1% of the dry matter, and the seed coat consists of 9.9% of the entire dry matter (Moraghan et al., 2006). In the seeds of two common bean cultivars, black beans from the Middle American gene pool grown in the United States, it was observed that the seed coat contains approximately 84% of the calcium, 24% of the iron, 8% of the potassium and only 7% of the zinc content of the whole seed (Moraghan and Grafton, 2002). Therefore, most of the calcium is in the seed coat and more iron, potassium and zinc is in the embryo. In addition, there is an inverse relationship between the amount of iron present in the embryo and seed coat. For instance, the iron

---

[*] Corresponding author. Tel.: +55 55 32208899; fax: +55 55 32208899.
E-mail addresses: nerineia@pq.cnpq.br, nerineia@hotmail.com (N.D. Ribeiro).

0889-1575/$ – see front matter. Crown Copyright © 2012 Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.jfca.2012.03.003

Table 1
Meteorological data collected at the 8th District of Meteorology, Meteorological Station of Santa Maria, installed at the Santa Maria Federal University (lat 29°42′S, long 53°49′W, 95 m asl), Rio Grande do Sul State, Brazil.

Month	Crop cycle 2007/2008			Crop cycle 2008/2009		
	T maximum (°C)	T minimum (°C)	Rainfall (mm)	T maximum (°C)	T minimum (°C)	Rainfall (mm)
October	26.0	16.6	113	24.6	15.0	255
November	27.1	14.6	105	29.2	17.2	44
December	31.3	18.3	202	30.8	18.1	32
January	30.4	19.1	96	29.9	18.1	162

content in embryos was highest in the Voyager cultivar, intermediate in T39, and least in UI911 (Moraghan et al., 2002). In contrast, the iron content in the seed coat was highest in UI911, intermediate in T39 and least in the Voyager cultivar.

Both genotype and environment affect the accumulation of minerals in common bean seeds (Quenzer et al., 1978; Moraghan and Grafton, 2001). Mineral content can be correlated with the mass of seeds. The calcium content of the seeds was negatively correlated with the mass of seeds, whereas the phosphorus content was positively correlated (Moraghan and Grafton, 2001). These authors found no significant correlation between potassium, iron and zinc content and the mass of seeds.

The mineral accumulation in the embryo and seed coat of the common bean is not yet known for cultivars grown in Brazil. Therefore, we studied the distribution of minerals, calcium, potassium, iron, zinc and copper in the embryo and seed coat of common bean cultivars of the Andean and Middle American gene pools obtained in different field experiments.

2. Materials and methods

2.1. Origin of common bean seeds

Seeds of 16 common bean cultivars (*P. vulgaris*) were obtained from field experiments carried out in the normal rainy season (with sowing in October), in two consecutive years, 2007/2008 and 2008/2009. Of those, 14 cultivars are part of the black bean commercial group: Rio Tibagi, Guateian 6662, Macanudo, Minuano, Macotaço, Guapo Brilhante, IAPAR 44, TPS Nobre, Diamante Negro, BRS Valente, IPR Uirapurú, BRS Campeiro, FEPAGRO 26 and BRS Supremo; the two remaining cultivars are part of the colored bean commercial group: Pérola, with carioca type seeds (beige with brown streaks), and Iraí, with cranberry type seeds (beige with red spots). All cultivars are in the Middle American gene pool, except for Iraí, which is in the Andean gene pool.

All field experiments were conducted in experimental areas of the Common Bean Breeding Program at the Plant Science Department of Santa Maria Federal University (UFSM), Brazil. Santa Maria lies in the Central Depression region of Rio Grande do Sul, southern Brazil (lat 29°42′S, long 53°49′W, 95 m asl). The climate type is temperate and rainy, with well-distributed rainfall throughout the year and subtropical temperatures. The meteorological data of the 2007/2008 and 2008/2009 crop cycles are shown in Table 1.

The soil was typical alitic Argisol, Hapludalf (Embrapa, 2006), with the following chemical composition in the 2007/2008 cycle: pH (H_2O) = 5.5; organic matter = 2.2%; phosphorus = 6.8×10^{-9} kg m^{-3}; potassium = 6.8×10^{-8} kg m^{-3}; calcium = 5.5×10^{-5} mol m^{-3}; and magnesium = 2.7×10^{-5} mol m^{-3}. The soil was prepared in a conventional manner, and fertilization was performed at two separate times based on the chemical soil analysis. The fertilizers used in the sowing furrow were 0.0250 kg m^{-2} of the 5–20–20 formula (nitrogen, phosphorus and potassium) and 0.0081 kg m^{-2} of triple superphosphate (42% P_2O_5). In addition, nitrogen fertilization was split in the growth stage of the first trifoliolate leaves (V3), at 0.0020 kg m^{-2} urea (45% of nitrogen).

In the 2008/2009 cycle, the experiment was conducted in the same experimental area with a similar chemical composition consisting of pH (H_2O) = 5.8; organic matter = 2.0%; phosphorus = 1.09×10^{-8} kg m^{-3}; potassium = 6.8×10^{-8} kg m^{-3}; calcium = 5.9×10^{-5} mol m^{-3}; and magnesium = 3.2×10^{-5} mol m^{-3}. The amount and type of fertilizer used and the application method in the 2008/2009 cycle were identical to those described for the 2007/2008 cycle, except that phosphorus was applied as 0.0054 kg m^{-2} of triple superphosphate in the sowing furrow. Micronutrients were not added to the fertilizer.

A randomized block experimental design was used, with three repetitions. Plots consisted of two rows 4 m long, spaced 0.50 m apart, and with a useable area of 4 m^2. Cultural treatments and insect and weed control were carried out whenever necessary to rule out competition. Disease control and irrigation were not performed.

In two crop cycles, plants were harvested and threshed by hand when mature. Agricultural machinery and equipment were not used in the harvesting and processing of seeds to avoid the contamination of samples with heavy metals. After manual removal of impurities and broken seeds, the seeds obtained were dried in a drying and sterilization oven (Odontobras1.5; Odontobras, São Paulo, Brazil) with forced air circulation (65–70 °C) until they reached an average moisture content of 13%. The seeds were then packaged in paper bags. A homogeneous sample of 250 g of the seeds per repetition was obtained for each cultivar each cycle.

2.2. Determination of mineral concentrations in embryo and seed coat

2.2.1. Sample preparation

Calcium, potassium, iron, zinc and copper concentrations were determined in the embryo (cotyledons and embryonic axis) and seed coat fractions. Sixty seeds of each cultivar were randomly collected from one plot. Two sets of 60 seeds were collected each crop cycle. The seeds (60) were placed between two sheets of paper towel lightly moistened with distilled water and covered with plastic bags. The seeds remained under these conditions for a 24-h period at room temperature (18 ± 2 °C). The seeds were then removed from the moistened sheets of paper, and the seed coat was removed by hand to reduce potential contamination. The samples were dried in an oven (65–70 °C) until they reached an average moisture content of 13% and were then weighed. The samples of the embryo and seed coat fractions were ground separately in an analytical knife micromill (Q298A21; Quimis, São Paulo, Brazil) to produce particles smaller than 1 mm.

2.2.2. Sample digestion

For sample digestion, we used nitric acid (HNO_3) and perchloric acid ($HClO_4$) (Suprapure, Merck, Darmstadt, Germany). The embryo and seed coat fractions were digested with concentrated perchloric/nitric acid (1/5, v/v) at 60 °C for 12 h, according to the method described by Souza et al. (2012), with some modifications. The resultant solution was transferred to a volumetric flask, diluted 10 times with Milli-Q high purity water device (18.2 MV MΩ cm quality; Millipore, USA). The blank solutions were prepared in the same manner as the samples.

2.2.3. ICP-OES determination

The analysis was carried out using an optical emission spectrometer with inductively coupled plasma (ICP-OES) equipped with a Cross Flow nebulizer (ICPE-9000; Shimadzu, São Paulo, Brazil). Argon (purity higher than 99.995%) was employed as the plasmogen and carrier gas. The operating conditions of the ICP-OES equipment were previously established, and the selected analytical emission lines were automatically determined by the instrument.

A standard solution of 1000 mg L^{-1} of calcium, potassium, iron, zinc and copper dissolved in 0.5% nitric acid (HNO_3) supplied by SpecSol® (High Purity, São Paulo, Brazil) was used as the stock solution for calibration. The final calibration range for the elements determined was from 0.1 to 100 mg L^{-1} for calcium; 1 to 200 mg L^{-1} for potassium; 0.05 to 5 mg L^{-1} for iron and 0.01 to 1 mg L^{-1} for zinc and copper. These standards were chosen based on the expected concentration range of the different studied elements. The spectral lines (nm) used were as follows: Ca 183.801; K 793.867; Fe 238.204; Zn 213.856; Cu 324.754.

The limit of detection (LOD) was calculated as the concentration corresponding to signals equal to three times the standard deviation of ten replicates of a blank solution. Additionally, LODs were calculated in the original samples, taking into consideration the amount of sample and the final dilution employed in the procedure. The repeatability was calculated as the relative standard deviation (%RSD) for ten independent analyses of an elemental standard solution (Analytical Methods Committee, 1987). Acceptable recovery values were obtained for all elements in the samples, indicating that this methodology is suitable for the determination of mineral concentrations in the embryo and seed coat.

The concentrations of calcium and potassium were expressed as g per 100 g^{-1} of sample dry matter, whereas the iron, zinc and copper concentrations were expressed as mg per 100 g^{-1} of sample dry matter.

2.3. Statistical analysis

The mineral data retrieved were analyzed by a completely randomized design, with two repetitions of the each crop cycles, in the laboratory. Treatments were combined into 2 × 16 × 2 three-factor schemes in which the variables were the two crop cycles (2007/2008 and 2008/2009), sixteen common bean cultivars (Rio Tibagi, Guateian 6662, Macanudo, Minuano, Macotaço, Guapo Brilhante, IAPAR 44, TPS Nobre, Diamante Negro, BRS Valente, IPR Uirapurú, BRS Campeiro, FEPAGRO 26, BRS Supremo, Pérola and Iraí) and two seed fractions (embryo and seed coat).

Calcium and potassium concentrations, initially expressed in g kg^{-1}, were transformed into g% (concentration initially in 100 g of dry matter of embryo and in 100 g of dry matter of seed coat), whereas iron, zinc and copper concentrations were transformed into mg% (concentration initially in 100 g of dry matter of embryo and in 100 g of dry matter of seed coat). This procedure is necessary because the embryo and seed coat fractions quantitatively represent very different portions of the total dry matter (DM) of seeds.

The obtained data were subjected to analysis of variance using the *F* test, with a significance cutoff at the 5% level of probability, in order to test the hypotheses of main effects and interactions of crop cycles × common bean cultivars, crop cycles × seed fractions, common bean cultivars × seed fractions and crop cycles × common common bean cultivars × seed fractions. All of the sources of variation were considered fixed. Regarding the variables with significant interaction, a comparison of means was performed using Scott–Knott's test for cultivars and Tukey's test for crop cycles and seed fractions, with a significance cutoff at the 5% level of probability. Analyses were performed with the aid of Microsoft Office Excel spreadsheets and SOC (Embrapa, 1997) and Genes (Cruz, 2001) software.

3. Results and discussion

3.1. General results

In the variance analysis, a significant interaction between crop cycles × cultivars × seed fractions was obtained in relation to calcium, potassium, iron and copper (Table 2), indicating that the concentrations of these minerals in the embryo and seed coat fractions vary among cultivars and crop cycles. The existence of genetic variability among common bean cultivars in regard to mineral concentrations allows the identification of cultivars with higher nutritional quality for use in foods. However, the selection must be performed by evaluating a larger number of environments due to the influence of uncontrollable factors in the expression of these characters. Moraghan and Grafton (2001), when assessing seeds of eight common bean cultivars of different gene pools in five locations in the United States, found significant cultivar × location interaction for calcium, potassium and iron concentrations.

3.2. Calcium distribution

The calcium content was much higher in the seed coat than in the embryo of the common bean cultivars assessed over two crop cycles (Fig. 1). The seed coat represented 8.2–10.7% of the total dry

Table 2
Variance analysis of calcium, potassium, iron, zinc and copper concentrations (mg per 100 g of dry matter) determined in the embryo and seed coat of 16 common bean cultivars obtained in two crop cycles.

Sources of variation	DF	Calcium		Potassium		Iron		Zinc		Copper	
		MS[a]	*P* val.[b]	MS	*P* val.	MS	*P* val.	MS	*P* val.	MS	*P* val.
Cycle (C)	1	210	0.022	767	0.000	619,997	0.000	4020	0.152	17	0.075
Genotype (G)	15	151	0.000	65	0.000	357,874	0.000	13,066	0.000	50	0.000
Fraction (F)	1	292,050	0.000	5386	0.000	57,476,058	0.000	672,171	0.000	9001	0.000
C × G	15	87	0.024	41	0.000	80,511	0.000	1194	0.115	10	0.269
C × F	1	204	0.011	459	0.000	591,162	0.000	4899	0.845	7	0.051
G × F	15	151	0.000	59	0.000	373,877	0.000	13,112	0.000	50	0.000
C × G × F	15	87	0.011	47	0.000	82,396	0.000	1151	0.863	10	0.038
Residue	64	38		11		12,049		1914		5	
Total	127	–		–		–		–		–	
Mean		945		1703		19.4		4.15		1.78	
CV (%)		12.79		14.87		14.88		43.63		21.33	

[a] Mean square.
[b] *P* value.

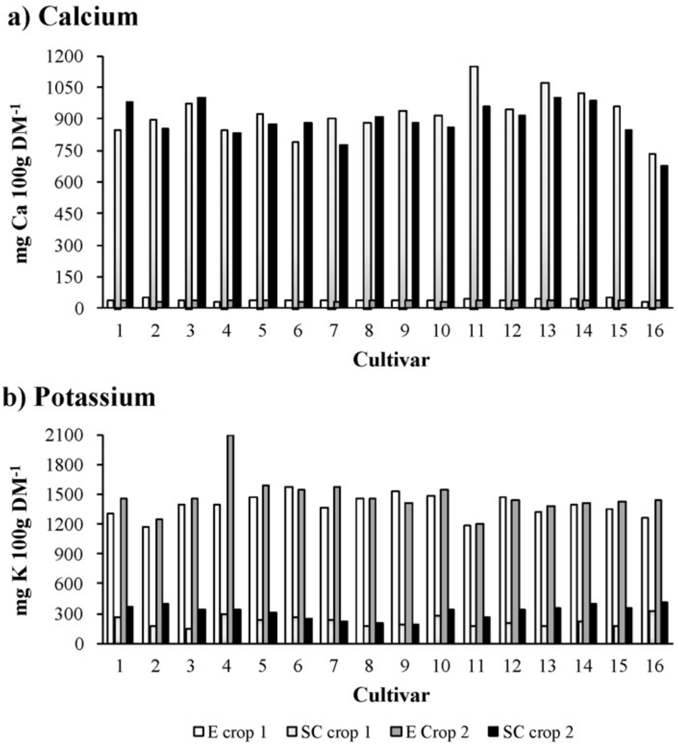


Fig. 1. Calcium and potassium concentrations (mg per 100 g of DM) determined in the embryo (E) and seed coat (SC) of 16 common bean cultivars (1: Rio Tibagi, 2: Guateian 6662, 3: Macanudo, 4: Minuano, 5: Macotaço, 6: Guapo Brilhante, 7: IAPAR 44, 8: TPS Nobre, 9: Diamante Negro, 10: BRS Valente, 11: IPR Uirapuru, 12: BRS Campeiro, 13: FEPAGRO 26, 14: BRS Supremo, 15: Pérola and 16: Iraí) obtained in two crop cycles.

matter of seeds, and 94.5 (Guateian 6662, 2007/2008 cycle) to 96.8% (Macanudo, 2008/2009 cycle) of the total calcium was located in the seed coat (Table 3). Therefore, removing the seed coat of the common bean in the preparation of meals is not recommended because it substantially reduces the amount of calcium, reducing the nutritional value of the bean. The percentage of calcium obtained in the seed coat of common bean cultivars under cultivation in Brazil (Table 3) was much higher than the values observed in the seed coat of cultivars grown in the United States and Mexico, which have shown variation from 81.4 to 86.0% (Moraghan and Grafton, 2002), 80 to 85% (Moraghan et al., 2002) and 67 to 81% (Moraghan et al., 2006). The differences between this study and those previously published, in terms of the level of calcium in the common bean seed, can be explained by genetic differences among cultivars in addition to variations in the calcium concentration in the soil. Additionally, calcium is a mineral that has restricted movement between the seed coat and embryo of common bean seeds, probably due to the presence of insoluble calcium oxalate crystals in the mature seed coat (Barnabas and Arnott, 1990).

In the embryo fraction, there were no significant differences among common bean cultivars regarding calcium concentration within each crop cycle (Fig. 1). However, in the seed coat fractions, the Macanudo, FEPAGRO 26 and the BRS Supremo cultivars had the highest calcium concentration in both crop cycles. In 2007/2008, Macotaço, IAPAR 44, Diamante Negro, BRS Valente, IPR Uirapurú, BRS Campeiro and Pérola cultivars also had significantly higher calcium concentrations. As the cooking process can be influenced by the calcium concentration in the seed coat (Quenzer et al., 1978), the cooking time of such cultivars should be evaluated to determine whether there is an increase in the time required for the seeds to reach suitable softness for consumption.

A wider range in variation was observed in the calcium concentration in the seed coat of the 2008/2009 cycle, ranging from 679 (Iraí, Andean gene pool) to 1000 mg 100 g^{-1} DM (Macanudo, Middle American gene pool) (Fig. 1). Moraghan and Grafton (1997) also observed that the common bean seeds of the Middle American gene pool, such as navy beans, had a higher calcium concentration when compared to cultivars of the Andean gene pool, such as cranberry beans.

3.3. Potassium distribution

There was little variation in the potassium concentration between the seed embryo and seed coat fractions of common bean cultivars (Fig. 1). In the embryos, the values obtained ranged from 1169 (Guateian 6662, 2007/2008 cycle) to 2100 mg 100 g^{-1} DM (Minuano, 2008/2009 cycle), and for the seed coat, they ranged from 160 (Macanudo, 2007/2008 cycle) to 409 mg 100 g^{-1} DM (Iraí, 2008/2009 cycle). However, as the embryo represented 89.3–91.8% of the total dry matter of seeds, the percentage of potassium was higher in the embryo (76–89.7%) when compared to the seed coat (Table 3). Therefore, more potassium is concentrated in the embryo of common bean seeds. A similar result was observed by Moraghan and Grafton (2002) in two black bean cultivars of the Middle American gene pool, where the highest percentage of potassium accumulated in the embryo (92%), which accounted for 90% of the total dry matter of the seeds.

The common bean cultivars did not significantly differ from each other regarding the potassium concentration in the embryo within each crop cycle (Fig. 1). Differences were observed in the seed coat fraction. The Iraí cultivar had a high potassium concentration in its seed coat during both crop cycles. In the 2008/2009 cycle, the Guateian 6662, BRS Valente, FEPAGRO 26 and BRS Supremo cultivars also stood out, with potassium concentrations greater than 346 mg 100 g^{-1} DM. As the common bean has fewer calories than the banana and also has low fat and sodium concentrations and does not contain cholesterol (Hosfield, 1991; Morrow, 1991), the use of common bean cultivars with high potassium concentration in the diet is a good alternative for people who practice intense physical activity and therefore require higher potassium concentrations (Lindinger, 1995). In contrast, the identification of common bean cultivars with low potassium concentration becomes important in patients with renal impairment. In that case, Louis and Dolan (1970) recommended the restriction of potassium in the diet. Therefore, the use of the common bean as part of a balanced diet should be based on knowledge of the cultivars' mineral composition and individual nutritional needs.

3.4. Iron distribution

The iron concentration was higher in the seed coat of common bean seeds in both crop cycles (Fig. 2) and ranged from 6.40 (Macanudo, 2007/2008 cycle) to 23.75 mg 100 g^{-1} DM (Rio Tibagi, 2007/2008 cycle). As the variation range was wide, the percentage of accumulated iron in seed coat ranged from 47.8 (Macanudo) to 83.2% (Rio Tibagi), in the 2007/2008 cycle (Table 3). The Macanudo cultivar presented the lowest iron accumulation in the seed coat (47.8%), whereas Rio Tibagi was the highest iron accumulator (83.2%). However, Macanudo had the highest iron accumulation in embryos (52.2%), whereas Rio Tibagi had the lowest accumulation levels in embryos (16.8%). This finding suggests that there is an inverse relationship between the iron accumulated in the embryo and the seed coat of common bean seeds, as has been observed in previous studies by Moraghan et al. (2002).

Cultivars did not significantly differ from each other regarding the iron concentration in embryo fractions within each crop cycle

Table 3
Dry matter and calcium, potassium, iron, zinc and copper percentages of partition determined in the embryo (E) and seed coat (SC) of 16 common bean cultivars obtained in two crop cycles (1st cycle: 2007/2008 and 2nd cycle: 2008/2009).

Cultivar	Dry matter (%)[a]				Calcium (%)				Potassium (%)				Iron (%)				Zinc (%)				Copper (%)			
	Cycle 1		Cycle 2		Cycle 1		Cycle 2		Cycle 1		Cycle 2		Cycle 1		Cycle 2		Cycle 1		Cycle 2		Cycle 1		Cycle 2	
	E	SC	E	SC	E	SC	E	SC	E	SC	E	SC	E	SC	E	SC	E	SC	E	SC	E	SC	E	SC
Rio Tibagi	90.1	10.0	89.9	10.1	3.8	96.2	3.9	96.1	82.9	17.1	79.9	20.1	16.8	83.2	20.4	79.6	62.6	37.4	62.6	37.4	54.3	45.7	57.0	43.0
Guateian 6662	89.3	10.7	89.9	10.2	5.6	94.5	3.7	96.3	86.4	13.6	76.0	24.0	46.0	54.0	31.4	68.6	63.2	36.8	64.5	35.5	52.2	47.8	55.6	44.4
Macanudo	91.4	8.6	91.0	9.0	3.8	96.2	3.2	96.8	89.7	10.3	81.0	19.0	52.2	47.8	46.5	53.5	76.1	23.9	64.4	35.6	63.2	36.8	61.5	38.5
Minuano	91.1	8.9	90.6	9.4	3.8	96.3	4.3	95.7	82.3	17.7	86.2	13.8	24.0	76.0	41.0	59.0	55.3	44.7	45.4	54.6	58.4	41.6	59.7	40.3
Macotaço	91.0	9.1	90.5	9.5	3.8	96.2	3.7	96.3	85.9	14.1	84.0	16.1	22.7	77.3	33.4	66.6	67.7	32.3	68.0	32.1	60.6	39.4	54.6	45.5
Guapo Brilhante	90.0	10.0	91.0	9.0	4.1	95.9	3.6	96.4	85.4	14.6	86.5	13.5	28.7	71.3	28.5	71.5	76.3	23.7	68.0	32.0	72.0	28.1	60.0	40.0
IAPAR 44	89.9	10.1	90.6	9.5	3.8	96.2	3.9	96.1	85.0	15.0	87.5	12.5	26.1	73.9	31.3	68.7	75.9	24.2	61.9	38.1	57.5	42.5	60.6	39.4
TPS Nobre	91.0	9.0	91.8	8.2	4.3	95.7	3.9	96.1	89.2	10.9	87.4	12.6	37.7	62.3	36.4	63.6	67.0	33.0	62.7	37.4	60.3	39.7	56.5	43.5
Diamante Negro	89.8	10.2	90.4	9.7	4.1	95.9	4.1	95.9	88.3	11.7	88.3	11.7	36.6	63.5	46.6	53.4	75.0	25.0	60.6	39.4	56.8	43.2	48.8	51.3
BRS Valente	90.1	9.9	90.0	10.0	3.7	96.3	3.7	96.3	84.0	16.0	81.8	18.3	36.6	63.4	39.2	60.8	63.0	37.0	56.7	43.3	56.6	43.4	54.1	46.0
IPR Uirapuru	90.7	9.3	91.1	8.9	3.4	96.6	3.5	96.5	86.5	13.6	81.8	18.2	27.1	72.9	32.6	67.4	59.7	40.3	64.3	35.7	51.4	48.7	50.8	49.3
BRS Campeiro	89.9	10.1	90.7	9.3	4.1	96.0	3.7	96.3	87.5	12.5	81.2	18.8	29.7	70.3	28.4	71.6	57.5	42.5	58.8	41.2	50.0	50.0	47.7	52.3
FEPAGRO 26	90.4	9.7	91.0	9.0	3.7	96.3	3.6	96.4	87.5	12.5	79.8	20.2	24.3	75.7	27.0	73.0	69.4	30.6	54.6	45.4	55.7	44.3	48.8	51.2
BRS Supremo	89.9	10.1	90.2	9.8	4.1	95.9	3.4	96.7	86.2	13.8	78.3	21.7	23.2	76.8	22.7	77.4	71.5	28.5	62.3	37.7	61.3	38.7	57.1	42.9
Pérola	91.3	8.7	91.4	8.6	4.8	95.2	4.4	95.6	88.3	11.7	80.4	19.6	34.2	65.8	38.1	61.9	59.3	40.7	57.2	42.8	47.7	52.3	44.9	55.1
Iraí	90.0	10.0	90.2	9.8	4.1	95.9	5.1	95.0	79.2	20.9	77.9	22.1	46.4	53.7	48.6	51.4	36.6	63.4	39.3	60.7	40.4	59.6	45.7	54.4
Mean	90.4	9.7	90.6	9.4	4.0	96.0	3.8	96.2	85.9	14.2	82.4	17.6	30.1	69.9	33.1	66.9	63.3	36.7	58.2	41.8	55.5	44.5	53.5	46.5

[a] The data represent seed coat and embryo mass in relation to the entire seed mass. The percentage of partitions of the minerals was calculated considering that data were expressed in mg 100 g^{-1} of dry matter (DM) and that the seed coat and embryo represent different portions of the total DM.

(Fig. 2). However, in the seed coat fractions, there were differences; the cultivars that had the highest iron concentration were the BRS Supremo (2007/2008 and 2008/2009 cycle), Rio Tibagi and Macotaço (2007/2008 cycle). The iron present in the seed coat of common bean seeds is more bioavailable than the iron present in embryos (Lombardi-Boccia et al., 1995). Hence, one should avoid removing the seed coat to prepare beans, as this will compromise the amount of iron readily available for absorption in the human body. The identification of common bean cultivars with a high iron concentration in the seed coat may represent a significant contribution to the quality of nutritional assessment and recommendations, especially in the prevention of anemia, which is currently a serious public health problem and affects approximately two billion people worldwide (UNICEF, 2011).

The BRS Supremo, Rio Tibagi and Macotaço cultivars are part of the black bean commercial group. According to Beebe et al. (2000), the higher iron concentration in the seed coat of common bean cultivars with black seeds can be explained by the high concentration of tannins, substances that are capable of complexing iron. Moraghan et al. (2002) also observed that the amount of iron accumulated in the seed coat of black common bean cultivars was superior to the amounts found in navy bean cultivars. In the present study, we determined that not all the black seed cultivars showed higher iron concentration in their seed coats. In the 2007/2008 cycle, the Guateian 6662 and the Macanudo (black beans of the Middle American gene pool) cultivars presented iron concentrations in the seed coat that were lower than the values obtained in the Pérola (carioca-type seeds of the Middle American gene pool) and Iraí (cranberry-type seeds from the Andean gene pool) cultivars. Therefore, factors other than tannin concentration affect the iron concentration in the seed coat of common bean seeds. In addition, the iron concentration in the seed coat of the Iraí (larger seeds) cultivar in the 2007/2008 cycle did not differ from the values observed in the TPS Nobre, Diamante Negro and BRS Valente (smaller seeds) cultivars, which indicates that there is no relationship between seed size and iron concentration. Moraghan and Grafton (2001), assessing the seeds of eight common bean cultivars of different gene pools in five locations in the United States, also found no association between iron content and seed size.

3.5. *Zinc distribution*

In terms of zinc concentration, the only significant interactions observed were cultivars × seed fractions (Table 2), indicating that, for this mineral, the environment had a lesser influence on its absorption, which could be convenient for the selection of common bean cultivars with high zinc concentration in seeds. In the 2008/2009 cycle, the rainfall intensity was high near the harvest, and this seems have affected the translocation of minerals to seeds in the common bean, except for zinc and copper, which showed no significant difference between crop cycles.

The zinc concentration in seed coat fractions was higher than the concentration obtained in embryo fractions in most cultivars (Fig. 2). The average zinc concentration in the seed coat ranged from 1.09 (Diamante Negro) to 4.03 mg 100 g^{-1} DM (Iraí). The Iraí cultivar presented the highest zinc concentration in the seed coat, revealing differences between Andean and Middle American cultivars regarding this mineral. As the Iraí cultivar has a greater seed mass, the hypothesis of an association between seed size and zinc content in the common bean may be proposed. However, Moraghan and Grafton (2001) found no significant correlation between seed size and zinc content in the common bean. Given the high zinc concentration assessed in the Iraí cultivar, its inclusion in the diet may be helpful in the prevention of malnutrition symptoms, which currently affects approximately 49% of the

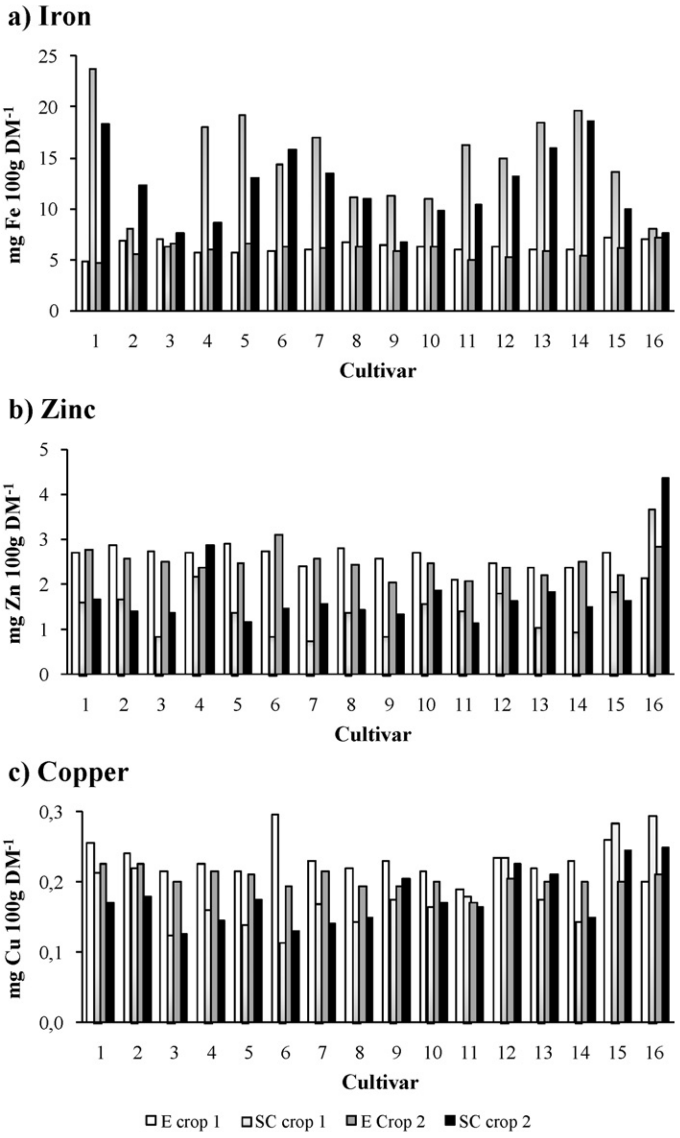


Fig. 2. Iron, zinc and copper concentrations (mg 100 g^{-1} DM) determined in the embryo (E) and seed coat (SC) of 16 common bean cultivars (1: Rio Tibagi, 2: Guateian 6662, 3: Macanudo, 4: Minuano, 5: Macotaço, 6: Guapo Brilhante, 7: IAPAR 44, 8: TPS Nobre, 9: Diamante Negro, 10: BRS Valente, 11: IPR Uirapuru, 12: BRS Campeiro, 13: FEPAGRO 26, 14: BRS Supremo, 15: Pérola and 16: Iraí) obtained in two crop cycles.

population who are at risk of developing zinc deficiency due to the low intake of the mineral in individual diets (Brown et al., 2001).

The average zinc concentration in embryos ranged from 2.08 (IPR Uirapurú) to 2.92 mg 100 g^{-1} DM (Guapo Brilhante), but common bean cultivars did not significantly differ from each other (Fig. 2). As the embryo represented more than 89.3% of the total dry mass of common bean seeds, the zinc content was higher in embryos in almost all cultivars, ranging from 55.3 to 76.3% (Table 3). However, the Iraí cultivar had a higher percentage of zinc accumulation in the seed coat in both crop cycles (63.4% and 60.7%, respectively), and the Minuano cultivar had a higher percentage of zinc localized in the seed coat in the 2008/2009 cycle (54.6%).

The amount of zinc that accumulated in the seed coat ranged from 23.7 to 63.4% (Table 3). These percentages were much higher than those observed in common bean cultivars grown in the United States and Mexico (Moraghan and Grafton, 2002; Moraghan et al., 2002). Moraghan and Grafton (2002) observed an average of 7% zinc accumulation in the seed coat of two black common bean (Middle American) cultivars. Moraghan et al. (2002) verified that there is a variation of 4–12% in the zinc content accumulated in the seed coat of three common bean cultivars of the Middle American gene pool that have different seed colors and were grown in different soil types. The differences between this study and those previously published, in regards to the migration of zinc to the common bean seed embryos, can be explained by genetic differences among cultivars of different gene pools, soil types and climates.

3.6. Copper distribution

The copper concentration in the common bean seeds was higher in the seed coat fractions, ranging from 0.115 (Guapo Brilhante, 2007/2008 cycle) to 0.295 mg 100 g^{-1} DM (Iraí, 2007/2008 cycle) (Fig. 2). Significant differences were only observed for the copper concentration in the seed coat of common bean cultivars in each crop cycle. In the 2007/2008 cycle, the cultivars that had the highest copper concentration in the seed coat fractions were the Pérola (0.285 mg 100 g^{-1} DM) and the Iraí (0.295 mg 100 g^{-1} DM). In the 2008/2009 cycle, the Pérola and the Iraí cultivars also stood out due to their copper concentration in the seed coat, but they did not significantly differ from the Diamante Negro, BRS Campeiro and FEPAGRO 26 cultivars. The identification of common bean cultivars with a high copper concentration is of interest because this micronutrient is vital for optimal immune system function, the transportation of iron, the metabolism of glucose and cholesterol, treating myocardial contractility and brain development, in addition to being a cofactor for many enzymes (Cunha and Cunha, 1998).

The percentage of copper that accumulated in the embryos ranged from 40.4 (Iraí, 2007/2008 cycle) to 72.0% (Guapo Brilhante, 2007/2008 cycle) and in the seed coat from 28.1 (Guapo Brilhante, 2007/2008 cycle) to 59.6% (Iraí, 2007/2008 cycle) (Table 2). The migration of copper to the embryo was quite different among cultivars. In the 2007/2008 cycle, the accumulation of copper in the embryo was 72% for the Guapo Brilhante, 50% for the BRS Campeiro and 40.4% for the Iraí cultivars. Additionally, it was found that there is an inverse relationship between the copper accumulated in the seed coat and embryos of common bean seeds, similar to that observed for iron content in this study and by Moraghan et al. (2002).

Genetic and environmental factors contributed to the observed differences in the accumulation of calcium, potassium, iron, zinc and copper in the embryo and seed coat fractions of common bean seeds (Table 3). More than 94.5% calcium was accumulated in the seed coat, whereas 76.0–89.7% of the potassium was concentrated in the embryos. The accumulation of iron, zinc and copper varied between the embryo and seed coat fractions in common bean cultivars. However, some cultivars presented very different concentrations of certain minerals when comparing both crop cycles, allowing the identification of cultivars with higher nutritional value. The BRS Supremo cultivar has high calcium ($1044\ \text{mg}\ 100\ \text{g}^{-1}$ DM) and iron ($24.88\ \text{mg}\ 100\ \text{g}^{-1}$ DM) concentrations, so its use is recommended in diets because it provides nutrients that are beneficial for the prevention of osteoporosis and anemia. The Iraí cultivar has high potassium ($1720\ \text{mg}\ 100\ \text{g}^{-1}$ DM), zinc ($6.51\ \text{mg}\ 100\ \text{g}^{-1}$ DM) and copper ($0.47\ \text{mg}\ 100\ \text{g}^{-1}$ DM) concentrations, and its use as part of a balanced diet can significantly improve the nutritional value of a menu.

4. Conclusions

Genetic factors affect the accumulation of calcium, potassium, iron, zinc and copper in the embryo and seed coat fractions of common bean seeds. Common bean seeds contain over 94.5% of the total calcium in the seed coat and 76.0–89.7% of the total potassium in the embryo. The accumulation of minerals such as iron, zinc and copper varies widely between the seed coat and embryo fractions in common bean cultivars. The BRS Supremo and the Iraí cultivars have high nutritional value and are recommended for use in human diets.

Acknowledgments

We thank the National Council of Technological and Scientific Development (CNPq) and the Coordination for the Improvement of Higher Level (CAPES) for the grants awarded. The authors would also like to thank Fabiano Kauer (Shimadzu) for technical support in the ICP-OES analyses.

References

Analytical Methods Committee, 1987. Analyst 112, 199.

Barnabas, A.D., Arnott, H.J., 1990. Calcium oxalate crystal formation in the bean (*Phaseolus vulgaris* L.) seed coat. Botanical Gazette 151, 331–341.

Beebe, S., Gonzalez, V., Rengifo, J., 2000. Research on trace minerals in the common bean. Food and Nutrition Bulletin 21, 387–391.

Brown, K.H., Wuehler, S.E., Peerson, J.M., 2001. The importance of zinc in human nutrition and estimation of the global prevalence of zinc deficiency. Food and Nutrition Bulletin 22, 113–125.

Broughton, W.J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., Vanderleyden, J., 2003. Beans (*Phaseolus* spp.): model food legumes. Plant Soil 252, 55–128.

Cunha, D.F., Cunha, S.F.C., 1998. Microminerais. In: Dutra-de-Oliveira, J.E., Marchini, J.S. (Eds.), Ciências nutricionais. Sarvier, São Paulo, (in Portuguese), pp. 141–165.

Cruz, C.D., 2001. Programa Genes: aplicativo computacional em genética e estatística: versão Windows. Universidade Federal de Viçosa, Viçosa (in Portuguese).

Embrapa, 2006. Centro Nacional de Pesquisa de Solos. Sistema Brasileiro de Classificação de Solos, 2. ed. Embrapa Produção de Informação/Embrapa Solos, Brasília/Rio de Janeiro, 306 pp. (in Portuguese).

Embrapa, 1997. Empresa Brasileira de Pesquisa Agropecuária. SOC: ambiente de software NTIA, versão 4.2.2: manual do usuário—ferramental estatístico. Centro Nacional de Pesquisa Tecnológica em Informática para a Agricultura, Campinas (in Portuguese).

Hosfield, G.L., 1991. Genetic control of production and food quality factors in dry bean. Food Technology 45, 98–103.

Lindinger, M.I., 1995. Potassium regulation during exercise and recovery in humans: implications for skeletal and cardiac muscle. Journal of Molecular and Cellular Cardiology 27, 1011–1022.

Lombardi-Boccia, G., De Santis, N., Di Lullo, G., Carnovale, E., 1995. Impact of processing on Fe dialysability from bean (*Phaseolus vulgaris* L.). Food Chemistry 53, 191–195.

Louis, C.J., Dolan, E.M., 1970. Removal of potassium in potatoes by leaching. Journal of the American Dietetic Association 57, 42–43.

Mesquita, F.R., Corrêa, A.D., Abreu, C.M.P., Lima, R.A.Z., Abreu, A.F.B., 2007. Linhagens de feijão (*Phaseolus vulgaris* L.): composição química e digestibilidade protéica. Ciência e Agrotecnologia 31, 1114–1121 (in Portuguese, with English abstract).

Moraghan, J.T., Grafton, K., 1997. Accumulation of calcium in bean cultivars differing in seed size. Journal Science of Food Agriculture 74, 251–256.

Moraghan, J.T., Grafton, K., 2001. Genetic diversity and mineral composition of common bean seed. Journal of the Science of Food and Agriculture 81, 404–408.

Moraghan, J.T., Grafton, K., 2002. Distribution of selected elements between the seed coat and embryo of two black bean cultivars. Journal of Plant Nutrition 25, 169–176.

Moraghan, J.T., Padilha, J., Etchevers, J.D., Grafton, K., Acosta-Gallegos, J.A., 2002. Iron accumulation in seed of common bean. Plant and Soil 246, 175–183.

Moraghan, J.T., Etchevers, J.D., Padilha, J., 2006. Contrasting accumulations of calcium and magnesium in seed coats and embryos of common bean and soybean. Food Chemistry 95, 554–561.

Morrow, B., 1991. The rebirth of legumes. Food Technology 45, 96–121.

Quenzer, N.M., Huffman, V.L., Burns, E.E., 1978. Some factors affecting pinto bean quality. Journal of Food Science 43, 1059–1061.

Reyes-Moreno, C., Paredes-López, O., Gonzalez, E., 1993. Hard-to-cook phenomenon in common beans—a review. Critical Reviews in Food Science and Nutrition 33, 227–286.

Sathe, S.K., Deshpande, S.S., Salunkhe, D.K., 1984. Dry beans of phaseolus: a review. Part 2. Chemical composition: carbohydrates, fiber, minerals, vitamins and lipids. Critical Reviews in Food Science and Nutrition 21, 41–93.

Souza, M., Madari, B.E., Sena, M.M., 2012. Aplicação de métodos quimiométricos na otimização da extração de Ca, Mg, K, Fe, Zn, Cu E Mn em folhas de Braquiária. Química Nova XY, 1–5.

Talukder, Z.I., Anderson, E., Miklas, P.N., Blair, M.W., Osorno, J., Dilawari, M., Hossain, K.G., 2010. Genetic diversity and selection of genotypes to enhance Zn and Fe content in common bean. Canadian Journal of Plant Science 90, 49–60.

UNICEF. United Nations Children's Fund. 18 Nations Fortify Foods. Retrieved from: http://www.unicef.org/pon96/nufortif.htm (19.06.11).

Wolswinkel, P., 1992. Transport of nutrients into developing seeds: a review of physiological mechanisms. Seed Science Research 2, 59–73.