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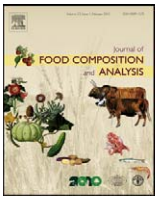

Original Article

Diversity of seed mineral composition of *Phaseolus vulgaris* L. germplasm

Carla Pinheiro [a], José P. Baeta [b], Ana M. Pereira [c], Hermínia Domingues [c], Cândido P. Ricardo [a,d,*]

[a] *Plant Biochemistry, Instituto de Tecnologia Química e Biológica, Apartado 127, 2781-901 Oeiras, Portugal*
[b] *Estação Florestal Nacional, Quinta do Marquês, 2780-159 Oeiras, Portugal*
[c] *Estação Agronómica Nacional, Quinta do Marquês, 2784-505 Oeiras, Portugal*
[d] *Instituto Superior de Agronomia, Tapada da Ajuda, 1349-017 Lisboa, Portugal*

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ABSTRACT

A collection of 155 accessions of ancient Portuguese common beans (*Phaseolus vulgaris* L.) was evaluated in relation to the content of 8 minerals (Zn, Cu, Fe, Mn, Ca, Mg, P and K) important for human nutrition. A high degree of variability for P, Fe, Zn, Cu, Mn and Ca was observed in the collection. Total correlation matrix analysis revealed the existence of two important sets of strong positive correlations ($P \leq 0.0001$), one involving P, Fe, Zn, Cu and protein, and the other Ca and Mn. The principal component analysis showed that Zn, Fe and Cu are highly correlated to the first component (27% of variability) and Mn and Ca to the second component (22% of the variability). The high mineral variability observed in the seeds of this common bean collection could be useful for the selection of cultivars with higher nutrition value and for the improvement of seed nutrition quality traits.

1. Introduction

Legume seeds are an important staple food and source of dietary minerals that potentially provide all of the 15 essential minerals required by humans (Welch et al., 2000). The common bean (*Phaseolus vulgaris* L.) is the most important grain legume for direct human consumption and is an extremely diverse crop in terms of morphological variability, uses and cultivation (Broughton et al., 2003). At average levels of usual consumption by people of reduced economic means (15–20 kg yr^{-1}), beans can provide 10–20% of the adult requirement for a number of nutrients, namely iron, phosphorus, magnesium, manganese, and in lesser degree, zinc, copper and calcium (Broughton et al., 2003). However, the concentrations of Fe, Zn, and Ca are low when compared to animal food products (Wang et al., 2003). Therefore, increasing the content of those minerals in plant food through breeding is considered a suitable strategy to combat mineral deficiency in human populations (Moraghan and Grafton, 2001). Searching for high mineral content cultivars is thus fundamental.

The Iberian Peninsula was an important region of introduction of the American *P. vulgaris* in Europe, becoming a secondary centre of diversity (Santalla et al., 2002). *P. vulgaris* rapidly conquered all of Portugal, partially replacing *Vigna* and the Asian *Phaseolus* species. As agriculture and society have evolved together, the current state of farming systems is the result of the interaction of climatic, edaphic, biotic and social factors (Broughton et al., 2003); consequently, crop seed composition has been modulated by genotype and environmental interactions. Due to its cleistogamic nature and the diversity of edafic climatic regions in Portugal, a natural selection and genetic drift of *P. vulgaris* led to the appearance of numerous local forms still in cultivation. Since these local forms were grown in the same soil for centuries they are somehow the result of the farmer's selection, who, year after year, chose varieties of high production.

This observation implies that *P. vulgaris* forms originating from very different soil types (reflecting the geological substrates) may have quite distinct genetic characteristics in relation to mineral uptake and use efficiency. This fact together with the suggestion that *P. vulgaris* seeds are a good nutritional source of several minerals prompted us to evaluate the mineral composition of a collection of local accessions from all over the country.

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[*] Corresponding author at: ITQB, Apartado 127, 2781-901 Oeiras, Portugal. Fax: +351 214433644.
E-mail address: ricardo@itqb.unl.pt (C.P. Ricardo).

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2. Material and methods

2.1. Plant sampling

The 155 accessions studied in this work belong to the *P. vulgaris* germplasm collection stored at the EAN Germplasm Bank (Oeiras, Portugal), and were originated from the regions shown in Fig. 1.

All the seeds assayed resulted from plants grown at Oeiras (Quinta do Marquês) in the same Anthrosol (WRB, 2006) fertilised at sowing with 300 kg/ha 1:3:3 (N:P:K) and with the characteristics shown in Table 1. The main soil characteristics were determined through the methods adopted by the Soil Conservation Service (1972): Soil PO_4 and K_2O availability was determined by the Egner–Riehm method (Egner et al., 1960), and Cu, Zn, Fe and Mn were extracted by the diethylenetriaminepentaacetic acid (DTPA) method (Lindsay and Norvell, 1978), and analysed by atomic absorption spectrometry as described below for the seeds.

From each accession, 20 plants were grown in a row and care was taken to check that each plant pertained to that specific accession. At harvest, each plant was collected individually and the seeds of 3 of its representative pods were gathered. A biological sample refers to 3 seeds from an individual plant.

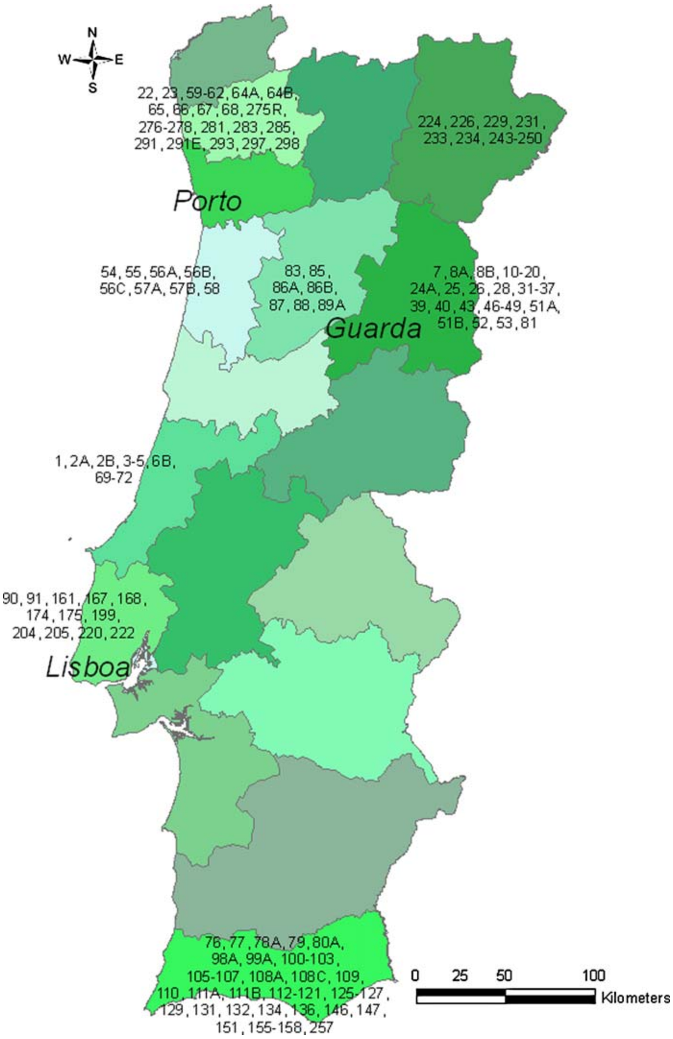

Fig. 1. Map of Portugal showing the collection sites of the several *P. vulgaris* accessions studied in the present work (ESRI® ArcMapTM 9.1).

Table 1
Physical characteristics and chemical data (0–30 cm depth layer) from the Anthrosol (WRB, 2006) where the 155 *P. vulgaris* accessions were grown.

Coarse sand (%)	12.8
Fine sand (%)	29.7
Silt (%)	24.5
Clay (%)	33.0
pH (H_2O)	7.9
$CaCO_3$ (%)	6.0
Organic matter (%)	1.51
Organic C (%)	0.88
Total N (%)	0.14
Available PO_4 (mg kg^{-1})	422
Available K_2O (mg kg^{-1})	378
Available Cu (mg kg^{-1})	1.35
Available Fe (mg kg^{-1})	3.81
Available Mn (mg kg^{-1})	7.77
Available Zn (mg kg^{-1})	0.87

2.2. Mineral composition analysis

For the determination of the seed minerals, Zn, P, Cu, Fe, Mg, Mn, Ca and K, triplicate biological samples ($n = 3$) from each accession were analysed. The seeds were washed with deionised water, dried at 80 °C, weighed and ashed at 450 °C in a muffle furnace. The ashes were dissolved in 5 mL of 20% (v/v) HCl and diluted to a volume of 100 mL with deionised water. This solution was analysed for Cu, Zn, Fe, Mn, Ca, Mg and K using a Perkin–Elmer 5000 flame (air–acetylene) atomic absorption spectrometer with hollow-cathode lamp tubes (Norwalk, Connecticut, USA), according to Chapman and Pratt (1961) and Anon. (1971). Phosphorus was measured in 5 mL of the same solution by the colorimetric molybdenum ammonium vanadate method (Black et al., 1965), using a Hitachi Perkin–Elmer Model-139 UV–vis spectrophotometer (Tokyo, Japan), at the wavelength of 470 nm.

For the atomic absorption spectrophotometer analyses, the linear ranges (mg l^{-1}) were: Ca = 7; Cu and Fe = 5; Mn = 3; K = 2; Zn = 1 and Mg = 0.5; the detection limits (mg l^{-1}) were: Fe = 0.01; K = 0.005; Mn, Cu, and Zn = 0.002; Ca = 0.001; Mg = 0.0001; the sensitivities for 1% absorption (mg l^{-1}) were: Fe and Cu = 0.1; Ca = 0.07; Mn = 0.05; K 0.02; Zn = 0.015 and Mg = 0.007. For the UV–vis spectrophotometer P determination, the linear range was 20.0 mg l^{-1}.

2.3. Protein analysis

Seed protein content was determined by Palha et al. (1988), and calculated from the total nitrogen measured by the Kjeldhal method.

2.4. Statistical analysis

The data were analysed by principal component analysis (PCA), using the correlation matrix, to determine the variables containing the maximum possible variance (first, second and third coordinate axes). The statistical software utilised was the JMP In 5.1 (SAS Institute, Cary, NC, USA).

3. Results

Table 2 shows the concentration of Zn, Cu, Fe, Mg, Mn, Ca, K and P in the seeds of the 155 accessions of *P. vulgaris* representative of the Portuguese cultivation regions shown in Fig. 1. Table 2 also contains the seed protein content, as determined by Palha et al. (1988).

High diversity in the mineral composition was found for the accessions of this germplasm collection. For each of the 8 minerals

Table 2

Mineral (Zn, P, Cu, Fe, Mg, Mn, Ca and K) and protein content, and weight of 100 seeds, in 155 common bean accessions of a Portuguese germplasm collection. Average values (and standard errors) from three independent samples are represented.

Accession	Weight of 100 seeds (g)	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Ca (%)	Mg (%)	K (%)	P (%)	Protein[a] (%)	Region
1	70.9 ± 2.1	36.9 ± 0.5	11.8 ± 0.7	65.3 ± 9.3	12.7 ± 0.4	0.089 ± 0.006	0.191 ± 0.005	1.85 ± 0.07	0.542 ± 0.026	24.9	Leiria
2A	46.2 ± 0.7	27.5 ± 0.8	9.5 ± 0.5	37.5 ± 3.9	11.9 ± 0.5	0.102 ± 0.017	0.208 ± 0.002	1.97 ± 0.05	0.465 ± 0.046	27.6	Leiria
2B	60.6 ± 1.9	33.6 ± 1.9	11.6 ± 1.4	55.2 ± 5.4	10.6 ± 0.6	0.101 ± 0.015	0.206 ± 0.021	1.95 ± 0.02	0.519 ± 0.033	25.7	Leiria
3	68.6 ± 2.4	36.2 ± 3.8	9.2 ± 0.6	53.9 ± 4.8	11.8 ± 0.7	0.089 ± 0.015	0.216 ± 0.005	1.81 ± 0.10	0.552 ± 0.058	27.6	Leiria
4	58.3 ± 1.5	41.4 ± 1.9	10.5 ± 0.4	84.6 ± 3.7	10.6 ± 0.5	0.112 ± 0.013	0.220 ± 0.102	1.84 ± 0.05	0.489 ± 0.010	29.9	Leiria
5	57.5 ± 1.8	29.4 ± 1.0	8.9 ± 2.1	45.4 ± 3.7	8.4 ± 0.3	0.095 ± 0.007	0.177 ± 0.030	1.75 ± 0.08	0.514 ± 0.026	27.0	Leiria
6B	39.2 ± 2.5	30.6 ± 4.0	9.1 ± 0.8	53.5 ± 3.4	10.4 ± 0.4	0.090 ± 0.023	0.242 ± 0.020	1.74 ± 0.10	0.496 ± 0.038	25.8	Leiria
7	56.3 ± 1.8	31.6 ± 2.8	11.5 ± 0.7	50.8 ± 5.1	10.1 ± 0.6	0.137 ± 0.048	0.226 ± 0.022	1.73 ± 0.01	0.528 ± 0.041	26.2	Guarda
8A	68.7 ± 2.8	32.8 ± 2.3	8.9 ± 1.1	49.6 ± 3.0	10.4 ± 0.6	0.124 ± 0.008	0.221 ± 0.012	1.65 ± 0.11	0.568 ± 0.045		Guarda
8B	67.0 ± 2.7	32.0 ± 1.7	7.5 ± 0.1	52.9 ± 3.9	10.3 ± 0.5	0.151 ± 0.028	0.210 ± 0.006	1.64 ± 0.08	0.498 ± 0.013		Guarda
10	55.4 ± 0.9	42.8 ± 4.5	10.5 ± 0.6	63.8 ± 1.5	12.2 ± 0.6	0.125 ± 0.009	0.192 ± 0.001	1.64 ± 0.05	0.557 ± 0.050	27.5	Guarda
11	41.7 ± 2.5	29.4 ± 3.5	8.7 ± 0.2	39.3 ± 7.4	10.0 ± 0.7	0.107 ± 0.029	0.204 ± 0.005	1.66 ± 0.03	0.478 ± 0.035	25.3	Guarda
12	62.0 ± 0.9	35.4 ± 6.5	11.0 ± 0.6	54.1 ± 2.9	11.2 ± 0.6	0.098 ± 0.006	0.180 ± 0.001	1.66 ± 0.13	0.510 ± 0.025		Guarda
13	45.5 ± 1.2	24.7 ± 3.7	9.7 ± 1.2	48.6 ± 9.0	11.3 ± 0.3	0.136 ± 0.018	0.201 ± 0.009	1.74 ± 0.06	0.445 ± 0.045	27.2	Guarda
14	51.8 ± 0.8	27.2 ± 0.7	9.5 ± 0.8	42.1 ± 4.9	12.9 ± 1.2	0.152 ± 0.033	0.186 ± 0.014	1.61 ± 0.05	0.493 ± 0.095	25.3	Guarda
15	47.1 ± 2.4	31.3 ± 4.9	8.3 ± 0.1	52.0 ± 4.8	10.2 ± 0.4	0.076 ± 0.012	0.193 ± 0.005	1.91 ± 0.05	0.487 ± 0.039		Sabugal
16	59.8 ± 0.3	31.6 ± 0.4	10.5 ± 0.6	52.4 ± 2.5	10.6 ± 0.7	0.112 ± 0.020	0.202 ± 0.009	1.83 ± 0.13	0.511 ± 0.014	25.0	Sabugal
17	50.2 ± 3.3	34.4 ± 2.1	11.1 ± 0.9	59.9 ± 3.5	11.1 ± 0.1	0.137 ± 0.019	0.217 ± 0.008	1.77 ± 0.05	0.519 ± 0.026	24.0	Sabugal
18	37.5 ± 2.9	41.4 ± 4.6	12.6 ± 0.6	70.2 ± 8.4	16.2 ± 1.5	0.199 ± 0.037	0.253 ± 0.026	1.65 ± 0.05	0.658 ± 0.061	26.9	Sabugal
19	48.9 ± 1.0	29.5 ± 2.4	9.3 ± 0.6	46.6 ± 4.4	11.2 ± 0.8	0.110 ± 0.005	0.202 ± 0.003	1.86 ± 0.07	0.484 ± 0.016	23.5	Sabugal
20	66.0 ± 3.1	26.8 ± 1.8	10.0 ± 1.0	39.8 ± 3.0	8.6 ± 0.7	0.101 ± 0.015	0.158 ± 0.006	1.60 ± 0.08	0.479 ± 0.041	25.3	Sabugal
22	28.1 ± 1.4	27.6 ± 1.0	9.0 ± 0.3	48.4 ± 5.2	13.1 ± 1.1	0.106 ± 0.010	0.200 ± 0.008	1.66 ± 0.03	0.462 ± 0.034	25.9	Sabugal
23	43.4 ± 0.5	34.4 ± 2.9	10.6 ± 0.7	59.3 ± 2.3	9.8 ± 1.2	0.126 ± 0.021	0.179 ± 0.006	1.65 ± 0.06	0.505 ± 0.028	23.5	Braga
24A	53.2 ± 0.9	34.5 ± 3.2	11.3 ± 0.6	67.1 ± 3.5	10.9 ± 0.7	0.122 ± 0.022	0.187 ± 0.010	1.73 ± 0.01	0.563 ± 0.038	28.2	Guarda
25	43.4 ± 1.7	28.9 ± 2.2	8.8 ± 0.2	41.1 ± 4.8	9.8 ± 0.2	0.125 ± 0.011	0.200 ± 0.004	1.67 ± 0.01	0.492 ± 0.010	24.6	Guarda
26	67.8 ± 1.0	33.9 ± 1.4	12.2 ± 0.4	52.2 ± 5.8	10.4 ± 0.5	0.133 ± 0.016	0.212 ± 0.005	1.64 ± 0.12	0.560 ± 0.021	27.6	Guarda
28	49.1 ± 4.0	37.4 ± 5.2	10.3 ± 0.8	46.4 ± 2.9	9.4 ± 0.4	0.121 ± 0.014	0.246 ± 0.031	1.64 ± 0.11	0.527 ± 0.019	25.1	Guarda
31	66.5 ± 3.6	31.9 ± 2.9	10.8 ± 0.3	49.0 ± 7.7	10.5 ± 0.4	0.122 ± 0.016	0.224 ± 0.006	1.56 ± 0.05	0.501 ± 0.052		Guarda
32	57.3 ± 0.6	35.4 ± 1.6	10.0 ± 0.4	56.7 ± 6.7	8.7 ± 0.3	0.088 ± 0.003	0.196 ± 0.006	1.59 ± 0.03	0.528 ± 0.022	25.5	Guarda
33	46.6 ± 1.1	41.3 ± 8.1	11.4 ± 0.2	57.1 ± 3.6	10.5 ± 0.6	0.095 ± 0.017	0.211 ± 0.006	1.81 ± 0.05	0.583 ± 0.023	28.8	Guarda
34	61.1 ± 2.1	37.7 ± 2.6	11.1 ± 0.3	57.5 ± 4.9	11.4 ± 0.8	0.124 ± 0.018	0.206 ± 0.006	1.54 ± 0.08	0.596 ± 0.004	25.5	Guarda
35	38.7 ± 1.0	32.6 ± 2.4	10.2 ± 0.1	51.0 ± 1.5	9.9 ± 0.5	0.109 ± 0.013	0.176 ± 0.003	1.59 ± 0.01	0.510 ± 0.027	24.0	Guarda
36	32.4 ± 0.7	38.1 ± 1.9	12.7 ± 0.7	56.3 ± 4.2	10.6 ± 0.6	0.136 ± 0.019	0.201 ± 0.008	2.04 ± 0.08	0.572 ± 0.039	26.6	Guarda
37	46.1 ± 1.1	36.4 ± 0.9	12.8 ± 0.6	59.8 ± 4.7	11.6 ± 0.6	0.130 ± 0.017	0.169 ± 0.005	1.60 ± 0.03	0.592 ± 0.022	30.0	Guarda
39	65.9 ± 4.1	32.1 ± 2.2	11.2 ± 0.2	42.6 ± 2.1	9.8 ± 0.2	0.097 ± 0.004	0.155 ± 0.005	1.34 ± 0.05	0.528 ± 0.007	29.7	Guarda
43	56.8 ± 3.0	36.2 ± 1.7	11.5 ± 0.4	54.5 ± 2.8	11.4 ± 1.8	0.158 ± 0.020	0.185 ± 0.015	1.48 ± 0.04	0.516 ± 0.005	27.5	Guarda
46	51.5 ± 0.3	32.3 ± 2.1	9.9 ± 0.5	39.7 ± 4.3	10.8 ± 0.6	0.120 ± 0.006	0.161 ± 0.001	1.26 ± 0.02	0.474 ± 0.013		Guarda
47	51.3 ± 1.6	27.5 ± 0.6	9.0 ± 0.7	37.2 ± 1.8	12.3 ± 1.5	0.180 ± 0.017	0.191 ± 0.004	1.58 ± 0.05	0.453 ± 0.002	25.4	Guarda
48	42.6 ± 0.6	43.7 ± 10.2	11.7 ± 1.6	48.7 ± 6.6	11.1 ± 0.9	0.105 ± 0.003	0.178 ± 0.009	1.55 ± 0.10	0.629 ± 0.089	29.3	Guarda
49	33.8 ± 0.8	37.7 ± 5.6	10.7 ± 0.6	51.5 ± 6.7	11.2 ± 1.8	0.139 ± 0.039	0.171 ± 0.005	1.35 ± 0.06	0.596 ± 0.033	25.5	Guarda
51A	43.2 ± 1.8	40.1 ± 1.6	9.6 ± 0.4	60.3 ± 4.2	12.6 ± 1.0	0.135 ± 0.015	0.175 ± 0.001	1.60 ± 0.03	0.498 ± 0.031	28.7	Guarda
51B	36.2 ± 0.9	42.5 ± 2.2	10.4 ± 0.3	64.5 ± 4.6	12.9 ± 0.3	0.137 ± 0.002	0.168 ± 0.015	1.65 ± 0.07	0.547 ± 0.009	27.7	Guarda
52	54.8 ± 0.5	35.7 ± 1.4	11.0 ± 0.4	55.5 ± 3.4	11.0 ± 0.2	0.121 ± 0.009	0.152 ± 0.005	1.72 ± 0.03	0.557 ± 0.045		Guarda
54	52.5 ± 0.9	36.5 ± 1.0	13.5 ± 1.0	61.7 ± 2.2	12.8 ± 0.7	0.087 ± 0.010	0.172 ± 0.009	1.63 ± 0.11	0.537 ± 0.010	28.5	Aveiro
55	50.8 ± 2.9	28.2 ± 1.3	8.4 ± 0.3	52.7 ± 3.8	14.1 ± 0.1	0.188 ± 0.004	0.156 ± 0.005	1.49 ± 0.05	0.461 ± 0.025	27.8	Aveiro
56A	58.0 ± 0.5	42.3 ± 2.0	10.2 ± 1.0	50.2 ± 3.4	12.4 ± 0.8	0.157 ± 0.009	0.228 ± 0.002	1.64 ± 0.02	0.615 ± 0.046	26.7	Aveiro
56B	52.4 ± 0.6	36.5 ± 2.0	12.9 ± 1.8	67.5 ± 12.3	15.8 ± 1.4	0.138 ± 0.012	0.189 ± 0.010	1.82 ± 0.05	0.554 ± 0.030	25.5	Aveiro
56C	47.5 ± 1.6	41.3 ± 2.3	11.8 ± 0.5	74.5 ± 9.7	14.3 ± 2.0	0.167 ± 0.035	0.186 ± 0.010	1.71 ± 0.06	0.487 ± 0.046		Aveiro
57A	42.0 ± 1.2	35.1 ± 3.0	11.6 ± 0.2	68.9 ± 4.1	12.9 ± 1.3	0.150 ± 0.010	0.156 ± 0.001	1.66 ± 0.11	0.521 ± 0.018	26.4	Aveiro
57B	36.7 ± 1.6	34.4 ± 0.2	11.7 ± 0.6	54.4 ± 5.1	13.1 ± 0.5	0.101 ± 0.022	0.179 ± 0.008	1.68 ± 0.10	0.513 ± 0.034	26.6	Aveiro
58	41.7 ± 0.7	36.6 ± 3.2	11.4 ± 0.7	67.2 ± 5.6	11.7 ± 0.6	0.107 ± 0.015	0.161 ± 0.003	1.58 ± 0.04	0.533 ± 0.038	24.9	Aveiro
59	47.0 ± 0.6	36.0 ± 2.2	11.5 ± 0.1	70.3 ± 3.8	11.9 ± 0.2	0.114 ± 0.012	0.175 ± 0.002	1.70 ± 0.03	0.585 ± 0.016	26.0	Minho
60	58.2 ± 1.5	41.8 ± 3.1	12.2 ± 0.3	45.8 ± 1.8	9.8 ± 0.7	0.082 ± 0.002	0.150 ± 0.004	1.69 ± 0.06	0.576 ± 0.053	28.7	Minho
61	59.4 ± 1.0	35.4 ± 2.7	8.8 ± 0.3	62.6 ± 6.4	14.7 ± 1.8	0.200 ± 0.010	0.166 ± 0.006	1.57 ± 0.05	0.424 ± 0.015	25.4	Minho

Table 2 (*Continued*)

Accession	Weight of 100 seeds (g)	Zn (ppm)	Cu (ppm)	Fe (ppm)	Mn (ppm)	Ca (%)	Mg (%)	K (%)	P (%)	Protein[a] (%)	Region
62	24.9 ± 0.3	37.5 ± 1.0	9.9 ± 2.0	77.6 ± 6.1	16.0 ± 0.9	0.158 ± 0.007	0.217 ± 0.017	1.48 ± 0.03	0.545 ± 0.015	23.6	Minho
64A	40.5 ± 0.5	33.5 ± 1.6	8.7 ± 1.0	68.6 ± 2.7	13.2 ± 0.4	0.195 ± 0.027	0.208 ± 0.005	1.47 ± 0.06	0.513 ± 0.043	24.4	Minho
64B	42.0 ± 1.3	37.6 ± 2.5	10.8 ± 0.2	75.9 ± 3.5	15.7 ± 2.4	0.151 ± 0.033	0.187 ± 0.008	1.50 ± 0.03	0.544 ± 0.029		Minho
65	54.6 ± 0.2	32.0 ± 0.9	9.7 ± 0.6	54.5 ± 1.6	12.5 ± 0.4	0.123 ± 0.013	0.185 ± 0.001	1.41 ± 0.08	0.466 ± 0.025	26.0	Minho
66A	47.3 ± 1.2	34.4 ± 1.4	11.9 ± 0.4	56.3 ± 4.0	10.2 ± 0.7	0.094 ± 0.020	0.202 ± 0.002	1.40 ± 0.03	0.580 ± 0.033	27.4	Minho
66B	43.6 ± 3.0	35.8 ± 0.9	12.4 ± 0.5	53.6 ± 3.9	11.4 ± 0.3	0.129 ± 0.018	0.181 ± 0.005	1.33 ± 0.03	0.566 ± 0.034	27.4	
67A	58.6 ± 1.1	32.9 ± 1.2	11.0 ± 0.9	52.7 ± 3.8	12.3 ± 0.5	0.143 ± 0.007	0.161 ± 0.002	1.31 ± 0.02	0.516 ± 0.030	23.8	Minho
67B	49.3 ± 2.5	33.4 ± 1.2	8.7 ± 0.4	49.0 ± 1.2	9.9 ± 0.6	0.125 ± 0.007	0.194 ± 0.003	1.36 ± 0.10	0.499 ± 0.063	23.8	
68	50.7 ± 1.2	34.1 ± 4.4	7.8 ± 0.6	55.5 ± 6.1	11.1 ± 0.5	0.127 ± 0.015	0.179 ± 0.003	1.58 ± 0.10	0.459 ± 0.063	23.5	Minho
69	70.9 ± 1.8	44.0 ± 1.6	12.1 ± 1.5	56.9 ± 9.3	11.0 ± 0.2	0.103 ± 0.001	0.168 ± 0.011	1.53 ± 0.02	0.543 ± 0.036	27.6	Oeste
71	45.3 ± 1.3	35.9 ± 0.7	10.2 ± 0.8	64.6 ± 3.7	10.5 ± 0.4	0.117 ± 0.014	0.184 ± 0.003	1.55 ± 0.05	0.512 ± 0.017	25.4	Oeste
72	50.9 ± 0.6	32.5 ± 2.8	8.1 ± 0.5	59.0 ± 4.2	14.1 ± 2.1	0.152 ± 0.014	0.178 ± 0.003	1.59 ± 0.14	0.486 ± 0.058	24.0	Oeste
76	69.0 ± 5.6	41.1 ± 4.5	10.5 ± 0.5	71.3 ± 5.0	10.8 ± 1.1	0.093 ± 0.012	0.192 ± 0.012	1.45 ± 0.03	0.601 ± 0.035	25.4	Algarve
77	54.6 ± 1.8	37.8 ± 1.4	9.8 ± 0.3	73.9 ± 6.5	9.9 ± 0.2	0.102 ± 0.021	0.149 ± 0.001	1.71 ± 0.10	0.529 ± 0.020	28.3	Algarve
78A	59.8 ± 1.8	36.8 ± 0.8	10.5 ± 0.5	71.6 ± 3.8	10.2 ± 1.0	0.074 ± 0.005	0.146 ± 0.004	1.80 ± 0.08	0.564 ± 0.023	29.6	Algarve
79	44.8 ± 1.1	33.1 ± 3.6	9.3 ± 1.4	50.8 ± 6.8	12.4 ± 1.3	0.116 ± 0.026	0.176 ± 0.003	1.62 ± 0.14	0.474 ± 0.033	26.5	Algarve
80A	58.9 ± 0.4	36.2 ± 0.8	6.5 ± 0.7	67.2 ± 8.6	14.4 ± 1.0	0.122 ± 0.012	0.178 ± 0.007	1.46 ± 0.02	0.538 ± 0.035	27.8	Algarve
80B	52.6 ± 2.4	40.6 ± 5.9	7.7 ± 1.3	76.1 ± 3.8	15.2 ± 1.1	0.123 ± 0.017	0.163 ± 0.005	1.75 ± 0.18	0.564 ± 0.034	27.8	
81	48.5 ± 0.5	41.9 ± 2.1	12.4 ± 0.7	83.7 ± 5.0	10.1 ± 0.6	0.117 ± 0.016	0.168 ± 0.002	1.81 ± 0.04	0.570 ± 0.017	29.5	Guarda
82	31.2 ± 2.6	26.7 ± 3.0	7.7 ± 1.1	49.2 ± 5.2	12.0 ± 1.4	0.123 ± 0.032	0.208 ± 0.012	1.33 ± 0.06	0.458 ± 0.044	25.7	Açores
83	43.1 ± 3.5	28.8 ± 2.1	7.2 ± 0.9	54.6 ± 5.2	11.4 ± 0.9	0.174 ± 0.014	0.188 ± 0.009	1.34 ± 0.02	0.432 ± 0.036	26.0	Viseu
85	41.4 ± 0.6	36.3 ± 2.8	10.1 ± 0.8	63.2 ± 9.7	16.1 ± 1.3	0.090 ± 0.016	0.192 ± 0.009	1.60 ± 0.02	0.535 ± 0.056	28.1	Viseu
86A	60.2 ± 0.6	33.8 ± 0.7	10.5 ± 0.2	63.8 ± 2.1	10.7 ± 0.8	0.120 ± 0.010	0.160 ± 0.004	1.64 ± 0.03	0.421 ± 0.019	25.6	Viseu
86B	42.3 ± 2.2	37.7 ± 2.1	8.7 ± 0.9	59.9 ± 3.1	10.1 ± 0.2	0.102 ± 0.005	0.179 ± 0.004	1.78 ± 0.13	0.438 ± 0.033		Viseu
87	71.7 ± 1.3	35.7 ± 2.6	12.4 ± 1.0	52.7 ± 1.0	10.2 ± 0.3	0.124 ± 0.018	0.148 ± 0.005	1.76 ± 0.28	0.496 ± 0.015	25.5	Viseu
88	64.3 ± 0.9	34.8 ± 1.6	11.0 ± 0.3	61.8 ± 3.7	11.1 ± 0.5	0.125 ± 0.004	0.151 ± 0.001	1.80 ± 0.03	0.534 ± 0.025	26.4	Viseu
89A	61.1 ± 2.5	36.0 ± 1.5	10.5 ± 1.3	64.5 ± 5.1	12.5 ± 1.1	0.125 ± 0.016	0.177 ± 0.005	1.36 ± 0.07	0.480 ± 0.044	25.4	Viseu
90	38.1 ± 0.5	43.4 ± 2.6	10.9 ± 0.2	88.4 ± 4.0	12.7 ± 0.3	0.157 ± 0.024	0.154 ± 0.001	1.59 ± 0.11	0.585 ± 0.034	27.9	Lisboa
91	86.0 ± 4.7	27.4 ± 2.3	5.4 ± 0.3	41.9 ± 5.4	15.5 ± 1.3	0.121 ± 0.008	0.228 ± 0.013	2.07 ± 0.14	0.458 ± 0.051		Faro
98A	65.0 ± 3.6	28.4 ± 1.9	8.7 ± 1.4	52.3 ± 4.6	13.3 ± 0.5	0.086 ± 0.021	0.192 ± 0.009	1.75 ± 0.03	0.558 ± 0.169	28.9	Faro
99A	40.0 ± 0.9	37.1 ± 3.0	6.5 ± 0.6	61.7 ± 4.7	20.0 ± 2.1	0.137 ± 0.014	0.193 ± 0.011	1.76 ± 0.01	0.498 ± 0.025		Faro
100	35.9 ± 4.8	37.4 ± 1.5	10.9 ± 0.9	67.3 ± 1.0	13.2 ± 1.0	0.142 ± 0.028	0.172 ± 0.008	1.58 ± 0.08	0.550 ± 0.019	27.0	Tavira
101	37.7 ± 1.5	31.4 ± 0.4	5.7 ± 0.5	54.1 ± 2.1	11.9 ± 0.4	0.099 ± 0.006	0.202 ± 0.005	1.29 ± 0.03	0.540 ± 0.128	23.3	Faro
102	53.1 ± 4.5	29.7 ± 1.8	4.7 ± 1.1	49.2 ± 3.6	15.0 ± 1.5	0.158 ± 0.011	0.182 ± 0.006	1.69 ± 0.14	0.353 ± 0.085	–	Faro
103	48.4 ± 1.9	36.3 ± 1.7	12.7 ± 0.4	68.9 ± 7.6	11.7 ± 0.6	0.110 ± 0.016	0.207 ± 0.006	1.39 ± 0.4	0.604 ± 0.011	28.9	Loulé
105	43.0 ± 0.5	33.7 ± 1.8	11.2 ± 0.2	66.5 ± 6.6	11.8 ± 1.1	0.132 ± 0.007	0.211 ± 0.014	1.46 ± 0.08	0.470 ± 0.019	23.4	Tavira
106	42.7 ± 1.0	35.3 ± 0.9	13.3 ± 0.5	59.3 ± 3.6	16.5 ± 0.6	0.174 ± 0.023	0.204 ± 0.004	1.65 ± 0.02	0.537 ± 0.027	27.0	Faro
107	41.1 ± 0.4	45.3 ± 2.7	10.8 ± 0.3	80.0 ± 6.9	15.6 ± 0.5	0.176 ± 0.020	0.185 ± 0.006	1.47 ± 0.07	0.649 ± 0.029	28.5	Tavira
108A	33.6 ± 2.5	34.0 ± 1.8	10.4 ± 0.4	52.1 ± 3.4	15.0 ± 1.2	0.216 ± 0.025	0.170 ± 0.005	1.35 ± 0.08	0.513 ± 0.006	23.9	Tavira
108C	42.4 ± 2.0	33.9 ± 0.8	12.2 ± 0.3	60.6 ± 6.3	11.8 ± 0.9	0.205 ± 0.009	0.167 ± 0.002	1.36 ± 0.03	0.487 ± 0.015	24.0	Loulé
109	36.3 ± 2.3	36.0 ± 3.3	9.9 ± 1.0	66.0 ± 10.0	12.9 ± 0.6	0.172 ± 0.014	0.175 ± 0.001	1.57 ± 0.08	0.797 ± 0.402	27.1	Faro
111B	28.2 ± 2.1	43.5 ± 2.0	10.9 ± 1.2	63.1 ± 4.3	11.2 ± 0.4	0.135 ± 0.021	0.197 ± 0.018	1.85 ± 0.16	0.554 ± 0.030	26.7	Faro
112	27.0 ± 1.4	33.7 ± 1.8	9.0 ± 1.0	49.3 ± 1.4	12.9 ± 0.6	0.111 ± 0.010	0.187 ± 0.012	1.52 ± 0.09	0.475 ± 0.019	25.5	Tavira
113	42.9 ± 0.5	36.0 ± 1.0	10.4 ± 0.6	62.0 ± 11.5	10.5 ± 1.1	0.132 ± 0.016	0.168 ± 0.012	1.67 ± 0.18	0.470 ± 0.034	21.7	Tavira
114	35.5 ± 3.0	26.2 ± 3.5	10.5 ± 1.0	61.9 ± 1.4	12.4 ± 0.1	0.116 ± 0.012	0.242 ± 0.009	1.44 ± 0.07	0.496 ± 0.034	25.9	Faro
116	35.1 ± 0.3	31.6 ± 0.5	10.5 ± 0.6	55.1 ± 6.3	10.7 ± 1.0	0.156 ± 0.007	0.217 ± 0.013	1.42 ± 0.08	0.476 ± 0.017	23.7	Loulé
118	65.3 ± 2.2	34.5 ± 2.6	9.2 ± 1.1	55.6 ± 4.4	10.0 ± 0.8	0.139 ± 0.002	0.175 ± 0.005	1.62 ± 0.05	0.493 ± 0.042	24.6	Faro
119	32.8 ± 3.8	41.0 ± 1.5	10.1 ± 0.3	56.2 ± 1.2	11.0 ± 0.5	0.107 ± 0.016	0.213 ± 0.014	2.12 ± 0.14	0.589 ± 0.028	25.8	Tavira
120	45.4 ± 2.2	29.3 ± 3.0	9.5 ± 1.0	54.9 ± 7.1	13.5 ± 2.1	0.131 ± 0.015	0.211 ± 0.019	1.66 ± 0.10	0.430 ± 0.039	25.3	Algarve
121	66.9 ± 2.6	29.1 ± 2.6	9.6 ± 1.1	53.6 ± 9.7	8.9 ± 0.6	0.109 ± 0.004	0.192 ± 0.008	1.70 ± 0.03	0.543 ± 0.071	26.0	Algarve
125	42.7 ± 3.0	35.1 ± 2.7	8.2 ± 1.0	60.3 ± 9.2	11.8 ± 0.6	0.171 ± 0.018	0.187 ± 0.016	1.52 ± 0.14	0.480 ± 0.054		Algarve
126	46.3 ± 1.7	30.8 ± 1.9	8.7 ± 0.3	54.1 ± 10.3	17.8 ± 2.7	0.165 ± 0.028	0.175 ± 0.007	1.55 ± 0.09	0.472 ± 0.040		Faro
127	56.2 ± 2.5	34.8 ± 1.5	8.8 ± 1.0	43.9 ± 1.6	14.9 ± 2.1	0.093 ± 0.012	0.165 ± 0.008	1.62 ± 0.11	0.439 ± 0.050	25.4	Faro
129	40.4 ± 1.2	26.8 ± 2.5	8.4 ± 0.4	39.5 ± 3.6	15.0 ± 0.1	0.247 ± 0.060	0.206 ± 0.012	1.77 ± 0.12	0.432 ± 0.027	26.4	Faro
131	41.3 ± 0.7	38.9 ± 4.9	8.6 ± 1.0	57.1 ± 11.8	18.6 ± 7.2	0.136 ± 0.015	0.200 ± 0.001	1.71 ± 0.05	0.528 ± 0.032	–	Faro
132	63.8 ± 0.8	33.7 ± 1.2	10.2 ± 0.4	58.2 ± 3.7	10.7 ± 0.4	0.104 ± 0.001	0.179 ± 0.014	1.69 ± 0.09	0.540 ± 0.005	27.0	Faro

134	79.0 ± 0.3	23.8 ± 2.5	8.8 ± 0.3	54.2 ± 4.7	15.7 ± 1.5	0.174 ± 0.004	0.173 ± 0.002	1.66 ± 0.06	0.524 ± 0.035		Faro
136	75.1 ± 2.2	23.9 ± 1.9	8.2 ± 0.7	34.0 ± 4.1	11.4 ± 0.8	0.143 ± 0.009	0.173 ± 0.009	1.54 ± 0.04	0.478 ± 0.044		Faro
146	53.0 ± 1.9	41.1 ± 2.1	7.9 ± 0.8	50.4 ± 1.7	17.2 ± 2.3	0.186 ± 0.023	0.202 ± 0.003	1.34 ± 0.09	0.441 ± 0.067		Faro
147	47.2 ± 1.1	30.0 ± 0.8	9.8 ± 1.0	44.6 ± 1.9	17.4 ± 1.6	0.153 ± 0.020	0.195 ± 0.005	1.72 ± 0.10	0.604 ± 0.021		Faro
151	80.0 ± 5.7	29.4 ± 5.8	7.2 ± 0.8	47.0 ± 14.6	17.5 ± 8.6	0.122 ± 0.013	0.205 ± 0.021	1.89 ± 0.13	0.535 ± 0.025		Faro
155	53.0 ± 1.4	35.9 ± 2.3	10.7 ± 1.3	55.3 ± 7.3	16.9 ± 2.1	0.116 ± 0.012	0.209 ± 0.009	1.71 ± 0.05	0.418 ± 0.038	26.0	Faro
156	63.9 ± 1.4	32.7 ± 3.0	8.9 ± 1.0	55.5 ± 8.0	11.1 ± 0.6	0.093 ± 0.013	0.207 ± 0.010	1.80 ± 0.05	0.389 ± 0.030	26.4	Faro
157	46.0 ± 1.5	34.8 ± 1.6	10.3 ± 0.7	57.6 ± 4.6	16.2 ± 1.6	0.141 ± 0.009	0.200 ± 0.008	1.59 ± 0.07	0.412 ± 0.027	25.5	Faro
158	50.0 ± 1.4	31.4 ± 1.8	11.5 ± 0.1	42.9 ± 2.1	12.5 ± 1.7	0.145 ± 0.026	0.215 ± 0.007	1.76 ± 0.09	0.493 ± 0.203	29.7	Faro
161	54.6 ± 1.8	38.0 ± 3.9	8.5 ± 1.0	72.7 ± 1.2	16.5 ± 2.4	0.168 ± 0.027	0.227 ± 0.002	1.52 ± 0.05	0.476 ± 0.088	26.2	Oeste
167	49.3 ± 1.8	31.1 ± 2.9	10.2 ± 0.9	43.1 ± 3.4	13.2 ± 1.4	0.167 ± 0.009	0.185 ± 0.008	1.69 ± 0.07	0.428 ± 0.032	28.4	Oeste
168	43.2 ± 2.4	33.0 ± 2.5	7.5 ± 1.1	51.7 ± 8.9	10.2 ± 1.2	0.114 ± 0.007	0.199 ± 0.015	1.71 ± 0.07	0.458 ± 0.020	29.4	Oeste
174	66.4 ± 3.2	36.0 ± 0.8	11.2 ± 1.8	47.2 ± 2.5	10.8 ± 0.2	0.119 ± 0.016	0.182 ± 0.009	1.52 ± 0.03	0.496 ± 0.055	26.9	Oeste
175	63.6 ± 2.1	33.2 ± 4.5	10.7 ± 1.6	40.1 ± 4.6	11.2 ± 0.7	0.144 ± 0.026	0.174 ± 0.006	1.69 ± 0.06	0.467 ± 0.038		Oeste
199	48.9 ± 2.2	28.4 ± 3.4	12.6 ± 0.8	56.7 ± 4.1	11.3 ± 1.0	0.106 ± 0.012	0.186 ± 0.006	1.63 ± 0.06	0.472 ± 0.029	22.6	Oeste
205	58.6 ± 5.5	33.6 ± 1.1	6.8 ± 0.2	56.2 ± 2.4	11.4 ± 0.5	0.099 ± 0.003	0.192 ± 0.008	1.64 ± 0.13	0.454 ± 0.048	24.1	Oeste
220	55.4 ± 1.4	19.7 ± 2.7	6.4 ± 0.3	52.0 ± 3.5	9.7 ± 0.6	0.068 ± 0.013	0.178 ± 0.007	1.46 ± 0.06	0.502 ± 0.047	28.0	Oeste
222	32.3 ± 2.2	36.7 ± 0.3	10.9 ± 0.9	85.5 ± 2.9	18.8 ± 1.2	0.293 ± 0.040	0.212 ± 0.010	1.34 ± 0.10	0.383 ± 0.021	21.1	Oeste
224	56.6 ± 0.4	37.2 ± 2.3	8.9 ± 1.1	65.5 ± 4.6	12.5 ± 0.6	0.153 ± 0.004	0.184 ± 0.008	1.57 ± 0.16	0.552 ± 0.019	29.2	Bragança
226	49.8 ± 1.6	28.0 ± 0.9	9.3 ± 0.5	38.8 ± 3.8	13.4 ± 0.5	0.141 ± 0.022	0.178 ± 0.001	1.39 ± 0.09	0.410 ± 0.010	24.0	Bragança
229	46.5 ± 2.0	35.0 ± 2.1	11.9 ± 0.7	83.5 ± 8.1	16.7 ± 0.8	0.174 ± 0.021	0.228 ± 0.028	1.24 ± 0.67	0.478 ± 0.093	26.2	Bragança
233	63.3 ± 4.4	11.5 ± 1.1	5.1 ± 0.6	32.2 ± 0.8	8.0 ± 0.3	0.126 ± 0.014	0.199 ± 0.010	1.43 ± 0.03	0.613 ± 0.023	22.6	Bragança
234	55.8 ± 2.5	20.3 ± 2.5	9.3 ± 1.6	66.5 ± 1.8	13.3 ± 1.6	0.123 ± 0.010	0.192 ± 0.014	1.52 ± 0.09	0.541 ± 0.019	22.2	Bragança
243	45.6 ± 1.7	16.1 ± 2.4	10.3 ± 1.6	41.9 ± 4.6	15.3 ± 2.0	0.142 ± 0.045	0.217 ± 0.011	1.35 ± 0.15	0.564 ± 0.039	27.2	Bragança
245	60.8 ± 0.8	32.9 ± 2.9	9.3 ± 0.3	60.6 ± 2.2	11.0 ± 0.8	0.125 ± 0.015	0.193 ± 0.010	1.41 ± 0.09	0.438 ± 0.028	24.6	Bragança
246	70.6 ± 6.0	35.8 ± 4.9	10.5 ± 1.3	73.9 ± 3.0	15.2 ± 2.0	0.196 ± 0.006	0.179 ± 0.027	1.56 ± 0.16	0.487 ± 0.114	24.6	Bragança
248	45.0 ± 0.5	37.5 ± 2.4	9.9 ± 1.1	61.5 ± 8.1	13.3 ± 2.3	0.154 ± 0.026	0.184 ± 0.002	1.49 ± 0.13	0.493 ± 0.008	26.6	Miranda do Douro
249	59.5 ± 2.1	34.2 ± 3.1	9.7 ± 1.1	85.8 ± 8.1	12.7 ± 2.1	0.087 ± 0.028	0.198 ± 0.004	1.49 ± 0.05	0.507 ± 0.060	26.0	Miranda do Douro
250	46.8 ± 2.2	34.7 ± 2.0	9.3 ± 0.7	60.2 ± 9.3	15.6 ± 1.0	0.162 ± 0.019	0.165 ± 0.013	1.45 ± 0.08	0.462 ± 0.026	26.1	Miranda do Douro
257	36.2 ± 1.4	36.3 ± 1.4	9.0 ± 0.1	49.6 ± 3.4	16.3 ± 0.9	0.162 ± 0.007	0.222 ± 0.019	1.59 ± 0.03	0.528 ± 0.024	26.2	Algarve
262	23.9 ± 1.4	35.3 ± 2.3	8.2 ± 1.3	66.0 ± 8.2	19.5 ± 1.0	0.152 ± 0.004	0.208 ± 0.006	1.57 ± 0.14	0.479 ± 0.038		Cabo Verde
275A	100.4 ± 3.1	29.8 ± 1.2	8.5 ± 0.7	48.4 ± 1.3	10.2 ± 1.4	0.076 ± 0.002	0.198 ± 0.005	1.56 ± 0.06	0.339 ± 0.021		Barcelos
275B	97.3 ± 4.0	27.8 ± 1.7	7.0 ± 0.3	41.4 ± 4.7	9.8 ± 1.3	0.120 ± 0.023	0.183 ± 0.020	1.59 ± 0.01	0.332 ± 0.030		Barcelos
275R	57.8 ± 0.9	28.9 ± 0.7	8.8 ± 1.1	46.0 ± 5.3	10.7 ± 0.2	0.127 ± 0.010	0.169 ± 0.006	1.54 ± 0.04	0.405 ± 0.010		Barcelos
276	34.4 ± 1.0	30.0 ± 1.5	8.2 ± 0.4	43.7 ± 5.6	10.6 ± 0.2	0.138 ± 0.003	0.197 ± 0.006	1.45 ± 0.09	0.426 ± 0.020	23.2	Barcelos
277	35.5 ± 1.4	31.0 ± 0.3	10.0 ± 0.3	53.3 ± 9.4	12.2 ± 1.1	0.136 ± 0.013	0.128 ± 0.010	1.70 ± 0.04	0.483 ± 0.012	22.9	Barcelos
278	36.3 ± 1.6	35.2 ± 0.8	9.2 ± 0.6	61.8 ± 3.5	16.1 ± 2.2	0.154 ± 0.029	0.224 ± 0.015	1.71 ± 0.07	0.507 ± 0.137	21.9	Barcelos
281	63.9 ± 4.2	32.9 ± 2.3	10.3 ± 0.5	60.2 ± 2.4	17.3 ± 0.6	0.189 ± 0.007	0.198 ± 0.014	1.65 ± 0.04	0.393 ± 0.149	22.3	Barcelos
283	56.5 ± 1.0	33.1 ± 1.6	10.8 ± 1.0	48.4 ± 5.8	12.5 ± 1.0	0.145 ± 0.030	0.198 ± 0.009	1.74 ± 0.09	0.537 ± 0.155		Barcelos
285	44.9 ± 2.2	31.1 ± 1.3	8.0 ± 1.4	50.1 ± 6.1	14.3 ± 1.4	0.172 ± 0.028	0.197 ± 0.009	1.57 ± 0.12	0.411 ± 0.046	26.5	Barcelos
291	61.3 ± 2.7	42.6 ± 1.7	8.6 ± 1.6	63.3 ± 5.6	20.1 ± 1.1	0.174 ± 0.028	0.187 ± 0.009	1.66 ± 0.11	0.491 ± 0.057		Barcelos
291E	54.5 ± 2.0	30.5 ± 2.3	9.8 ± 1.0	41.3 ± 6.4	16.3 ± 1.3	0.099 ± 0.004	0.204 ± 0.013	1.77 ± 0.04	0.557 ± 0.067		Barcelos
293	40.5 ± 2.4	34.4 ± 0.9	10.3 ± 1.0	51.6 ± 6.1	13.0 ± 0.7	0.125 ± 0.036	0.198 ± 0.009	1.85 ± 0.10	0.457 ± 0.028		Barcelos
297	24.0 ± 0.4	34.0 ± 1.9	11.6 ± 0.7	61.1 ± 6.1	18.5 ± 1.3	0.165 ± 0.014	0.239 ± 0.008	1.81 ± 0.13	0.503 ± 0.026	22.5	Barcelos
298	24.8 ± 0.1	34.2 ± 1.2	10.7 ± 0.2	62.7 ± 3.5	15.9 ± 0.2	0.137 ± 0.001	0.247 ± 0.010	1.57 ± 0.02	0.489 ± 0.025		Barcelos
Ratio percentile[b]	3.0	2.2	3.8	2.3	2.2	2.700	1.600	1.5	1.700	1.4	
Max	100.4	45.3	13.5	88.4	20.1	0.293	0.253	2.12	0.797	30.0	
Min	3.0	11.5	4.7	32.2	8.0	0.068	0.128	1.24	0.497	21.1	

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[a] Protein content as determined by Palha et al. (1988) in 124 accessions.

[b] Ratio percentile 97.5%/2.5%.

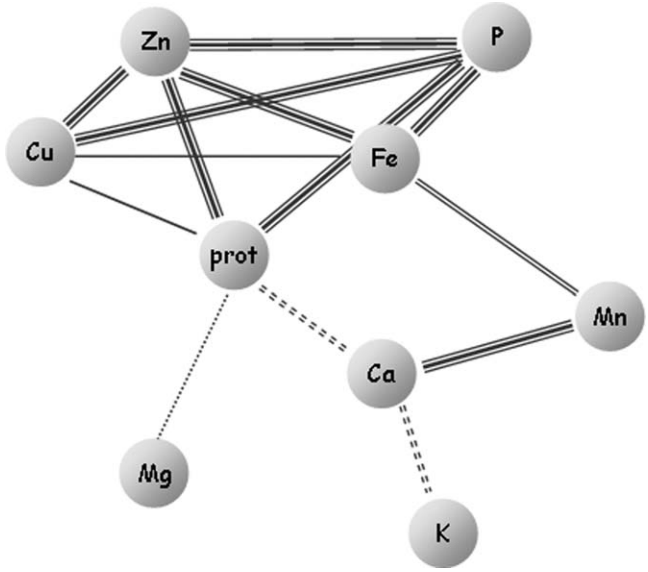

Fig. 2. Graphical representation of the correlations between the several elements analysed in the *P. vulgaris* accessions ($n = 155$), which results from the total correlation matrix: $P \leq 0.0001$ (triple line), $P \leq 0.001$ (double line) and $P \leq 0.05$ (single line). Negative correlations are similarly illustrated by dotted lines.

analysed it was possible to detect many accessions with very high seed concentration of that mineral. Particularly high levels of Fe, Zn, P and Ca were observed, but several accessions with low levels of seed minerals were also identified. For the micronutrient concentrations, accessions varied from 2.5-fold for Mn to 3.9-fold for Zn, while for the macronutrient concentrations, accessions varied from 1.6-fold for P to 4.3-fold for Ca. Taking into consideration the coefficient of variation, for micronutrients the observed variation ranged from 15.3% for Zn to 20.6% for Mn, and for macronutrients from 10.0% for K to 25.5% for Ca. These results indicate the existence in the germplasm collection of a significant degree of genetic variability that seems particularly relevant for Cu, Ca, Fe, Zn and Mn.

A total correlation matrix analysis (Fig. 2) revealed two strong sets of correlations, one associating protein, Zn, Fe, P and Cu ($P \leq 0.0001$), and the other Ca and Mn ($P \leq 0.0001$). When partial correlations were considered, the very strong associations Zn–Fe, Zn–Cu, Cu–P and Ca–Mn were confirmed and it was possible to classify the Fe–P association as a strong correlation "forced" by the other very strong ones. We could not find any correlation between mineral composition and the geographical origin of the accessions, which might be due to the high soil heterogeneity in Portugal. We could not find any correlation between mineral composition and seed size either.

A PCA showed that Zn, Fe and Cu are highly correlated to the first component (27% of the variability), Ca and Mn to the second component (22% of the variability) and Mg and K to the third component (15% of the variability) (Fig. 3).

4. Discussion

Considering the great value of traditional plant germplasm collections, it is important to characterise them with respect to their nutritional value. Studies on Portuguese grain legume germplasm (Palha et al., 1988; Pereira and Tavares-de-Sousa, 1996; Pereira et al., 1998, 2006; Rodiño et al., 2001, 2003; Vaz et al., 2004) have focused on growth habits, physiological traits and seed protein content. We have now analysed the seed mineral content of an important Portuguese germplasm collection of common bean (*P. vulgaris*). The high variability in Fe and Zn concentration found in Mesoamerican and Andean landraces (Beebe et al., 2000; Moraghan and Grafton, 2001; Moraghan et al., 2002) is also observed in the Portuguese collection, which, additionally, displays high variability in relation to P, Mn, Ca and Cu. This information is potentially important for breeding programs since some accessions have high values of P, Zn, Fe, Cu and protein. It is also relevant that besides the Fe–Zn positive correlation previously reported (Welch et al., 2000) we find strong positive correlations of P–Cu, P–protein and Ca–Mn.

Despite the detection of these correlations, little information exists on the biochemical processes that underlie them. Concerning the P–proteins correlation we might speculate that it could reflect some kind of association existing in the protein bodies, where protein and phosphorus (as phytate) are accumulated. The Ca–Mn correlation highlights the problems associated with Mn metabolism in grain legumes. Considerable variability can occur in the Mn concentration of seeds, influencing plant growth and development, crop yield and seed quality (Longnecker and Uren, 1990). Manganese toxicity is a major constraint for the production of common bean in tropical and subtropical soils (Gonzalez and Lynch, 1999), but it can be avoided if the soil Ca/Mn ratio is higher than 80 (Bekker et al., 1994). Ca may alleviate Mn phytotoxicity

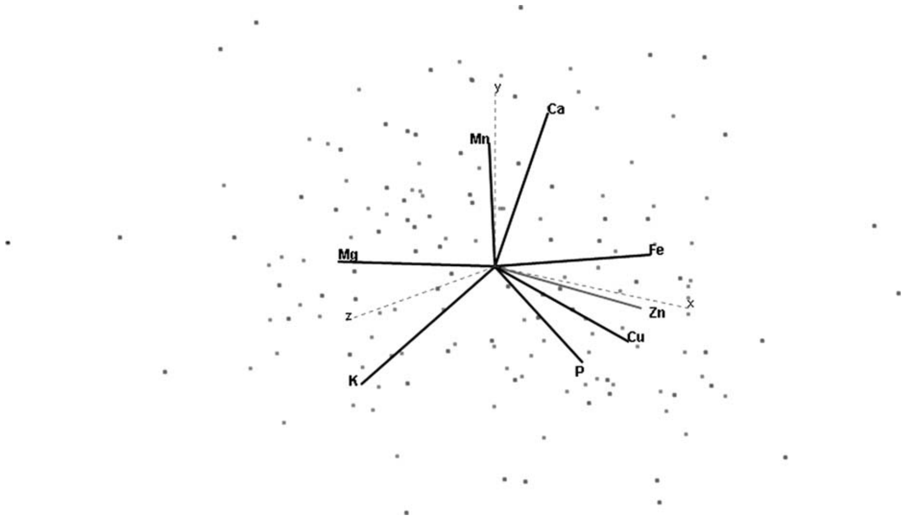

Fig. 3. Principal component analysis of the several elements (Zn, Cu, Fe, Mn, Ca, Mg, P and K) analysed in the *P. vulgaris* accessions ($n = 155$).

through the inhibition of Mn absorption (Bekker et al., 1994) and translocation to the shoots (Alam et al., 2006). In our study, Mn toxicity during common bean development was not expected because the soil Ca/Mn ratio was ~3000. Interestingly, Mn accumulation in seeds does not seem to be negatively affected by Ca. On the contrary, we observed a strong positive Ca–Mn correlation that was similarly found in Arabidopsis seeds (Vreugdenhil et al., 2004).

When considering the nutrition potentialities of this common bean collection we should emphasise the importance of legumes (in particular common beans) for direct human consumption worldwide (Broughton et al., 2003) and the relevance of grain legumes as mineral suppliers (Welch et al., 2000). Deficiencies in essential mineral cations affect large populations in several parts of the world, as it is well known for Fe and Zn. The importance for the human nutrition of P, Cu, Ca and Mn, in addition to Zn and Fe should also be taken into consideration (Solomons and Ruz, 1998). For instance, Mn deficiency has been detected in animals, and it was also observed that high dietary intake of Ca, P and Fe reduces Mn absorption (Hathcock, 2004). So, the relevance of the observed Ca–Mn strong positive correlation in the common beans should be evaluated in terms of nutritional Mn bioavailability.

Our results, besides expressing the importance of the Portuguese common bean germplasm collection, raise several questions of a physiological and biochemical nature, indicating that processes that culminate in the mineral storage in the seeds are poorly understood. Additional studies are needed to understand the integration of all those processes and their implications regarding animal and human nutrition.

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