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Intrinsic variability of mineral composition of chickpea (*Cicer arietinum*, L.)

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The influence of genetic biotype on the mineral composition of chickpea was studied. Experimental design included 37 cultivars of both Desi and Kabuli biotypes cultivated under the same climatic and agronomic conditions in order to exclude the variability of the results due to environment and genotype × environment interaction effects. The biotype, as source of variance in mineral composition, was a significant factor in explaining differences between Ca, Mg and K contents. Cu, Fe, Mn, Na and Zn contents did not show differences between biotypes. According to data previously published, differences may be explained by differences in the coat thickness and composition between biotypes. Two homogeneous subgroups of chickpea cultivars were identified, one having relatively high calcium contents and the other having relatively high iron contents. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Chickpea is a legume which is widely consumed all over the world. According to the size, shape and colour of the seed, two biotypes are usually acknowledged. The Kabuli chickpea, large-seeded with a salmon-white testa, is grown mainly in the Mediterranean area, the Near East, Central Asia and America, and the Desi chickpea, small-seeded with a light brown testa, is cultivated mostly in India and East Africa (Gil and Cubero, 1993). It is generally accepted that the Kabuli biotype was derived from the Desi biotype through a mutation followed by conscious selection (Jana and Singh, 1993). Moreover, a polymorphism has been reported between *Cicer arietinum* and its wild relative *Cicer reticulatum* (Udupa *et al.*, 1993). Elsewhere, major environmental influences have often caused genotype–environmental interactions (Hosfield, 1991; Singh *et al.*, 1993), yet the differences between cultivars are less pronounced than those due to cultivation in different agroclimatic regions (Attia *et al.*, 1994*a*). Thus, genetic and biotype differences in chemical composition must be evaluated while excluding the agroclimatic effect. For instance, Dodd and Pushpamma (1980) found great variations in mineral content due to the effect of the growing location and differences have also been reported in the Cu and Zn contents for both biotypes due to the effects of their location (Jambunathan and Singh, 1981). The aim of this paper was to study differences in mineral composition between Desi (#16 cultivars) and Kabuli (#21 cultivars) chickpea biotypes grown under the same environmental agronomic conditions. Therefore, this experimental design tried to exclude the genotype–environmental interaction as a very important source of the variance in the mineral composition of the chickpea, thus reducing the genotype–environmental interaction to inhomogeneity of soils.

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MATERIALS AND METHODS

Seed samples

Table 1 shows the 37 cultivars of both Desi and Kabuly biotipes included in the study. All were cultivated in land belonging to the Centro de Investigacion y Desarrollo Agrario (CIDA), to the west of the city of Córdoba (37° 52′ N, 4° 47′ W, elevation 137 m), and harvested in July 1995. Samples were ground in a high speed mill (Cyclotec-200, Tecator, Höganäs, Sweden) and fitted with a 0.75 mm screen.

Mineral composition analysis

For the ashing of the samples, the method described by Periago (1993) was followed. The crucibles containing 1 g of the ground, dried samples were incinerated in a furnace at 525°C applying the following heating stages

Table 1. Cultivars included in Desi (D and Kabuli (K) chickpea batches

Cultivar	Biotype	Cultivar	Biotype	Cultivar	Biotype
CA 2223	K	CA 2156	K	CA 2219	K
CA 2033	K	CA 2164	K	CA 2065	K
JG 62	D	CA 2222	K	CA 2149	K
CA 1276	D	P 678	D	CA 2137	K
CA 2206	K	CA 2075	K	CA 1778	D
P 25	K	CA 2095	K	CA 2133	K
CA 2016	K	P 39	K	CA 2082	K
CA 2227	K	CA 2225	K	CA 2087	D
CA 2138	D	CA 1449	D	CA 1410	D
CA 2047	D	CA 2077	D	CA 1540	D
CA 1776	D	CA 1351	D	CA 1713	D
CA 2161	K	ICCCL 81001	K	WR 315	D
CA 1796	D				

to prevent mineral losses by volatilization: 90–250°C (ramp time 1 h, hold time 2 h), 525°C (ramp time 6 h, hold time 9 h) and 525–100°C (ramp time 2 h). To check that there were no losses of mineral elements, studies of recovery were made in spiked ashed samples at different temperatures/time and it has been verified that at 525°C the best recovery percentages were obtained. After cooling, 2 ml nitric acid (Suprapur®, Merck, Darmstadt, Germany) was added, and the solutions were dried on a thermostatic hotplate. They were subsequently placed once again in the furnace where they remained at 525°C for 1 h. The recovery of the white ash was carried out by adding 2 ml of nitric acid (Suprapur®) in a 50 ml volumetric flask made up to volume with deionized water, and subsequently stored in polypropylene flasks under refrigeration conditions. For Ca and Mg, the solution was diluted 1/10 and 1% lanthanum chloride (LaC13.H20) was added to overcome potential anionic interferences.

Analyses were performed using a Perkin–Elmer Model 2380 atomic absorption spectrophotometer with an air/acetylene flame and spoiler nebulizer. Single-element hollow cathode lamps were used for all elements except Na and K which were determined by emission using the same instrument. In order to calculate the detection limit ($\bar{X}_{blank} + 3SD$), the criteria of IUPAC were followed (IUPAC, 1987), as the lowest concentration of an element that the analytical process can reliably detect using a confidence limit for $1-\alpha=0.95$, where α is the significance level or probability of committing a Type I error. Once the detection limit was obtained, the concentration limit could be defined as being the minimum detectable concentration in mg kg^{-1} dry weight. The entire analytical procedure was tested for both measurement precision and accuracy in order to assess the degree of reliability of the data generated. The precision of the method was established by the calculation of between-assay variation coefficients from the data from ten independent analyses carried out at different times on a chickpea flour sample (Alegria *et al.*, 1988; Barberá *et al.*, 1990; Coni *et al.*, 1994). The level of accuracy was continuously monitored by a spiked recovery test of the minor elements. The instrument settings and other experimental conditions were in accordance with the manufacturer's specifications and are shown in Table 2, together with the results of the detection and concentration limits, sensitivity, precision and spiked recovery test.

Statistical analysis

The differences in mineral content for each element between both biotypes were evaluated statistically using analyses of variance (ANOVA). In order to determine relationships within the quantitative parameters set, a principal component analysis (PCA) was carried out (SAS, 1989), using normalized varimax as a rotational strategy. In order to identify the homogeneous groups according to their mineral content, a cluster analysis was carried out (SAS, 1989).

RESULTS AND DISCUSSION

The biotype factor as a source of variance of Cu, Fe, Zn, Mn and Na contents did not show any significant effect (Table 3 and Fig. 1(A)). So these results will be discussed by comparing with results obtained by other authors.

The mean Cu content obtained was 1.22 mg/100 g (Table 3) and ranged from 0.95 to 1.69 mg/100 g. Similar results were obtained by Dodok *et al.* (1993) (1.17 mg/100 g), Attia *et al.* (1994*a*) for Kabuli chickpeas (1.08 mg/100 g), and Sika *et al.* (1995) (1.06 mg/100 g), but all these values are much higher than those reported by Meiners *et al.* (1976) (0.47 mg/100 g). This wide variability in the results may be due to the immobility of

Table 2. Operating specifications used and analytical parameters obtained for each element in the methodological procedure followed

Element	λ (nm)/slit	Sensitivity (mg l^{-1})	Detection limit (mg l^{-1})	Concentration limit (mg kg^{-1})	Precision (%)
Cu	324.7/0.7	0.93	0.019	0.65	3.76
Fe	248.3/0.2	0.12	0.088	4.40	1.09
Zn	213.9/0.7	0.01	0.019	0.96	0.61
Mn	279.5/0.2	0.37	0.035	1.75	2.31
Ca	422.7/0.7	0.19	0.035	17.39	1.98
Mg	285.2/0.7	0.10	0.0185	9.26	1.04
Na	589.0/0.2	0.79	—	—	4.70
K	766.5/0.2	3.24	—	—	2.59

Table 3. Mineral composition (mg/100 g^{-1}) of both chickpea biotypes, as X ± SD (range)

Element	All samples (n = 37)	Desi (n = 16)	Kabuli (n = 21)	ANOVA F-value (1)
Cu	1.22 ± 0.15 (0.74)	1.25 ± 0.17 (0.63)	1.20 ± 0.13 (0.53)	1.09
Fe	4.48 ± 0.52 (2.67)	4.51 ± 0.34 (1.42)	4.46 ± 0.63 (2.67)	0.10
Zn	3.53 ± 0.36 (1.42)	3.57 ± 0.30 (0.96)	3.50 ± 0.41 (1.42)	0.25
Mn	1.68 ± 0.23 (0.92)	1.72 ± 0.25 (0.91)	1.65 ± 0.22 (0.83)	0.81
Ca	178 ± 40.44 (153.87)	210 ± 30.07 (95.36)	154 ± 29.56 (102)	31.38
Mg	125 ± 7.42 (35.12)	128 ± 7.90 (31.42)	122 ± 6.28 (25.9)	5.14
Na	21.9 ± 6.32 (25.05)	22.9 ± 6.51 (23.03)	21.07 ± 6.21 (24)	0.73
K	905 ± 71.09 (306)	878 ± 64.17 (207)	926 ± 70.7 (306)	4.39

copper in soils. Its uptake by plants depends on the extent of root interception with copper-enriched zones (Gilkes, 1981) and the physical properties of the soil, particularly the presence of clay-sized particles and oxides of iron and manganese, which may adsorb copper ions from the soil solutions. Thus, soil characteristics such as organic matter, irregular distribution or particle soil features, can obscure the copper variability due to genetic factors, such as a different biotype.

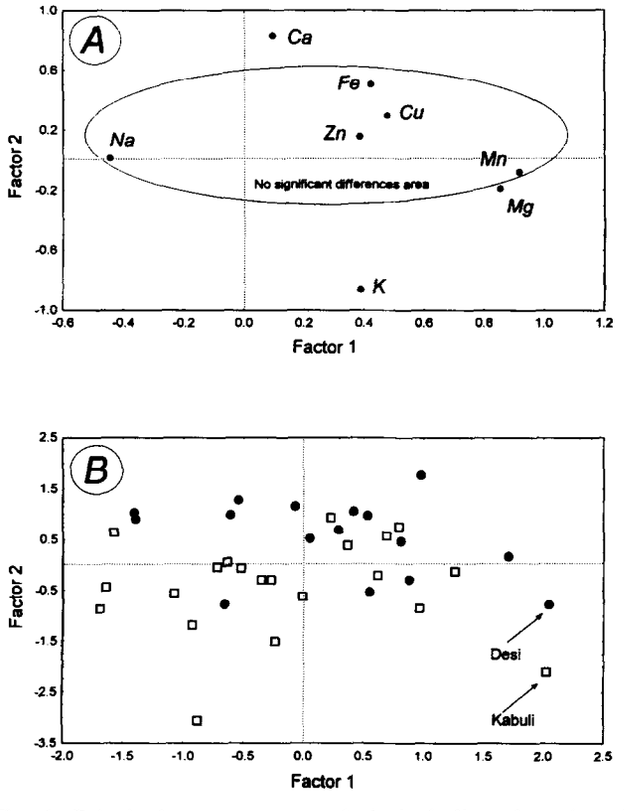

Fig. 1. Principal component analysis including the mineral composition variables showing factor loadings (A) and the first and second principal component scores of the 37 chickpea cultivars (B). The no significant differences area in (A) was obtained according to ANOVA results.

The mean Fe content obtained was 4.48 mg/100 g (Table 3) and ranged from 3.20 to 5.88 mg/100 g. Higher iron levels have been reported by other authors such as Gáborcík (1994) (from 6.42 to 7.10 mg/100 g) or Sika *et al.* (1995) (6.45 mg/100 g), and even values twice as high as those found by us have been reported by Dodok *et al.* (1993), from 8.24 to 11.41 mg/100 g. In agreement with our results, Jambunathan and Singh (1981) also found no differences in the contents of Fe in the seeds of either biotype but they found a higher Fe content in the Kabuli seed coat. However, when comparing the Fe results found by other authors, it should be noted that there are factors dependent on the composition of the soil exercising a significant effect on the amount of Fe that the plant can absorb. Thus, over 90% of Fe (III) in the soil is complexed with organic ligands and the uptake depends on the concentration of such ligands, (Benians *et al.*, 1977) and some inorganic ions such as nitrate exhibit a synergistic effect with iron (Pendias and Pendias, 1984). Therefore, any comparison with the results of different authors should be made with some caution. Inherent factors in the soil significantly affect the content of iron found in the chickpea. In addition, the state of the legume's development at the moment it is harvested should also be taken into account because higher Fe contents (× 1.54) have been reported in immature stages of pea seed, at 14–18 days after flowering, than in mature stages when, after 10 days on the plant, the green colour of the pods disappears (Geervani and Devi, 1988).

The mean Zn content obtained was 3.53 mg/100 g (Table 3) and ranged from 2.96 to 4.38 mg/100 g. These results are similar to those obtained by Sika *et al.* (1995) (3.63 mg/100 g) and Avancini *et al.* (1992) (from 3.86 to 4.42 mg/100 g).

The mean Mn content obtained was 1.68 mg/100 g (Table 3) and ranged from 1.41 to 2.33 mg/100 g. These results coincide with those reported by Sika *et al.* (1995) but are lower than those previously obtained by Avancini *et al.* (1992) (from 3.23 to 5.15 mg/100 g). Many items, such as soil factors, climate, soil pH, crop management, have been reported as affecting the Mn content in crops (McDowell, 1992).

The mean Na content obtained was 21.9 mg/100 g (Table 2) and ranged from 14.1 to 39.2 mg/100 g. A wide variability in Na contents reported in the literature was found, ranging from 2.06 mg/100 g (Avancini *et al.*, 1992) to 108 mg/100 g (Attia *et al.*, 1994*b*). This great variability might be determined by the different types of fertilizers used. For instance, it has been reported that the use of KCl as a K fertilizer increases plant Cl but depresses the Na content (Reid and Horvath, 1980). This and other agronomic factors can be used to explain the wide Na content range in chickpea found in the literature.

The biotype factor was a significant one to explain the differences between the Desi and Kabuli biotypes in Ca, Mg and K contents. Thus, the Desi biotype displayed a higher Ca and Mg content but a lower K content than the Kabuli biotype (Table 3). Figure 1 shows the principal component analysis (PCA), including the eight quantitative variables considered and the non-significant differences area, excluding Ca, Mg and K contents. However, Jambunathan and Singh (1981) reported that the mean mineral and trace element compositions of whole seed chickpeas do not differ significantly when comparing eight Desi and seven Kabuli cultivars and only found differences when the seed coat mineral content was compared, such as a higher Ca, Zn, Cu, Fe and Mn content for Kabuli cultivars.

A higher content of Ca was found in Desi (× 1.36) than in the Kabuli biotype (Table 3). A higher content in the seed coat had been reported (Sosulski and Gadan, 1988) and at the same time, it has been observed that Ca is the main mineral present in the chickpea seed-coat (Jambunathan and Singh, 1981). Therefore, the differences in Ca content for both biotypes might be explained as being a consequence of the seed-coat development, because it has been reported that the seed coat percentage clearly permits the distinguishing of both biotypes (Jambunathan and Singh, 1980). Therefore, while Kabuli cultivars contained only 4.3% hulls, Desi cultivars contained 11.5% (Sosulski and Gadan, 1988). Even on comparing Kabuly cultivars, a higher Ca content (× 1.58) has been related to a higher seed coat percentage (× 1.4) (Attia *et al.*, 1994*b*).

A lower but significant difference in the Mg contents was found between biotypes (Table 3). The differences found between biotypes for the Mg content have previously been demonstrated (Dodok *et al.*, 1993; Sika *et al.*, 1995). However, before that, Jambunathan and Singh (1981) did not find any differences between either biotype for the Mg content of whole seed but they found twice the content in the seed coat than in dhal so that the differences in the Mg content may be due to a higher seed coat percentage in Desi than in Kabuli (Sosulski and Gadan, 1988).

A higher K content was obtained in the Kabuli biotype, possibly due to the fact that the K concentration in the coat was significantly higher in Kabuli (1.22 mg/100 g) than in Desi (830 mg/100 g), as shown by Jambunathan and Singh (1981). This shows that, although Desi displayed a higher percentage of seed coat (Sosulski and Gadan, 1988) the K content in the whole seed was higher in the Kabuli (Table 3). It has been reported that the K coat content is influenced significantly by the location factor (Jambunathan and Singh, 1981), but in our experimental design this factor did not influence the results.

As a consequence of decortication, Attia *et al.* (1994*a*) obtained appreciably significant decreases in Ca, Mg, Zn and K contents. In addition, we obtained a higher Ca and Mg content in the Desi cultivar (Table 3) which showed a higher seed coat percentage. These results suggested that some mineral contents may be related to quantitative seed coat characteristics. Table 4 shows the statistically significant relations obtained between different minerals. For instance, a significant relation between Mg and Mn was always found. In order to explain this, it must be considered that the principal function of the magnesium is an activation of numerous essential enzymes, such as the many enzymes which are known to use the energy of ATP, and it is known that magnesium or manganese activate all of them (Salisbury and Ross, 1969). A principal component analysis (PCA), involving the eight quantitative variables, was performed (Fig. 1(A)) in order to determine the mineral divergences between the two biotypes (Fig. 1(B)). Figure 1B shows the first and second principal component scores of the 37 chickpea cultivars. Samples of both Desi and Kabuli biotypes are not very well distinguished along the Factor 1 axis because the highest factor loading is for Mn (0.9166) and the Mn content is similar for both biotypes, (Table 3). In contrast, along the Factor 2 axis, both biotype populations are better distinguished because the factor loadings are very high for Ca (0.8272) and K−0.8618) and contents of Ca and K are different for the biotypes (Table 3). However, the results did not produce any clear separation and the first two factors accounted only for 53.9% of the total variance. Thus, a classification was performed using a cluster analysis (CA) by a single linkage as an amalgamation strategy (SAS, 1989) in order to determine the grouping of the chickpea cultivars. The results are shown in Fig. 2. Two homogeneous groups were obtained. Group 1 includes those Desi biotype cultivars with a higher Ca content (231 mg/100 g). In processed vegetables and legumes, divalent ions, mainly Ca, inhibit softening or in some cases increase firmness (McFeeters, 1989), so it is possible that this group of chickpeas, has common cooked characteristics. Group II comprises those cultivars with a very high Fe content (4.93 mg/100 g). It has been asserted that human iron absorption is better in chickpea-supplemented diets (Sangha and Dhaliwal, 1994). This could be explained by the high concentration of Fe detected in some lines included in the cluster group II. Thus, lines CA 2164, CA 2227, CA 1276, CA 2225 and CA 2033 displayed an iron content > 5 mg/100 g (Fig. 2).

Table 4. Significant relationships obtained between different elements at 0.05 significance level

Significant ($p < 0.05$) Pearson's correlation coefficients					
All samples ($n = 37$)		Desi ($n = 16$)		Kabuli ($n = 21$)	
Mn/Mg	0.80	Mn/Mg	0.88	Mn/Mg	0.74
Mn/K	0.52	Mn/K	0.51	Mn/K	0.66
Mn/Fe	0.34	Mg/K	0.70	Mn/Na	−0.52
Mn/Na	−0.38	Ca/K	−0.52	Ca/K	−0.64
Ca/K	−0.63	Na/Cu	−0.51	Mg/Zn	0.54
Mg/K	0.49	Na/Zn	0.56		

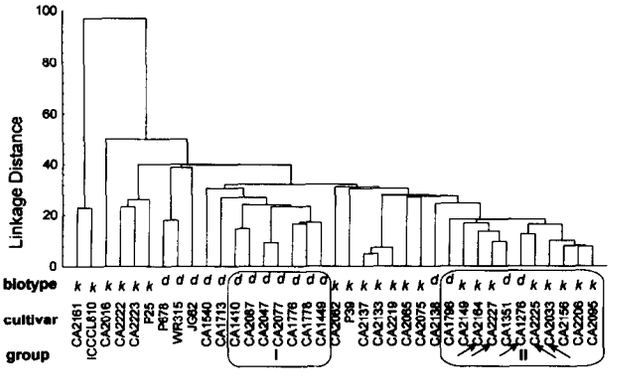

Fig. 2. Cluster analysis results performed in order to find out chickpeas cultivar homogeneous groups. Arrows in group II indicate an iron content $> 5\,mg/100\,g^{-1}$.

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