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RESEARCH ARTICLE

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Genotype and environment effects on sensory, nutritional, and physical traits in chickpea (*Cicer arietinum* L.)

Maria J. Cobos[1]*, Inmaculada Izquierdo[2], Miguel A. Sanz[3], Antonio Tomás[2], Juan Gil[4], Fernando Flores[5] and Josefa Rubio[6]

[1] *CSIC, Instituto de Agricultura Sostenible. 14080 Córdoba, Spain.* [2] *SCA Campo de Tejada. Escacena del Campo, 21870 Huelva, Spain.* [3] *Instituto Tecnológico Agrario de Castilla y León. 47071 Valladolid, Spain.* [4] *Universidad de Córdoba, ETSIAM, Dpto. Genética, CeiA3. Campus de Rabanales. 14071 Córdoba, Spain.* [5] *Universidad de Huelva, Dpto. Ciencias Agroforestales. 21819 Huelva, Spain.* [6] *IFAPA, Área de Mejora y Biotecnología. 14080 Córdoba, Spain*

Abstract

The development of chickpea cultivars with high quality grains for human consumption is an important objective in breeding programs. Genotype and environment effects on seed quality traits (sensorial, nutritional and physical) were studied in chickpea dry grain. Twenty genotypes were grown in winter and spring sowings over two campaigns in four different locations in southern Spain. Significant differences were observed in oil, acid detergent fiber (ADF) and protein content between sowing times (S). In winter, oil and ADF content were higher, while protein content was lower. Although, in general, highly significant variation was detected for genotype (G), environment (E) and single interactions (GE, GS and ES), the genotype effect was stronger for ADF, neutral detergent fiber (NDF), oil, starch and protein content, and for physical and sensory traits (r^2>27%). In contrast, environment played an important role in variation in the content of amylose and amylopectin (r^2=71.7%). No high relationships were found between the sensory and nutritional or physical characteristics studied. In general, our results suggest a high genetic gain for seed quality in nutritional, physical and sensory traits in chickpea. Genotypes with good seed sensory quality should be selected in the final stages of the breeding program, because it is not feasible to evaluate very large numbers of samples. However, in some cases, moderate correlations were found between sensory and either nutritional or physical traits. Therefore, indirect selection to increase the frequency of genes for sensory traits in an early stage should be considered.

Additional key words: seed quality; sowing time; organoleptic traits; principal component analysis; genotype-environment interaction.

Abbreviations used: ADF (acid detergent fiber); CT (seed coat thickness); E (environment); ES (environment-sowing time interaction); G (genotype); GE (genotype-environment interaction); GES (genotype-environment-sowing time interaction): GS (genotype-sowing time interaction); HI (hydration index); NDF (neutral detergent fiber); NIRS (near infrared reflectance spectroscopy); PCA (principal component analysis); r^2 (determination coefficient of the cross validation); R^2 (determination coefficient of the calibration); S (sowing time); SEC (typical error of the calibration); SECV (typical error of cross validation); SW (seed size).

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Correspondence should be addressed to María-José Cobos: covamj@gmail.com

Introduction

Chickpea (*Cicer arietinum* L.) is a widely used legume in the world because it is considered an excellent source of dietary protein (Frias *et al.,* 2000). Chickpea's protein quality is better than other legumes such as dry bean, pigeonpea, black gram and green gram (Friedman, 1996; Kaur *et al.,* 2005). Overall, chickpea seed has good nutritional value; it has low levels of anti-nutritional factors and it is rich in some minerals and vitamins (thiamine and niacin). The fatty acid composition and high amounts of unsaturated fatty acids in chickpea make it a special legume, suitable for many nutritional applications, potentially including a role in the prevention and treatment of chronic health problems such as cardiovascular disease (Zia-Ul-Haq *et al*., 2007; Jukanti *et al*., 2012). Furthermore, it can be considered a cheap source of high quality protein in developing countries. The increased interest in chickpea has triggered the publication of many review papers concerning various aspects of chickpea seed quality (Williams & Singh, 1987; Wood & Grusak, 2007; Jukanti *et al.,* 2012).

Two main market classes of chickpea are recognized, based primarily on seed color: kabuli (white flower with large and cream-colored seeds) and desi (purple flower with smaller, angular and dark seeds). Desi chickpeas have a thicker seed coat, and this influences seed composition particularly fiber content (Jambunatan & Singh, 1980; Singh, 1984; Gil *et al*., 1996). The difference in coat thickness between desi and kabuli is controlled by a major gene (Gil & Cubero, 1993).

Chickpea is mainly used for human consumption in various forms, as fresh immature (green) seeds, whole dry seeds, dhal (split seeds) and flour. Desi types are mainly cultivated in the Indian subcontinent, East Africa and Australia and are usually dehulled and split before cooking. Kabuli types are cultivated principally in the Mediterranean Basin, the Near East and America, where whole seeds are eaten after soaking and boiling. In the Mediterranean Basin, where soaking and cooking are the traditional forms of processing, the cooked chickpeas are presented in various different ways, in soups, hummus, salads, etc. In addition, today, precooked chickpea grains are packaged in tins or glass bottles, in order to satisfy the demand of a segment of the population. The most important criteria for the consumer in order of priority are: appearance, taste, texture in the mouth, price and nutritional value (Williams & Singh, 1987). For this reason, it is important to evaluate physicochemical properties and sensory characteristics, to guide the development of new varieties with desirable quality traits.

In Spain, chickpea is considered a traditional crop; however, its cultivated area has significantly decreased in the last 50 years. Even though Spain is the major producer as well as the major consumer of chickpea in Europe, nowadays Spain needs to import approximately double than its chickpea production (Faostat: http://faostat.fao.org/). Spanish chickpea landraces have high quality seed, reaching a high price in the market; being mainly consumed as whole boiled grain in different dishes. However, traditionally it is a spring crop and, consequently, plants develop poor biomass during their growing season resulting in low yield. Winter sowing in the Mediterranean basin is limited due to chickpea susceptibility to blight, a disease caused by *Ascochyta rabiei* (Pass.) Lab. The recent development of blight resistant lines has made possible the introduction of winter sowing in this region with the prospect of significantly increasing chickpea production (it could be doubled). Therefore, it is possible to develop more productive cultivars without sacrificing good grain quality, in the chickpea breeding programs.

The protein and carbohydrate content of chickpea has been shown to vary widely depending on genotype, growing conditions during grain maturation, cultural practices and sowing time (autumn or spring) (El-Adawy, 2002; de Almeida Costa *et al*., 2006; Gül *et al*., 2008; Tayyar *et al*., 2008). Significant genotype (G) × environment (E) interactions have also been reported for some grain nutritional quality traits in chickpea (Ghirardi *et al*., 1974; Berger *et al*., 2006; Frimpong *et al*., 2009), fatty acids and tocopherols (Gül *et al*., 2008), grain canning quality (Nleya *et al*., 2002) and milling traits, such as dehulling efficiency and splitting yield (Wood *et al*., 2008). These results suggest that the GE interaction for quality traits has important implications in developing selection strategies for plant breeding programs. However, for sensory traits, no studies have so far been reported concerning the effects of G, E or GE interactions in chickpea. Sensory analysis has traditionally played an important role in quality control for food products. Nevertheless, in breeding programs, sensory traits can only be evaluated in the final stages when a relatively small number of genotypes have been selected, because a trained tasting panel is necessary and limited resources mean that it is not feasible to evaluate very large numbers of samples. Hence, it would be useful to identify relationships between sensory traits with physicochemical properties in order to base selection on more objectives, quicker, and easier to measure characteristics that could be evaluated at earlier stages of the breeding program. This would improve breeding efficiency and maximize the chances of obtaining new cultivars containing the traits desired by the marketplace. In this work, we studied the genotype and environmental effects on sensory traits of boiled grains and the nutritional and physical traits of dry grains in chickpea. The relationship between sensory and physicochemical traits was also examined.

Material and methods

Plant material

The experimental material used included 13 advanced breeding lines (CA2984, CA3026, CA3045,

CA3049, CA3050, CA3051, CA3052, CA3057, CA3058, CA3060, CA3062, CA3063 and CA3065) of chickpea resistant to ascochyta blight obtained from our breeding program in Córdoba (Spain) plus 6 Spanish cultivars (‘Zoco’, ‘Fardón’, ‘Cavir’, ‘Saborio’, ‘Pringao’ and ‘Patio’) and the landrace ‘Blanco Lechoso’. ‘Blanco Lechoso’ and ‘Cavir’ were used only in spring sowings and ‘Zoco’ only in winter sowings. All genotypes were kabuli type, except for CA3026, which was desi type derived from a kabuli × desi cross.

These genotypes were sown in winter (December) and spring (March) for two campaigns (2006/2007 and 2007/2008) in four locations in the south of Spain, representative of the areas where chickpea is cultivated: (i) Alameda del Obispo Centre of the Andalusian Institute of Agricultural and Fisheries Research and Training (IFAPA), Córdoba; (ii) Venta del Llano, IFAPA, Mengibar (Jaén); (iii) Escacena (Huelva) and (iv) Conil de la Frontera (Cádiz). Sowing at each location was performed following a randomized complete block design with three replicates per location. The unit plot was four 5 m long rows, with inter-row spacing of 0.5 m and a density of 20 plants/m^2 (with a plant-to-plant distance of 0.1 m in the row). The crop was harvested in June and July for the winter and spring sowings, respectively. The climatic conditions at each growing location are summarized in Table 1.

Characters evaluated

Nutritional analysis

The content of protein, oil, linoleic and oleic acid, acid detergent fiber (ADF) and neutral detergent fiber (NDF) were measured using Foss-NIRSystems 6500 System I spectrophotometer (Foss-NIR Systems Inc., Silver Spring, MD, USA) in the Central Service for Research Support at the University of Córdoba (Spain). The samples were ground in a Cyclotec 1093 Sample Mill with a 0.3 mm mesh screen. The flour samples were analyzed by reflectance using a ring cup. The spectra were acquired with Win-ISI software 1.5 (Infrasoft International, Port Matilda, PA, USA). The reflectance (log1/R) spectra were collected in duplicate. Calibration statistics are shown in Table 2.

Chickpea grain was milled in a Tecator Cyclone mill to pass through a 0.5 mm mesh screen. After milling, the full-fat flour (non-defatted samples) was used directly for measurements. The total starch content was measured using a commercial kit (Megazyme, Bray, Co. Wicklow, Ireland) by the AOAC method 996.11, AACC method 76-13.01, ICC standard Method No. 168, and RACI Standard Method. Total starch was expressed as grams of starch per 100 g of dried flour. Amylose and amylopectin content was also evaluated using a commercial kit (Megazyme) and results were expressed as a percentage of total starch. For all samples, the absorbance was measured at 510 nm.

Physical analysis

The following physical traits were evaluated: Seed size (SW) in terms of 100-seed weight (g); seed coat thickness (CT), defined as weight per unit surface area (mg/mm^2), measured for five seeds per genotype and location following the method described by Gil & Cubero (1993); hydration index (HI), determined by the method reported by Williams *et al.* (1988) with the following formula: [(Weight of 100 seeds after 18 hours soaking – Weight of 100 seed before soaking)/100] / Seed weight.

Sensory analysis

The soaking, cooking, and preparation of the samples were performed as in Sanz & Atienza (1999). The

Table 1. Environmental characteristics of the locations (Spain) where chickpea genotypes were evaluated during two campaigns under winter and spring sowing conditions.

Campaign	Location[1]	Geographical coordinates[2]	Precipitation (mm)		Mean temperature (ºC)	
			Oct-Feb	Mar-June	Oct-Feb	Mar-June
2006/07	Córdoba*†	37º53’N/4º47’O/117	62.8	38	13.7	18
	Mengibar*†	37º57’N/3º48’O/280	173.6	202.8	10.8	16.5
	Escacena*†	37º24’N/6º23’O/173	410.4	81.6	13.3	16.9
	Conil	36º16’N/6º05’O/41	415.4	113.2	14.2	16.7
2007/08	Córdoba†		57.4	59.6	12.7	17.5
	Mengibar*†		179.8	198.4	9.7	17
	Escacena*†		228.8	248.4	13.5	17.6
	Conil*†		292.6	124	14.3	17.6

[1] *Location for winter sowing; †Location for spring sowing. [2] latitude/longitude/altitude

Table 2. Near infrared (NIR) spectroscopy calibration statistics of chickpea chemical composition used for predictions.

Characters[1]	SEC[2]	R^2 [3]	SECV[4]	r^2 [5]
Protein	0.43	0.97	0.57	0.95
Oil	0.29	0.88	0.32	0.85
ADF	0.55	0.98	0.80	0.96
NDF	1.00	0.95	1.16	0.93
Oleic acid	0.79	0.97	1.33	0.91
Linoleic acid	0.51	0.98	1.01	0.93

[1] ADF, acid detergent fiber; NDF, neutral detergent fiber. [2] SEC, typical error of the calibration. [3] R^2, determination coefficient of the calibration. [4] SECV, typical error of cross validation. [5] r^2, determination coefficient of the cross validation

chickpeas were soaked for 12 hours in distilled water at 30ºC. After that, they were drained and immediately boiled for approximately 120 min at atmospheric pressure, until they reached the optimum organoleptic texture (indicated by the gelatinisation of the starch and corresponding color change). Boiled grain samples then underwent sensory evaluation. Ten trained judges assessed the samples in terms of appearance, taste, and texture, using a 0 to 5 point scale.

The following organoleptic traits were scored: Mouth thickness, described as the sensation produced by the grain skin in contact with the tongue and palate, 0 (smooth) - 5 (rough); hardness, response to deformation forces once inside the mouth, 0 (soft) - 5 (hard); buttery texture and graininess, related to the sensation during mastication, 0 (not at all buttery) - 5 (very buttery) and 0 (not at all grainy) - 5 (very grainy); broken and loose skin based on visual appearance of the grain appearance before eating and related to its entirety, 0 (very broken) - 5 (not at all broken) and 0 (very loose) - 5 (not at all loose).

Statistical analysis

As locations and campaigns were unbalanced, we considered each campaign-location combination as an environment. Combined analysis of variance was conducted over sowing time and environments for each nutritional, physical, and sensory trait (McIntosh, 1983). Genotype (G) and environment (E) were considered as random effects and sowing time (S) as the fixed effect. As the data were unbalanced, a general linear model was built.

Due to the difficulty of evaluating a large number of samples for sensory traits, only one sample was evaluated per genotype, this containing a mixture of the three replicates. Without analysis of the replicates, the double interactions genotype-environment (GE) and genotype-sowing time (GS) cannot be tested (McIntosh, 1983). However, we have used triple interaction (GES) effects as a conservative test (F test) to assess both GE and GS interactions.

The determination coefficient (r^2) was calculated to ascertain the proportion of the variance explained by each trait out of the total variance (square sum of each variation source/total square sum). Specifically, this coefficient (× 100) represents the percentage of variance due to G, E, S or interactions for each trait in the model.

Principal component analysis (PCA) with varimax rotation was performed to explore relationships between traits. PCA was applied starting from the phenotypic correlation matrix based upon genotype means. In order to identify the best genotype for each sensory quality, the principal components related to sensory traits were plotted on scatter plots. For this, we selected all principal components that had eigenvalues of greater than 1. All analysis were calculated using IBM SPSS Statistics for Windows version 17 (IBM, Armonk, NY, USA).

Results

This study was carried out in a wide range of chickpea cultivated areas in the south of Spain, representing different growing conditions in terms of temperature and precipitation (Table 1). Due to large numbers of missing values, data from the winter sowing in the second campaign (2007/2008) in Córdoba and both seasons of the first campaign (2006/2007) in Conil de la Frontera (Cádiz) were excluded from the analysis. This does not affect the other analysis, as we considered each campaign-location combination to be a unique environment.

In general, the combined analysis of variance for all nutritional and physical characters evaluated revealed that both genotype and environment effects were highly significant (p<0.001) (Table 3). The sowing time was strongly significant (p<0.01) for ADF and oil content, and more weakly significant for protein content (p<0.05), ADF and oil content being higher and protein content lower in winter sowings. Although significant interaction effects (SE, SG, and GE) were detected, they were not very strong, as suggested by the low values of the determination coefficients (r^2), except in the case of SE for linoleic and oleic acids, and starch content with r^2 values of 15, 21 and 12% respectively (Table 3). The genotype effect was stronger than the effects of the environment and sowing time for some nutritional (ADF, NDF, oil, protein and starch content) and physical (coat thickness, HI and seed size) traits (r^2>30%). In contrast, environment played an important role in the variation in

Table 3. Mean squares from combined analysis of variance for nutritional and seed physical characters of chickpea genotypes growing under two sowing time (winter and spring) in different environments in southern Spain.

Characters[1]	Mean squares								
	Sowing time (S) df = 1	Environment (E) df = 6	Interaction SE df = 5	Repetition SE df = 26	Genotype (G) df = 19	Interactions			Error df = 340
						SG df = 87	EG df = 15	SEG df = 50	
Nutritional characters									
ADF	36.8	10.8	2.0	0.7	62.6	0.8	0.3	0.3	0.2
	(*0.005*; 2.5)*	(*0.000*; 4.5)	(*0.062*; 0.7)	(1.3)	(*0.000*; 82.3)	(*0.000*; 0.8)	(*0.008*; 1.9)	(1.0)	(4.9)
NDF	12.8	19.0	3.7	3.0	57.2	1.6	0.5	0.7	0.4
	(*0.145*; 0.8)	(*0.000*; 7.3)	(*0.376*; 1.2)	(5.0)	(*0.000*; 70.1)	(*0.000*; 1.6)	(*0.024*; 3.1)	(2.3)	(8.5)
Oil	11.57	0.45	0.71	0.06	1.05	0.08	0.03	0.04	0.01
	(*0.009*; 23.3)	(*0.000*; 5.5)	(*0.000*; 7.1)	(3.0)	(*0.000*; 40.1)	(*0.000*; 2.4)	(*0.000*; 5.7)	(4.2)	(8.8)
Linoleic acid	205.6	412.8	201.9	5.0	85.0	9.2	4.7	4.4	1.5
	(*0.364*; 3.1)	(*0.000*; 36.9)	(*0.000*; 15.0)	(1.9)	(*0.000*; 24.0)	(*0.000*; 2.0)	(*0.000*; 6.1)	(3.3)	(7.6)
Oleic acid	904.5	399.3	360.1	6.0	100.0	9.9	5.6	4.4	1.5
	(*0.177*; 10.6)	(*0.000*; 28.1)	(*0.000*; 21.1)	(1.8)	(*0.000*; 22.3)	*0.000*; 1.7)	(*0.000*; 5.7)	(2.6)	(6.0)
Protein	249.1	15.8	22.4	1.9	43.4	2.0	0.8	0.8	0.3
	(*0.024*;15.7)	(*0.000*; 6.0)	(*0.000*; 7.1)	(3.2)	(*0.000*; 52.2)	(*0.000*; 1.9)	(*0.000*; 4.3)	(2.6)	(7.0)
Starch	193.8	36.5	44.4	4.2	29.2	2.8	2.0	1.6	0.8
	(*0.097*; 10.5)	(*0.000*; 11.8)	(*0.000*; 12.0)	(5.6)	(*0.000*; 30.0)	(*0.075*; 2.2)	(*0.000*; 9.5)	(4.4)	(13.1)
Amylose	67.2	2035.6	23.3	35.5	20.7	9.4	4.5	7.7	7.1
	(*0.164*; 0.4)	(*0.000*; 71.7)	(*0.687*; 0.7)	(5.4)	(*0.000*; 2.3)	(*0.178*; 0.8)	(*0.994*; 2.3)	(2.3)	(14.1)
Amylopectin	67.2	2035.6	23.3	35.5	20.7	9.4	4.5	7.7	7.1
	(*0.164*; 0.4)	(*0.000*; 71.7)	(*0.687*; 0.7)	(5.4)	(*0.000*; 2.3)	(*0.178*; 0.8)	(*0.994*; 2.3)	(2.3)	(14.1)
Seed physical characters									
CT (×10^{-6})	9.7	9.1	0.9	0.3	46.7	0.6	0.4	0.2	0.2
	(*0.025*; 0.9)	(*0.000*; 5.0)	(*0.038*; 0.4)	(0.7)	(*0.000*; 81.6)	(*0.001*; 0.8)	(*0.000;* 3.0)	1.0	(6.6)
HI (×10^{-3})	156.1	50.7	45.7	1.1	69.4	0.7	2.0	2.1	0.4
	(*0.124*; 6.3)	(*0.000*; 12.3)	(*0.000*; 9.2)	(1.2)	(*0.000*; 53.4)	(*0.986*; 0.4)	(*0.008*; 7.0)	4.3	(5.9)
SW (100 seeds)	242.0	290.2	158.9	6.3	1256.3	5.8	10.4	10.4	3.0
	(*0.272*; 0.8)	(*0.000*; 5.9)	(*0.000*; 2.7)	(0.6)	(*0.000*; 81.4)	(*0.896*; 0.3)	(*0.008*; 3.1)	1.8	(3.4)

[1] ADF, acid detergent fiber; NDF, neutral detergent fiber; CT, coat thickness; HI, hydration index; SW, seed weight. df: degrees of freedom. * In brackets, *p* values (italics, left) and determination coefficient (r^2) (right).

content of amylose and amylopectin and to a lesser extent that of oleic and linoleic acids.

In the current study, sensory traits were evaluated in grains from all environments in the first campaign but considering only one replicate per location due to the difficulty of evaluating a large number of samples. The analysis of variance of these characters showed that environment and genotypic effects showed significant variation in most cases (p<0.05) (Table 4). No significant differences were found between winter and spring sowing for sensory traits. The genotype effect was the most important (r^2>27%, with a maximum of 80% for mouth thickness) indicating that there is variability among genotypes for these traits. In general, simple interaction effects were not significant. Regarding visual characters, the samples tested had high mean scores for broken and loose skin (>4.57), near the top of the scale, with a narrow range of variation, indicating that the boiled grains generally had a good visual appearance.

Correlations between sensory and other quality traits could offer breeders the possibility of indirect selection in the early generations of breeding programs. For these reasons, in the current study, several nutritional, physical and sensory characters were studied to explore the relationships between sensory traits of boiled grains and both chemical and physical properties in dry chickpea grains. It would be particularly useful to detect strong correlations between a targeted character and some other more easily measured characteristics. Considering that the genotype effect was in general the most important for the characters studied, mean scores per genotype were estimated across all the environments and sowing times to study their relationships by principal component analysis (Table S1 [suppl.]). This type of analysis, used to represent relationships among sets of many interrelated variables, is based on the correlation matrix (Table S2 [suppl.]). Amylose, amylopectin and linoleic acid contents were excluded, the first two because they had low genetic variation and

Table 4. Combined analysis of variance for sensorial characters of chickpea genotypes grown under two sowing times (winter and spring) in different environments in southern Spain.

Characters	Mean squares						
	Sowing time (S) df = 1	Environment (E) df = 2	Interaction SE df = 2	Genotype (G) df = 19	Interactions SG df = 29	EG df = 15	SEG df = 19
Hardness	2.45 (*0.138*; 7.5)*	2.41 (*0.000*; 14.7)	0.54 (*0.159*; 3.3)	0.62 (*0.000*; 36.1)	0.25 (*0.521*; 11.7)	0.13 (*0.961*; 11.3)	0.26 (15.3)
Mouth thickness	0.15 (*0.874*; 0.2)	0.33 (*0.148*; 1.0)	1.17 (*0.003*; 3.4)	2.92 (*0.000*; 80.9)	0.16 (*0.399*; 3.6)	0.16 (*0.415*; 6.8)	0.15 (4.0)
Buttery	0.26 (*0.584*; 0.7)	2.43 (*0.000*: 13.6)	0.39 (*0.210*; 2.2)	0.75 (*0.002*; 39.8)	0.29 (*0.314*; 12.2)	0.23 (*0.496*; 19.1)	0.23 (12.3)
Grainy	3.01 (*0.349*; 4.8)	4.30 (*0.000*: 13.8)	2.18 (*0.018*; 6.9)	0.89 (*0.027*; 27.1)	0.64 (*0.220*; 15.3)	0.40 (*0.587*; 18.8)	0.44 (13.3)
Broken	0.07 (*0.401*; 2.6)	0.05 (*0.058*; 3.5)	0.08 (*0.076*; 5.5)	0.06 (*0.000*; 41.1)	0.03 *0.519*; 13.5)	0.02 (*0.899*; 16.1)	0.03 (17.7)
Loose skin	0.49 (*0.091*;5.9)	0.25 (*0.027*; 6.0)	0.05 (*0.280*; 1.1)	0.17 (*0.008*; 38.0)	0.11 (*0.010*; 19.5)	0.06 (*0.095*; 21.6)	0.03 (7.9)

df: degrees of freedom, * in brackets, *p* values (italics, left) and determination coefficient (r^2) (right).

the third one due to its strong correlation with oleic acid.

The first six principal components selected explained 91.25% of the total variance (Table 5). The first component (PC1) was highly related to fiber content. This component explained a high percentage of the total variance in ADF, NDF and coat thickness (square loading × 100 > 81%), with a positive relation between them. This result could be expected, because fiber is mainly found in the seed coat in chickpea (Singh, 1984). Starch content showed a moderate-to-high negative loading (-0.68) on PC1. This could be due to competition for the same substrate, since the same component, glucose, is involved in the biosynthesis of both fiber and starch.

The second component (PC2) was strongly represented by the sensory characters, buttery texture, graininess and hardness (Table 5), buttery texture having the highest loading on this component. Hardness was represented to a lesser extent (loading = -0.82) and a moderate-to-low percentage of variation was shared with PC1 (loading = 0.41). The loading of hardness in PC1 was positively related to fiber content. Neither high nor moderate loadings were found for nutritional or physical traits in PC2.

The variation in the sensory character mouth thickness was shared mainly with PC3. Mouth thickness was strongly positively related to hydration index (HI) in this component and in less extension with seed size, with loadings of 0.88, 0.80 and 0.59 respectively (Table 5); that is, the smoother the seed, the lower the HI and smaller the grain size. Seed protein content is an important quality factor for chickpea breeding programs. In the current study, the most heavily weighted character in PC4 was protein content (-0.84), and this was negatively related to both oil and starch content, which had loadings of 0.72 and 0.65 respectively.

The visual characters showed the highest loading in the fifth component (PC5): broken and loose skin (0.79 and 0.92 respectively) (Table 5). They were positively related to each other and not related to any other traits. As mentioned above, these traits obtained high mean scores suggesting that the visual appearance of boiled grains is generally good in the analyzed material. Oleic acid had the highest loading in PC6 (0.84), showing a certain positive relation with oil content and seed size.

In our data, there was a moderate relation between sensory traits and other physicochemical properties, except for mouth thickness and HI with a higher relation (Table 5). As it was cited above amylase and amylopectin contents were excluded of PCA, because of their low genetic variation, nonetheless they had a moderate-high correlation with buttery, hardness and loose skim (0.43, 0.63 and 0.70 respectively) (Table S2 [suppl.]). Nevertheless, these correlations have provided us with a good knowledge base to understand the associations between quality components and to consider how changing one component might affect others.

As we mentioned above, two principal components (PC2 and PC3) explained a high percentage of variation

Table 5. Loading coefficients after varimax rotation of seed quality characters of 20 chickpea genotypes on the six principal components (PC).

Characters[1]	PC1	PC2	PC3	PC4	PC5	PC6
ADF	**0.90**	–0.13	0.37	0.03	–0.01	–0.09
NDF	**0.94**	–0.14	0.03	0.03	–0.07	–0.21
Oil	–0.30	0.09	0.26	**0.72**	0.11	**0.51**
Oleic acid	–0.12	0.26	0.04	–0.11	–0.32	**0.84**
Protein	**–0.46**	0.00	–0.01	**–0.84**	–0.04	0.24
Starch	**–0.68**	0.02	0.03	**0.65**	–0.02	–0.26
CT (×10^{-3})	**0.94**	–0.19	0.12	0.05	0.20	–0.06
HI	–0.21	–0.01	**–0.80**	–0.17	–0.06	–0.24
SW (100 seeds)	**–0.47**	0.01	**–0.59**	–0.26	0.09	**0.46**
Hardness	**0.41**	**–0.82**	0.07	0.10	0.14	0.02
Skin surface	–0.04	–0.27	**–0.88**	0.09	–0.20	0.12
Buttery	–0.12	**0.94**	0.04	0.02	0.10	0.15
Grainy	–0.01	**–0.87**	–0.31	–0.12	0.06	–0.10
Broken	0.17	0.21	0.12	–0.04	**0.79**	–0.38
Loose skin	–0.05	–0.18	0.09	–0.09	**0.92**	0.02
Explained variation (%)	25.80	17.24	14.09	12.06	11.42	10.64
Cumulative variation	25.80	43.04	57.13	69.19	80.61	91.25

[1] ADF, acid detergent fiber; NDF, neutral detergent fiber; CT, coat thickness; HI, hydration index; SW, seed weight. In bold: loading coefficients > 0.40

in sensory traits related to taste and texture of boiled grains: from 77% of mouth thickness to 88% of buttery texture. These components were used to study the variability of sensory characters between genotypes with a scatter plot, allowing us to select adequate genotypes for these characters. A genotype having positive values in these two principal components indicates that the grains were not hard or grainy and relatively buttery and smooth in the mouth.

The distribution of genotypes on the PC2 against PC3 plot (Fig. 1) revealed that the genotypes ‘Fardon’, ‘Blanco Lechoso’ and CA3060 were the best in terms of quality related to PC2 (grains being relatively buttery and not hard or grainy). However, the cultivar ‘Fardon’ was smoother in the mouth than the others. The ‘Zoco’ variety and CA3058 displayed the poorest quality related to PC2, ‘Zoco’ having rougher grains. The rest of the lines and varieties showed moderate values in terms of quality related to PC2, with the greatest variation in mouth thickness (PC3).

Discussion

Our study indicates that genotype has important significant effects on ADF, NDF, oil, protein and starch content. This is in agreement with the findings of other authors who have studied some of these components (Gil *et al*., 1996; Tayyar *et al*., 2008; Frimpong *et al*., 2009; Ali *et al*., 2011). For amylose and amylopectin content, two types of glucose polymer with linear and branched molecules respectively, the variation observed in the current study could be attributed mainly to environmental effects (r^2>71%). The proportion of both components is responsible for many of the differences in seed, flour and dough behavior during processing (Wood & Grusak, 2007). Regarding differences in nutritional components with sowing time, Tayyar *et al*. (2008) reported higher protein content in chickpea from spring than autumn plantings, which is consistent with our results. They attributed this higher protein concentration to the shorter period for pod filling and less starch accumulation under spring sowing. In our data, a negative relation was found between protein and both starch and oil content in PC4.

Seed coat thickness, HI and seed size could be related to the seed mechanism for inhibiting water uptake. Seed soaking reduces the time necessary to obtain an adequate texture in the cooking process. The mechanism for inhibiting water uptake could be related to seed chemical composition or seed coat (husk) components. Clemente *et al*. (1998) reported a high initial hydration of seeds and suggested that the seed coat did not hinder the water uptake. According to our data, HI was not correlated with CT. Regarding seed chemical composition, no correlation was found between HI and any of the nutritional traits. Gil *et al*. (1996) did not find HI to be associated with other physical or chemical traits of seed in a collection of 50 chickpea genotypes. In our study, a moderate positive correlation was found with seed size.

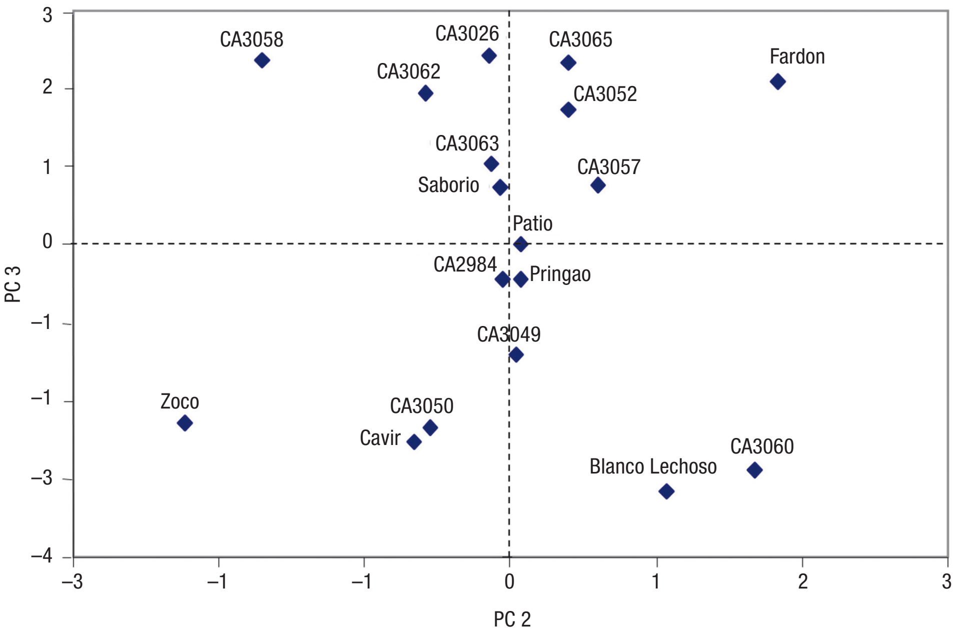

Figure 1. Plotting of chickpea genotypes grown under winter and spring sowing conditions on two principal components (PC2 and PC3) related with sensory traits of boiled seeds.

The whole seed of chickpea possesses the necessary properties to be packaged precooked in tins or jars, in order to meet consumer demands. The aspects of grain quality that relate to consumer appeal, eating quality and processing quality, have not been documented to the same extent as those of nutritional quality. Sensory analysis provides the most reliable information about the textural qualities of chickpea but panel testing is laborious and time-consuming. In the current study, great efforts have been made to obtain this useful type of data. According to our results, the sensory traits analyzed do vary significantly between genotypes. Further, we found that, of the sensory traits considered, visual characteristics do not have a great impact on the overall acceptability of chickpea genotypes, while taste and texture are more important.

In order to facilitate early selection for sensory quality, we explored the relation of sensory traits with both chemical components and physical properties of the grains. Although, in the current study, we did not find strong association between sensory traits and other physical or chemical characteristics, a lower fiber content or coat thickness could have some positive effect on cooked grain hardness. Hardness has been reported to be high and positively correlated with texturometer values in chickpea (Clemente *et al.*, 1998). However, it is possible to reduce cooked grain hardness by increasing the cooking time (Clemente *et al.*, 1998). In order to select for sensory traits in early generations, it is necessary to develop quick and reliable methods that allow us to evaluate a larger number of accessions. On the basis of the current results, it could be feasible to improve sensory traits by indirect selection in an early stage discarding lines with high values for fiber content and coat thickness or low values for HI and amylopectin content. In a more refined sense, sensorial traits should be evaluated in the final stages of the breeding program, after the number of breeding lines has been markedly reduced. Because starch content is an important seed component, around 50%, it may be interesting to explore in the future its physical characteristics (granule size, levels of organization, gelatinization, etc.) and their relationship with sensorial traits.

The positive association between mouth thickness and HI indicate that the rougher the seed, the higher the HI. This may be because rougher seeds have a higher surface area for water uptake than smother ones. However, consumers are likely to prefer a smooth sensation produced by the skin in the mouth. The ‘Fardon’ cultivar showed good sensory quality, with seeds that are relatively smooth and buttery, not hard or grainy, while the ‘Blanco Lechoso’ landrace, which has a high acceptance in the Spanish market, also had seeds that are relatively buttery, and not hard or grainy seeds but they are rougher. These results suggest it is likely that buttery texture, hardness, and graininess might be enough to select genotypes with good sensory quality boiled grains. In this sense, the advanced breeding line CA3060 could be a good genotype to be released as new cultivar.

In conclusion: (i) the genotype effect was the greatest source of variation in all grain quality studies, and hence a high genetic gain of grain quality is expected for nutritional, physical and sensory traits in chickpea; (ii) as no clear relation has been found between the sensory and physicochemical properties analyzed in the current study, selection should be based on both, grain nutritional quality and sensory quality; and (iii) it may be enough to consider the sensory traits, buttery texture, graininess and hardness, to evaluate sensory quality in chickpea in order to minimize the work and time required for panel testing.

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