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Culinary and sensory traits diversity in the Spanish Core Collection of common beans (*Phaseolus vulgaris* L.)

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Abstract

The Spanish National Plant Genetic Resource Center's core collection of bean germplasm includes 202 accessions selected from more than 3000 accessions in function of passport data, seed phenotype, genetic background, and agronomic traits. To acquire more useful information about these accessions, we cultivated and characterized them for sensory and culinary traits. We found considerable variation for culinary and sensory traits of the cooked beans (mean coefficients of variation: 41% for the sensory traits and 40% for the culinary traits). The large dataset enabled us to study correlations between sensory and culinary traits and among these traits and geographic origin, seed color, and growth habit. Greater proportion of white in the seed coat correlated positively with brightness and negatively with mealiness (r=0.60, r=-0.60, p<0.001, respectively). Mealiness correlated negatively with seed-coat roughness and rate of water absorption (r=-0.60, r=-0.53, p<0.001, respectively). Materials of Andean origin had lower seed-coat brightness (p<0.01) and seed-coat roughness, and greater seed-coat perceptibility, mealiness, flavor, and aroma (p<0.001) than materials of Mesoamerican origin. Growth habit failed to correlate with culinary or sensory traits. Breeders can benefit from the information about this core collection available at www.crf.inia.es/crfesp/paginaprincipaljudia.asp.

Additional key words: genetic resources; variability in beans; organoleptic characters; culinary characterization; core collection; domestication region.

Abbreviations used: CV (coefficient of variation); PCA (principal component analysis)

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Introduction

The Spanish National Plant Genetic Resource Center has collected and conserved over 3000 accessions of bean (www.inia.es/inventarionacional/). To make it easier to use the material from this vast repository, a core collection was compiled based on the passport data and seed phenotype (De la Rosa *et al.*, 2000). Later, the information about the accessions was expanded to include agronomic and morphological traits of the plants, type of phaseolin (Pérez-Vega *et al.*, 2009), and some resistances (Pérez-Vega *et al.*, 2006; Asensio *et al.*, 2010; Pascual *et al.*, 2010).

Beans are a good source of proteins, starch, dietary fiber, vitamins and minerals, and other nutritional components, as well as of polyphenolic compounds, and they might help prevent cardiovascular disease, obesity, and cancer (Hayat *et al.*, 2014). Furthermore, in association with some rhizobium strains, beans can replace fertilization with chemical N (Mulas *et al.*, 2011). These health and environmental benefits have led to renewed interest in beans in developed countries, where demanding consumers force growers to consider culinary traits and sensory value, as well as good environmental practices in plant cultivation.

Culinary traits (rate of hydration, cooking time, percentage of whole beans after cooking, etc.) can be

characterized in the laboratory with considerable repeatability, but this process is time consuming (Romero-del-Castillo et al., 2012). Fine characterization of sensory traits in cooked beans requires trained panels and appropriate conditions (Romero-del-Castillo et al., 2008) that allow only limited sets of samples to be managed. For these reasons, systematic information about the culinary and sensory traits in the cooked beans of the germplasm banks' accessions is lacking (Romero-del-Castillo et al., 2010). The appearance of the seeds (external visual traits) remains the primary sensory criterion for classification and guiding consumers' choices (Jahns et al., 2001; Costa et al., 2011). Therefore, it would be useful to have a complete description of materials that includes information about culinary traits, sensory traits in cooked beans, and external visual traits in the raw beans.

In a previous study, our group found considerable variability in culinary and sensory traits in a set of accessions from Catalonia (Rivera *et al.*, 2013). In the same study, we correlated the color of the seeds with some of their sensory and culinary traits. Previously, Singh *et al.* (1991) reported that beans' genetic background, which depends on where they were originally domesticated (the Andes *vs.* Middle America), correlates with several traits of the plant, seed, and phaseolin seed protein patterns. Furthermore, the most appreciated bean landraces in Spain have indeterminate growth habit (Casquero *et al.*, 2006), suggesting some kind of relationship between growth habit and sensory quality.

Studying the Spanish core collection of bean germplasm, a broad collection of materials with proven genetic variation, could help us to better understand the relationships between all these traits. Moreover, complementing the information already compiled with information about culinary and sensory traits should help breeders to make better use of the collection while paving the way for similar studies in other collections.

In the present study, we characterized the accessions of this core collection on culinary and sensory traits to: i) determine the variation of these traits in the Spanish core collection, and ii) analyze the relations among these traits and between them and domestication origin, seed coat color, and growth habit.

Material and methods

Plant material and field trials

The Spanish core collection of beans includes 202 accessions belonging to 51 market classes (Santalla *et al.*, 2001) from all areas of Spain where beans are cul-

tivated. About 75% are of Andean origin and 25% are of Mesoamerican origin (Pérez-Vega *et al.*, 2009).

The 202 accessions were sown in Sabadell (Northeast Spain: 41° 32' 50.7" N, 2° 4' 14.7" E), a location with loam soil with abundant Ca, low P, and a mild Mediterranean climate that allows both short- and long-cycle materials to develop to maturity. We used a randomized two-block design with 21 effective plants per plot. The experiment was conducted at a low density (29,167 plants/ha) to facilitate individualized recording of data. A vertical plastic net was used to trellis the accessions with indeterminate growth. Plants were cultivated with the traditional management in the area, including drip irrigation and fertilizer when necessary. During cultivation, growth habit (Singh, 1982) was recorded.

Seeds were harvested by plots, but owing to the lack of significance of the block factor for agronomic traits, the seeds from the two blocks for each accession were combined to ensure a sufficient sample for the culinary and sensory analyses.

Characterization

Raw seed traits

Seed-coat color was visually classified in the following categories: yellow, white, cream, gray-brownish to greenish, brown, vinous brown, black, ochre, purple, rosy, green, bicolor, or tricolor. The % of colored surface of the seed was estimated by scanning samples of 100 seeds per accession and processing the data using WinSeedle Pro v2005b (Regent 156 Instruments, Inc., Quebec, Canada) that yields the percentage of white colour in the seed surface. The same procedure was used to estimate the length, width, projected area, and curvature of the seed. Thickness (perpendicular to length and width) was measured in 10 seeds per accession with digital calipers. The length/width index was calculated to determine seed shape (Puerta-Romero, 1961). Volume (V) and surface area (SA) were calculated using geometric formulas adjusted to seed shape: spherical, $V = \frac{4}{3}\pi r^3$ and $SA = 4\pi r^2$; rectangular, V = lwhand SA = 2(wh + lw + lh); or ellipsoid, $V = \frac{4}{3}\pi abc$ and $SA = \frac{\pi^2}{\Lambda} a \left(b + c + \sqrt{(b^2 + c^2)} \right)$, where w represents width; l, length; h, height; a, length-axis radius; b, height-axis radius and c, width-axis radius. We also

Culinary traits

A sample of 100 g of beans of each accession was soaked in 300 mL of distilled water. At 3 h, 6 h, 9 h,

recorded the 100-seed weight for each accession.

and 24 h the beans were drained and weighed to determine water absorbed during soaking, measured as 100 \times (weight at time t_1 -weight at time t_0)/ weight at time t_0 . The resulting data were used to calculate the rate of water absorption during soaking: 100 × (water absorption at 9 h/water absorption at 24 h). After the last weight measurement, beans were cooked as described by Romero-del-Castillo et al. (2012). Cooking time was recorded as the time elapsed from breaking a boil to the end of cooking process. The sample of beans was weighed after being allowed to drain for 10 min; the result was recorded as wet weight and was used to estimate the percentage of whole seeds after cooking: $100 \times (weight \ of \ the \ whole \ seeds/\ wet \ weight)$. This sample was then dried in an oven at 60°C for 48 h and weighed again; the result was recorded as dry weight. Water absorption during cooking, expressed as a percentage of the dry mater, was calculated using the following formula 100 × [(wet weight - dry weight)/dry weight] - water absorption at 24 h soaking.

Sensory traits in cooked seeds

The sensory traits seed-coat perceptibility, seed-coat roughness and mealiness (Romero-del-Castillo et al., 2008), flavor [complex combination of the olfactory, gustatory, and trigeminal sensations perceived during tasting (ISO 5492, 2008)], seed-coat brightness (from dull to very bright), and aroma [detected when the volatiles enter the nasal passage and are perceived by the olfactory system (Meilgaard et al., 1999)], were calculated using regression models from near-infrared spectroscopy (NIR) measurements. The ground, cooked samples were analyzed and recorded using a model 5000 spectrophotometer (Foss-NIRSystems, Silver Spring, MD, USA) equipped with a rapid content analyzer module. The lab procedures and the regression models used are described in detail in Plans et al. (2014). All the results are given in a scale from 0 (minimum expression of the trait) to 10 (maximum expression of the trait).

Statistical analysis

To estimate the variation, we calculated the means, variances, and covariances of the recorded traits.

Accessions were grouped successively in function of the type of phaseolin (indicating Andean or Mesoamerican origin of domestication), seed-coat color, and growth habit. For each type of classification, analysis of variance (ANOVA) was done based on the linear model $y_{ij} = \mu + \alpha_i + \epsilon_{ij}$, where y_i was an individual level for a

specific trait, μ was the grand mean, α_i was the effect of i group based on type of phaseolin, seed-coat color, or growth habit, and ϵ_{ij} was the random error for i groups with j replications of the model following a N ~ $(0, \sigma^2)$. All factors were considered fixed. Finally, for the type of phaseolin and seed-coat color, we performed normalized principal component analysis (PCA) and we calculated 95% confidence ellipses around each cluster of accessions. We used the R software (R Development Core Team, 2007) Agricolae, pcaMethods, and Ellipse packages for all statistical analyses (Murdoch & Chow, 2007; Stacklies *et al.*, 2007; Mendiburu, 2010).

Results

Sensory and culinary traits in the core collection

At the end of the trial, complete datasets were available for 174 of the 202 accessions in the core collection. For visual traits of the raw seeds, the coefficients of variation (CVs) ranged from 11% for width to 162% for the percentage of white color (Table 1). For the culinary traits, the CVs ranged from 13% for water absorption at 24 h to 81% for water absorption at 3 h (Table 1). For the sensory traits after cooking, the CVs ranged from 24% for aroma to 76% for seed-coat roughness (Table 1).

Having data about a large number of accessions with high variation in all traits enabled us to perform a robust study of the correlations among the sensory and culinary traits. Although r values ≥ 0.25 were significant different from zero at p < 0.001, for practical purposes, we discuss only absolute r values ≥ 0.50 (Table 2).

The beans with the greatest proportion of white surface were also the brightest (r=0.60); these beans were also less mealy (r=-0.60) than darker beans (Table 2). The beans with rougher seed coats and faster water absorption were also less mealy (r=-0.60, r=-0.53). The size of the seeds (100-seed weight) correlated positively with seed-coat perceptibility (r=0.67). Water absorption during cooking correlated positively with cooking time (r=0.57) and negatively with the percentage of whole beans after cooking (r=-0.54). Flavor correlated negatively with seed-coat roughness (r=-0.55) and positively with aroma, seed-coat perceptibility, and mealiness (r=0.71, r=0.57, r=0.54).

Domestication origin and sensory and culinary traits

Our study comprises 131 accessions domesticated in the Andes and 43 domesticated in Mesoamerica

(Pérez-Vega *et al.*, 2009). The results showed significant differences between the two origins for the visual traits of the raw seeds (p<0.05 for length/width and p<0.001 for the others), except seed curvature and percentage of white surface (Table 3). The PCA also showed that Mesoamerican materials tended to have smaller seeds (Fig. 1a).

With regard to the sensory traits of the cooked beans (Table 3), we found that materials of Andean origin had lower seed-coat brightness (p<0.01) and seed-coat roughness and higher seed-coat perceptibility, mealiness, flavor, and aroma (all significant at p<0.001) than materials of Mesoamerican origin. These results are reflected in the PCA done on the sensory traits of the cooked beans and

Table 1. External visual traits of the raw beans, culinary and sensory traits of the cooked beans. Mean, coefficient of variation (CV, %), and extreme values for the traits studied.

	Mean	Max	Min	CV
External visual traits of the raw beans				
100-seed weight (g)	41.1	79	16	26.4
Width (mm)	8	10.2	5.8	11.4
Length (mm)	13	21.6	8.2	16.9
Thickness (mm)	6.1	8	4.1	15.2
Length/width	1.7	2.4	1.1	17.7
Volume (mm ³)	541.6	1346.4	129.7	41.1
Surface area (mm ²)	421.7	876.3	123	34.8
Projected area (mm ²)	84.2	166.3	37.5	24.2
Curvature	0	0.1	0	36.6
White surface color (%)	26.9	100	0	162.4
Culinary traits				
Water absorption at 3h (%)	33.4	93.7	0.7	80.6
Water absorption at 6h (%)	55.9	113	2.9	59.8
Water absorption at 9h (%)	71.9	122.1	5.3	45.2
Water absorption at 24h (%)	102.5	134.3	38.2	12.9
Rate of water absorption	67.9	97.9	8.4	41.5
Water absorption during cooking (%)	75.6	147	41.3	26.2
Cooking time (min)	65.3	159	34	33.3
Whole beans (%)	86.4	100	30.7	16.4
Sensory traits of the cooked beans				
Seed-coat roughness	2.5	10	0	76.4
Seed-coat perceptibility	5.3	10	0	38.1
Seed-coat brightness	5.1	10	0	36.8
Mealiness	5.9	10	0	31.4
Flavor	5.2	10	0	39.2
Aroma	6.6	10	0	24.5

Table 2. Correlations among sensory traits of the cooked beans, culinary traits and external visual traits of the raw beans.

	Rate of water absorption	Cooking time (min)	Whole beans (%)	Seed-coat brightness	Seed-coat roughness	Aroma	Seed-coat perception	Mealiness	Flavor
White surface color (%)	0.48***	0.17*	-0.22**	0.60***	0.4***	-0.18*	-0.15*	-0.60***	-0.18*
Volume (mm³)	-0.19*	0.13	0.1	0	-0.25***	0.35***	0.49***	0.26***	0.41***
Surface area (mm²)	-0.12	0.24**	0.08	-0.13	-0.15*	0.25***	0.35***	0.19*	0.28***
100-seed weight (g)	-0.25***	0.16 *	-0.06	0.03	-0.22**	0.38***	0.67***	0.27***	0.45***
Rate of water absorption		0.02	-0.20**	0.30***	0.24**	-0.31***	-0.23**	-0.53***	-0.33***
Water absorption during cooking (%)		0.57***	-0.54***	0.29***	0.06	0.07	0.20**	-0.37***	0.12
Whole beans (%)				-0.19*	-0.06	0	-0.43***	0.23**	-0.07
Seed-coat brightness					0.54***	-0.20**	-0.05	-0.73***	-0.25***
Seed-coat roughness						-0.38***	-0.36***	-0.60***	-0.55***
Aroma							0.44***	0.48***	0.71***
Seed-coat perception								0.33***	0.57***
Mealiness									0.54***

^{***}p<0.001; **p<0.01; *p<0.05.

Table 3. Comparisons of means for external visual traits of the raw beans and sensory traits of the cooked beans, according to region of domestication.

	Andean	Mesoamerican	Significance ANOVA	
External visual traits of the raw beans				
100-seed weight (g)	44.03	31.36	***	
Width (mm)	8.1	7.55	***	
Length (mm)	13.39	11.81	***	
Length/width	1.67	1.56	*	
Thickness (mm)	6.41	5.27	***	
Projected area (mm ²)	87.75	71.97	***	
Curvature	0.04	0.04	NS	
White surface color (%)	24.21	35.71	NS	
Sensory traits of the cooked beans				
Seed-coat roughness	2.01	3.91	***	
Seed-coat perceptibility	5.79	3.52	***	
Seed-coat brightness	4.89	5.81	**	
Mealiness	6.4	4.35	***	
Flavor	5.73	3.42	***	
Aroma	7.03	5.19	***	

^{***}p<0.001; **p<0.01; *p<0.05; NS, not significant.

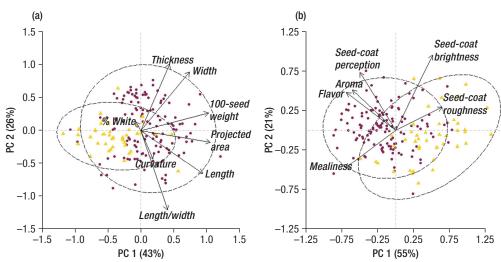


Figure 1. Principal component (PC) analysis in the external visual traits of the raw beans (a) and sensory traits of the cooked beans (b), considering domestication origin. Accessions with phaseolins of Mesoamerican origin are represented as yellow triangles and those with phaseolins of Andean origin are represented as purple circles. The dashed lines represent the 95% confidence ellipses. The percentages between brackets refer to the variation explained by each principal component.

phaseolin type (Fig. 1b). No significant differences were found in any culinary trait, and the PCA failed to reveal any clear clustering among materials of Mesoamerican or Andean origin based solely on culinary traits.

Relations between the color of raw seeds and culinary and sensory traits of the cooked seeds

The ANOVA on the means of the sensory and culinary traits of the accessions distributed into 13 color categories of the raw seeds revealed few significant differ-

ences between groups for most traits, although the rate of water absorption was generally lower (40-62, p<0.001) in brown, vinous brown, gray-brownish to greenish, purple, and black seeds and higher (84-93, p<0.001) in yellow and white seeds. Nevertheless, some black beans showed a high rate of water absorption.

In the PCA performed on sensory traits of the cooked seeds in function of the color classification of the accessions, white beans tended to have rougher and brighter seed coats and to be less mealy than more pigmented beans, while pigmented beans tended to have a stronger aroma and flavor than white beans (Fig. 2a).

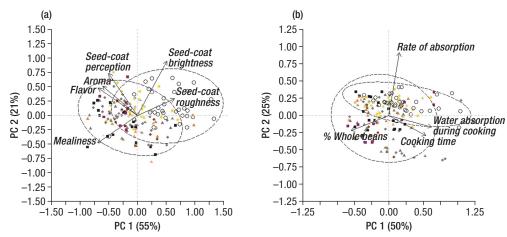


Figure 2. Principal component (PC) analysis in the sensory traits recorded in cooked beans (a) and in culinary traits (b), considering the color of the raw seed coat. Accessions with white seed coats are represented as white circles and accessions with seed coats of other colors are represented as color icons. The dashed lines represent the 95% confidence ellipses. The percentages between brackets refer to the variation explained by each principal component.

On the other hand, the PCA of the culinary traits (Fig. 2b) also confirmed that white beans tended to have higher rates of water absorption and to yield a lower percentage of whole beans after cooking (Fig. 2b).

Growth habit and sensory and culinary traits

Our study includes 29 accessions with determinate growth (Type I) (Singh, 1982) and 111 accessions with indeterminate growth (Type IV). The ANOVA to compare the mean values of the sensory and culinary traits between materials with determinate and indeterminate growth found significant differences only in seed-coat perceptibility (p<0.001) and flavor (p<0.01), but these differences were small (for seed-coat perceptibility: 3.7 in indeterminate growth vs. 2.8 in determinate growth; for flavor: 6.6 in indeterminate growth vs. 6.1 in determinate growth).

Discussion

Sensory and culinary traits in the core collection

The Spanish core collection of beans, derived from screening more than 3000 accessions fundamentally through the criteria of collection area and appearance of the raw seeds and later fine-tuned with information from molecular markers of genetic background, also has wide variability in sensory and culinary traits. The especially high variation in the traits recorded in the cooked beans suggests that the cooking process generates variation in the characteristics of the seed that is added to the variation that was present before cooking.

The traits 100-seed weight and seed width, length, and thickness were included in the selection criteria for the core collection. The CVs for these traits were among the lowest for all the traits studied, coinciding with the findings reported by Casquero *et al.* (2006). The high CVs in culinary and sensory traits of the cooked seeds support the idea that the current core collection encompasses a large proportion of the variation for these traits that were not initially included in the selection criteria.

For the trait water absorption, the CVs were lower at 24 h than at 3 h, 6 h, or 9 h. This suggests that differences between accessions for this trait are more substantial in the early part of the soaking process. The rate of water absorption is a variable that we wanted to explore as a synthetic value for the differences found at different times in the process. To this end, we analyzed the correlations between this variable and two absorption constants proposed by Peleg (1988): K_1 (a constant related to mass transfer rate) and K_2 (a constant related to maximum water absorption capacity). The rate of water absorption correlated negatively with K_1 (r=-0.81, p<0.001) and positively with K_2 (r=0.80, p<0.001), showing that the rate of water absorption is a good synthetic trait that summarizes the relevant information.

In the overall set of the core collection, we found a wide variety of combinations between visual traits of the raw seeds, sensory traits of the cooked seeds and culinary traits, although some combinations were more common than others. The strongest correlations were found between seed-coat brightness and mealiness (r=-0.73, p<0.001) and between flavor and aroma (r=0.71, p<0.001). Plans *et al.* (2014) reported similar results for flavor and aroma (r=0.62, p<0.001) and also reported that flavor correlated with seed-coat roughness (r=-0.4, p<0.01) and mealiness (r=0.68, p<0.001). In our study, flavor also correlated with

seed-coat roughness (r=-0.55, p<0.001) and with mealiness (r=0.54, p<0.001). We found that 100-seed weight correlated with seed-coat perception (r=0.67, p<0.001), % of white correlated with mealiness (r=-0.60, p<0.001), and with seed-coat brightness (r=0.60, p<0.001). Mealiness also correlated negatively with seed-coat roughness (r=-0.60, p<0.001), as previously reported by Rivera *et al.* (2013) in an earlier study done in Catalan landraces.

The relation between seed size and rate of water absorption is controversial (Sefa-Dedeh & Stanley, 1979; Del Valle *et al.*, 1992; Abdel Kader, 1995; Berrios *et al.*, 1999). We found a very low correlation between the rate of water absorption and seed volume (r=-0.19, p<0.05), suggesting that factors other than surface area and volume are also involved; these factors might include the shape of the hilum and micropyle or the structure and composition of the seed coat and cotyledon and their thickness (Sefa-Dedeh & Stanley, 1979).

Domestication origin and sensory and culinary traits

Phaseolus vulgaris L. was domesticated independently in the Andean and Mesoamerican regions, resulting in two highly differentiated gene pools that can be distinguished by the phaseolin content (Gepts & Blis, 1986; Gepts et al., 1986; Koenig et al., 1990). Using this approach, these authors found that seeds of Mesoamerican origin tend to be smaller than those of Andean origin. We found significant differences between the two origins for all traits related to size and shape (p<0.05 for length/width and p<0.001 for the others), except seed curvature, a trait not included in previous studies and percentage of white in the seed surface, although the mean values for this trait differed greatly between Andean and Mesoamerican materials, these differences were not significant (Table 3).

Our results showed significant differences between the two origins for all the sensory traits of the cooked beans, but none for the culinary traits. Materials of Andean origin had lower seed-coat brightness (p<0.01) and seed-coat roughness, and greater seed-coat perceptibility, mealiness, flavor, and aroma (all significant at p<0.001). These differences had not been reported before and add new criteria for the description of the two groups.

Relations between the color of raw seeds and culinary and sensory traits of the cooked seeds

The color of beans is attributed to the presence and quantity of polyphenols, such as condensed tannins

(Espinosa-Alonso *et al.*, 2006), which reside mainly in the seed coat and are found in greater concentrations in black, red, and bronze seeds (Bressani & Elias, 1980). Seed-coat color has been related with other traits of interest like *Pythium ultimum* resistance (Lucas & Griffiths, 2004; Campa *et al.*, 2010) or seed composition (Casañas *et al.*, 2013; Hacisalihoglu & Settles, 2013).

We found few significant differences in culinary and/ or sensory properties in relation to color. Beans with a high proportion of white surface area tended to be brighter, rougher, and less mealy; they also absorbed water faster and yielded a low percentage of whole beans after cooking (Table 2; Figs. 2a and 2b). Stanley (1992) attributed the lower rate of water absorption in colored seeds to the formation of macromolecules from tannins and other molecules such as proteins and pectins. These macromolecules strengthen the cell wall and reduce the absorption of water, impeding the separation of cells and softening. However, Espinosa-Alonso et al. (2006) reported that the variation in polyphenolic contents found in their studies was more related to the rest of the genetic background than to color. In a previous study (Rivera et al., 2013), our group found a similar correlation between the percentage of white in raw seeds and the percentage of whole beans after cooking (r=-0.47, p<0.05) to those found in the present study.

Growth habit and sensory and culinary traits

Growth habit did not correlate with culinary or sensory traits. These results do not explain the predominance of beans with indeterminate growth among the most prestigious varieties in Spain (PGI Judías de El Barco de Ávila, PGI Alubia de La Bañeza-León, PGI Faba Asturiana, PGI Faba de Lourenzá, and PDO Mongeta del Ganxet) (Casquero, 2012). This suggests that we should probably refer to agronomical, historical, or cultural reasons to explain this point.

Our results add information on the Spanish core collection of beans, increasing its value as a source of material that can be used to obtain new varieties. Breeding programs benefit greatly from more complete characterization of the accessions, including information about culinary aspects, the appearance of the raw seed, texture, and flavor of the cooked seed or resistance to disease. Moreover, crossing the materials in the collection will help identify genes related to sensory and culinary quality. The recent sequencing of the genome of *Phaseolus vulgaris* L. (Schmutz *et al.*, 2014) and the identification of quantitative trait loci related to sensory and culinary quality (Posa-Macalincag *et al.*, 2002; Garcia *et al.*, 2011; Casañas *et al.*, 2013) should

pave the way for the identification of genes and the development of internal markers that will allow them to be managed easily.

The traits detailed in the accessions of the Spanish core collection can be consulted on the Spanish National Plant Genetic Resource Center's website www.crf.inia.es/crfesp/paginaprincipaljudia.asp.

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