

Agronomical and Morphological Diversity of Carob (*Ceratonia Siliqua* L.) Cultivars: A Case of a Semi-arid Region in Majorca (Spain)

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Abstract

There is an increasing awareness of the potential usefulness of the carob tree (*Ceratonia siliqua* L.) as a crop, due to its adaptability to agriculture in marginal lands and its capacity to confront foreseeable adverse climatic conditions. Moreover, in the food industry there is an increasing interest in this fruit as a functional ingredient, due to its physicochemical components and nutritional benefits. The species is traditionally cultivated in the Mediterranean region and a wide range of carob cultivars have been developed. The present work aims to evaluate yield and morphological pod and seed characteristics from 9 different cultivars from Majorca (Spain). Statistically significant differences were found among the cultivars for the characteristics examined, indicating a high genetic diversity. A correlation analysis between yield and morphological parameters exhibited a negative correlation between seed yield and pod width, length, weight and thickness; a positive correlation of pod weight with pod length, width and thickness, and negative correlation between seed weight and carob sex. The Principal Component Analysis (PCA) was applied in order to separate the different carob cultivars and establish three main groups. In the first of them, a great phenotypic diversity is evident. A two-dimensional model was found to be significant and explained 77.66% of the observed variability, the first component being mainly dominated by pod characteristics and seed yield (57.72%) and the second component mainly by seed parameters (19.94%).

INTRODUCTION

One of the most promising alternatives, given the need for agriculture to confront the challenge of climate change, is the use of marginal lands for sustainable production [1, 2]. Local varieties reappear as good candidates for profitable production when low-input techniques are applied and they are cultivated in nutrient-poor soils and harsh climatic conditions [3]. The situation is worse in arid and semiarid countries, that covers a third of the world's continental areas [4], and in coastal regions where a significant portion of the land may have been affected by salinity [1]. Thus, agriculture in the Mediterranean regions could be significantly affected by the global climate changes [5, 6]. The term local cultivar has been applied, taking into consideration its association in marginal conditions, stress conditions, and also the direct lack of competition with highly improved varieties [2]. For this reason, the potential adaptation of these cultivars to the edaphoclimatic context of each territory has awakened interest in the recovery of native cultivars as potential contributors of interesting genes [7]. The ability to preserve the biodiversity of agricultural species is key to establishing the basis of research in future varietal selection programs [2, 8].

The carob tree (*Ceratonia siliqua* L.) is native to regions of the Mediterranean basin [9, 10], and grows on poor calcareous soils and areas where there may be high salinity [11, 12]. Due to its rusticity and its resistance to drought by decreasing leaf water potential and probably enhancing its root system depth [13], it is well adapted to the Mediterranean climate with a minimum care [14, 15, 16]. Main limitation of this crop is sensitivity to frost [11, 17]. The ability of this species to grow in contrasting climatic and edaphic conditions suggests a high degree of adaptability and a significant variability among its

members [18]. Thus, the potential for the carob crop to adapt to adverse climatic conditions as well as its proven resistance to the bacterium *Xylella fastidiosa* [19], has given it back the attention it deserves. The carob tree flower is sometimes hermaphrodite and rarely monoecious, the male tree being sterile and unproductive [20]. The carob fruit is divided into two parts, pod and seeds (90% and 10%, respectively) [21, 22]. The pods vary in morphological characteristics, such as dimension, size, weight, shape, and seed-pulp ratio, due to the differences between cultivated varieties and climatic conditions [23]. The carob pulp is rich in sugar, dietary fibers, tannins and is an important source of macroelements which make it interesting for the production of functional ingredients for the agri-food industry [9, 24]. Furthermore, carob seeds are highly valued for their gum content, extracted from the seed endosperm after the removal of the cuticle and germ, and containing between 80 and 85% of galactomannans which is a food additive (E410) used as stabilizer and gelling agent [25, 26]. This is the why carob fruit has recently become of significant importance to the food industry due to its physicochemical components with functional, technological and flavoring properties along with its nutritional benefits [21, 27].

Between 2012 and 2021 the average carob production was 183,915 tons on an area of 74,229 ha, with Spain being the main producer with 44,114 tons, followed by Portugal, Italy and Morocco [28]. The number of carob trees has significantly increased in the Mediterranean region and has continued progressing over the last few decades because of the importance and usefulness of its raw pods which have been progressively employed in various industries [20]. Majorca is a Balearic Island (Spain) in the middle of the Mediterranean region characterized by a typical Mediterranean climate with hot and dry summers and mild winters. The region is also rich in fruit biodiversity with a wide range of local varieties, such as carob cultivars.

The present work aims to evaluate the agronomic production and morphological characters of carob pods and seeds from 9 different cultivars from Majorca (Spain) to establish correlations between morphological parameters, and to classify the cultivars in their main groups.

MATERIALS AND METHODS

Experimental field and meteorological data

The research was carried out at the experimental field of Son Mulet (UTM coordinates, X; 494566.85, Y; 4368489.20) in Majorca (Balearic Islands). The carob orchard was cultivated in marginal non-irrigated conditions, without any agronomic input. The soil characteristics are summarized in Table 1. All the determinations were carried out following the official laboratory methods of the Spanish Ministry of Agriculture, Fishery and Food (MAPA 1994) [36]. Meteorological data were collected from the government platform SIAR (Agroclimatic Information System for Irrigation) from 2003 to 2023 choosing the nearest meteorological station, located in Felanitx (Fig. 1). The climate is typically Mediterranean (BSk) according to the Köppen-Geiger climate classification system (Britannica 2023), with hot and dry summers and mild winters, precipitation being concentrated from autumn through to spring.

Table 1
Physical and chemical properties of the soil (soil depth: 40–80 cm) where the assays were carried out.

Soil Properties			
Organic matter (%)	2.23	Na (mg/kg DM)	36
CaCO ₃ equivalent (%)	53.89	Clay (%)	27.2
Active lime (%)	16	Silt (%)	33.7
N Kjeldahl (% DM)	0.14	Sand (%)	39.1
P (Olsen) (mg/kg DM)	6.60	Texture	Clay loam
K (mg/kg DM)	336	CCE (% DM)	11.4
Ca (mEq/100 g DM)	7084	pH	8.5
Mg (mEq/100 g DM)	133		
DM: dry matter.			

Plant material

For this study, a total of nine cultivars from Majorca were evaluated, seven of them traditional ones (Bajoca, Bauçana, Bugadera, D'en Pau, Des Mestre, Duraió and Lloseta) and two open-pollinated selections (Granja and H2-12 (commercially called E-13P)).

Cultural practices

The carob trees were planted in 2013 with a spacing of 8 x 8 meters (156 trees ha⁻¹). Crop management was carried out without any irrigation system, pest treatment or disease control. However, during the first year of planting, support irrigation was applied to assist the establishment of the plants. Every year, two surface ploughings (spring and autumn) were carried out in order to facilitate water infiltration in the rainy season.

Agronomic and morphological analyses

Sex, tree vigor, growth habit and foliage density

Sex, vigor, growth habit and foliage density parameters were evaluated for each cultivar, following basic descriptors present in Batlle and Tous, 1997. Trunk section was measured by obtaining first trunk perimeter and as:

$$TS = TP^2 \cdot 4^{-1} \cdot \pi^{-1} \text{ (cm}^2\text{)}.$$

Carob production and seed yield

The carob fruits were harvested when they reached the RS5 ripening stage according to Kyriacou et al. (2021) [37], during 3 different seasons (2021, 2022 and 2023). Tree production was obtained weighing each individual separately. The parameters evaluated were: mean pod production ($\text{kg}\cdot\text{tree}^{-1}$), mean agronomic seed yield (%), mean seed production ($\text{kg}\cdot\text{tree}^{-1}$) and accumulative pod and seed production efficiency ($\text{g} \cdot \text{cm}^{-2}$). Agronomic seed yield (ASY) was obtained by grinding 5.00 kg pods with a M-10 CIMAG carob seed classifier and dividing seed weight by the total pod quantity. Mean seed production was estimated with the mean pod production and the mean ASY. Cumulative production efficiency correlates tree production with its size. The sum of the annual production from 2021 to 2023 (g) and its trunk section in 2023 (cm^2) were the values used to establish this parameter.

Morphological fruit and seed description

For the morphological characteristics, a homogeneous and representative sample of 20 individual carob pods per cultivar and year were selected, along with 25 seeds from each of the same pods. The parameters evaluated in pods and seeds were: length, width and thickness, using a digital caliper (Kemmler Präzisionswerkzeuge GmbH, Mössingen, Germany), and weight using a Highgland® portable precision HCB1002 balance (Adam Equipment Co LTD, Kingston, United Kingdom). Individual pod seed yield (ISY) was obtained, as well, by considering the relation between seed weight and pod weight.

Statistical analysis

When the data exhibited a normal distribution and homogeneity of variance, a factorial ANOVA was conducted to determine significant differences among the cultivars. The Tukey test was performed to evaluate the statistical differences between means ($p < 0.05$) indicated by the ANOVA analysis. Alternatively, when the data did not follow a normal distribution, the Kruskal-Wallis analysis was conducted. A principal component analysis (PCA) was carried out to identify the primary variations in morphological characteristics within the cultivars. In addition, hierarchical cluster analysis (HCA) was used to investigate the similarities and dissimilarities among the cultivars. Finally, correlation between morphological parameters was evaluated using Pearson's correlation coefficient. All data analysis was carried out using the IBM SPSS software package.

RESULTS AND DISCUSSION

Sex, tree vigor, growth habit and foliage density

The results of sex, vigor, growth habit and foliage density in trees of different cultivars are presented in Table 2. Figure 2 shows the pictures of those parameters.

Table 2
Carob tree characteristics of the different carob cultivars studied.

Cultivar	Sex	Vigor	Growth habit	Foliage density
Bajoca	Female	Medium	Upright	Dense
Bugadera	Female	Medium	Upright	Dense
Bauçana	Female	High	Open	Less Dense
D'en Pau	Female	Medium	Upright	Dense
Des Mestre	Hermaphrodite	Very high	Open	Dense
Duraió	Female	Medium	Upright	Most dense
Granja	Female	High	Weeping	Less dense
H2-12	Hermaphrodite	Medium	Upright	Dense
Lloseta	Hermaphrodite	High	Open	Less dense

Three of the nine cultivars, Des Mestre, Lloseta and H2-12, presented hermaphroditic flowers, the frequency of this condition being extraordinarily high in the Balearic Island [23, 29]. Vigor varied from medium to very high among all the cultivars, (Fig. 2A) with Des Mestre presenting the highest values (Table 2).

The carob cultivars exhibited mainly an upright or an open growth habit except for the Granja cultivar (Fig. 2B), which displayed a weeping habit. In terms of foliage density, Duraió was the densest cultivar (Fig. 2C), while Bauçana, Lloseta (Fig. 2D) and Granja were the least dense.

Trunk section results of the different cultivars are shown in Fig. 3. Values ranged from 112.98 cm² in Lloseta to 180.26 cm² in Granja. However, no significant differences among cultivars ($p < 0.05$) could be detected for trunk section in 2023, nine years after planting.

Carob production and seed yield

Results of carob tree production among the cultivars studied are reflected in Fig. 4.A. Mean carob tree production collected during the whole experiment was 8.41 ± 0.50 pod kg·tree⁻¹, not showing significant differences ($p < 0.05$) from year to year. Similar mean productive values were observed in non-fertile traditional carob orchards in southern Portugal [30]. Considering these cultivars, the only significant differences in the growing season were detected in two cultivars: H2-12 and Bugadera. Those differences reflected a considerable decrease in production in 2022. The mean carob production

between the cultivars ranged from 3.60 ± 0.76 pod $\text{kg} \cdot \text{tree}^{-1}$ to 16.45 ± 3.03 pod $\text{kg} \cdot \text{tree}^{-1}$ and presented significant differences, being H2-12 the most productive, and Duraió, Granja and Bajoca, the least.

With regard to the agronomic seed yield values (ASY), the results are presented in Table 3. The average ASY for Majorcan carobs, without considering the different cultivars, was $13.33 \pm 0.71\%$. No differences were found between years, but significant differences were found among cultivars ($p > 0.05$), the values ranging from $8.17 \pm 0.85\%$ in Des Mestre to $18.74 \pm 0.56\%$ in Granja.

Table 3
Agronomic seed yield values (ASY) among cultivars studied.
Black letters express significant differences ($p < 0.05$).

Cultivar	ASY (%)			Mean ASY (%)
	2021	2022	2023	
Granja	19.25	19.37	17.62	18.74 ± 0.56 a
H2-12	18.26	13.24	19.88	17.13 ± 1.73 ab
Bugadera	16.23	14.96	16.89	16.03 ± 0.49 abc
Bauçana	13.82	12.90	13.68	13.47 ± 0.29 bcd
D'en Pau	12.14	12.24	14.14	12.84 ± 0.56 bcde
Duraió	14.02	10.30	14.07	12.80 ± 1.08 bcde
Bajoca	11.98	8.91	13.86	11.58 ± 1.25 cde
Lloseta	9.48	8.58	9.57	9.21 ± 0.27 de
Des Mestre	8.75	6.25	9.52	8.17 ± 0.85 e

These values agree with other cultivar seed yield values from Spain [31] and Portugal [32], but not with others from the Mediterranean coastal basin like Morocco [18] or Algeria [20], which have both greater (17.47 to 29.44%) and lower (4.58 to 7.35%) seed yield values, respectively. According to Barracosa et al. (2007) [32] it is preferable to use thin, narrow carobs which are not too heavy and contain a high number of seeds, in order to obtain an incremental yield of seeds.

Average seed production among the cultivars studied is shown in Fig. 4.B. With regard to seed yield production, obtained with ASY data and mean pod production data, the mean value was 1.18 ± 0.07 seed $\text{kg} \cdot \text{tree}^{-1}$. In 2022, seed production per tree was significantly ($p < 0.05$) lower (0.83 ± 0.09 seed $\text{kg} \cdot \text{tree}^{-1}$), than 2021 (1.28 ± 0.14 seed $\text{kg} \cdot \text{tree}^{-1}$) and (1.18 ± 0.13 seed $\text{kg} \cdot \text{tree}^{-1}$). Once again, H2-12 and Bugadera cultivars exhibited significant variation in terms of seed production year on year (Fig. 4B). Cumulative pod and yield efficiency in terms of pod are exhibited in Fig. 5. Cumulative pod and seed yield efficiencies were significantly different ($p < 0.05$) among the cultivars, and mean values were 189.86 ± 14.38 pod

$\text{g}\cdot\text{cm}^{-2}$ and 24.96 ± 2.06 seed $\text{g}\cdot\text{cm}^{-2}$, respectively. H2-12 was the most efficient cultivar in both cases with 333.85 ± 59.76 pod $\text{g}\cdot\text{cm}^{-2}$ and 43.10 ± 4.09 seed $\text{g}\cdot\text{cm}^{-2}$, respectively. Meanwhile, in terms of pod efficiency this cultivar did not show significant differences from Lloseta, D'en Pau, Bauçana and Bugadera (Fig. 5.A). This last cultivar exhibited similar seed efficiency values to H2-12. The least efficient cultivar in pod production was Granja with 55.17 ± 10.18 pod $\text{g}\cdot\text{cm}^{-2}$, and in seed production, Bajoca with 9.27 ± 1.77 seed $\text{g}\cdot\text{cm}^{-2}$ (Fig. 5.B).

Fruit morphometric description

Table 4 presents the morphometric parameters of carob pods from the different cultivars, along with Fig. 6, which show pictures of the pods. Considerable variation in pod morphology were observed in the different carob cultivars. Thick, large fruits from the Lloseta cultivar could be easily identified, compared with the thin, small fruits from Granja (Fig. 6). The mean pod length value ranges from 15.74 ± 0.24 to 20.45 ± 0.32 cm, for Granja and Lloseta cultivars, respectively. Significantly ($p < 0.05$), the Lloseta cultivar produced the largest fruits meanwhile Granja, H2-12 and Bauçana, the smallest. Similarly, mean pod weight was significantly different between the cultivars; Lloseta being the heaviest (27.55 ± 1.07 g), and the fruits from Granja and H2-12 the lightest (13.19 ± 0.29 g and 10.73 ± 0.26 g, respectively). With regard to width and thickness, the same pattern was observed, Lloseta being the cultivar with the significantly wider fruits (25.80 ± 0.31 mm) and thicker (12.27 ± 0.30 mm) than the others. Granja was the cultivar that displayed narrower fruits (16.99 ± 0.18 mm) and H2-12 the thinnest (6.55 ± 0.12 mm).

Table 4
Morphometric pod parameters between the cultivars studied. Black letters express significant differences ($p < 0.05$).

Cultivar	Length (cm)	Weight (g)	Width (mm)	Thickness (mm)	Individual seed yield (%)
Bajoca	19.00 ± 0.31 b	17.66 ± 0.46 c	22.54 ± 0.23 b	8.29 ± 0.17 de	11.88 ± 0.46 d
Bauçana	16.38 ± 0.27 ef	16.88 ± 0.43 cd	20.50 ± 0.23 d	8.75 ± 0.12 d	13.52 ± 0.27 c
Bugadera	17.29 ± 0.23 de	15.63 ± 0.28 d	21.84 ± 0.16 bc	7.76 ± 0.11 ef	14.12 ± 0.30 c
D'en Pau	17.87 ± 0.20 cd	19.54 ± 0.43 b	21.09 ± 0.15 cd	9.52 ± 0.18 c	12.69 ± 0.26 cd
Des Mestre	18.62 ± 0.25 bc	21.41 ± 0.54 b	21.91 ± 0.24 b	10.32 ± 0.16 b	7.82 ± 0.28 e
Duraió	17.88 ± 0.26 cd	19.82 ± 0.43 b	21.06 ± 0.21 cd	9.79 ± 0.12 bc	13.47 ± 0.31 c
Granja	15.74 ± 0.24 f	13.19 ± 0.29 e	16.99 ± 0.18 f	7.52 ± 0.10 f	20.19 ± 0.36 a
H2-12	15.80 ± 0.24 f	10.73 ± 0.26 f	18.98 ± 0.17 e	6.55 ± 0.12 g	17.83 ± 0.45 b
Lloseta	20.45 ± 0.32 a	27.55 ± 1.07 a	25.80 ± 0.31 a	12.27 ± 0.30 a	9.28 ± 0.46 e

In comparison to other studies, our results about pod length were broadly in agreement to those reported by Barbagallo et al. (1997) [33] for Sicilian cultivars, ranging from 12.0 to 20.5 cm, Barracosa et al. (2007) [32] for Portuguese cultivars, ranging from 12.9 to 20.3 cm, and by Tous et al. (2009) [31] for other Spanish cultivars (between 15.99 and 17.55 cm). However, Majorcan mean carob length was slightly higher than that reported by Bolaric et al. (2021) [34] for Croatia carob pods (12.63 to 15.36 cm), Boublenza et al. (2019) [20] in Algerian carob pods (10.30 to 18.21 cm) and also to those of Sidina et al. (2009) [18] in Moroccan carobs (10.09 to 14.36 cm).

Regarding the carob weight, Boublenza et al. (2019) [20] obtained similar values for Algerian cultivars (between 13.71 to 30.57 g) and also a similar interval was published by Barracosa et al. (2007) [32] (between 11.46 to 26.39 g) and Tous et al. (2009) [31]. Furthermore, the results for width and thickness are in agreement to those reported by Baragallo et al. (1997) [33], Barracosa et al. (2007) [32], Tous et al. (2009) [31] and Bolaric et al. (2021) [34], but greater than those reported by Boublenza et al. (2019) [20].

Seed morphometrical description

Results of the seed morphometrical description are presented in Table 5, as well as Fig. 7 showing pictures of carob pods from the different cultivars. According to these results, seed morphological parameters were significantly different among the cultivars (Table 5). However, these differences, as opposed to the fruit, were not clearly visible (Fig. 7). Seed length varied from 8.97 ± 0.03 mm in Granja to 10.91 ± 0.07 mm in Bajoca, a significant difference.

Table 5
Morphometric seed parameters between the cultivars studied. Black letters express significant differences ($p < 0.05$).

Cultivar	Length (mm)	Weight (g)	Width (mm)	Thickness (mm)
Bajoca	10.91 ± 0.07 a	0.21 ± 0.003 b	7.24 ± 0.04 de	3.76 ± 0.05 de
Bauçana	9.86 ± 0.06 de	0.21 ± 0.002 b	7.35 ± 0.05 cd	3.87 ± 0.04 cd
Bugadera	10.02 ± 0.06 cd	0.20 ± 0.002 c	7.07 ± 0.04 f	3.86 ± 0.03 cd
D'en Pau	10.32 ± 0.05 b	0.21 ± 0.002 b	7.16 ± 0.03 ef	3.73 ± 0.03 de
Des Mestre	9.70 ± 0.06 ef	0.19 ± 0.004 c	7.30 ± 0.04 cde	3.63 ± 0.04 e
Duraió	9.88 ± 0.05 de	0.23 ± 0.002 a	7.61 ± 0.04 a	4.16 ± 0.03 b
Granja	8.97 ± 0.03 g	0.22 ± 0.002 b	7.54 ± 0.04 ab	4.71 ± 0.04 a
H2-12	9.47 ± 0.04 f	0.19 ± 0.003 c	7.24 ± 0.04 def	3.95 ± 0.04 c
Lloseta	10.16 ± 0.05 bc	0.19 ± 0.004 c	7.44 ± 0.03 bc	3.42 ± 0.05 f

Mean seed length was significantly different among cultivars ranging from 8.97 ± 0.03 for Granja cultivar to 10.91 ± 0.07 cm for Bajoca cultivar. Those results were in agreement with other Mediterranean cultivars reported by Barbagallo et al. (1997) [33] for Sicilian cultivars, Barracosa et al. (2007) [32] for Portuguese cultivars, Tous et al. (2009) [31] for other Spanish cultivars and Bolaric et al. (2021) [34] for Croatian cultivars. As regards seed weight, mean value ranged between 0.19 ± 0.004 g and 0.23 ± 0.002 g, being these in agreement with observed in other mediterranean carob cultivars [18, 20, 31, 32, 33, 34]. Three groups of cultivars could be identified according to seed weight: the heaviest group composed exclusively of the Duraió cultivar, a second group, a medium seed weight cultivar group, comprising the Bajoca, Bauçana, D'en Pau and Granja cultivars, and finally, the lightest seed weight group constituted by Bugadera, Des Mestre, H2-12 and Lloseta. The differences were statistically significant ($p < 0.05$), and a same relation between tree sex and seed weight according to Turnbull et al. (2006) [35] was found, the seeds from hermaphroditic cultivars being lighter than the female ones. Width and thickness in Majorca carob cultivars were also different among the cultivars, and values were coincident with those reported by Barbagallo et al. (1997) [33], Barracosa et al. (2007) [32], Tous et al. (2009) [31] and Bolaric et al.

(2021) [34]. The first parameter fluctuated between 7.61 ± 0.04 mm in Duraió and 7.07 ± 0.04 mm in Bugadera cultivars respectively, and the second one between 4.71 ± 0.04 mm in Granja and 3.63 ± 0.04 mm in Des Mestre.

Correlation analysis

The correlation analysis of all morphological characteristics from pods, their seeds, and tree sex is summarized in Table 6. In the present study, pod weight exhibited a negative correlation with seed yield. This observation is explained by Batle and Tous (1997) [15], who suggest that traditional selection was primarily based on fruit quality factors such as pod size, pulp weight, and sugar content. Furthermore, seed yield displayed a negative correlation with pod width. Barracosa et al. (2007) [32], Tous et al. (2009) [31], and Boublenza et al. (2019) [20] obtained similar results. Additionally, seed yield exhibited a negative correlation with pod length and pod thickness. These findings align with those reported by Sidina et al. (2019) [18], who indicated that seed yield demonstrated a negative correlation with all pod characteristics. Moreover, according to our results, seed yield showed a positive correlation with seed thickness, and pod length displayed positive correlations with pod width, pod thickness, and pod weight. Similar results were obtained by Sidina et al. (2019) [18]. Seed weight was related with carob tree sex, being consistent with Turnbull et al. (2006) [35].

Table 6. Pearson coefficient correlation between agronomic and morphological characters of pods and seeds of Majorcan carob cultivars.

Characters	1	2	3	4	5	6	7	8	9	10
Seed length (1)	1									
Seed width (2)	-0.067	1								
Seed thickness (3)	-.551**	.152**	1							
Seed weight (4)	.151**	.471**	.388**	1						
Pod length (5)	.303**	.057	-.305**	0.023	1					
Pod weight (6)	.223**	.134**	-.333**	-0.007	.722**	1				
Pod width (7)	.508**	.053	-.610**	-.106*	.574**	.690**	1			
Pod thickness (8)	.110*	.175**	-.302**	-0.030	.490**	.840**	.546**	1		
Seed yield (9)	-.377**	0.092	.593**	.190**	-.456**	-.666**	-.681**	-.615**	1	
Sex (10)	-.143**	-0.004	-.339**	-.424**	.177**	.215**	.261**	.254**	-.296**	1

** Correlation is significant at the 0.01 level

** Correlation is significant at the 0.05 level

Principal component analysis and hierarchical cluster analysis

A principal component analysis (PCA) was performed to determine the relative importance of the different physical parameters and the variability within the studied cultivars.

PCA was performed using the collected data (sex, pod weight, pod length, pod width, pod thickness, seed yield, seed weight, seed length, seed width, and seed thickness). Data analysis revealed that the two primary components account for 77.66% of the observed variability among the studied cultivars (Fig. 8). PC1 (57.72%), in red, was positively significantly related to pod weight, pod length, pod width, pod thickness, while negatively significantly related to seed yield and seed thickness. PC2 (19.94%), in green, was positively explained by seed weight and seed width. PC3, instead, explained 16.19% of total variance dominated by sex and seed length.

The PCA (Fig. 8B) and hierarchical cluster analysis (HCA) (Fig. 9) revealed the differentiation of three groups based on morphological parameters. The first group consisted of the majority of the cultivars (D'en Pau, Duraió, Bajoca, Bugadera, Bauçana, Des Mestre). This group displayed a substantial degree of heterogeneity. The second group comprised H2-12 and Granja, cultivars characterized by the highest seed yield. Finally, the third group only included Lloseta, a cultivar characterized by lower seed yield and larger pod dimensions.

CONCLUSIONS

The use of marginal land for agriculture is seen as a practical measure in confronting climate change, mainly due to the carbon sequestration carried out by plants. Carob (*Ceratonia siliqua* L.) is an evergreen species present in semi-arid and arid regions, used in the pharmaceutical, cosmetic and food industries, and which has recently attracted much attention because of its beneficial properties. However, it can also be used for soil restoration purposes and as C sequestration due its great rusticity and few needs. In our study we consider H2-12 (E-13P) as a promising double aptitude cultivar, for the pharmaceutical-food industry and animal-human consumption, because of its elevated pod and high seed yield. However, cultivars such as Lloseta, which exhibits constant and high pod yield, and Granja, with a high seed yield, could also both be considered useful, depending on the final destination of the production. Furthermore, it would be worth considering hermaphroditic cultivars as pollen donors and as potential pod producers replacing non-producer male trees. Majorcan cultivars show a great diversity of morphological traits in pods and seeds distinguishing seed yield. The statistical analysis and principal component analysis (PCA) classify Majorcan cultivars in three groups; a first group with the majority of cultivars (Bajoca, Bauçana, Bugadera D'en Pau, Des Mestre and Duraió), a second with open pollinated cultivars, Granja and H2-12, and the third one only comprising Lloseta. This aggrupation and the variation of morphological traits could be influenced by selection processes. Cultivars H2-12 and Granja, which constitute the open-pollinated group, present fruits with higher values of seed yield than the traditional cultivars. This may be due to selection processes taking more into account actual economic interest in

seeds rather than in pulp. On the other hand, the Lloseta traditional cultivar, which is isolated in the PCA analysis, displays fruits morphologically very different from the other cultivars and with very high pulp content. This study gathers together part of the great richness of the carob species that is present in Majorca, and it could be the starting point of further investigation into local Majorcan carob cultivars.

Declarations

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AUTHOR CONTRIBUTIONS

Field data, J. P. and M.B.-A.; data analyses M.B.-A. and M. Ll.; writing—original draft preparation, M.B.-A., M. Ll. and M.C.G.; writing—review and editing, J.M.L.; M. Ll. and M.C.G.

ADDITIONAL INFORMATION

The authors declare no conflict of interests. Research was funded by Institute for Agri-food and Fishing Research and Training of the Balearic Island (Government of the Balearic Islands).

The authors declare no conflict of interests.

The authors declare that the provenance of the plant material originates from the germplasm bank of *Ceratonia siliqua* L. of the Government of the Balearic Islands.

The authors declare that the study was in accordance with relevant institutional, national, and international guidelines and legislation.

References

1. Hussain, M.I., Farooq, M. & Muscolo, A. Crop diversification and saline water irrigation as potential strategies to save freshwater resources and reclamation of marginal soils—a review. *Environ Sci. Pollut. Res.* 27, 28695–28729 (2020).
2. Marone, D. *et al.* Importance of Landraces in Cereal Breeding for Stress Tolerance. *Plants* 10, 1267 (2021).
3. Abos, B., Alquézar, J.M., Lafuente, V. & Vidal, M. *Recuperando variedades locales: tomate zaragozano tipo “San Pedro”*. (Cuadernos de CERAÍ núm. 3, 2016).
4. Verstraete, M. M. & Shwartz, S. A. Desertification and global change. *Vegetatio* 91, 3–13 (1991).

5. Santillán, D., Garrote, L., Iglesias, A. & Sotes, V. Climate change risks and adaptation: new indicators for Mediterranean viticulture. *Mitig. Adapt. Strat. Glob. Chang.* 25, 881–899 (2020).
6. Varotsos, K.V. *et al.* Near future climate change projections with implications for the agricultural sector of three major Mediterranean islands. *Reg. Environ. Change* 21, 1–15 (2021).
7. Dwivedi, S.L., Blair, M.W., Upadhyaya, H.D. & Rodomiro Ortiz, A.K. Landrace Germplasm for Improving Yield and Abiotic Stress Adaptation. *Trends Plant Sci.* 21, 31–42 (2016).
8. Botu, M. *et al.* Conservation of Fruit Tree Genetic Resources and Their Use in The Breeding Process. *Ann. "Valahia" Univ. Targoviste, Agric.* 11 (2017).
9. Benković, M., Belščak-Cvitanović, A., Bauman, I., Komes, D., Srećec, S. Flow properties and chemical composition of carob (*Ceratonia siliqua* L.) flours as related to particle size and seed presence. *Int. Food Res. J.* 100, 211–218 (2017).
10. Rodríguez-Solana, R., Salgado, J.M., Pérez-Santín, E., Romano, A. Effect of carob variety and roasting on the antioxidant capacity, and the phenolic and furanic contents of carob liquors. *J. Sci. Food Agric.* 99, 2697–2707 (2019).
11. Gubbuk, H., Guven, D. & Gunes, E. Physical features of some Turkish carob (*Ceratonia siliqua* L.) pods. *Bull. UASVM Hortic.* 66, 685 (2009).
12. Tetik, N. *et al.* Physical and chemical characterization of *Ceratonia siliqua* L. germplasm in Turkey. *Sci. Hort.* 129, 583–589 (2011).
13. Ezzine, H., Metougui, M. L., Boukcim, H., & Abbas, Y. Physiological responses of three field-grown species (*Ceratonia siliqua*, *Eucalyptus camaldulensis*, and *Moringa oleifera*) to water deficits in a Mediterranean semi-arid climate. *Sci. Rep.* 13, 4536 (2023).
14. Öztürk, M., Seçmen, Ö., Gücel, S. & Sakcali, S. An Overview of Economic and Medicinal Importance of Carob Plants (*Ceratonia siliqua* L.) in the Mediterranean Basin. *Acta Hort.* 964:197–204 (2012).
15. Tous, J. *et al.* Agronomic and Commercial Performance of Four Spanish Carob Cultivars. *HortTechnology* 19, 465–470 (2009).
16. Krokou, A., Stylianou, M. & Agapiou, A. Assessing the volatile profile of carob tree (*Ceratonia siliqua* L.) *Environ. Sci. Pollut. Res.* 26, 35365–35374 (2019).
17. Santos, M., Fraga, H., Belo-Pereira, M. & Santos, J.A. Assessment of Growing Thermal Conditions of Main Fruit Species in Portugal Based on Hourly Records from a Weather Station Network. *Appl. Sci.* 9, 3782 (2019).
18. Sidina, M.M. *et al.* Fruit and seed diversity of domesticated carob (*Ceratonia siliqua* L.) in Morocco. *Sci. Hort.* 123 (2009).
19. Olmo, D. *et al.* Landscape epidemiology of *Xylella fastidiosa* in the Balearic Islands. *Agronomy* 11, 473 (2021).
20. Boublenza, *et al.* Algerian carob (*Ceratonia siliqua* L.) populations. Morphological and chemical variability of their fruits and seeds. *Sci. Hort.* 256 (2019).

21. Cantalejo, M. J. Effects of Roasting Temperature on the Aroma Components of Carob (*Ceratonía siliqua* L.). *J. Agric. Food. Chem.* 45, 1345–1350 (1997).
22. El Bouzdoudi, B. *et al.* Mineral Composition of Mature Carob (*Ceratonía siliqua* L.) Pod: A Study. *Int. J. Food Sci. Nutr. Eng.* 7, 91–103 (2017).
23. Batlle, I. & Tous, J. *Carob tree. Ceratonía siliqua* L. *Promoting the conservation and use of underutilized and neglected crops* (eds.) 17 (International Plant Genetic Resources Institute, 1997).
24. El Batal H. *et al.* Sugar composition and yield of syrup production from the pulp of Moroccan carob pods (*Ceratonía siliqua* L.). *Arab. J. Chem.* 9, S955 – S959 (2016).
25. López-Sánchez, J. I., Moreno, D. A. & García-Viguera, C. D-pinitol, a highly valuable product from carob pods: Health-promoting effects and metabolic pathways of this natural super-food ingredient and its derivatives. *Agric. Food* 3, 41–63 (2018).
26. Papaefstathiou, E., Agapiou, A., Giannopoulos, S., & Kokkinofa, R. Nutritional characterization of carob and traditional carob products. *Food Sci. Nutr.* 1–11 (2018).
27. Goulas, V., Stylos, E., Chatziathanasiadou, M.V., Mavromoustakos, T. & Tzakos, A.G. Functional Components of Carob Fruit: Linking the Chemical and Biological Space. *Int. J. Mol. Sci.* 17, 1875 (2016).
28. Mahdad, Y. M. & Gaouar, S. B. S. Origin, distribution and domestication of the carob tree (*Ceratonía siliqua* L.). *Turk. J. Bot.* 47, 89–96 (2023).
29. Zohary, D. Domestication of the carob (*Ceratonía siliqua* L.), *Isr. J. Plant Sci.* 50, 141–145 (2022).
30. Correia, P. J. & Pestana, M. Exploratory Analysis of the Productivity of Carob Tree (*Ceratonía siliqua*) Orchards Conducted under Dry-Farming Conditions. *Sustainability* 10, 2250 (2018).
31. Tous, J., Romero, A. & Battle, I. The Carob Tree: Botany, Horticulture, and Genetic Resources. *Hortic. Rev.* 41, 385–456 (2013).
32. Barracosa, P., Osório, J. & Cravador, A. Evaluation of fruit and seed diversity and characterization of carob (*Ceratonía siliqua* L.) cultivars in Algarve region. *Sci. Hort.* 114, 250–257 (2007).
33. Barbagallo, M. G., Lorenzo, R. D., Meli, R. & Crescimanno, F.G. Characterization of carob germplasm (*Ceratonía siliqua* L.) in Sicily. *J. Hortic. Sci.* 72, 537–543 (1997).
34. Bolaric, S. *et al.* Morphological and molecular characterization of Croatian carob tree (*Ceratonía siliqua* L.) germplasm. *Turk. J. Agric. For.* 45 (2021).
35. Turnbull, L. A., Santamaria, L., Martorell, T., Rallo, J. & Hector, A. Seed size variability: from carob to carats. *Biol Lett.* 22 (2006).
36. Ministerio de Agricultura Pesca y Alimentación. *Métodos Oficiales de Análisis de Suelos y Aguas para Riegos Tomo III* (Servicio de Publicaciones del Ministerio de Agricultura, Pesca y Alimentación, 1994).
37. Kyriacou, M.C., Antoniou, C., Roupheal, Y., Graziani, G. & Kyrtzsis, A. Mapping the Primary and Secondary Metabolomes of Carob (*Ceratonía siliqua* L.) Fruit and Its Postharvest Antioxidant Potential at Critical Stages of Ripening. *Antioxidants* 10, 57. (2021)

Figures

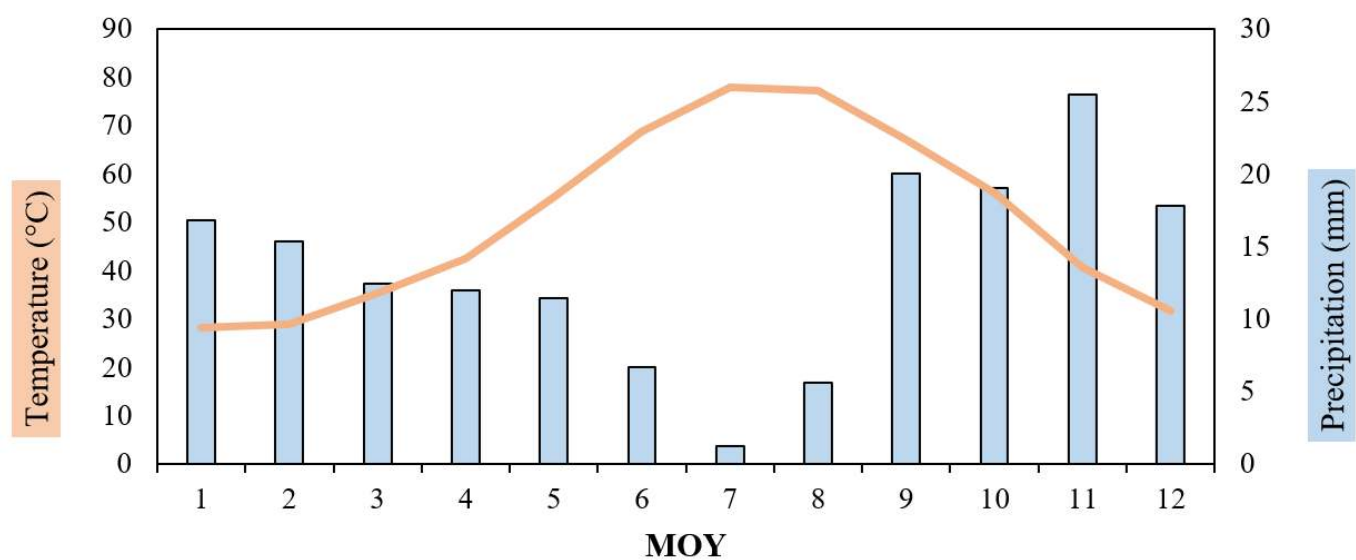


Figure 1

Average precipitation (blue bars) and temperature (orange line) from 2003 to 2023 of every month of the year (MOY) in Felanitx, Majorca.

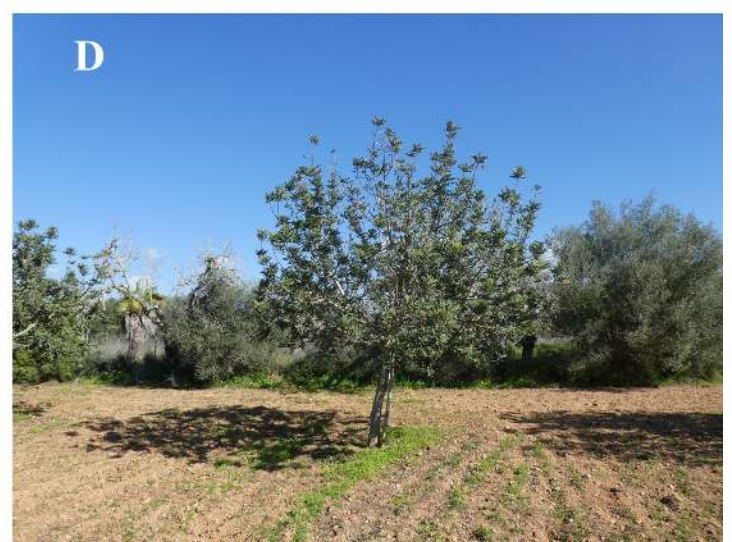


Figure 2

Vigor, grow habit and foliar density in trees of different cultivars. A. Des Mestre. B. Granja. C. Duraió. D. Lloseta.

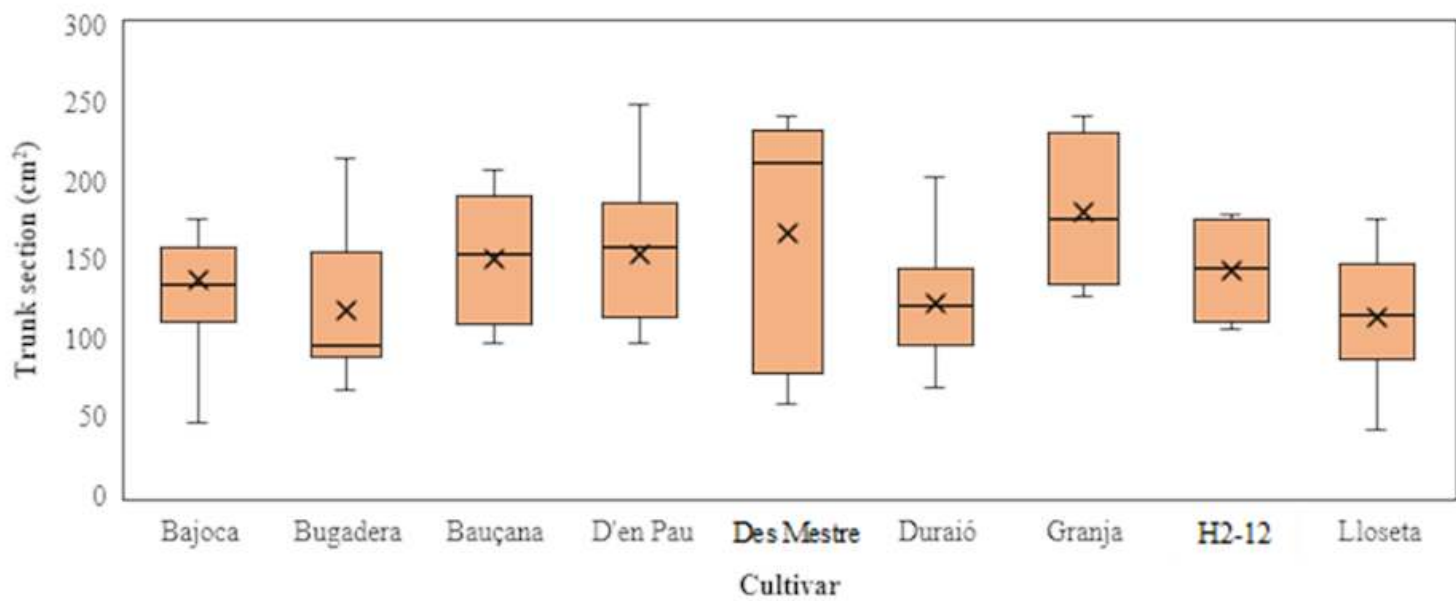


Figure 3

Trunk section values measured in 2023 among the cultivars studied. The central cross in the box represents the mean value.

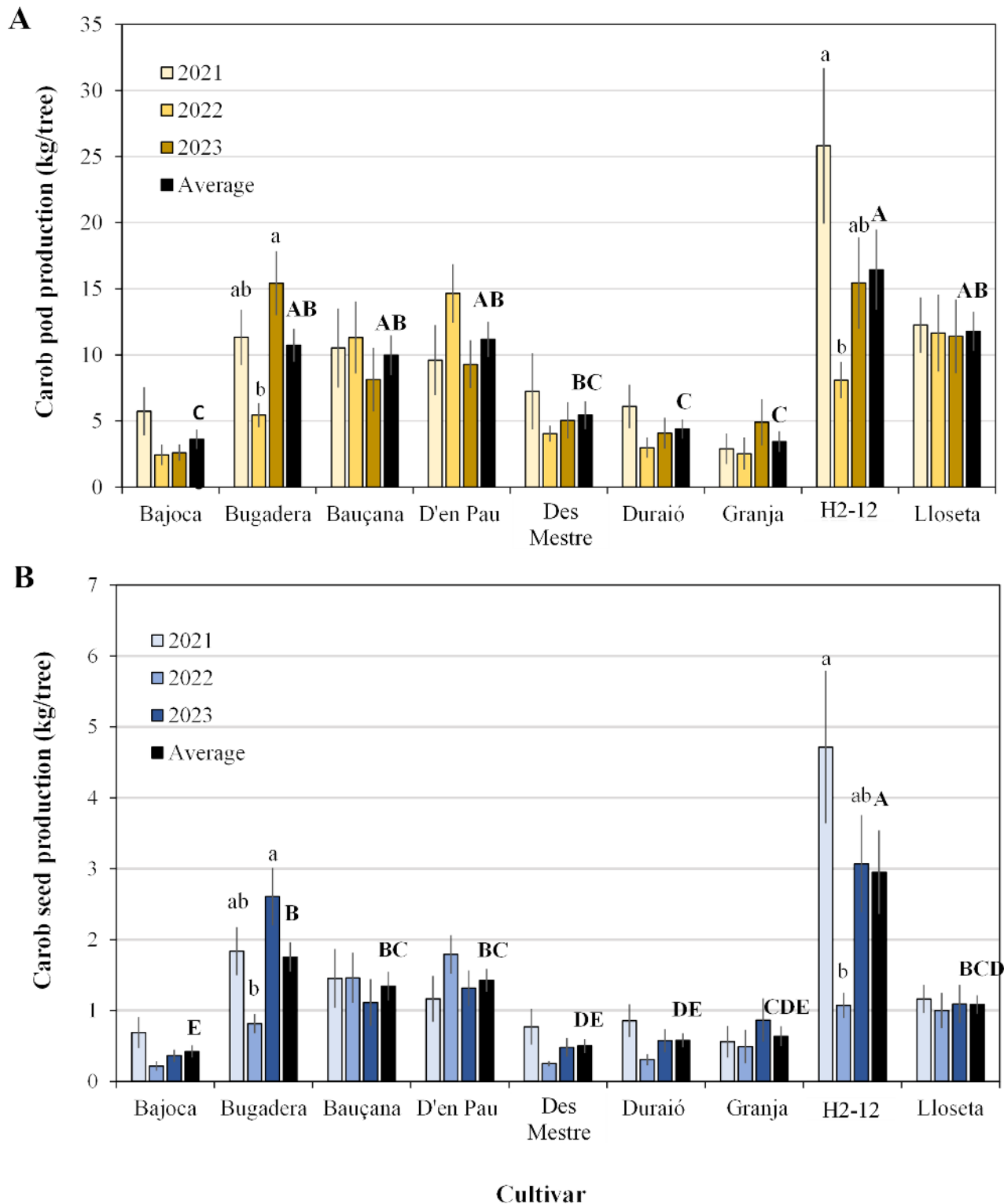


Figure 4

A. Average pod production among the cultivars studied. B. Average seed production among the cultivars studied. Capital letters above bars express significant differences ($p < 0.05$) between cultivars in terms of average production, while small ones express significant differences between years in the same cultivar.

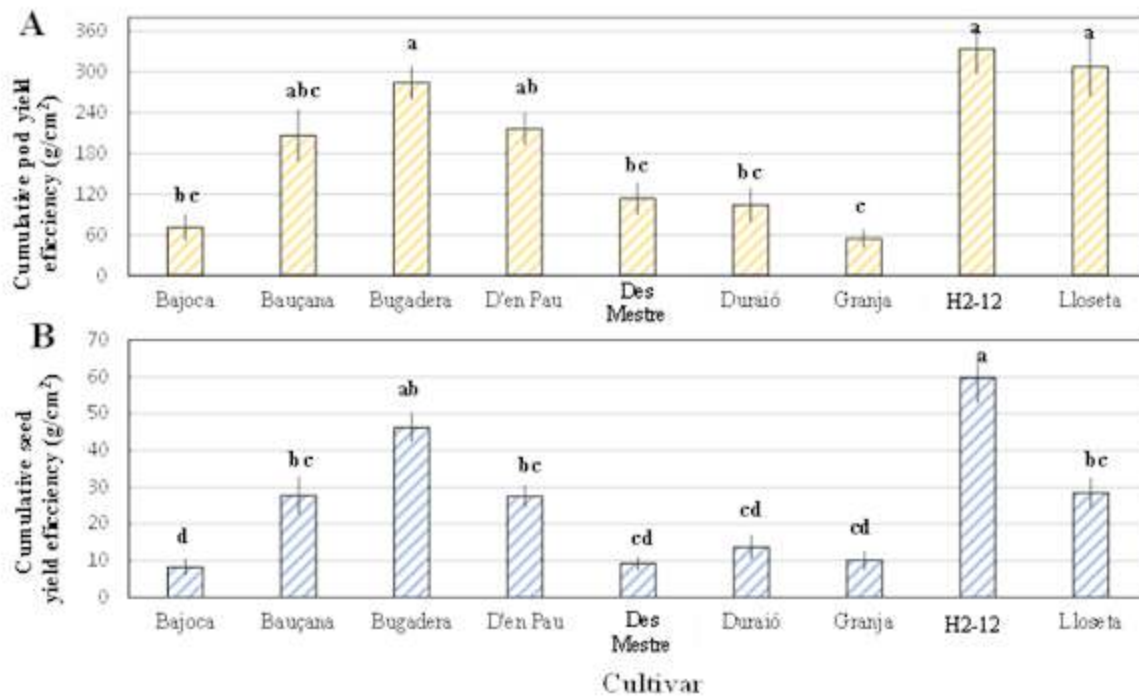


Figure 5

Cumulative yield efficiency in terms of pod (A) and seed (B) between the cultivars studied. Small letters represent significant differences ($p < 0.05$) between cultivars.

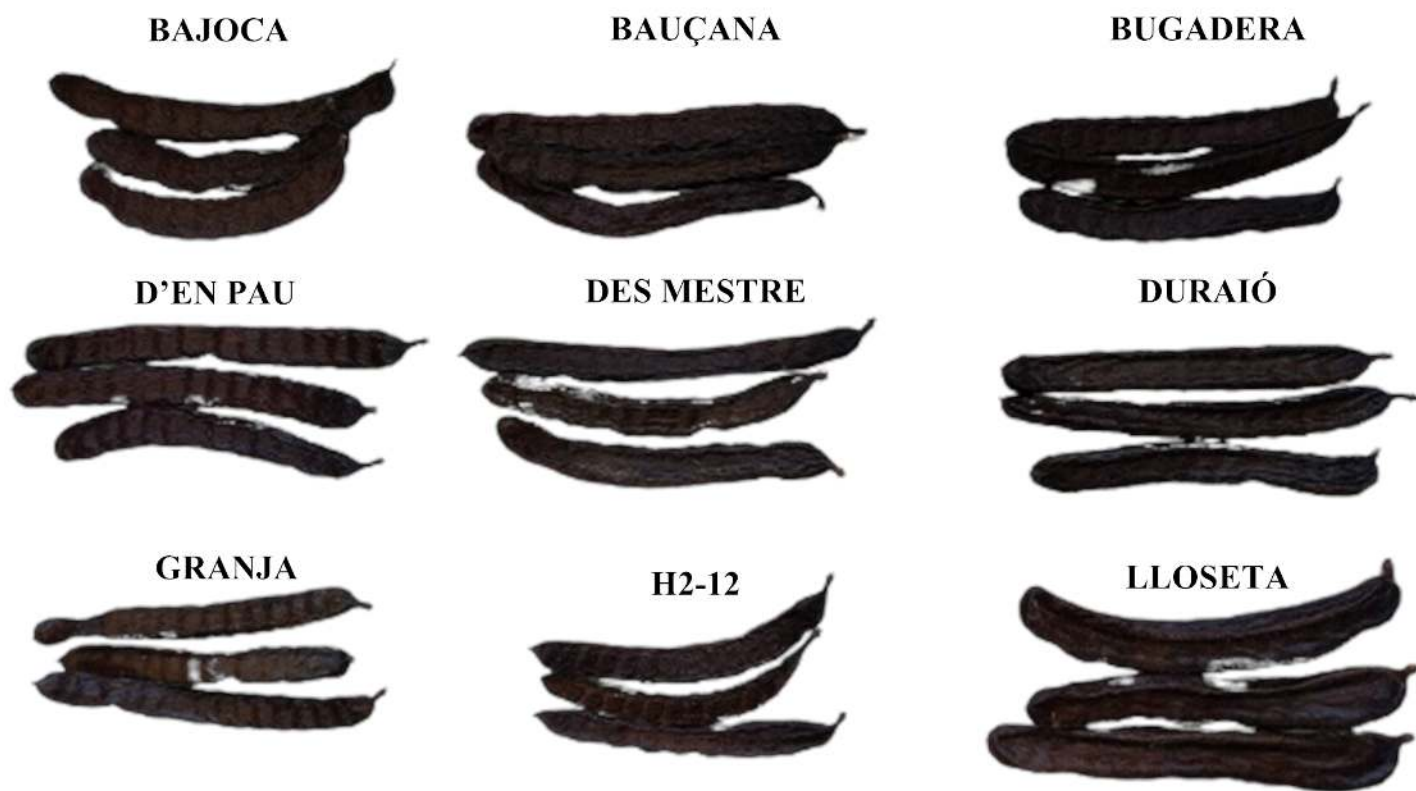


Figure 6

Carob pods from cultivars studied.

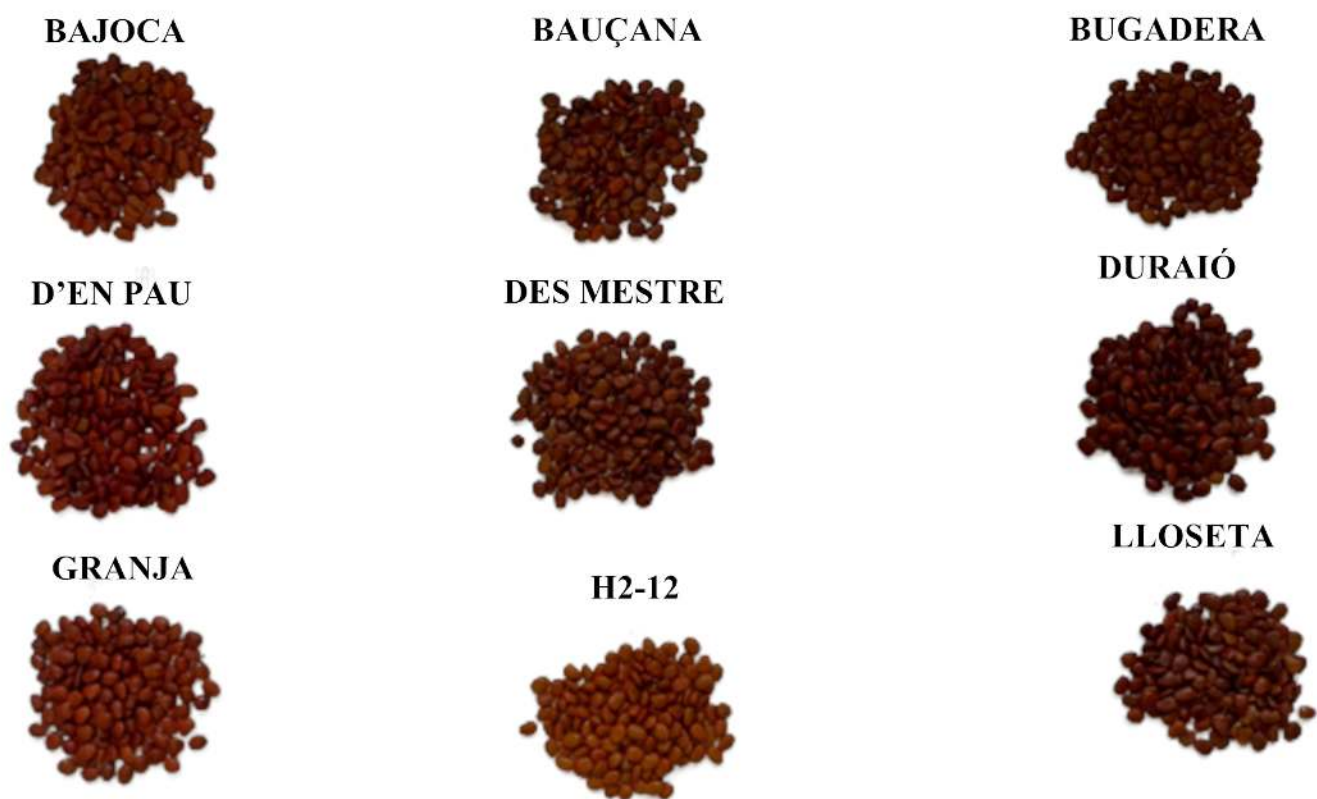


Figure 7

Carob seeds from the cultivars studied.

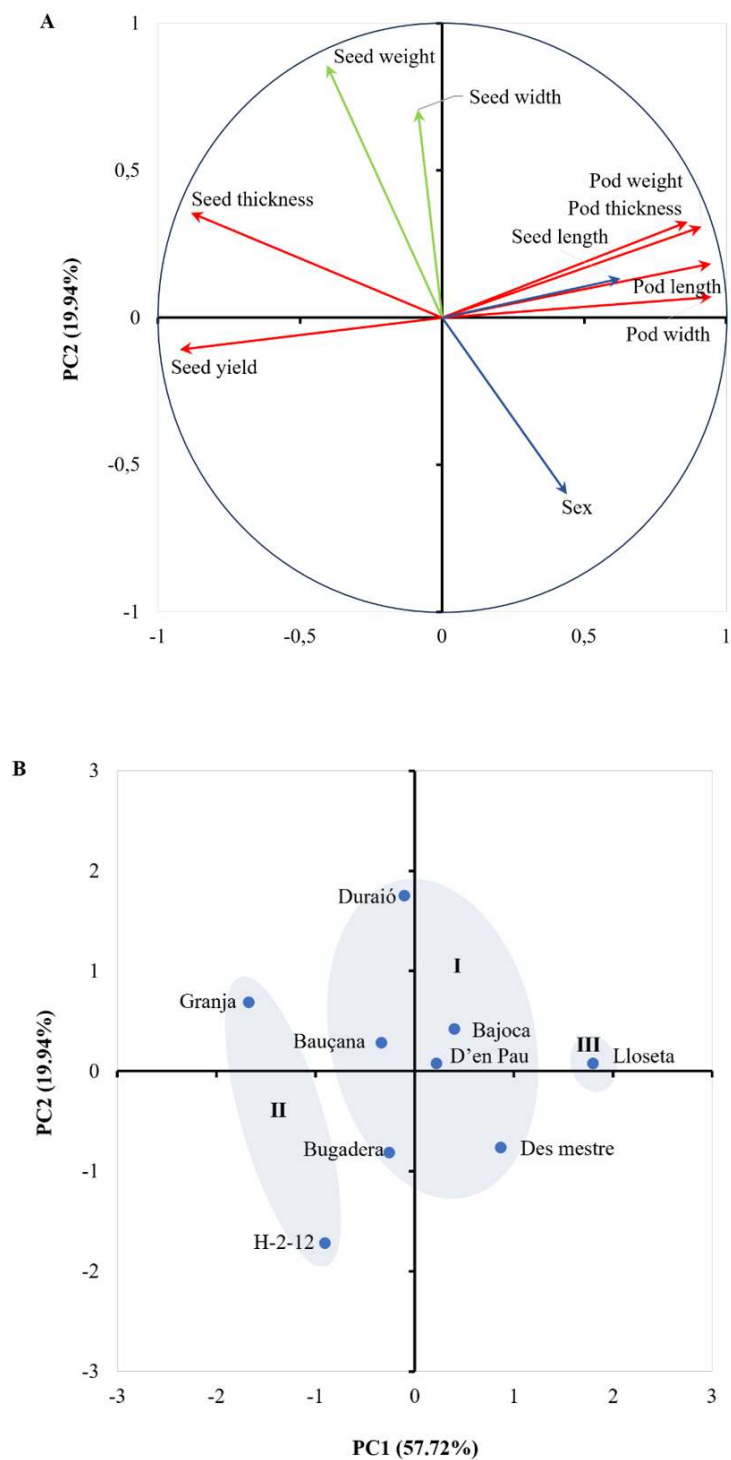


Figure 8

Principal component analysis (PCA) without rotation for the 9 cultivars based on physical parameters. A. Biplot for the relationships between parameters for component PC1 and PC2. B. Biplot representing 9 cultivars along the axes PC1 and PC2

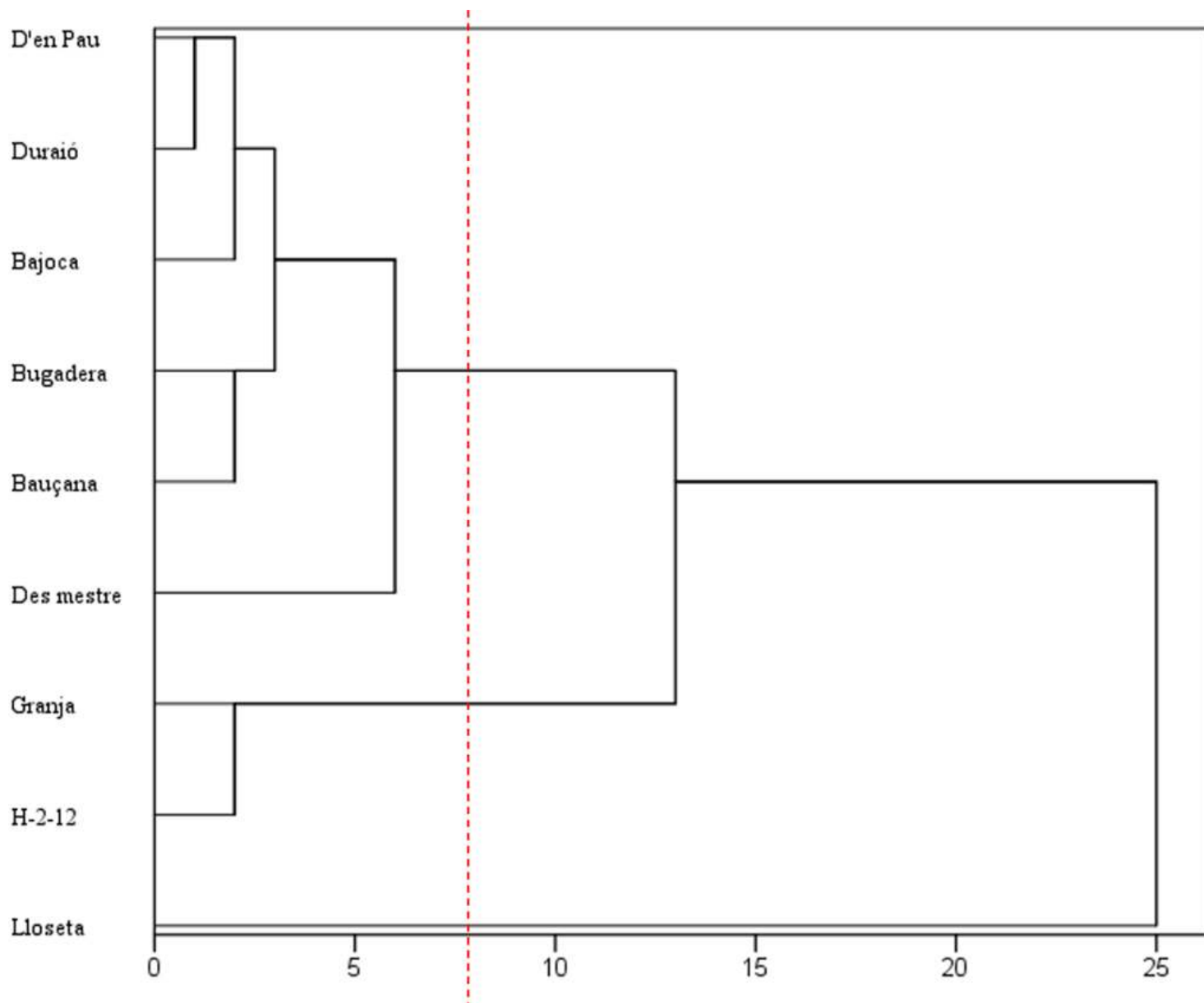


Figure 9

Dendrogram for physical parameters obtained by the hierarchical cluster analysis (HCA).