

## Article

# Evaluation of the Morphological and Physicochemical Diversity of Carob (*Ceratonia siliqua*, Fabaceae) Germplasm from Algeria

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**Abstract:** Despite the economic and ecological importance of the carob tree, few studies are available on the morphological characterization and chemical composition of carob in Algeria. This has resulted in the lack of selection of commercial cultivars of high seed and sugar content. The present study aims to assess the diversity of 11 wild and 1 cultivated carob populations in various regions of Algeria, characterized by diverse geographical and climatic conditions. The final objective is to assess the diversity of neglected carob plants that exhibit superior fruit quality traits and/or are well adapted to different pedoclimatic conditions. The current study focuses on analyzing pod and seed diversity by investigating 12 morphological and 5 physicochemical traits. The analysis of variance revealed significant differences between populations ( $p < 0.001$ ) for all traits studied, indicating high morphological and chemical variability among these carob populations. Principal component analysis (PCA) was employed to study the relationship between these traits and the geographical origin. High seed yields characterized wild populations, whereas cultivated populations were rich in sugar. Based on morphological traits of pods, seeds and chemical compositions, a hierarchical clustering grouped the carob populations into four groups. The findings of this study represent a significant advancement towards establishing effective cultivar breeding programs in Algeria. They demonstrate that the germplasm examined in this research exhibits an optimal level of morphological and chemical diversity, which is instrumental in the identification of specific traits of both commercial and environmental significance.

**Keywords:** carob tree; Algeria; diversity; morphological characterization; germplasm



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## 1. Introduction

Carob (*Ceratonia siliqua* L.) is a diploid ( $2n = 24$ ) evergreen perennial tree belonging to the Fabaceae family, subfamily Caesalpinioideae [1]. This species is dioecious but may occasionally exhibit hermaphroditic or monoecious conditions. Carob originates from the pre-Mediterranean tropical area and has been dispersed throughout the world's Mediterranean-climate regions [2]. The carob tree can thrive in natural and highly intensive cultivated environments [3]. This plant has a broad, semispherical corona, a sturdy trunk covered in rough brown crust, and strong branches. It can reach a height of 8 to 17 m [4]. It is characterized by its high adaptive potential at various altitudes and in humid, sub-humid, semi-arid, and arid climates [5].

In Mediterranean areas, various components of the carob plant (seeds, pods, leaves, flowers, wood, roots, and bark) have historically held significant value. They are used not only for food production but also in traditional medicinal practices to address various

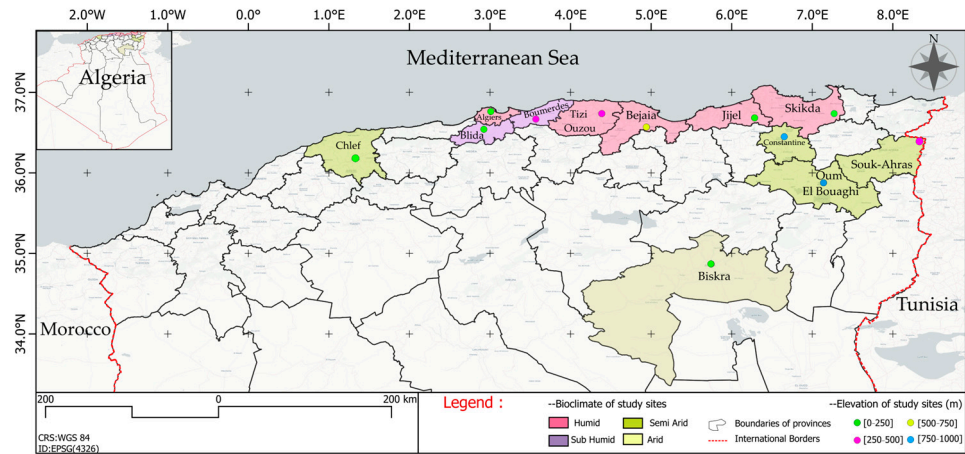
health issues, including gastrointestinal disorders, hypertension, and diabetes [6]. The unit of weight for gemstones, known as the carat, is thought to have originated from the uniform weight of carob seeds [7].

Currently, carob tree products have various applications in different industrial sectors, such as food and beverage, pharmaceuticals, cosmetics, animal feed, and agroforestry systems, serving as a significant economic resource [8]. The richness in sugar content of carob pods [9–12], along with their high fiber content [13,14], and the presence of secondary metabolites such as polyphenols, flavonoids, and tannins, largely account for this high economic and industrial potential [15–17]. Moreover, carob pods contain calcium, phosphate, and potassium [9,18], making them an alternative source of minerals. Furthermore, these seeds are rich in galactomannan, a food additive (E410) [19] widely used by the food industry as a stabilizer and thickener [20]. To date, the main products made from seedless pods are flour and syrup, which can be used as food substitutes for chocolate or cocoa [21]. Additionally, it offers valuable environmental and ecological benefits. Its crucial role in halting soil degradation and erosion positively impacts the mitigation of desertification [22]. The great variability within carob accessions has been assessed through morphological [1,5,23–25], physicochemical [10,11], and molecular analysis [20,26–31]. According to Mahdad and Gaouar [2], world carob production has declined significantly over the past seven decades, dropping by 72% from 650,000 to 183,915 tonnes. The most significant loss has been in Spain, where production has fallen from 420,000 to 48,756 tonnes, representing a loss of 88%. In Algeria, carob production was reduced by 86% from 24,000 to 3219 tonnes [2]. This significant drop in production is mainly due to a reduction in the total harvested area, the lack of reforestation programs and the creation of new plantations. Carob tree farming in the European Union has become unprofitable due to the introduction of less expensive locust bean gum alternatives, for instance, guar gum, xanthan gum, and tara gum, which are also used as thickeners, polymers for food packaging, and binding agents [32]. In Algeria, the carob tree has recently been registered by the Ministry of Agriculture as a new field crop. It has been added to the species list used to contrast desertification in the Green Dam project. The Algerian carob variety catalog has not been updated to date [5], and there is a need to enhance and identify new carob accessions that can meet the needs of the carob production sector. The objective of this study is to characterize twelve populations of *Ceratonia siliqua* in Algeria from both a morphological and physicochemical perspective. The aim is to identify the most suitable accessions for different climatic areas. The focus is on uncharacterized wild carob populations to showcase the potential of Algerian carob germplasm. Through detailed analyses of pods and seeds, we aim to identify and characterize the most valuable carob population with optimal traits for industrial applications. These populations will then be selected to meet the Algerian Ministry of Agriculture requirements and the international market.

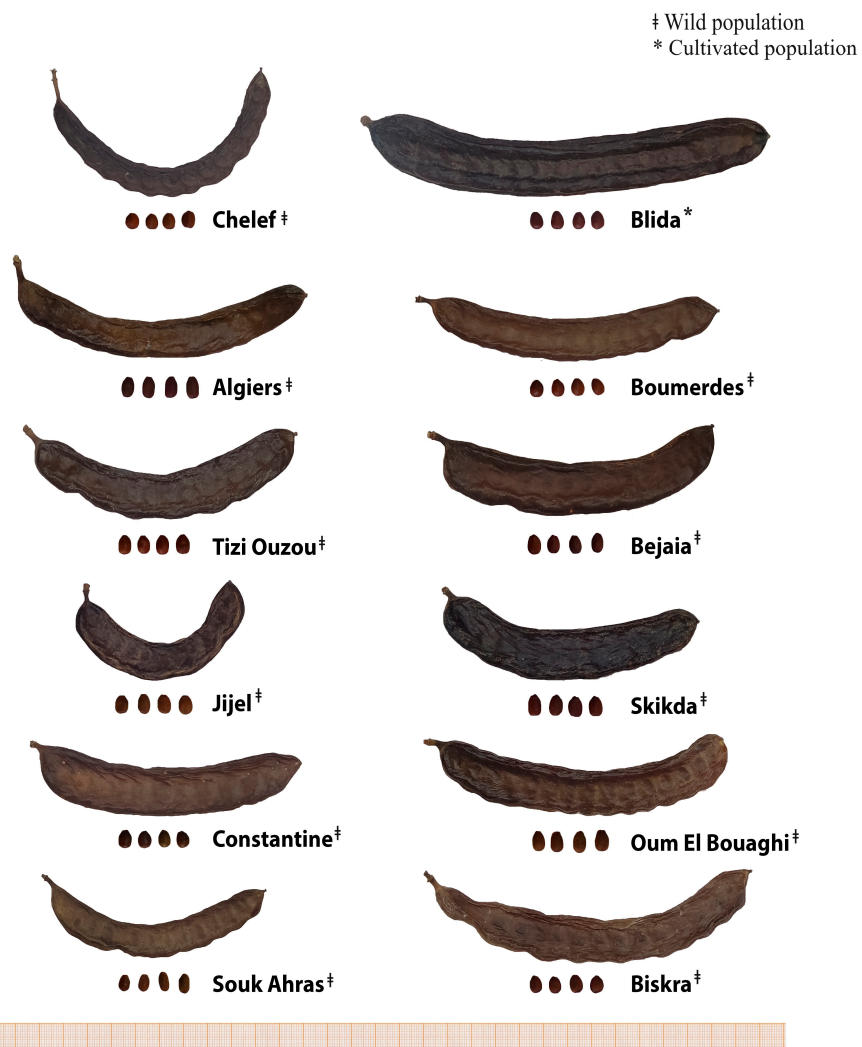
## 2. Materials and Methods

### 2.1. Plant Material

A total of twelve populations of *Ceratonia siliqua* were sampled at twelve geographical sites across Algeria, characterized by different altitudes and bioclimates (arid, semi-arid, subhumid, and humid) according to Emberger [33] (Figure 1). Out of these 12 populations, 11 are considered wild and have never been characterized before (Chlef, Algiers, Boumerdes, Tizi Ouzou, Bejaia, Jijel, Skikda, Constantine, Oum El Bouaghi, Souk Ahras, and Biskra), while only 1 (Blida, also known as Tlemseni by the inhabitants of this region) is a cultivated variety (Figure 2). The geographical coordinates of the 12 carob tree populations for each ecoregion are presented in Table 1.



**Figure 1.** Geographical distribution of 12 populations of *Ceratonia siliqua* from Algeria on several bioclimatic stages [34].



**Figure 2.** Pods and seeds of 12 populations of *Ceratonia siliqua* from Algeria.

**Table 1.** Geographical coordinates and bioclimatic conditions of 12 populations of *Ceratonia siliqua* from Algeria.

Ecoregions	Population	Latitude	Longitude	Altitude (m)	Bioclimate
Chlef	P1	36°09'52" N	1°20'10" E	132	Semi Arid
Blida	P2	36°31'51" N	2°55'46" E	112	Sub Humid
Algiers	P3	36°45'09" N	2°59'43" E	245	Humid
Boumerdes	P4	36°39'27" N	3°32'02" E	392	Sub Humid
Tizi Ouzou	P5	36°45'32" N	4°22'14" E	356	Humid
Bejaia	P6	36°31'47" N	4°57'51" E	515	Humid
Jijel	P7	36°40'12" N	6°16'21" E	73	Humid
Skikda	P8	36°43'22" N	7°16'28" E	45	Humid
Constantine	P9	36°23'45" N	6°38'59" E	901	Semi Arid
Oum El Bouaghi	P10	35°52'01" N	7°08'18" E	900	Semi Arid
Souk Ahras	P11	36°24'31" N	8°21'51" E	255	Semi Arid
Biskra	P12	34°50'38" N	5°44'03" E	109	Arid

## 2.2. Sampling Method

The pods were harvested in August 2021. Ten trees were randomly selected for each population, and thirty pods were randomly taken from each tree's harvest. After the pods had been measured, three seeds per pod (90 seeds per population) were randomly selected from each tree for each population.

## 2.3. Morphological Analysis of Pod and Seed

Twelve morphological traits for the silique, eight for the pods and four for the seeds were measured to characterize populations of *Ceratonia siliqua* from Algeria (Table 2) using a digital caliper and a digital balance with an accuracy of 0.01 mm and 0.001 g, respectively. The traits measured included pod length (cm), pod width (mm), pod thickness (mm), pod weight (g), pulp weight (g), pulp percentage (%), seed length (mm), seed width (mm), seed thickness (mm), seed weight per pod (g), number of seeds, and seed yield (%). These traits were measured in accordance with the methodology described by Tous et al. [4].

## 2.4. Physicochemical Analysis

Upon measuring the pods, the pulp was subsequently crushed and ground into a powder with particles not exceeding 500 µm in size. All physicochemical analyses were conducted on these samples, encompassing assessments of moisture content, ash content, fiber composition, total sugars, and protein content. The fresh samples were oven-dried at 60 °C for 48 h until a constant mass was obtained before assaying. The moisture content was determined in accordance with the AOAC standard 925.10 by drying the sample to constant weight at 105 °C [35]. The ash content was determined in accordance with AOAC 972.15 by incinerating two grams of dry sample overnight in a controlled muffle furnace preheated to 600 °C [36]. Protein content was calculated using a nitrogen-to-protein conversion factor of 6.25 per gram of sample [37], in accordance with the procedures prescribed by Kirk [38] and the micro-Kjeldahl method. The fiber content of carob powder was determined using the Weende AOAC 978.10 method [39], which involves etching two grams of the sample with 0.128 M sulfuric acid, followed by 0.313 M NaOH. Total sugars were analyzed using the sulfuric phenol method and determined by colorimetry at 485 nm [40].

## 2.5. Statistical Analysis

The results obtained in this study were subjected to a one-factor analysis of variance ANOVA after testing for normality (Shapiro–Wilk test  $p$ -value > 0.05) and homogeneity of variance (Bartlett's test  $p$ -value < 0.001). The statistical significance of the differences between the population means was evaluated using Duncan's post hoc tests at a level of significance  $\alpha = 0.05$ , employing the SPSS version 26 software.

Multivariate analyses were conducted for all the quantitative parameters using RStudio software (v. 4.2.1), and the principal component analysis (PCA) was carried out using R packages “FactoMineR” and “factoextra” package within the R environment [41,42]. Hierarchical clustering (HC) was performed using the “ward.D2” method and Euclidean distance as the dissimilarity measure using the *hclust* function and *corrplot* [43] for correlation analyses using the Pearson method.

### 3. Results

#### 3.1. Morphological and Physicochemical Diversity

The results of morphological analyses conducted on various carob populations are summarized in Table 2.

The carob pods from the Blida population were the longest, widest, thickest, and heaviest and had the highest pulp yield compared to all the other populations examined. The measurements of the pods were found to be as follows: length of  $19.03 \pm 0.18$  cm, width of  $25.63 \pm 0.12$  mm, thickness of  $11.41 \pm 0.07$  mm, weight of  $33.34 \pm 0.52$  g, and pulp yield of  $91.55 \pm 0.10\%$ .

On the other hand, pods from the Souk Ahres population are the shortest, narrowest, thinnest, and lightest; they measure  $12.32 \pm 0.08$  cm in length,  $17.52 \pm 0.08$  mm in width,  $5.29 \pm 0.04$  mm in thickness, and  $6.77 \pm 0.11$  g in weight.

Furthermore, carob pods from the Biskra population exhibit similar characteristics to those from the Blida population, with the pods measuring  $16.66 \pm 0.11$  cm long. Carob pods from the Skikda population also share similar characteristics with pods from the Blida region, including the widest pods and seeds, as well as the highest pulp yield, with mean values of  $23.58 \pm 0.13$  mm,  $7.11 \pm 0.03$  mm, and  $88.49 \pm 0.23\%$ , respectively. Chlef, Biskra, Blida, and Algiers populations exhibited the highest average number of seeds per pod at  $13.27 \pm 0.16$ ,  $13.09 \pm 0.18$  and  $13.05 \pm 0.16$ , respectively. The Jijel, Chelef, and Souk Ahres populations showed high average seed yields, with mean values of  $25.95 \pm 0.33\%$ ,  $23.89 \pm 0.43\%$ , and  $21.27 \pm 0.45\%$ , respectively.

Regarding seed traits, the Skikda and Constantine populations exhibited the highest values for length, with a mean value of  $9.98 \pm 0.03$  mm and  $9.32 \pm 0.03$  mm, respectively. In contrast, the widest, thickest, and heaviest seeds were found in the Blida population, with a mean value of  $7.35 \pm 0.02$  mm,  $4.63 \pm 0.01$  mm, and  $2.77 \pm 0.04$  g, respectively. The Blida population exhibited the greatest seed size, with a mean length of  $7.35 \pm 0.02$  mm, width of  $4.63 \pm 0.01$  mm, and weight of  $2.77 \pm 0.04$  g. Seeds from the Chelef population exhibited similar characteristics to those of the Blida population, with a thickness of  $4.19 \pm 0.02$  mm and a weight of  $2.37 \pm 0.03$  g. Concerning the physicochemical analysis results (Table 3), the Boumerdes, Tizi Ouzou, and Bejaia populations exhibited the highest moisture content, with values of  $12.70\% \pm 0.13$ ,  $12.16\% \pm 0.08$ , and  $12.26\% \pm 0.11$ , respectively. However, the Souk Ahres population pods showed the lowest moisture content,  $8.29 \pm 0.09\%$ . The Algiers and Chelef populations showed the highest levels ( $3.22 \pm 0.09\%$  and  $3.66 \pm 0.20\%$ , respectively). However, the Tizi Ouzou population had the lowest ash content, with a value of  $1.89\%$ . Protein levels were also found to be higher in the populations of Skikda, Biskra, Alger, Chlef, and Oum El Bouaghi with mean values of  $5.54 \pm 0.15\%$ ;  $5.34 \pm 0.42\%$ ;  $4.97 \pm 0.21\%$ ;  $4.73 \pm 0.41\%$ , and  $4.54 \pm 0.16\%$ , respectively. The fiber content of the samples ranged from  $5.69 \pm 0.33\%$  to  $10.86 \pm 0.48\%$ . The populations with the highest rates were Jijel, Boumerdes, and Souk Ahras, with mean values of  $10.86 \pm 0.48\%$ ,  $8.23 \pm 0.85\%$ , and  $7.94 \pm 0.40\%$ , respectively. The carob populations from the Algiers, Bejaia, and Blida ecoregions exhibited higher levels of sugar content, with values of  $47.08 \pm 1.65\%$ ,  $47.50 \pm 1.76\%$ , and  $55.51 \pm 2.31\%$ , respectively. However, the values reported for the Skikda and Constantine populations are lower, at  $40.33 \pm 0.40\%$  and  $41.25 \pm 0.20\%$ , respectively.

**Table 2.** Morphological characterization of pods and seeds of 12 populations of *Ceratonia siliqua* from Algeria.

Population	Pods							Seeds				
	Length (cm)	Width (mm)	Thickness (mm)	Weight (g)	Pulp Weight (g)	Number of Seeds	Seed Yield (%)	Pulp Percentage (%)	Length (mm)	Width (mm)	Thickness (mm)	Weight per Pod (g)
P1	15.63 ± 0.11 <sup>cd</sup>	18.59 ± 0.21 <sup>f</sup>	5.97 ± 0.08 <sup>f</sup>	10.38 ± 0.20 <sup>g</sup>	8.01 ± 0.20 <sup>g</sup>	<b>13.60 ± 0.15<sup>a</sup></b>	23.89 ± 0.43 <sup>b</sup>	76.11 ± 0.43 <sup>i</sup>	8.77 ± 0.03 <sup>f</sup>	6.81 ± 0.03 <sup>e</sup>	4.19 ± 0.02 <sup>b</sup>	2.37 ± 0.03 <sup>b</sup>
P2	<b>19.03 ± 0.18<sup>a</sup></b>	<b>25.63 ± 0.12<sup>a</sup></b>	<b>11.41 ± 0.07<sup>a</sup></b>	<b>33.34 ± 0.52<sup>a</sup></b>	<b>30.57 ± 0.49<sup>a</sup></b>	13.09 ± 0.18 <sup>b</sup>	<b>8.45 ± 0.10<sup>j</sup></b>	<b>91.55 ± 0.10<sup>a</sup></b>	8.91 ± 0.02 <sup>e</sup>	<b>7.35 ± 0.02<sup>a</sup></b>	<b>4.63 ± 0.01<sup>a</sup></b>	<b>2.77 ± 0.04<sup>a</sup></b>
P3	15.33 ± 0.17 <sup>d</sup>	20.94 ± 0.13 <sup>e</sup>	8.47 ± 0.11 <sup>b</sup>	14.52 ± 0.28 <sup>c</sup>	12.24 ± 0.25 <sup>c</sup>	13.05 ± 0.16 <sup>b</sup>	16.31 ± 0.25 <sup>f</sup>	83.69 ± 0.25 <sup>e</sup>	9.20 ± 0.04 <sup>c</sup>	6.66 ± 0.03 <sup>fg</sup>	4.08 ± 0.03 <sup>c</sup>	2.28 ± 0.04 <sup>c</sup>
P4	14.23 ± 0.10 <sup>f</sup>	20.68 ± 0.13 <sup>e</sup>	7.64 ± 0.09 <sup>d</sup>	13.21 ± 0.20 <sup>e</sup>	11.05 ± 0.20 <sup>d</sup>	12.41 ± 0.13 <sup>cd</sup>	17.10 ± 0.25 <sup>e</sup>	82.90 ± 0.25 <sup>f</sup>	9.29 ± 0.04 <sup>bc</sup>	6.60 ± 0.03 <sup>g</sup>	4.04 ± 0.03 <sup>c</sup>	2.17 ± 0.02 <sup>d</sup>
P5	13.38 ± 0.12 <sup>g</sup>	22.70 ± 0.13 <sup>c</sup>	7.74 ± 0.06 <sup>d</sup>	13.64 ± 0.19 <sup>de</sup>	11.95 ± 0.19 <sup>c</sup>	9.64 ± 0.13 <sup>g</sup>	12.79 ± 0.18 <sup>h</sup>	87.21 ± 0.18 <sup>c</sup>	8.93 ± 0.04 <sup>e</sup>	7.01 ± 0.02 <sup>c</sup>	4.09 ± 0.02 <sup>c</sup>	1.69 ± 0.02 <sup>f</sup>
P6	14.82 ± 0.07 <sup>e</sup>	22.13 ± 0.16 <sup>d</sup>	7.63 ± 0.07 <sup>d</sup>	14.23 ± 0.22 <sup>cd</sup>	12.06 ± 0.21 <sup>c</sup>	12.04 ± 0.11 <sup>d</sup>	16.24 ± 0.27 <sup>f</sup>	83.76 ± 0.27 <sup>e</sup>	9.08 ± 0.02 <sup>d</sup>	7.01 ± 0.02 <sup>c</sup>	3.95 ± 0.02 <sup>d</sup>	2.17 ± 0.02 <sup>d</sup>
P7	<b>11.75 ± 0.11<sup>i</sup></b>	17.95 ± 0.10 <sup>g</sup>	5.72 ± 0.06 <sup>g</sup>	<b>6.34 ± 0.10<sup>h</sup></b>	<b>4.74 ± 0.08<sup>h</sup></b>	10.35 ± 0.14 <sup>f</sup>	<b>25.95 ± 0.33<sup>a</sup></b>	<b>74.05 ± 0.33<sup>j</sup></b>	8.99 ± 0.04 <sup>de</sup>	6.76 ± 0.03 <sup>e</sup>	3.77 ± 0.02 <sup>e</sup>	1.60 ± 0.02 <sup>g</sup>
P8	13.65 ± 0.11 <sup>g</sup>	23.58 ± 0.13 <sup>b</sup>	8.17 ± 0.08 <sup>c</sup>	15.77 ± 0.26 <sup>b</sup>	14.06 ± 0.25 <sup>b</sup>	<b>9.35 ± 0.14<sup>g</sup></b>	11.51 ± 0.23 <sup>i</sup>	88.49 ± 0.23 <sup>b</sup>	<b>9.98 ± 0.03<sup>a</sup></b>	7.11 ± 0.03 <sup>b</sup>	3.70 ± 0.02 <sup>fg</sup>	1.72 ± 0.03 <sup>f</sup>
P9	15.25 ± 0.12 <sup>d</sup>	22.00 ± 0.14 <sup>d</sup>	5.63 ± 0.05 <sup>g</sup>	11.72 ± 0.18 <sup>f</sup>	10.04 ± 0.17 <sup>e</sup>	10.17 ± 0.12 <sup>f</sup>	14.81 ± 0.20 <sup>g</sup>	85.19 ± 0.20 <sup>d</sup>	9.32 ± 0.03 <sup>b</sup>	7.06 ± 0.03 <sup>bc</sup>	3.64 ± 0.02 <sup>g</sup>	1.68 ± 0.02 <sup>fg</sup>
P10	15.77 ± 0.15 <sup>c</sup>	20.99 ± 0.12 <sup>e</sup>	6.63 ± 0.07 <sup>e</sup>	13.59 ± 0.22 <sup>de</sup>	11.58 ± 0.22 <sup>cd</sup>	12.53 ± 0.14 <sup>c</sup>	15.69 ± 0.25 <sup>f</sup>	84.31 ± 0.25 <sup>e</sup>	9.05 ± 0.04 <sup>d</sup>	6.92 ± 0.03 <sup>d</sup>	3.65 ± 0.02 <sup>g</sup>	2.01 ± 0.03 <sup>e</sup>
P11	12.32 ± 0.08 <sup>h</sup>	<b>17.52 ± 0.08<sup>h</sup></b>	<b>5.29 ± 0.04<sup>h</sup></b>	6.77 ± 0.11 <sup>h</sup>	5.37 ± 0.11 <sup>h</sup>	11.09 ± 0.15 <sup>e</sup>	21.27 ± 0.45 <sup>c</sup>	78.73 ± 0.45 <sup>h</sup>	9.23 ± 0.05 <sup>bc</sup>	<b>6.21 ± 0.03<sup>h</sup></b>	<b>3.44 ± 0.02<sup>h</sup></b>	<b>1.40 ± 0.02<sup>h</sup></b>
P12	16.66 ± 0.11 <sup>b</sup>	18.61 ± 0.12 <sup>f</sup>	6.71 ± 0.06 <sup>e</sup>	11.24 ± 0.17 <sup>f</sup>	9.23 ± 0.16 <sup>f</sup>	13.27 ± 0.16 <sup>ab</sup>	18.67 ± 0.27 <sup>d</sup>	81.33 ± 0.27 <sup>g</sup>	<b>8.62 ± 0.03<sup>g</sup></b>	6.74 ± 0.03 <sup>ef</sup>	3.76 ± 0.02 <sup>ef</sup>	2.01 ± 0.02 <sup>e</sup>
Mean	14.81 ± 0.05	21.13 ± 0.06	7.29 ± 0.03	13.73 ± 0.13	11.75 ± 0.12	11.60 ± 0.05	16.54 ± 0.11	83.46 ± 0.11	9.13 ± 0.01	6.88 ± 0.01	3.90 ± 0.01	1.98 ± 0.01
CV%	18.27	14.54	26.19	51.51	57.66	23.57	37.85	7.50	7.35	7.09	11.60	28.40
Significance	***	***	***	***	***	***	***	***	***	***	***	***
<i>p</i> -value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
F	218.20	289.24	431.41	671.63	680.41	115.26	328.42	328.42	98.65	89.81	166.76	160.83

Data are expressed as means ± standard error. Different letters indicate significant differences among populations according to Duncan post hoc test ( $p < 0.05$ ). \*\*\* Statistically significant differences between populations at  $p < 0.001$ .

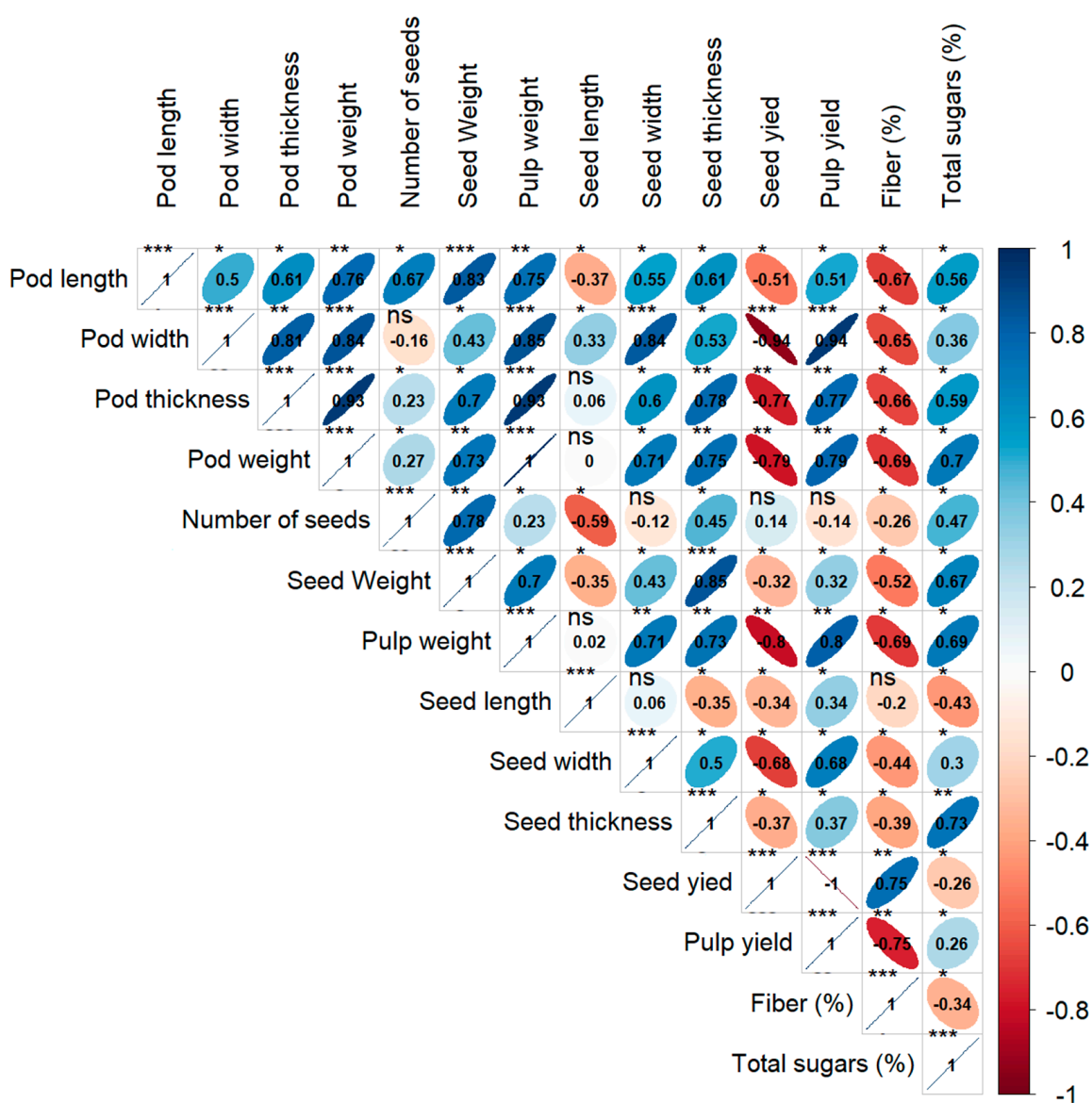
**Table 3.** Results of physicochemical measurements of pods of 12 populations of *Ceratonia siliqua* from Algeria.

Population	Moisture (%)	Ash (%)	Protein (%)	Fibre (%)	Total Sugars (%)
P1	9.89 ± 0.11 g	3.22 ± 0.09 b	4.73 ± 0.41 bc	7.41 ± 0.63 bcd	47.08 ± 1.65 bc
P2	11.74 ± 0.06 bcd	2.28 ± 0.03 ef	3.03 ± 0.16 de	<b>5.69 ± 0.33 d</b>	<b>55.51 ± 2.31 a</b>
P3	11.00 ± 0.19 ef	<b>3.66 ± 0.20 a</b>	4.97 ± 0.21 abc	6.89 ± 0.41 bcd	42.23 ± 0.36 de
P4	<b>12.70 ± 0.13 a</b>	2.07 ± 0.04 efg	3.31 ± 0.19 d	8.23 ± 0.85 b	44.75 ± 0.79 bcde
P5	12.16 ± 0.08 ab	<b>1.89 ± 0.07 g</b>	3.21 ± 0.13 de	7.67 ± 0.46 bc	44.71 ± 1.15 bcde
P6	12.26 ± 0.11 ab	2.02 ± 0.10 fg	<b>2.52 ± 0.20 e</b>	7.37 ± 0.52 bcd	47.50 ± 1.76 b
P7	11.70 ± 0.08 bcd	2.79 ± 0.07 c	3.13 ± 0.18 de	<b>10.86 ± 0.48 a</b>	42.70 ± 0.51 cde
P8	11.51 ± 0.27 cde	2.09 ± 0.05 efg	<b>5.54 ± 0.15 a</b>	6.03 ± 0.47 cd	<b>40.33 ± 0.40 e</b>
P9	11.33 ± 0.16 def	2.21 ± 0.05 efg	3.37 ± 0.18 d	7.86 ± 0.44 bc	41.25 ± 0.20 e
P10	12.00 ± 0.11 bc	2.68 ± 0.04 cd	4.54 ± 0.16 c	7.73 ± 0.57 bc	43.61 ± 2.14 bcde
P11	<b>8.29 ± 0.09 h</b>	2.41 ± 0.03 de	3.10 ± 0.07 de	7.94 ± 0.40 b	45.94 ± 2.65 bcd
P12	10.90 ± 0.15 f	2.27 ± 0.07 ef	5.34 ± 0.42 ab	7.17 ± 0.56 bcd	43.16 ± 1.55 bcde
Mean	11.46 ± 0.07	2.49 ± 0.05	3.99 ± 0.11	7.61 ± 0.19	44.41 ± 0.64
CV%	10.23	29.18	31.91	26.10	12.55
Significance	***	***	***	***	***
p-value	<0.001	<0.001	<0.001	<0.001	<0.001
F	27.26	31.08	25.80	5.92	7.95

Data are expressed as means ± standard error. Different letters indicate significant differences among populations according to Duncan post hoc test ( $p < 0.05$ ). \*\*\* Statistically significant differences between populations at  $p < 0.001$ .

### 3.2. Correlation Analysis

The results of the correlations among all morphological traits, fiber content and total sugars are presented in Figure 3. A strong negative correlation was found between pulp yield and seed yield ( $r = -1$ ). The correlation between pod weight and the variables pod length, width and thickness is highly positive, with correlation coefficients of  $r = 0.76$ ,  $0.84$ , and  $0.93$ , respectively. Moreover, seed yield is negatively correlated with the three morphological traits of the pod: length, width, and thickness. The correlation coefficients are  $r = -0.51$ ,  $-0.94$ , and  $-0.77$ , respectively. A negative correlation was observed between seed yield and pod weight ( $r = -0.79$ ). In addition, the total pulp sugar content was positively correlated with pod weight, pulp weight, pod thickness, and pod length, with correlation coefficients of  $r = 0.70$ ,  $0.67$ ,  $0.59$ , and  $0.56$ , respectively. Furthermore, a robust correlation was observed between seed weight and pod length ( $r = 0.83$ ). Finally, all pod traits show a negative correlation with fiber content, except for seed yield, which has a positive correlation ( $r = 0.75$ ).

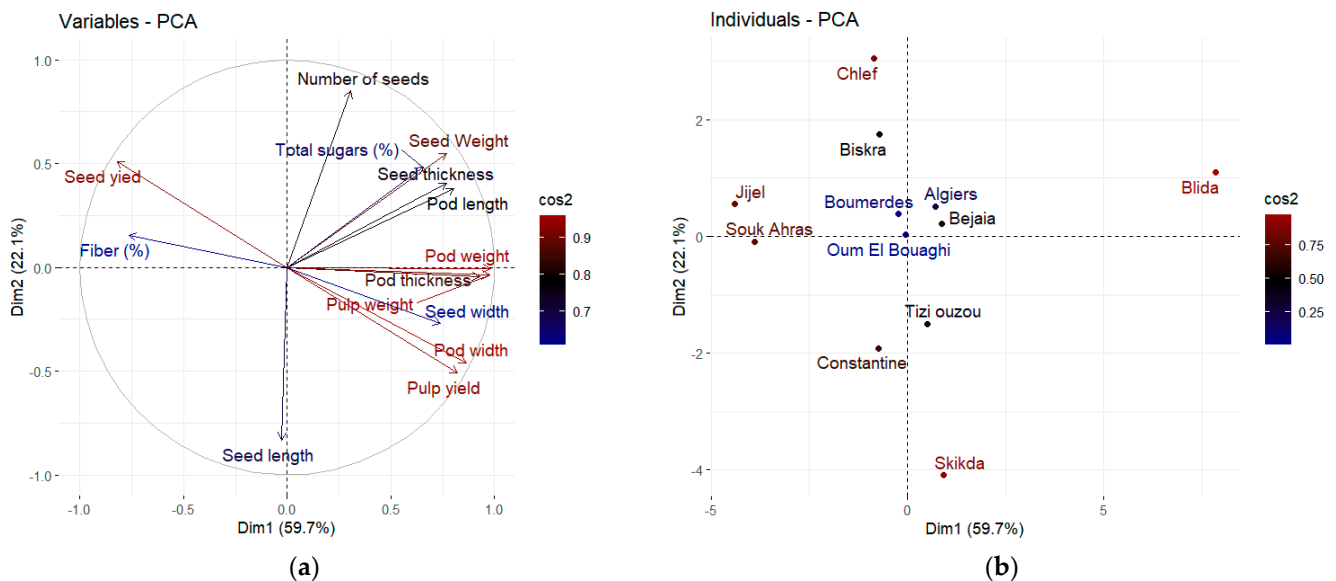


**Figure 3.** Pearson correlation plot depicting the morphological and chemical traits of the 12 populations of *Ceratonia siliqua* from Algeria (\*\*\*) statistical significance at  $p < 0.001$ ; \*\* statistical significance at  $p < 0.01$ ; \* statistical significance at  $p < 0.05$ ; ns not statistical significance).



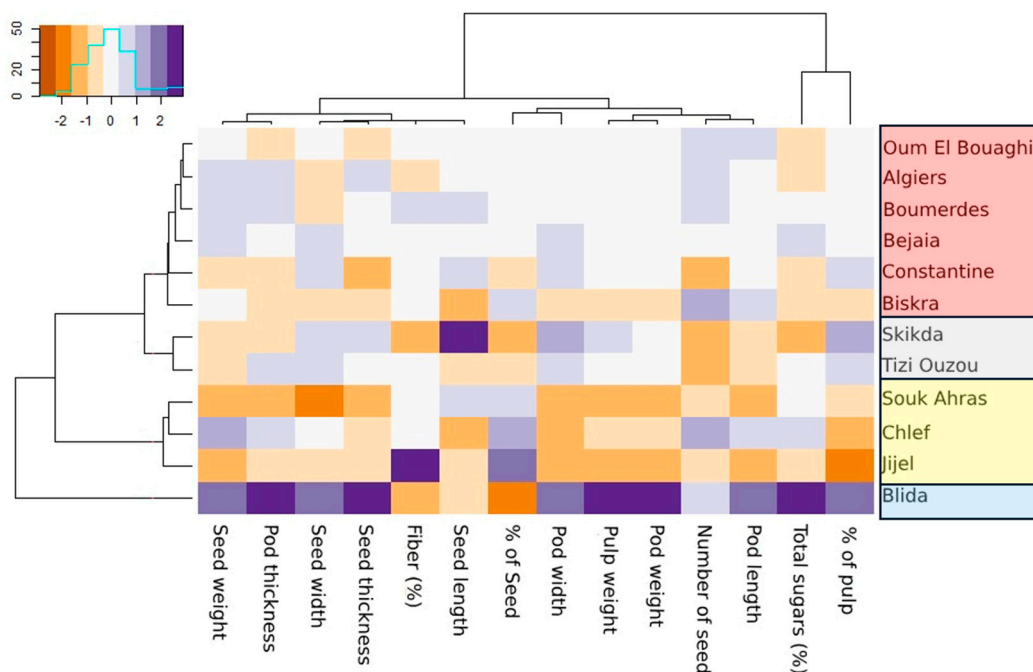
### 3.3. Multivariate Analyses

Figure 4 depicts the principal component analysis (PCA) of all morphological and chemical traits. The first two principal components, with eigenvalues greater than one (8.36% and 3.09%, respectively), explain 81.8% of the total variance, with the first axis explaining 59.7% and the second axis 22.1%. The first axis is primarily influenced by most variables, except for the number of seeds per pod and seed length, which are represented in the second axis. According to Figure 4, the populations can be divided into three groups, with an additional ungrouped population that stands out due to its high thickness, width, length, pulp weight, and sugar content relative to the others.



**Figure 4.** Principal component analysis (PCA) of 12 populations of *Ceratonia siliqua* from Algeria based on morphological and chemical traits. In the left panel, the variables plot (a) and, in the right panel, the individuals plot (b).

The analysis was completed by a hierarchical clustering analysis based on morphological and chemical results (Figure 5), which was conducted to investigate similarities between the 12 populations. Four clusters were identified: three groups and one ungrouped population cluster. This confirmed the results of the PCA. The first group comprises most of the populations, namely Oum El Bouaghi, Algiers, Boumerdes, Bejaia, Constantine, and Biskra, distributed across different bioclimatic areas. The average measurements for all parameters are compared with those of the other groups in Figure 5. The second group comprises the populations of Skikda and Tizi Ouzou, which are situated within the humid bioclimatic zone and exhibit a low number of seeds compared to the other groups. The third group includes populations from Souk Ahras, Chlef, and Jijel and is characterized by very short, narrow, light pods with a high seed yield if compared to the other groups. Finally, the ungrouped population is that of Blida, which belongs to the subhumid bioclimatic zone and is characterized by the largest pods with a high pulp yield, low seed yield, low fiber content, and high total sugar content.



**Figure 5.** Heatmap and hierarchical clustering of 12 populations of *Ceratonia siliqua* from Algeria by morphological and chemical characteristics.

#### 4. Discussion

The carob tree, which has always been considered neglected and of poor commercial value, represents a potentially key agricultural and industrial resource for many countries of the Mediterranean basin [44]. Despite this, research has not focused much on its characterization, and varietal catalogs do not comprehensively represent the biodiversity of this tree crop, particularly in Algeria [11]. The Blida carob population is among the most widely cultivated and characterized Algerian carob populations. The findings of the present study on the Blida carob population confirmed morphometrics characteristics that have been previously reported. Several studies have shown that longer, wider, and thicker pods with higher pulp weight lead to lower seed yields [5,23,24,45,46]. Furthermore, the results obtained from the analysis of the wild-type carob population are consistent with the findings of researchers who have shown that wild-type carob pods produce higher seed yields compared to cultivated varieties [11,47].

Also notable is the result of the mean seed yield that the Algerian carob populations of this study displayed (16.54%), which is higher compared to other Mediterranean countries, such as Spain with 12.11% [48], Portugal with 13.11% [1], Lebanon with 13.13% [46], and Turkey with 8.79% [49]. This morphological variability observed in the present study is dependent on the plant genotype, as evidenced by studies conducted in various countries within the Mediterranean region [5,14,50].

The moisture content results are similar to those found in other Mediterranean countries, ranging from 6% to 16.7%, such as Spain [51], Italy [52], and Morocco [53]. Ash contents varied from 2% to 6% for eleven distinct populations, with the Tizi Ouzou population having less than 2% ash content. Protein content ranged from 3% to 7.6%, except for the Bejaia population, aligning with the work of several authors [9,10,45,52–55]. Furthermore, the high protein content of the Biskra population might be attributed to the saline soil in this area. The fiber content results are consistent with other works, ranging from 4.20% to 11.85% [10,11,45,55–57]. The presented data for sugar content are in line with the findings of Lipumbu et al. [58] and Goulas et al. [13]. The chemical composition of carob can vary significantly and is influenced by various factors, including the plant's genotype, the geographical location of cultivation, and the specific climatic conditions in that region. Different genotypes can lead to variations in nutrient content, flavor, and

other chemical properties of the carob. The geographical origin is important because soil composition, altitude, and local agricultural practices can all impact the chemical profile of the carob pods. Climatic conditions, such as temperature, rainfall, and humidity, further contribute to these differences, affecting the plant's growth cycle and metabolic processes. A comprehensive study that analyzed carob samples from various Mediterranean countries confirmed this variability in chemical composition. The study revealed that carob plants from different areas have distinct chemical characteristics, emphasizing the significance of environmental and genetic factors. These findings highlight the complexity of carob's chemical composition and stress the need to consider these variables when studying and using carob in food and nutritional applications [11,23,24,44,59–61].

The correlation analysis results of the present study (Figure 3) agreed with those reported by Boublenza et al. [11] and Mahdad et al. [5], who identified a significant negative correlation between pulp yield and seed yield. Furthermore, the results from the populations under study were consistent with those reported by Sidina et al. [24], Haradach et al. [10], Chiami et al. [46], Mahdad et al. [5], and Barceló-Anguiano et al. [62] who indicated that the variable; seed yield is negatively correlated with the three morphological characteristics of the pod: length, width, and thickness. This explains why the pods with the highest seed yield are the shortest, thinnest and narrowest. Furthermore, these results concur with those of Barracosa et al. [1], who indicated that seed yield and pod weight were negatively correlated. In addition, total pulp sugar content is positively correlated with pod weight, pulp weight, pod thickness, and pod length, which agrees with the results of Boublenza et al. [11]. This explains why pods with a high sugar content are fleshy pods. Furthermore, fiber content is negatively correlated with pulp traits, which is in line with the results of Boublenza et al. [11] and explains why pods with a high fiber content are thin pods. In addition, a strong correlation between seed weight and pod length was found; these results agree with those of Boublenza et al. [11] and Bolaric et al. [29].

The PCAs (Figure 4) and the hierarchical clustering analyses (Figure 5) demonstrate that cultivated carob trees are distinguished by a high pod weight, which is positively correlated with pod length, width, and thickness, and are rich in sugar. In contrast, wild-type carob trees exhibit a high seed yield and a high crude fiber content. These findings are consistent with a study conducted by Fadel et al. [63], which compared the fruits of a private plantation and a forest carob tree. The study demonstrated that the carob in the private domain is heavier and wider than in the forest domain.

## 5. Conclusions

The results of the present study investigated the high degree of morphological and chemical variability in local Algerian carob germplasm. This variability is of great importance for selecting cultivars with desirable characteristics for future breeding programs. The results of the multivariate and correlation analyses between all the traits studied enabled the characterization of the 12 Algerian populations into three groups. The first group was represented by the populations of Oum El Bouaghi, Algiers, Boumerdes, Bejaia, Constantine, and Biskra; the second group included those of Skikda and Tizi Ouzou, and the third those of Souk Ahras, Chlef, and Jijel. The latter group included wild-type samples and accessions that exhibited high seed yields, a trait well-suited to the agri-food industry due to its richness in the food additive E410. In addition, a non-grouped cluster composed by Blida was identified. This carob population encompasses cultivated varieties characterized by large pods with a high sugar content. Finally, the results of this study have enabled the identification of poorly characterized wild carob accessions that exhibit high performance for future reforestation programs, with the objective of improving the quality and quantity of Algerian carob.

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