

Original Research Paper

Morpho-geometric identification of leaflets from male, female and hermaphrodite populations of the carob tree (*Ceratonia siliqua* L.) in Algeria

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Abstract

The carob tree is an agro-salvo-pastoral species that has an important socio-economic and ecological role. For many years, the horticultural in Algeria has been in regression, mainly because of anthropic factors. This regression has led to the increase in carob tree prices, which makes this speculation gainful for farmers and agro-industrialists. However, the problem of sexual determinism in carob remains an important difficulty for the creation of orchards and the development of genetic improvement programs for the species. So, the object of this study is to identify and distinguish by a morpho-geometric characterization of the leaflets, the carob trees of male, female and hermaphrodite sex, from 21 different regions of the Algerian north with 781 leaflets characterized through the selection of 16 landmarks. Once recorded in digital format (TPS), the morpho-geometric conformations are aligned and superimposed using the Procrustean superposition technique. Analysis of variance (ANOVA) of the data revealed highly significant differences in shape ($p=0.0001$), and significant centroid size ($p=0.0243$) between the three populations studied. Descriptive analysis of size showed only one significant difference ($p=0.035$) between the size of hermaphrodite and male leaflets. Principal component analysis (PCA) showed inertia of 70.45% between the first three components and revealed three different shapes. The analysis of canonical variants (CA) distinguished these different forms, the female leaflet so much towards an elliptical form with a base contracted in node downwards and with apex characterized by a closed angle. While the shape of the male leaflet is generally oval with a scattered base and with apex characterized by an open angle. The leaflets of hermaphrodites resemble the leaflets of males (oval) but with a flattened base. These results can be used to make a preliminary selection of young carob plants.

Keywords: Carob tree, populations, leaflets, characterization, morpho-geometry, elliptical shape, oval shape.

المخلص

تُعد شجرة الخروب من الأنواع الزراعية-الرعية-الزراعية التي لها دور اجتماعي-اقتصادي وبيئي مهم. منذ سنوات عديدة، شهدت الزراعة البساتينية في الجزائر تراجعاً في هذا النوع من الأشجار بسبب تأثير العوامل البشرية. وقد أدى هذا التراجع إلى ارتفاع أسعار أشجار الخروب مما يجعل هذه المضاربة مربحة للمزارعين والصناعيين الزراعيين. ومع ذلك، لا تزال مشكلة الحتمية الجنسية في الخروب تمثل صعوبة مهمة أمام إنشاء البساتين وتطوير برامج التحسين الوراثي لهذا النوع. لذا، فإن الهدف من هذه الدراسة هو تحديد وتمييز أشجار الخروب من الجنسين الذكر والأنثى والخنثى عن طريق التوصيف المورفولوجي الهندسي للورقات من 21 منطقة مختلفة من الشمال الجزائري مع 781 ورقة تم توصيفها من خلال اختيار 16 علامة مميزة. وبمجرد تسجيلها في شكل رقمي (TPS)، تتم محاذاة التطابقات المورفولوجية الهندسية وتركيبتها باستخدام تقنية التراكب البروكروستي. وكشف تحليل التباين (ANOVA) للبيانات عن وجود اختلافات ذات دلالة إحصائية كبيرة في الشكل ($p=0.0001$)، وحجم مركزي كبير ($p=0.0243$) بين المجموعات الثلاث التي خضعت للدراسة. وأظهر التحليل الوصفي للحجم اختلافًا واحدًا ذا دلالة إحصائية ($p=0.035$) بين حجم الخنثى والذكر. أظهر تحليل المكونات الرئيسية (PCA) قصورًا ذاتيًا بنسبة 57.15% بين المكونين الأولين وكشف عن ثلاثة أشكال مختلفة. وقد ميز تحليل المتغيرات الكنسية (CA) هذه الأشكال المختلفة، فالنشرة الأنثوية تميل كثيراً نحو الشكل الإهليلجي مع قاعدة متقلصة في العقدة إلى أسفل وقمة تتميز بزاوية مغلقة. بينما يكون شكل النشرة الذكرية بيضاوية الشكل بشكل عام ذات قاعدة مبعثرة وقمة تتميز بزاوية مفتوحة. تشبه ورقات الخنثى ورقات الذكور (بيضاوية الشكل) ولكن بقاعدة مقلطة. يمكن استخدام هذه النتائج كوسيلة لإجراء اختبار أولي لنباتات الخروب الصغيرة.

الكلمات المفتاحية: شجرة الخروب، المجموعات السكانية، المنشورات، التوصيف، هندسة التشكل، الشكل البيضاوي، الشكل البيضواوي.

Introduction

The carob tree (*Ceratonia siliqua* L.) is a fruit tree in the Fabaceae family, with a pre-Mediterranean tropical origin (Mahdad, 2022). This species has been domesticated since the dawn of time, dating back to 4000 BC. It has been cultivated extensively since at least 2000 BC for its by-products and also for its tolerance to water shortages (Ait Chitt et al., 2007; Biner et al., 2007; Mahdad and Gaouar 2023). It is a xerophilous, sclerophyllous, heliophilous, calcicolous and thermophilous species (Mahdad and Gaouar, 2016).

This hardy agroforestry species plays an essential socio-economic and ecological role, particularly in dry regions and areas where desertification processes are occurring at significant magnitudes, notably in Algeria (Mahdad et al., 2022). All parts of this precious plant (foliage, flowers, fruit, wood, bark, roots) are likely to be of real interest and have ornamental or landscape value (Hariri et al., 2009). As a result, it is one of the fruit and forest trees with the greatest value-adding potential, because of its high nutrient content, which has attracted the interest of several researchers, but above all because of its seeds, which are the subject of commercial transactions whose value far exceeds that of woody production. Whole pods, seeds, pulp and gum are widely sold in Europe and used in the food industry (Biner et al., 2007).

In Algeria, carob production is steadily declining due to the ageing of the few existing carob orchards, the repeated fires that ravage tens of thousands of carob trees every year, and the lack of programs to renew and establish new viable plantations (Mahdad, 2022). As a result, the urgent introduction of management strategies for the species is strongly recommended with a view to conserving its genetic diversity and improving its agronomic and commercial characteristics.

Among its agronomic characteristics, the late onset of flowering of female plants is considered a disadvantage not only for yield, since sowing carob, a dioecious species, results in a ratio of 50% females and 50% unproductive males (Mahdad, 2013), but also for genetic improvement programs based on crosses between selected parents, which take a considerable amount of time (up to 8 years) to select individuals for the F1 generation. These disadvantages, particularly that of sexual determinism can be overcome by the differences that may exist between the leaves of the different sexes of carob plants, hence the aim of this study, which is to identify the leaflets of the male, female and hermaphrodite populations by morpho-geometric characterization, to allow the selection of plants according to their sex from a very young age.

Objective

To study the difference that exists within the leaflets between the male, female and hermaphrodite populations of the carob tree in Algeria by means of a morpho-geometric analysis. The aim of this type of analysis is first to look for any morphological variation in the leaflets and to determine their specific characteristics to confirm the presence or absence of homogeneity within the leaf groups collected.

Materials and Methods

Sampling regions

Sampling was carried out in northern Algeria, more specifically in the wilayas of Tlemcen, Tipaza, Sétif, Relizane, Aïn defla, Boumerdés, Béjaïa, Jijel, Skikda, El Tarf, Guelma, Mila, M'sila, Blida, Sidi Bel Abbès, Annaba, Oran, Mostaganem, Mascara, Tizi- Ouzou and Chlef (Figure 1).

Harvesting of plant material

A total of 253 carob leaves were collected (June 2020) from 21 regions in northern Algeria. These leaves came from 118 male plants, 127 female plants and 4 hermaphrodite plants. The small number of hermaphrodite experimental units is explained by their rarity in Algeria. For each tree, three leaflets were characterized except for the hermaphroditic plants where all the leaflets were characterized. Where all leaflets were taken into consideration (37 leaflets), in all, 781 leaflets were characterized using two variables, size and shape

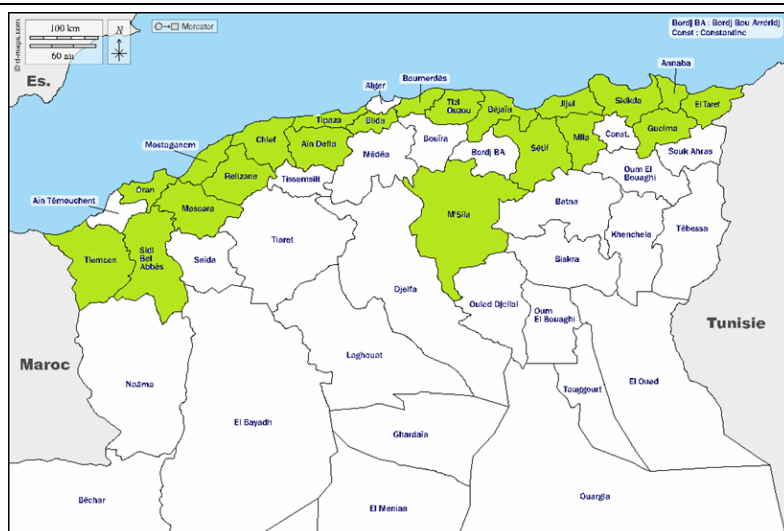


Figure 1. Geographical map of the regions sampled

Morpho-geometric characterisation method

Digital photography and image processing

High-resolution digital photographs were taken of the leaves using a Samsung S6 mobile phone camera. The leaves were placed on numbered white paper on which a ruler graduated in the same direction and distance was placed (Figure 2).



Figure 2. Photo of carob leaf N° 01, female (June 2020)

Folder organisation

The leaf image files were saved in a single folder, containing all the leaves (male, female, hermaphrodites) and Tps (Notepad): specimens in tps format, which groups together information on all the leaves (Figure 3).

Choice of landmarks

To analyze the conformation of an object, a careful choice must be made when selecting landmarks. We chose leaflets with undamaged margins. To do this, we chose 16 reference points.

Data collection

Before we can begin collecting data, i.e. digitizing the landmarks, we need to create a TPS file, which is essentially a list containing our specimens. This file is essential for setting up the landmarks with TpsDig2.



Figure 3. Organization of folders

Creating the TPS file

Firstly, the TPS files must be created using TpsUtil version 1.81. This file contains all the photographed specimens analyzed and is required for the subsequent implantation of marker points using TpsDig32 version 2.31 software.

After opening the tpsUtil software to create a TPS file, follow the steps below.

A window appears (Figure 4)

- ✓ Step 1: Click on "Select an operation» and choose "Build tps file" from the drop-down list (Figure 5).

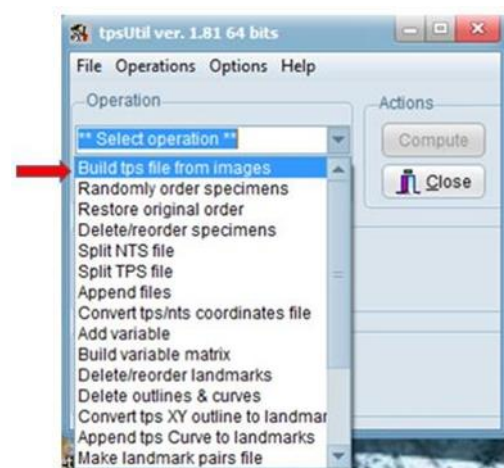


Figure 4. the main window of the TpsUtil software

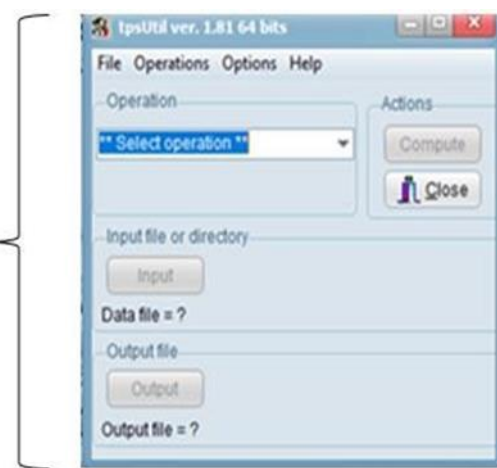


Figure 5. step 1 of file creation

TPS (Select an operation)

- ✓ Step 2: click on "Input", once you have found the image directory click on file, simply click on a single image in this directory and tpsUtil will automatically import the rest (Figure 6 and 7).

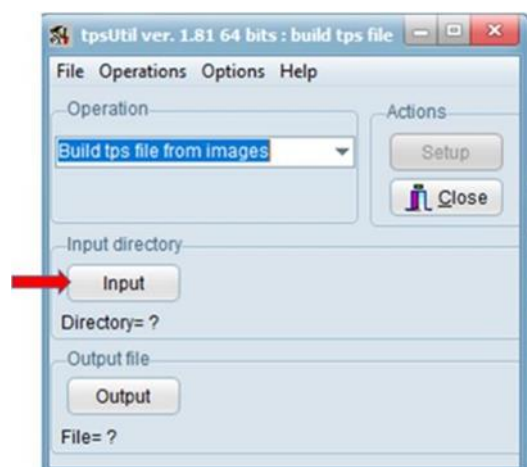


Figure 6. Step 2 in creating a TPS file (choice of input directory)

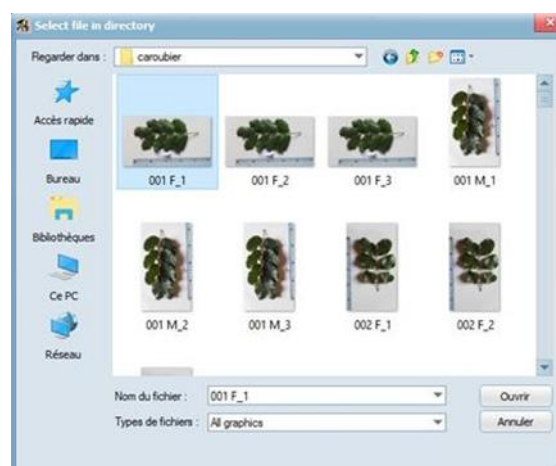


Figure 7. the image directory window

- ✓ Step 3: Click on "output" to create an output file with the extension "tps" and name it. We recommend that you save this file in the folder with the images (Figure 8 and 9).

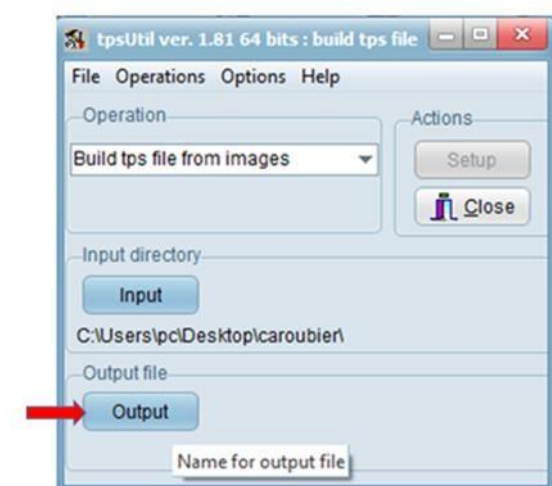


Figure 8. Step 3 in creating a TPS "file name" (assigning a name to the output file)

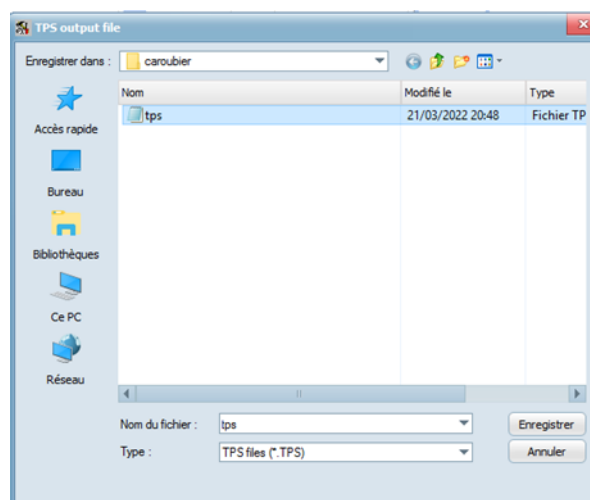


Figure 9. Naming the TPS file

- ✓ Step 4: Click on "Setup". A window will appear with the addresses of all the selected image files (check/uncheck as required), confirm the creation of the tps file and click on "Create" (Figure 10).
- ✓ Step 5: Click on "Close" to exit tpsUtil (Figure 11).

Digitizing the reference points

In this stage, the reference points are placed at the edge of the sheet. The digitized images are then saved in the tps file. There are five important criteria to consider when digitizing the reference points: Firstly, the reference points must be homologous points on all the samples analyzed. Secondly, the points must not change position relative to the other points. Thirdly, the reference point must cover the shape of the object under study. In addition, the repeatability of the marks that remain on the object must be high. Finally, the marks must be on the same plane. A key element in this process is to identify points with a high level of agreement so that the correspondences between the samples are clear (Bookstein, 1991; Zelditch et al., 2004).

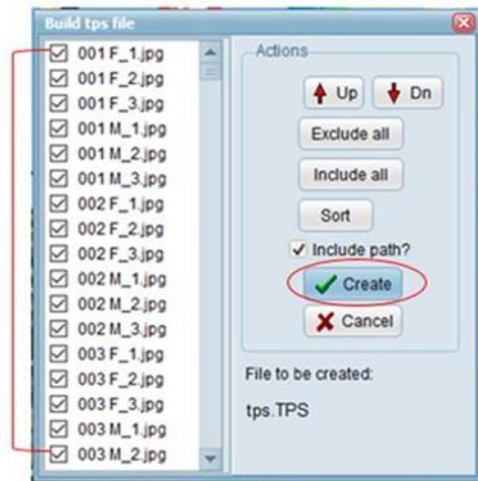


Figure 10. Step 4 creating the tps file

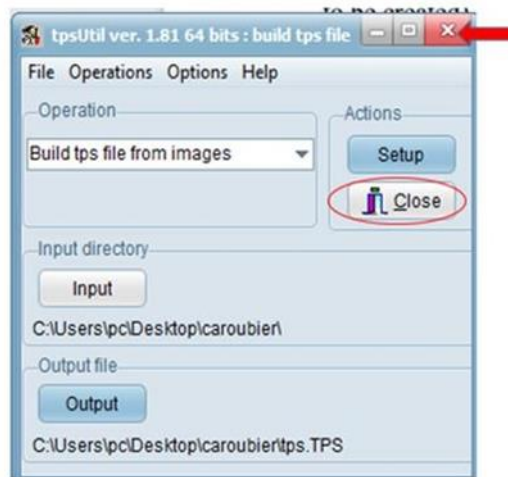


Figure 11. Step 5 "Close" to exit

tpsUtil

Before applying the coordinates, define the scale factor of the measurements in tpsDig using the ruler included in the background of each image (Figure 12).

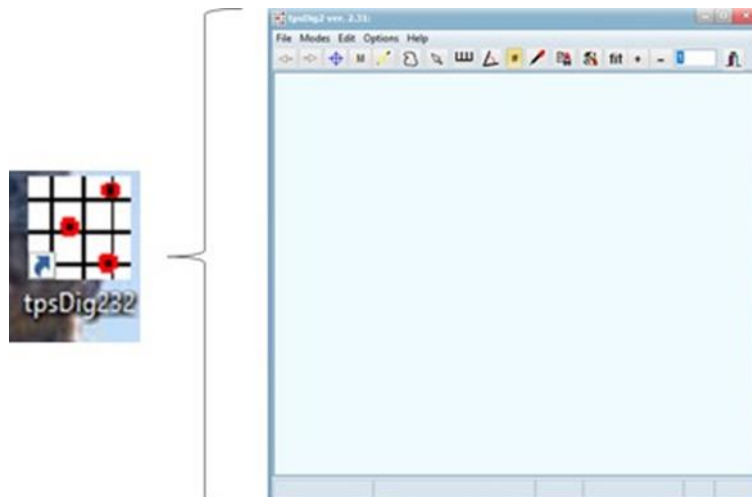


Figure 12. The main TpsDig 32 software window

Once TpsDig2 is open, we create the "tps" file. The scanning steps of the tpsDig2 software are described in detail by Lateb (2014) and do not need to be repeated here. However, Figure 13 shows a tpsDig2 window with the main options. Once the digitization step is complete, the tps files can be analyzed using various software packages such as MorphoJ, mophoR, ImageJ...etc. The software we used for our analyses is MorphoJ version 1.06 (Klingenberg, 2005).

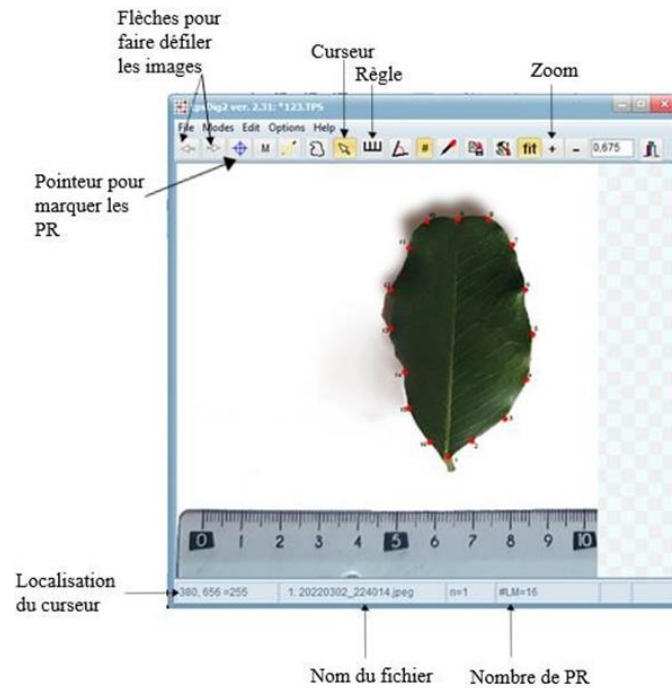


Figure 13. Graphical interface of the tpsDig2 software

Checking the data

Once the data has been collected, it must be analysed beforehand to check for compatibility and any digitisation errors.

Statistical analysis

In this study, we selected 16 reference points on each image (leaflet) in 2 dimensions. In this way, each homologous point is represented with its coordinates (x, y).

The first step in MorphoJ version 1.06 was to validate the data and eliminate outliers. In fact, careless errors can occur during digitization, particularly the digitization of interchangeable markers. Once recorded in digital format, the data is aligned and superimposed using the Procrustean superimposition technique. This step separates the size and conformation parameters.

After running a set of processes to digitize the leaves, statistical tests were carried out to analyze the data. An analysis of variance (ANOVA) was used to test the significance of size and shape. Size was tested using descriptive statistics

and a post-HOC (Honestly Significant Difference) Tukey HSD test to study the pairwise comparison between the different sex populations using R version 3.6.3. Principal component analysis (PCA) is a very common method in geometric morphometry used to explore shape trends and variability between specimens. The discrimination achieved by canonical variant analysis (CA) aims to explain the average differences in leaflet shape variation between the different sexes.

Results and Discussion

Checking for outliers

The "red" data in Figure 14 are similarly represented by the "blue" data, and we can conclude that there are no outliers.

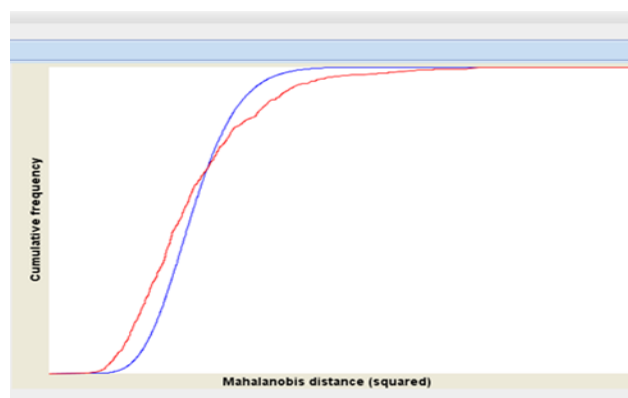


Figure 14. Cumulative distribution diagram of the distances of individual samples and the average shape of the complete sample

Procrustes overlay

After superimposing the Procrustes in MorphoJ, we obtained the graph shown in Figure 15. This procedure enabled us to observe the differences in shape between all the individuals by removing the three effects: position, size and rotation (Evin et al., 2008). These differences are expressed numerically as Procrustean distances.

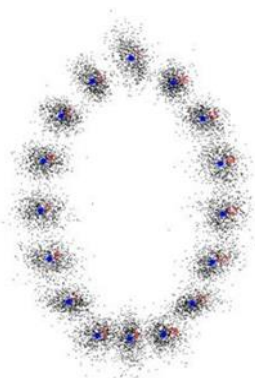


Figure 15. Result of the Procrustean overlay

Procrustes Overlay was used to create a consensus shape by considering all the individuals in the sample and identifying the point of origin or centroid from the reference points defined on each image. All morpho-geometric tools strictly require that all digitized landmarks are homologous across all specimens. Otherwise, they represent the same biological locations on each individual (Bookstein, 1991; Zelditch et al., 2004).

Each consensus landmark is shown in blue in Figure 15, surrounded by point clouds representing different coordinates for all specimens. The landmarks (3,4,5,6,7,11,12,13,14,15) with compact and dark clouds indicate that they have close coordinates, whereas the landmarks (1,2,8,9,10,16) with scattered and diffuse clouds indicate that the landmarks are moving away from the consensus (mean shape) which proves a difference at the apex and base of the leaflets. The coordinates of the "consensus" are shown in Table 1 below.

Table 1. Coordinates of consensus benchmarks (new variables)

Landmarks	Axis 1 (X)	Axis 2 (Y)
LM1	-0,00040972	0,32838338
LM2	-0,08106558	0,27829474
LM3	-0,14601930	0,20239170
LM4	-0,18321947	0,10428243
LM5	-0,18892559	-0,00334524
LM6	-0,17048870	-0,10889984
LM7	-0,13124217	-0,20256673
LM8	-0,07112533	-0,27578391

LM9	-0,00334731	-0,28320568
LM10	0,06709518	-0,27486273
LM11	0,12714541	-0,20645439
LM12	0,16890205	-0,11599381
LM13	0,19076631	-0,01144302
LM14	0,18563963	0,09834782
LM15	0,14923983	0,19661350
LM16	0,08705478	0,27424178

ANOVA (analysis of variance)

A hierarchical analysis of variance (ANOVA) was used to analyse morphological variation in leaflets in relation to sex. The results are shown in Tables 2 and 4.

✓ size

From Table 2, the sex factor is statistically significant, $p = 0.0243$ (<0.05), thus influencing leaflet size. Concerning the separation of means, only one significant difference was noted between the size of hermaphrodite and male leaflets -0.68 cm ($p = 0.035$). However, no significant difference was found between male and female leaflets ($p = 0.224$), female and hermaphrodite ($p = 0.176$) (Table 3).

The Pillai tr index equalled 0.14, showing a sex effect on shape at certain geometric points or anatomical parts, mainly the apex and base of the leaflets, as confirmed in the Procrustes superposition study.

Table 2. Results of the hierarchical analysis of variance for centroid size for carob leaflets.

Source of variation	SS	MS	DF	F	P(value)
Individual	17,088	8,544	2	3,74	0,024*
Residuals	1779,088	2,286	778		

SS: Sum Squares; MS: Mean Squares; df: degree of freedom (ddl). * Significant at the $\alpha=0.05$ threshold.

Table 3. Mean, minimum, maximum centroid leaflet size for each population.

populations	N	Mean $\pm$ Standard Error	Min	Max	P-value
Female	378	8.88 $\pm$ 0.08 ^{a,b}	4.09	14.16	0.224
Hermaphrodite	37	9.34 $\pm$ 0.20 ^a	7.51	13.07	0.030
Male	366	8.69 $\pm$ 0.08 ^b	4.76	13.07	0.176

NB: the averages followed by different letters are significantly different.

✓ shape

Statistical data processing using a single factor ANOVA shows that total shape is highly influenced by gender ($p=0.0001$) (Table 4).

Table 4. Results of the hierarchical analysis of variance for the shape (Shape) of carob leaflets.

Source of variation	SS	MS	Df (ddl)	F	P (val) ***	Pillai tr	P(val)
Individual	0,238	0,004	56	7,82	<0.0001	0,14	<0.0001
Residues	11,840	0,0005	21784				

SS: Sum Squares; MS: Mean Squares; df: degree of freedom (ddl). *** Highly significant at the $\alpha=0.05$ threshold.

Principal component analysis (PCA)

Inertia distribution

Principal Component Analysis (PCA) applied to the data obtained on all the samples enabled us to retain the first three principal components (PC) explaining respectively 32.408%, 24.749%, and 13.296% of the total variation in leaflet shape, i.e. a cumulative variance of 70.453% (Figure 16, Table 5).

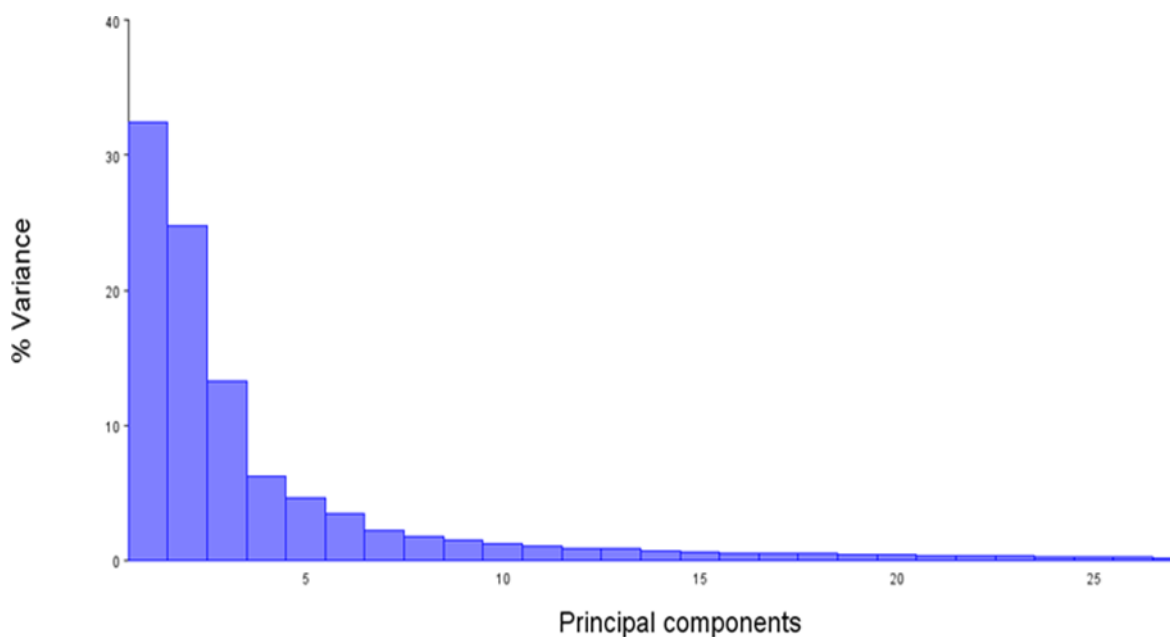


Figure 16. Percentage of variation explained by the principal axes

Table 5. Eigenvalues and percentages of variance explained by PCA variables

	Eigenvalues	% Variance	Cumulative %
1	0,00493241	32,408	32,408
2	0,00376679	24,749	57,157
3	0,00202350	13,295	70,453
4	0,00094644	6,218	76,671

Description of the PCA

The morphological differences of these conformations are highlighted in the Shape Graph (Figure 17).

Conformational change in the first axis

The first component (PC1) controls the deformation of a sheet according to the extreme of the positive and negative side points using the wireframe graph (Figure 18).

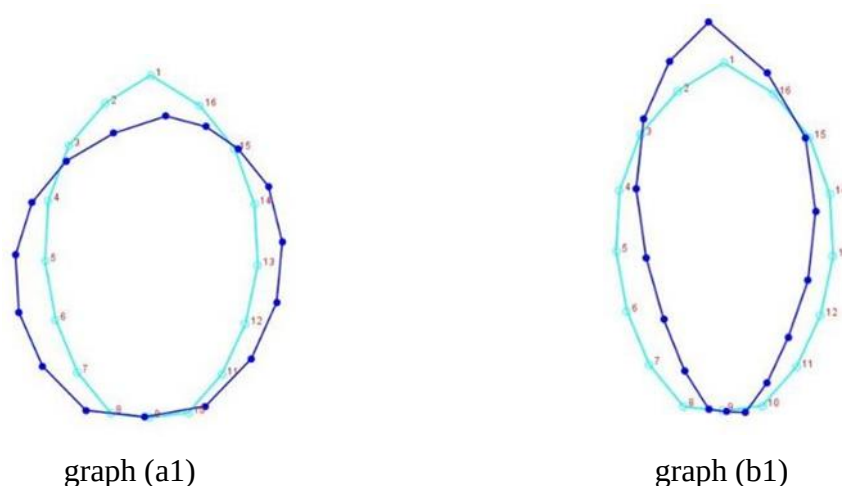


Figure 18. Visualization of the change in leaf conformation along the first axis

Characterization of the conformation on the left of the PCA graph (a1)

- ✓ general shape: oval
- ✓ thickness base of leaflet (landmarks 8.9.10): scattered base.

- ✓ apex: triangle (landmarks 2.1.156) open angle ($< 90^\circ$). (wide angle).

Characterization of the conformation on the right of the PCA graph (b1)

- ✓ general shape: elliptical.
- ✓ thickness base of leaflet (landmarks 8.9.10): base contracted (narrow).
- ✓ apex: triangle (landmarks 2.1.16), closed angle ($>90^\circ$).

Conformational change in the second axis

The second component of the PCA (PC2) detected a deformation of the leaf from one level to another using the wireframe graph (Figure 19).

The deformations at the base of the leaflet are characterized by relaxations with downward node directions for the left-hand PCA (a2) (PR: 8.9.10), and upward node directions for the right-hand PCA (b2)

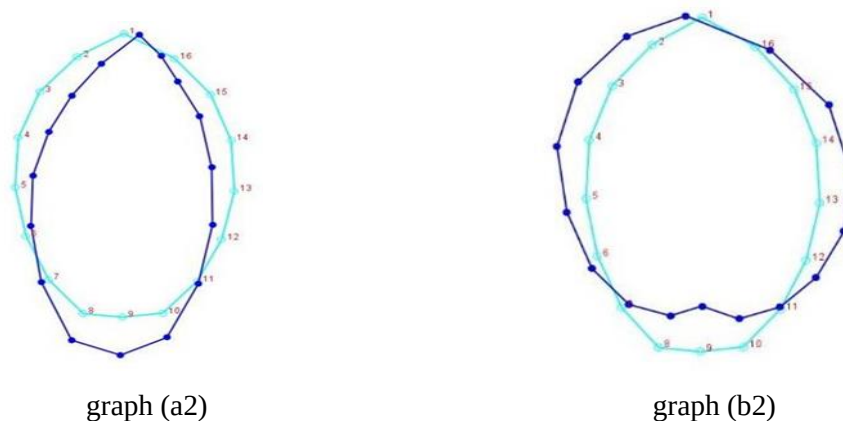


Figure 19. Visualization of the change in leaf conformation along the second axis

Principal component analysis detected leaf morphological variation in the carob tree along the first two principal axes according to several criteria. PC1 showed a deformation ranging from an elliptical shape with a contracted base and a closed-angle apex, to an oval shape with a dispersed base and an open-angle apex. PC2, on the other hand, mainly detected a relaxation of the leaf base triangle (the direction of the node, from top to bottom) (Figure 20).

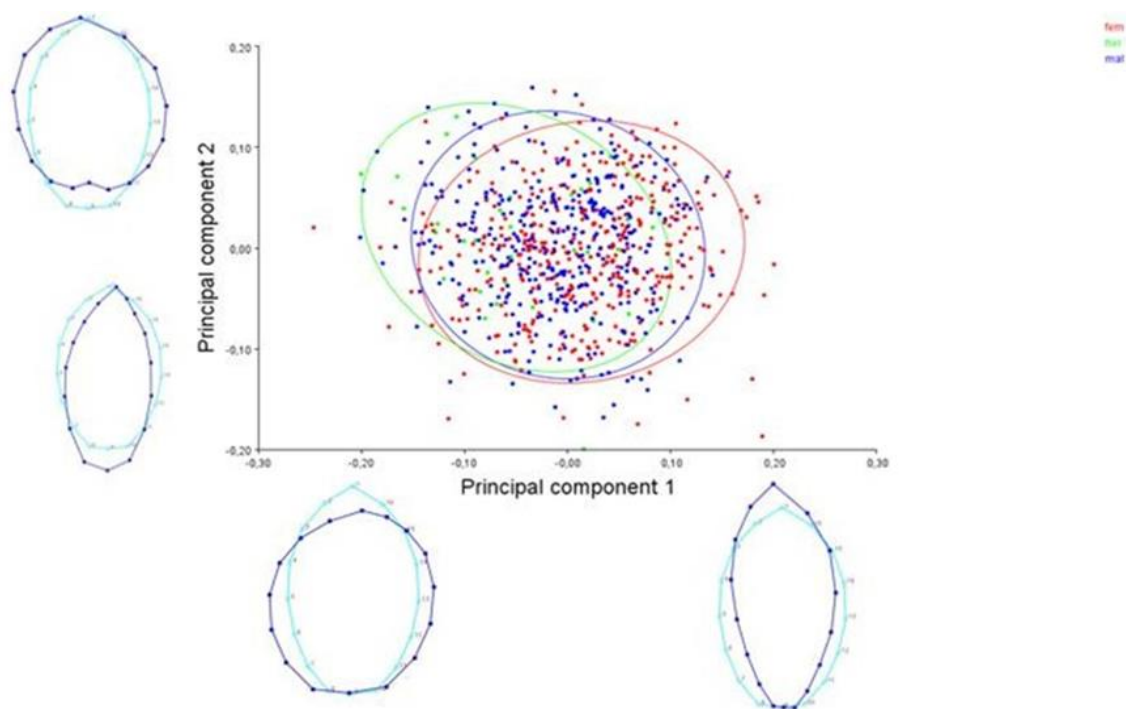
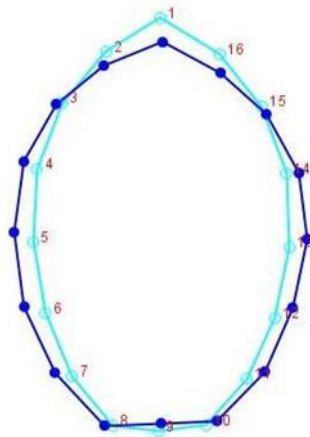


Figure 20. Projection of the different shapes of carob leaflets according to the first two principal components: female (red), hermaphroditic (green) and male (blue).

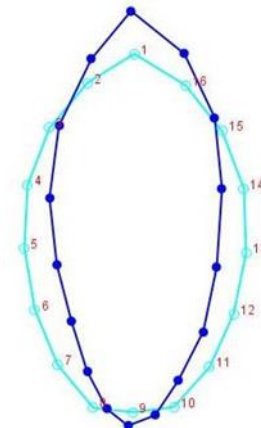
Canonical variant analysis

The aim of the canonical variant analysis is to explain the mean differences in leaf shape variation between the different sexes (male, female, hermaphrodite). The graphical representation in the [CV1, CV2] plane shows the difference between the sexes per reference individual (**Figure 21**).

CV1 shows that the base, the general shape of the leaf and the apex play a discriminating role.



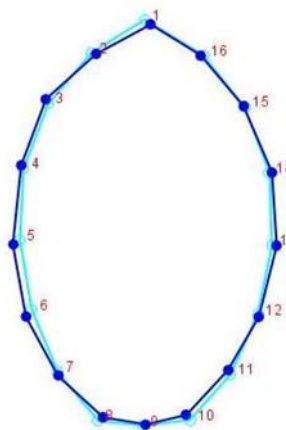
Graph c 1 (left)



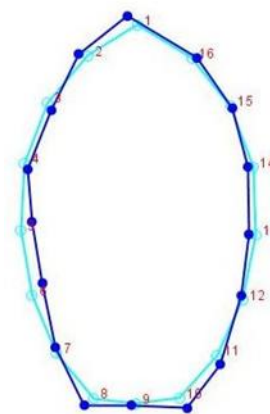
Graph d 1 (right)

Figure 21. Visualization of leaflet change along the first axis (CV1)

CV2 shows that the base of the leaf plays a discriminating role (Figure 22).



Graph c 2 (left)



Graph d 2 (right)

Figure 22. Visualization of leaflet change along the second axis (CV2)

The concept of populations as a function of observation groups made it possible to distinguish males from females after visualizing the change in the leaf along CV1. The female leaflet has an elliptical shape with a base contracted downwards into a node (narrow shape) and with an apex characterized by a closed angle (graph d1). The male leaflet, on the other hand, is generally oval with a dispersed base and a wide-angle apex (graph c2).

The second observation group CV2 was used to detect hermaphrodites. The leaflets of the hermaphrodites mainly resemble the leaflets of the males (oval) but with a flattened base (Graph d2) (PR: 8-9-16) (Figure 23).

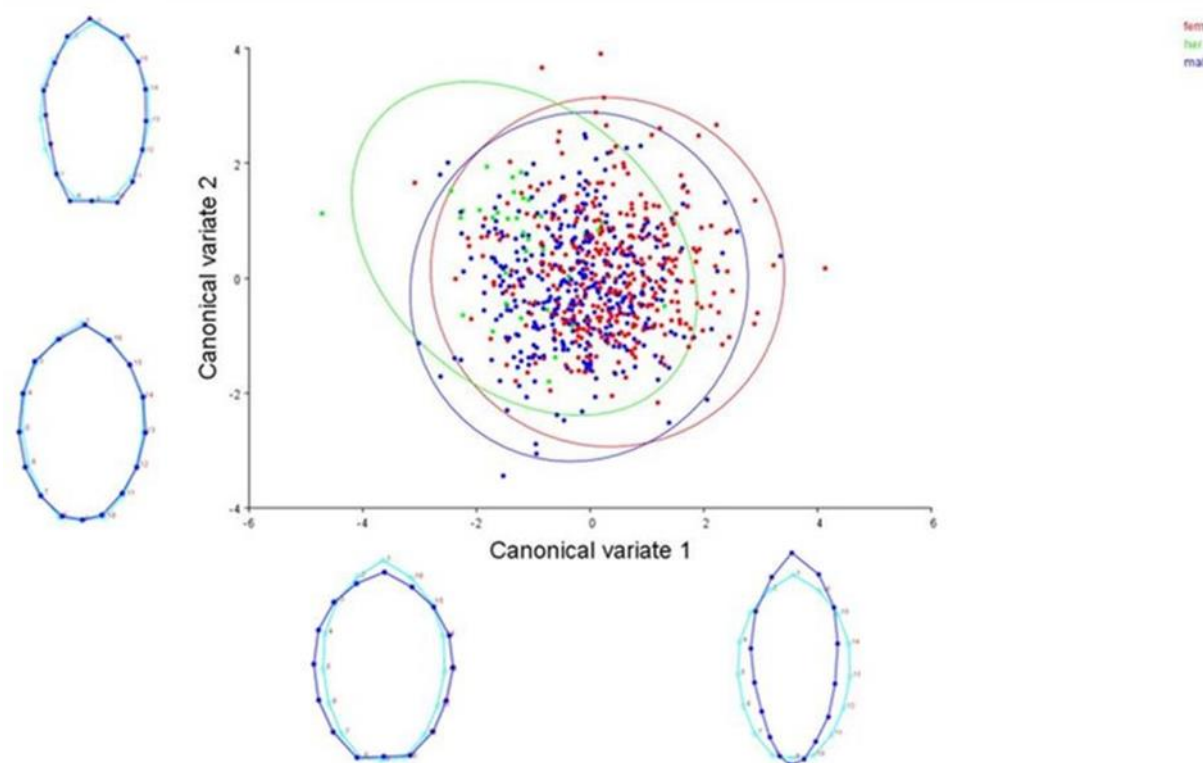


Figure 23. Graphical representation of canonical variants 1 and 2, female (red), hermaphrodite (green) and male (blue).

Mahalanobis distance (D^2)

A Mahalanobis distance (D^2) was used to examine the similarity between groups of individuals and show that all groups are significantly different from each other based on 1000 permutations (**Table 6**).

Table 6. Results of Mahalanobis distances between groups

	Female	Hermaphrodite
Hermaphrodite	1,51	
Male	0,55	1,16

The Mahalanobis distance showed a low geometric dissimilarity between female and male leaflets ($D^2 = 0.55$) but this does not exclude the existence of a difference between these two populations as demonstrated in the previous analysis, and an average dissimilarity between hermaphrodite and female leaflets ($D^2 = 1.51$), and between hermaphrodite and male leaflets ($D^2 = 1.16$), precisely a difference at the base of the leaflets.

Discussion

Our study shows morpho-geometric variability in carob leaflets according to their male, female and hermaphrodite sexes in Algeria. While female leaflets tend towards an elliptical shape, male leaflets are generally oval, while hermaphrodite leaflets resemble male leaflets (oval) but with a flattened base.

The results obtained are different from the study by Gharnit (2003) in Morocco, which focused on the characterisation of carob leaves using classical morphometry (length, width, number of leaflets and length of petiole). Our study shows that the non-productive male population has longer and wider leaves, while the female population has leaflets that are shorter, narrower and more elongated than those of the male population.

This study is based on the comparison of linear distances and angles between homologous points on different objects. These measurements are subjected to uni- or multivariate statistical tests for the analysis of morphological variation (Rohlf and Bookstein, 1990; Bookstein, 1996), and the geometric relationships between the variables are not preserved, making it impossible to represent the shape of the

object graphically. Geometric morphometry has been developed to overcome these limitations, by preserving and comparing very precisely the configurations of different objects (Perrard, 2012).

Because of these drawbacks, Zelditch et al. (2012) also claim that it is complex to reconstruct the conformation of an organism from linear measurements. Indeed, an object described by 16 landmarks requires 120 linear measurements to reconstruct its conformation. These limitations have led several authors to recommend the geometric morphometry method, in particular the landmark approach, which once the landmarks are well chosen, enables the conformation of the object to be analyzed to be visualized instantaneously (Cucchi et al., 2015). This method has proved its worth in many areas of biology, including botany, and several taxonomists and botanists have recognized the potential of this method in their field (Jensen, 2003).

Conclusion and outlook

This study contributes to our knowledge of the differences between male, female and hermaphrodite plants via the geometric characteristics of the leaflets. The study indicates significant variability in leaflet shape in the three populations (male, female and hermaphrodite) and also shows the different shapes according to overall trends.

The morpho-geometric analysis was essentially based on 16 landmarks. Of these, PR 1, 2, 8, 9, 10 and 16 were the most discriminating in identifying and characterizing these carob populations according to sex.

The results obtained show three different types of leaflets depending on their sex. The female leaflet has an elliptical shape with a base contracted downwards into a knot, and with an apex characterized by a closed angle. Male leaflets, on the other hand, are generally oval with a scattered base and an open-angled apex. As for hermaphrodite leaflets, they resemble male leaflets (oval) but with a flattened base.

In addition, a significant difference between the centroid size of hermaphrodite and male leaflets was recorded. The Mahalanobis distance revealed a low geometric dissimilarity between female and male leaflets, and a medium dissimilarity between hermaphrodite and female leaflets, and between hermaphrodites and males, precisely a difference at the base of the leaflets.

This study made it possible to distinguish relatively between adult male, female and hermaphrodite trees on the basis of the size and, above all, the shape of the leaflets. These results can be used as a means of making a preliminary selection of young carob seedlings. However, to make a precise selection, a genetic study is essential, with the aim of identifying and developing molecular markers (SSR and SNP) linked to sexual determinism in the species.

References

- Aafi, A. 1996.** Le caroubier : Caractères botaniques et écologiques, groupements végétaux, techniques d'élevage en pépinière, traitement et soins culturaux, utilisation et production. Centre national de la recherche forestière. Maroc, pp 1-10.
- Aafi, A. 1996.** Note technique sur le caroubier. Détermination of Chemical Composition of Carob (*Ceratonia siliqua*): Protein, Fat, Carbohydrates, and Tannins, Journal of food composition and analysis, Vol. 10, pp: 166-172.
- Ait Chitt, M., Belmir, H. & Lazrak, A. 2007.** Production de plants sélectionnés et greffés de caroubier. Transfert de technologie en agriculture. Maroc. N° 153: 1-4.
- Albanell, E. 1990.** Caracterización morfológica, composición química y valor nutritivo de distintas variedades de garrofa (*Ceratonia siliqua* L.) cultivadas en España. Tesis doctoral. Barcelona. España, pp. 209.
- Arribas, C., Pereira, E., Barros, L., Alves, M.J., Calhelha, R.C., Guillamón, E., & Ferreira, I.C. 2019.** Healthy novel gluten-free formulations based on beans, carob fruit and rice: Extrusion effect on organic acids, tocopherols, phenolic compounds and bioactivity. Food chemistry, 292: 304-313.
- Avallone, R., Plessi, M., Baraldi, M., & Monzani, A. 1997.** Determination of chemical composition of carob (*Ceratonia siliqua*): Protein, fat, carbohydrates, and tannins. Journal of Food Composition and Analysis, 10(2), 166–172, doi: 10.1006/jfca.1997.0528.

- Ayaz, F.A., Torum, H., Glew, R.H., Bak, Z.D., Chuang, L.T., Presley, J.M., & Andrews, R. 2009.** Nutrient Content of Carob Pod (*Ceratonia siliqua* L.) Flour Prepared Commercially and Domestically. *Plant Foods Hum Nutr* 64, 286–292.
- Barracosa, P., Osório, J., & Cravador, A. 2007.** Evaluation of fruit and seed diversity and characterization of carob (*Ceratonia siliqua* L.) cultivars in Algarve region. *Scientia Horticulturae*. 114: 250-257.
- Batista, M.T., Amaral, M.T., & Proença Da Cunha, A. 1996.** Carob fruits as source of natural antioxidant. In *Proceeding of the III International Carob Symposium*. Cabanas-Tavira, Portugal.
- Battle, I. 1997.** Current situation and possibilities of development of the carob tree (*Ceratonia siliqua* L.) in the Mediterranean region. Unpublished FAO Report. Rome. Italy.
- Battle, I., & Tous, J. 1997.** Carob tree. *Ceratonia siliqua* L. Promoting the conservation and use of underutilized and neglected crops. 17. Institute of Plant Genetic and Crops Plant.
- Baumel, A., Mirleau, P., Viruel, J., Bou Dagher Kharrat, M., La Malfa, S., Ouahmane, L., Diadema, K., Moakhar, M., Sanguin, H., & Médail, F. 2018.** Assessment of plant species diversity associated with the carob tree (*Ceratonia siliqua*, Fabaceae) at the Mediterranean scale. *Plant Ecology and Evolution*, 151(2), 185–193, doi: 10.5091/plecevo.2018.1423.
- Biernacka, B., Dziki, D., Gawlik-Dziki, U., Różyło, R., & Siastala, M. 2017.** Physical, sensorial, and antioxidant properties of common wheat pasta enriched with carob fiber. *LWT*, 77: 186- 192.
- Biner, B., Gubbuk, H., Karhan, M., Akus, M., & Pekmezei, M. 2007.** «Sugar profiles of the pods of cultivated and wild types of carob bean (*Ceratonia siliqua* L.) in Turkey». *Food chemistry* 100: pp. 1453-1455.
- Bolonos, M. 1955.** Rapport sur le caroubier. Instituto forestal de Investigaciones y experiencias Madrid (Espagne), pp.9.
- Bookstein, F.L. 1991.** Morphometric tools for landmark data: geometry and biology. 2e éd. Cambridge : Cambridge University Press.
- Bookstein, F.L. 1996.** Biometrics, biomathematics and the morphometric synthesis. *Bulletin of mathematical biology*, 58(2), 313-365.
- Boublenza, I. 2012.** Contribution à l'étude de multiplication du caroubier : *Ceratonia siliqua* L. Mémoire de magistère. Université Abou Bekr Belkaid-Tlemcen, Algérie.
- Boublenza, I. 2020.** Etude des populations spontanées et cultivées du caroubier (*Ceratonia siliqua* L.) en Algérie. PhD thèse. Univ. Tlemcen, Algérie, pp. 115.
- Boublenza, I., El Haitoum, A., Ghezlaoui, S., Mahdad, M., Vasai, F., & Chemat, F. 2019.** Algerian carob (*Ceratonia siliqua* L.) populations. Morphological and chemical variability of their fruits and seeds. *Scientia Horticulturae*, 256, 108537.
- Caja, G. 1985.** La Garrofa: Composición, procesado y usos agroindustriales. Jornadas de la Garrofa. LLiria, Valencia - España.
- Coit, J.E. 1962.** Carob culture in the semi-arid southwest. Vista, CA: J. Eliot Coit, pp 6.
- Correia, P.J., Gama, F., Pestana, M., & Martins-Loução, M.A. 2010.** Tolerance of young (*Ceratonia siliqua* L.) carob rootstock to NaCl. *Agricultural Water Management*, 97: 910- 916.
- Crosi, L., Avallone, R., Cosenza, F., Farina, F., Baraldi, C., & Baraldi, M. 2002.** Antiproliferative effects of *Ceratonia siliqua* L. on mouse hepatocellular carcinoma cell line. *Fitoterapia* 73, p.674-684.
- Cucchi, T., Baylac, M., Evin, A., Bignon-Lau, O., & Vigne, J-D. 2015.** Morphométrie géométrique et archéozoologie : Concepts, méthodes et applications. (Chap. 4).
- Dakia, P.A., Blecker, C., Robert, C., Wathelet, B., & Paquot, M. 2008.** Composition and physicochemical properties of locust bean gum extracted from whole seeds by acid or water dehulling pre-treatment. *Food Hydrocolloids*, 22, 807e818.
- Durazzo, A., Turfani, V., Narducci, V., Azzini, E., Maiani, G., Carcea, M. 2014.** Nutritional

- characterisation and bioactive components of commercial carobs flours. Food Chemistry, 153:109-113.
- Evin, A., Baylac, M., Pons, JM., & Cornette, R. 2008.** Apports de la morphométrie géométrique à l'étude du crane de chauves-souris : l'exemple d'un complexe d'espèces du genre *Myotis*. Symbiose. 21 : 7-9.
- Fournier, P. 1977.** Les quatre flores de la France (générale, alpine, méditerranéenne, littorale) Le Chevalier, Paris.
- García-Ochoa, F., & Casas, J.A. 1992.** Viscosity of locust bean (*Ceratonia siliqua*) gum solutions. J. Sci. Food Agri. 59: 97-100.
- Gharnit, N. 2003.** Caractérisation et essai de régénération in vivo du caoubier (*Ceratonia siliqua* L.) originaire de la province de chefchaouen (Nord-ouest du Maroc). Thèse de Doctorat en science. Université Abdelmalek Essaadi. Tanger.
- Gharnit, N., El Mtili, N., Ennabili, A., & Sayah, F. 2004.** Floral characterization of carob tree (*Ceratonia siliqua* L.) from the province of Chefchaouen (NW of Morocco). Moroccan J. Biol. 1:41-51.
- Gharnit, N., El Mtili, N., Ennabili, A., & Sayah, F. 2006.** Pomological characterization of carob tree (*Ceratonia siliqua* L.) from the province of Chefchaouen (NW of Morocco). Moroccan J. Bio., Vol. 2-3, P.1-11.
- Gonçalves, S. & Romano, A. 2005.** Locust bean gum (LBG) as a gelling agent for plant tissue culture media. Scientia Horticulturae, 106(1): 129-134.
- Gonçalves, S., Correia, P.J., Martins-Loução, M.A. & Romano, A. 2005.** A new medium formulation for in vitro rooting of carob tree based on leaf macronutrients concentrations. Biologia Plantarum. 49: 277-280.
- Hariri, A., Ouis, N., Sahnouni, F., & Bouhadi, D. 2009.** Mise en œuvre de la fermentation de certains ferments lactiques dans des milieux à base des extraits de caroube, rev. Microbiol. Ind. San et environ. Pp. 37-55.
- Haselberg, C. 1996.** Factors influencing flowers and fruit development in carob (*Ceratonia siliqua* L.). In III International Carob Symposium. Cabanas- Tavira, Portugal. (in press).
- Haselberg, C. 1998.** A contribution to the classification and characterisation of female and male varieties of *Ceratonia siliqua* L. Proceedings of the II International Carob Symposium. Valencia, pp. 137-151.
- Hillcoat, D., Lewis, G., & Verdcourt, B. 1980.** A new species of *Ceratonia* (Leguminosae-Caesalpinoideae) from Arabia and the Somali Republic. Kew Bulletin, 35, 261–271, doi: 10.2307/4114570.
- Jensen, LT., Ajua-Alemanji, M., & Culotta Jensen, VC. 2003.** The *Saccharomyces cerevisiae* high affinity phosphate transporter encoded by PHO84 also functions in manganese homeostasis. J Biol Chem. 278(43):42036-40.
- Kamal, E.M.Y., El-Manfaloty, M.M., & Hend, M.A. 2013.** Assessment of Proximate Chemical Composition, Nutritional Status, Fatty Acid Composition and Phenolic Compounds of Carob (*Ceratonia siliqua* L.). Food and Public Health. 3(6): 304-308.
- Klingenberg, C.P. 2005.** MorphoJ: an integrated software package for geometric morphometrics.
- Konate, I. 2007.** Diversité Phénotypique et Moléculaire du Caroubier (*Ceratonia siliqua* L.) et des Bactéries Endophytes qui lui sont Associées. PhD thèse. Univ. Mohammed v-Agdal. pp.196.
- Lateb, M. 2014.** Contribution à l'étude des hybrides des chênes de la forêt de l'Akfadou, via l'approche « Geometric morphometrics ». Mémoire Magistère, UMMTO, 71 p.
- Lozzi, A., Abdelwahad, R., Mentag, R., & Abousalim, A. 2019.** Development of a new culture medium and efficient protocol for in vitro micropropagation of *Ceratonia siliqua* L. In Vitro Cellular & Developmental Biology – Plant, 55, 615–624, doi: 10.1007/s11627-019- 09990-6.

- Mahdad, M.Y. 2013.** Situation et perspectives d'amélioration du caroubier (*Ceratonia siliqua* L.) dans le Nord-ouest de l'Algérie. Thèse de Magister, Université de Tlemcen, Algérie, pp. 83.
- Mahdad, M.Y. 2022.** Caractérisation par l'outil morphométrique, physico-chimique et moléculaire de la diversité génétique chez *Ceratonia siliqua* L. : identification et sélection des premiers cultivars de caroubier en Algérie. Thèse doctorale, Université de Tlemcen, Algérie, pp. 120.
- Mahdad, M.Y., & Gaouar, S.B.S. 2016.** Le Caroubier (*Ceratonia siliqua* L.) dans le Nord- ouest de l'Algérie : Situation et perspectives d'amélioration. Editions Universitaires Européennes. Germany. ISBN-13: 978-3639542035.
- MAHDAD, YASSINE MOUSTAFA and GAOUAR, SEMIR BECHIR SUHEIL 2023** "Origin, distribution and domestication of the carob tree (*Ceratonia siliqua* L.)," Turkish Journal of Botany : Vol. 47: No. 2, Article 2. <https://doi.org/10.55730/1300-008X.2748>
- Mahdad, Y.M., Mediouni, R.M., Viruel, J., Selka, N., and Gaouar, S. B. S. 2022.** Functional Diversity Based on Morphometric Analysis and Identification of the Algerian Carob Tree (*Ceratonia siliqua* L.) Cultivars. Genetics & Biodiversity Journal. 6(2), 1–20. <https://doi.org/10.46325/gabj.v6i2.238>.
- Makris, D.P., & Kefalas, P. 2004.** Carob pods (*Ceratonia siliqua* L.) as a source of polyphenolic antioxidant. Food Technol. Biotechnol. 42: 105-108.
- Melgarejo, P. & Salazar, D.M. 2003.** Tratado de fruticultura para zonas áridas y semiáridas.
- Missbah, M.E.I., Aujjar, N., Belabed, A., Dessaux, Y., & Filali-Maltouf, A. 1996.** Characterization of rhizobia isolated from carob (*Ceratonia siliqua* L.). J. App. Bacteriol. 80: 165-173.
- Naghmouchi, S., Khouja, M.L., Rejeb, M.N., & Boussaid, M. 2008.** Effect of growth regulators and explant origin on in vitro propagation of *Ceratonia siliqua* L. via cuttings. Biotechnology, Agronomy, Society and Environment, 12(3), 251–258.
- NAS. (1979).** Tropical Legumes: Resources for the Future. National Academy of Sciences. Washington DC, USA, pp. 109-116.
- Nassar-Abbas, S.M., e-Huma, Z., Khan, M.K., Esbenshad, H., & Jayasena, V. 2016.** Carob kibble: A bioactive-rich food ingredient. Comprehensive Reviews in Food Science and Food Safety, 15: 63-72.
- Neukom, H. 1988.** Carob bean gum: properties and application. In: Fito P. and Mulet A., (Eds) Proceedings of the II International Carob Symposium. Valencia, Spain, pp. 551-555.
- Noblet, J., Fortune, H., Dubois, S., & Henry, Y. 1989.** Nouvelles méthodes d'estimation de teneur en énergie digestible, métabolisable et nette des aliments pour le porc. INRA ed., Paris. 106p.
- Orphanos, P.I., & Papaconstantinou, J. 1969.** The carob varieties of Cyprus. Tech. Bull.5. Cyprus Agricultural Research Institute. Ministry of Agriculture and Natural Resources, Nicosia.
- Ortiz, P.L., Arista, M., & Talavera, S. 1996.** Produccion de nectar y frecuencia de polinizadores en *Ceratonia siliqua* L. (Caesalpinaceae). Anales del Jardin Botanico de Madrid 54, pp: 540-546.
- Owen, R.W., Haubner, R., Hull, W.E., Erben, G., Spiegelhalder, B., Bartsch, H., Haber, B. 2003.** Isolation and structure elucidation of the major individual polyphenols in carob fibre. Food and Chemical Toxicology, 41: 1727-1738.
- Perrard, A. 2012.** Systématique et morphométrie géométrique: l'évolution de la nervation alaire au sein du genre *Vespa* (Hyménoptères, Vespidae) (Doctoral dissertation, Paris, Muséum national d'histoire naturelle).21
- Priolo, A., Waghorn, G.C., Lanza, M., Biondi, L., & Pennisi, P. 2000.** Polyethylene glycol as a means for reducing the impact of condensed tannins in carob pulp: Effects on lamb growth performance and meat quality. J. Anim. Sci. 78: 810-816.
- Puhan, Z., & Wielinga, M.W. 1996.** Products derived from carob pods with particular emphasis on carob bean gum (CBG). Report Technical Committee of INEC (unpublished).

- Ramón-Laca, L., & Mabberley, D.J. 2004.** The ecological status of the carob-tree (*Ceratonia siliqua*, Leguminosae) in the Mediterranean. *Botanical Journal of the Linnean Society*, 144, 431–436, doi: 10.1111/j.1095-8339.2003.00254.x.
- Rejeb, M.N. 1995.** Le caroubier en Tunisie: Situations et perspectives d'amélioration. Dans: Quel avenir pour l'amélioration des plantes. Edit. AUPELF- UREF. John Libbey Eurotext. Paris. pp: 79-85.
- Rejeb, M.N., Laffray, D., & Louguet, P. 1991.** Physiologie du caroubier (*Ceratonia siliqua* L.) en Tunisie. *Physiologie des arbres et arbustes en zones arides et semi-arides*, Group d'étude de l'Arbre, Paris, France, pp: 417-426.
- Retana, J., Ramoneda, J., & Garcia del Pino, F. 1990.** Importancia de los insectos en la polinización del algarrobo. *Bol. San. Veg. Plagas*, 16:143-150.
- Retana, J., Ramoneda, J., García del Pino, F., & Bosch, J. 1994.** Flowering phenology of carob, *Ceratonia siliqua* L. (Caesalpinaceae). *J. Hort. Sci.* 69: 97-103.
- Rivière, C.H., & Leco, H. 1900.** Manuel pratique de l'agriculteur algérien. Editeur : Augustin CHALLAMEL. Paris, pp. 349-353.
- Rohlf, F.J. 1990.** Morphometrics. *Annual Review of Ecology and Systematics*, 21, 299– 316.
- Rohlf, F.J. 2015.** The tps series of software. Hystris, Ital J Mammal [Internet]. 26(1):9–12.
- Rohlf, F.J., & Slice, D. 1990.** Methods for comparison of sets of landmarks. *Systematic Zoology*. 39, 40-59.
- Saïdi, R., El bouzdoudi, B., El Kbiach, M.B, Maouni, A., Badoc, A., & Lamarti, A. 2016.** Micropropagation of Carob tree (*Ceratonia siliqua* L.) by cotyledonary buds. *J. Mater. Environ. Sci.* 7 (12) 4850-4859. ISSN : 2028-2508.
- Santos, M., Rodrigues, A., & Teixeira, J.A. 2005.** Production of dextran and fructose from carob pod extract and cheese whey by *Leuconostoc mesenteroides* NRRL B512 (f), *Biochemical Engineering Journal* 25, 1–6.
- Sbay, H. 2008.** Le caroubier au Maroc un arbre d'avenir : Centre de recherche forestière charia Omar Ibn Khattab, BP.763, Agdal, Rabat, Maroc. PP : 44. PP : 07-31.
- Sbay, H., & Abourouh, M. 2006.** Apport des espèces à usages multiples pour le développement durable : cas du pin pignon et du caroubier. Centre de recherche forestière haut-commissariat aux eaux et forêts et à la lutte contre la désertification, Rabat, pp. 1-9.
- Sidina, M.M., El Hansali, M., Wahid, N., Ouattmane, A., Boulli, A., & Haddioui, A. 2009.** Fruit and seed diversity of domesticated carob (*Ceratonia siliqua* L.) in Morocco. *Scientia Horticulturae*. 123: 110-116.
- Thomson, P. 1971.** The carob in California. *California Rare Fruit Growers Yearbook III*: 61- 102.
- Tsatsaragkou, K., Gounaropoulos, G., & Mandala, I. 2014.** Development of gluten free bread containing carob flour and resistant starch. *LWT - Food Science and Technology*, 58: 124- 129.
- Turhan, T., Bialka, K.L., Demirci, A., & Karhan, M. 2010.** Ethanol production from carob extract by using *Saccharomyces cerevisiae*. *Bioresource technology*, 101 (14), 5290-5296.
- Tucker, S.C. 1992.** The developmental basis for sexual expression in *Ceratonia siliqua* (Leguminosae:Caesalpinioideae: Cassieae). *Am. J. Bot.* 79 (3): 367-327.
- Tous, J. 1984.** Cultivo del Algarrobo. Hoja Divulgativa N2: 10. Ministerio de Agricultura. Madrid. 16 p.
- Winer, N. 1980.** The potencial of the carob (*Ceratonia siliqua* L). *Int. Tree Crops J.* 1: 15-26.
- Vourdoubas, J., Makris, P., Kefalas, J., & Kalia katsos, J. 2002.** Studies on the production of bioethanol from carob. In *The 12th National Conference and Technology Exhibition on Biomass for Energy. Industry and Climate Protection, Proceedings, Amsterdam* (pp. 489 - 493).
- Yousif, A.K., Alghzawi, H. M. 2000.** Processing and characterization of carob powder: *Food Chem.* 69, 283–287.
- Zelditch, M.L., Swiderski, D., Sheets, H.D., & Fink, W. 2004.** Geometric Morphometrics for

Biologists: A Primer. 10.1016/B978-012778460-1/50003-X.

Zelditch, M.L., Swiderski, D.L., & Sheets, H.D. 2012. Geometric morphometrics for biologists: a primer. Academic Press. 443 p.

Zouari, N., & El Mtili, N. 2020. In Vitro Propagation of Mature Carob Trees (*Ceratonia siliqua* L.) from the Axillary Buds. *American Journal of Plant Sciences*, 11, 1369–1382, doi: 10.4236/ajps.2020.119098.

Webography:

- ✓ <http://dx.doi.org/10.4404/hystrix-26.1-11264>
- ✓ <http://www.worldagroforestry.org/sea/products/afdbases/af/asp/speciesinfo.asp?spid=509>
www.faostat.fao.org.
- ✓ <https://d-maps.com/index.php?lang>