Morphometric and physicochemical characteristics of carob pods in three geographical regions of Morocco

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Received: 8 July 2020 / Accepted: 25 November 2020 / Published online: 9 December 2020
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Abstract

In three different regions of Morocco, i.e., the center, north and southwest, pulps and seeds from eight carob trees, *Ceratonia siliqua* L., were compared in order to characterize and promote the carob tree and its subspecies as new potential development for rural development and soil conservation. Twelve different morphometric parameters of the trees were investigated. The results showed significant differences between the pods of the three regions for most parameters ($p=0.05$). The pods and the seeds of the north are thicker, longer and wider for apical, median and basal width and hereby a mass of seeds per pod which are important. The differences between the trees in the three regions showed a clear separation of the northern region of Morocco from the two other regions. Phytochemical study for flavonoids and saponins in the pulps of the trees showed a significant difference between the three regions. However, the difference for total phenols was not significant. The carob pulp of the north was rich in fat and total sugars, while the carob of the center is rich in total nitrogen and total protein. For seeds, the difference was not significant between the three regions in water content, fatty acids and total flavonoids. However, the carob seeds of the southwest Morocco have the highest content of total nitrogen, total protein, total sugars and saponins.

Keywords *Ceratonia siliqua* · Pods · Seeds · Morphometric · Physicochemical analysis · Pulp

1 Introduction

The climate in Morocco is both Mediterranean and Atlantic with a hot, dry season associated with cold and rainy and wet periods. The ecogeographic and climatic factors play a significant role in the type of vegetation [1]. Moreover, erosion and desertification cause an annual loss of about 31,000 hectares of vegetation. For the rural populations, forest products are needed, and therefore substantial efforts are devoted to reforestation (10 to 15,000 hectares/year). However, according the Ministry of Forestry, reforestation failures are very common. Using species known for their adaptation strategies to climate change could

improve the success rate of reforestation operations. Populations of carob tree are widely distributed in arid and sub-arid area in Morocco (in the Rif and Atlas Mountains and in the valleys of southwestern Anti-Atlas); it plays a great role in the biodiversity of the forest's ecosystem. Several plants of carob tree from Mediterranean origin have been characterized [2–4]. The carob trees have a great potential for reforestation [5].

To identify the most valuable and suitable subspecies of the carob trees (*Ceratonia siliqua* L.) for plantation, the morphometric characterization and determination of chemical composition of carob pod from Chefchaouen (region in the north of Morocco) were studied.

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Furthermore, the chemical composition of carob pods was investigated. With respect to the scientific literature, no comparative morphometric characterization associated with physicochemical studies has been performed before on these areas. In the present study, at three different locations in Morocco, an evaluation of the effect of environmental conditions on the morphometric and the physicochemical properties of pulps and seeds of the carob trees has been studied.

2 Materials and methods

2.1 Plant material

From three regions of Morocco, samples of *Ceratonia siliqua* L. were harvested:

- In the north: in two localities: Al Hoceima and Chefchaouen.
- The center: Azilal, Demnate and Ouzoud.
- In the southwest: The samples were harvested from Izouika, Reggada and Imouzzar Ida Ou Tanane.

A geographic database (latitude, longitude, altitude and precipitations) was recorded for each location using a global positioning system (Table 1). The locations represent different soil conditions and a variety of climatic conditions corresponding to different eco-geographical situations. Randomly, ripe carob pods collections were carried out from 85 trees: 20 in the north, 30 in the center and 35 in the southwest. The samples were packed, labeled and brought to the laboratory for analyses and data collection.

2.2 Morphometric analyses

The morphometric analyses were performed using 30 pods and 30 seeds for each sampled tree from the three localities. Morphometric analysis was carried out on 30 cloves per tree based on the following parameters: pod

length (LG), apical width (LGa), median width (LGm) and basal (LGb), pod thickness (EG), number seeds per pod (NgG), pod mass (PG), seed mass per pod (PgG), mass of the pulp (PP), degree of curvature (C) and length of peduncle (Lp). Thirty seeds were chosen randomly, after crushing the pods. The morphometrics measures of the seed were carried out based on the following parameters: length (Log), width (lg) and thickness of the seed (Eg).

2.3 Physicochemical analyses

The pods and seeds were used for biometric study: determination of the moisture content, the crude ash and the physicochemical analyses: total nitrogen, total protein, crude fat, flavonoids and total saponins. Total sugar and total phenols contents were determined according to Dubois et al. [6] and Dai et al. [7] methods, respectively.

2.3.1 Humidity

Five grams (g) of powder of each carob sample was placed in a stove oven preheated at 103 °C. After 4 h of drying, the samples were covered and cooled in the desiccators for 40 min. Moisture content, as a percentage, was calculated for each sample according to the following formula:

$$H = (E - m) \cdot \frac{100}{E}$$

where E = mass in grams of the test sample, and m = mass in grams, of the dry test.

2.3.2 Crude ash

The determination of crude ash was performed following the instructions of the French standardization system (NF V18-101) [8]. The crude ash content (CA), as a percentage, was calculated using the following formula:

Table 1 Geographical and meteorological conditions in harvesting areas of carob pods

	Ecoregions	Geographic region	Latitude N	Longitude w	Altitude m	Rainfall mm/year	Temperature °C
North	El Hoceima	Rif	35°11'	3°57'	50–250	327	22
	Chefchaouen	Rif	35°12'	5°16'	350–550	700	17
Center	Azilal	High atlas	31° 58' 01"	6° 34' 10"	1 351	400 to 600	33
	Demnate	Middle atlas	31° 43' 52"	7° 02' 10"	968	300 to 500	33
	Ouzoud	Middle atlas	32° 1' 48"	6° 46' 48"	553		22.9
Southwest	Imouzzar Ida Ou Tanane	Ouest of High Atlas	30° 40' 12"	9° 28' 48"	1160	120 to 400	18.3
	Izouika	Anti atlas	29°54'36"	9°33'0"	123	150 to 200	19.2
	Reggada	Anti atlas	29° 34' 48"	9° 41' 60"	403		22

$$I. \quad CB = (m_2 - m_0) \cdot \frac{100}{m_1 - m_0}$$

where m_0 is the mass, in grams, of the empty crucible, m_1 is the mass, in grams, of the crucible containing the sample, and m_2 is the mass, in grams, of the crucible and crude ash.

2.3.3 Total nitrogen and total protein

Mineralization of 1 g of finely ground sample was performed by a digester 12 posts Selecta TC Type K in the presence of concentrated sulfuric acid and 10 g of catalyst (CuSO_4 , K_2SO_4 and Se). The heating was performed gently until the mass has carbonized and the foam disappears and then louder until 410 °C for 3 h. The distillation was carried out by a nitrogen-type distiller VELP UDK 127 and titration 1 N sulfuric acid. Proteins were calculated from the total Kjeldahl nitrogen using a conversion factor of 6.25 proteins per gram of sample.

2.3.4 Crude fats

A 5 g of each sample of carob powder was placed in the extractor type SER 148 VELP SCIENTIFICA RS-232. The crude fats were isolated using petroleum ether. The apparatus was heated at 115 °C in three steps: immersion for 30 min, washing for 60 min and finally recovery for 45 min. Then, the residue was dried in a stove oven with vacuum maintained at a temperature of 105 °C and then cooled in desiccators and weighed.

2.3.5 Total flavonoids

The assays were performed in UV-visible spectrophotometer Vectra 8453 diode array type HP. One gram of powdered sample was mixed with 100 ml of 80% methanol. After stirring and sonication, 2 ml of the extract was mixed with 100 ml of Neu's reagent. The absorption was determined at 404 nm and compared to the standard quercetol (0.05 mg/ml). The standard was treated with the same amount of reagent [9]. The percentage of total flavonoids was calculated as equivalent quercetol according to the following formula [10]:

$$F = (0,05 \times A_{\text{ext}}/A_{\text{q}}) \times 100/C_{\text{ext}} (\%)$$

where A_{ext} is the absorption of the extract; A_{q} is the absorption of the quercetol; and C_{ext} is the concentration of the plant extract which is 10 mg/ml.

The percentages of total flavonoids are converted and then calculated as mg of dry matter.

2.3.6 Saponins

The foam index was used to determine the presence of saponins. For this, 1 g of each sample powder was added to 100 ml of distilled water and boiled for 30 min. After cooling and filtration using Whatman paper, the volume was adjusted to 100 ml with distilled water. Ten test tubes containing 1 to 10 ml of decoction were prepared and adjusted to 10 ml with distilled water. Following a violent horizontal shaking, the tubes were allowed to stand for 15 min. Then, the height of the foam remaining in each tube was measured in cm and the foam index (I) was calculated according to the following formula:

$$I = \text{Foam height in the 9th tube} \times (10/0,09).$$

The presence of saponins in the plant is confirmed if the foam index is greater than 100.

2.3.7 Total sugars

An amount of 1 g of powdered sample was dispersed in 10 ml of dimethyl sulfoxide (DMSO) at 25% in water (v/v). After 15 min of incubation in a boiling water bath, a 0.1 ml of the mixture was diluted with 9.9 ml of water. The amount of 0.5 ml of the extract was added to 0.5 ml of 5% phenol. After homogenization, 2 ml of H_2SO_4 (75%) was added. This mixture was incubated in a boiling water bath for 15 min and then allowed to cool for 15 min in the dark. The optical density (OD) was determined at 292 nm.

2.3.8 Total phenols

A volume of 25 µl of each extract was mixed with 110 µl of Folin-Ciocalteu reagent [11] and homogenized, and then the mixture was left to stand for 3 min. After, 200 µl of sodium carbonate (20%) was added followed by 1.9 ml of distilled water. The mixture was placed for 30 min at 60 °C, and the optical density was read at 750 nm. The calibration curve used to calculate the total concentration of phenolic compounds was determined with caffeic acid.

2.4 Statistical analysis

The results of the morphometric and the physicochemical study were evaluated using EXCEL and STATISTICA 6. The statistical analyses followed were principal component analysis (PCA) and factorial discriminant analysis (FDA). FDA was made based on the sugar content, protein, fat, total flavonoids, total phenolic and saponins.

3 Results and discussion

3.1 Morphometric variability

Statistical analysis of the results from the morphometric analyses of the carob trees between the three regions of the carob trees showed significant differences for the following ten parameters: mass, length and thickness of the pod, apical, middle and basal width of fruit, number of seeds per pod, seed mass per pod and seed length and width (Table 2). Moreover, the carob pod in northern Morocco is heavier, longer, wider and thicker (Table 2). The seeds of the trees from this region were longer and wider than those in the south and central Morocco. The fruits of northern Morocco had a longer stalk (13.75 mm) and a heavier pulp (9.12 g) compared to other studied regions. A significant difference was found for the trees between the north and south for all studied morphometric parameters except for the degree of curvature. The difference between the south and the center is not significant for stem length, the mass of the pulp and seed thickness (Table 2). This difference between regions is confirmed by a dendrogram that shows a clear separation of the northern region from the two other regions (Fig. 1). These results are in agreement with those obtained by Makrem et al. [12] in Tunisia and by Barracosa et al. [2] in Portugal. The results concerning the seeds of the northern region of Morocco are similar to those reported in Fez (Morocco) by Elfazazi et al. [13]. On the other hand, cultivation practices of carob seem to improve the mass of the pod, the stem length, the mass of the pulp, the length of the seed and the apical and basal width of the pod. Rainfall and temperature are the most important climatic parameters influencing the carob tree distribution [14]. Rainfall in northern Morocco seems to favor the mass, length, thickness and width of the pod and length of the stalk. These results were confirmed by those found by Barracosa et al. [2] who reported that the Moroccan ecoregions north pods were longer, wider, heavier and thicker. If rainfall increases, the mass of the pulp, the length, the width and the thickness of the seeds increase, inducing the mass of seeds per pod.

With respect to pharmacological uses, the pulp is known to be used in the prevention and treatment of a wide variety of diseases, including diabetes, hyperlipidemia, irritable bowel syndrome and colon cancer [15].

3.2 Physicochemical analyses

The results of physicochemical analyses presented a significant difference for both total flavonoids and saponins between the carob pulp from the southern, the central

Table 2 Morphometric parameters of the carob pods and the seeds of the three studied locations

	PG (g)	LG (cm)	LGa (mm)	LGm (mm)	LGb (mm)	EG (mm)	C (cm)	Lp (mm)	PP (g)	NgG	PgGs (g)	Log (mm)	Lg (mm)	Eg (mm)
North	11.22 ± 4.75 ^c	15.17 ± 2.55 ^c	17.97 ± 2.71 ^c	18.68 ± 2.94 ^c	17.79 ± 3.88 ^c	6.71 ± 1.51 ^c	11.58 ± 3.52 ^a	13.75 ± 2.80 ^b	9.12 ± 2.18 ^b	10.82 ± 2.46 ^c	2.09 ± 0.72 ^c	9.11 ± 0.95 ^c	7.15 ± 0.85 ^c	4.55 ± 0.55 ^b
Center	6.85 ± 2.44 ^b	13.72 ± 2.9 ^b	15.8 ± 2.01 ^b	17.17 ± 2.17 ^b	15.93 ± 2.21 ^b	5.97 ± 1.09 ^b	10.78 ± 3.46 ^b	11.05 ± 2.76 ^a	5.04 ± 2.08 ^a	11.52 ± 2.74 ^b	1.53 ± 0.51 ^b	8.69 ± 0.84 ^b	6.29 ± 0.62 ^b	4.16 ± 0.50 ^a
Southwest	7.12 ± 2.37 ^a	14.19 ± 2.46 ^a	16.74 ± 2.71 ^a	11.73 ± 2.63 ^a	17.12 ± 1.81 ^a	5.28 ± 1.05 ^a	11.73 ± 2.63 ^a	11.03 ± 4.47 ^a	5.61 ± 2.06 ^a	9.62 ± 2.57 ^a	1.82 ± 0.58 ^a	8.80 ± 0.77 ^a	6.44 ± 2.05 ^a	4.15 ± 1.43 ^a

Means with the same letter(s) in the same column are not significantly different at the 0.05 level

For the same morphometric parameters, lines with values followed by the same letter(s) are not significantly different at the 0.05 level

and the northern Morocco. However, the pulps from the south have the highest percentages of these compounds (Table 3). The pulps of the trees from the three studied regions showed no significant difference in total phenols. The richness of flavonoids and saponins in pulps from trees of southwest may be due to environmental conditions. In fact, the carob trees in the southwestern regions, with high temperatures and less precipitations, probably reacted to this by increasing the pool of flavonoids [16]. Ruiz et al. [17] have reported that changes in the content of saponins depend mainly on the variety, origin and the region wherein the plant is growing. The high flavonoids content in carob pulp may increase the commercial value of the carob tree. It is well known that flavonoids have multiple biological activities: anti-carcinogenic, anti-inflammatory and anti-viral [18, 19]. They are also excellent antioxidants and free radical scavengers [20, 21].

Regarding the content of flavonoids in seeds, no significant difference was observed between the three regions despite the involvement of environmental conditions and genetic factors in their production rate [22]. However, the seeds from the trees of the north have a rate of total phenols higher than those from the trees in the center and southwest of Morocco. This may be due to the carob variety as confirmed by Nassourou et al. [22]. They showed that the content of phenolic compounds in plants was affected by germination, maturity, variety, processing and storage of seeds.

For the other studied parameters such as total nitrogen, total protein, total sugars and total phenols, the differences were not significant between the pulps of the three studied regions (Table 3). Also, no significant differences were observed between the carob seeds of the three regions for water and fat contents (Table 4). Thus, the physicochemical composition of the carob pulp from trees in north, center and southwest of Morocco differs only for the content of

Fig. 1 Dendrogram of morphometric traits of pods and seeds of the three studied carob locations

Table 3 Carob pulps' physicochemical parameters of the three studied locations

	Humidity in %	Ash in %	Total nitrogen in %	Total proteins %	Fats in %	Total sugars in mg/g	Total phenols in mg/g	Total flavonoids in mg/g	Saponins
North	13.28 ± 1.85 ^x	3.79 ± 0.71 ^x	0.53 ± 0.12 ^x	3.35 ± 0.79 ^x	0.58 ± 0.4 ^x	416.16 ± 130.53 ^x	8.07 ± 5.95 ^x	0.17 ± 0.13 ^x	83 ± 37 ^x
Center	13.21 ± 1.54 ^x	3.37 ± 0.65 ^y	0.62 ± 0.14 ^x	3.88 ± 0.93 ^x	0.38 ± 0.21 ^y	324.87 ± 116.05 ^x	9.41 ± 5.67 ^x	0.32 ± 0.13 ^y	56 ± 27 ^y
Southwest	11.5 ± 2.64 ^y	3.01 ± 1.09 ^y	0.53 ± 0.16 ^x	3.34 ± 1.02 ^x	0.5 ± 0.21 ^x	414.64 ± 142.32 ^x	7.35 ± 3.95 ^x	0.6 ± 0.26 ^z	111 ± 48 ^z

Means with the same letter(s) in the same column are not significantly different at the 0.05 level

Table 4 Physicochemical parameters of the carob seeds of the three studied locations

	Humidity in %	Ash in %	Total nitrogen in %	Total proteins %	Fats in %	Total sugars in mg/g	Total phenols in mg/g	Total flavonoids in mg/g	Saponins
North	12.45 ± 0.72 ^a	4.04 ± 0.77 ^a	3.59 ± 0.47 ^a	22.42 ± 2.98 ^a	2.54 ± 0.95 ^a	66.35 ± 18.38 ^a	0.67 ± 0.21 ^a	3.25 ± 1.57 ^a	96 ± 22 ^a
Center	12.7 ± 3.42 ^a	4.57 ± 0.62 ^b	3.85 ± 0.36 ^b	24.08 ± 2.28 ^b	2.57 ± 1.21 ^a	92.09 ± 39.5 ^b	0.86 ± 0.38 ^a	2.05 ± 1.08 ^b	101 ± 34 ^a
Southwest	12.19 ± 1.01 ^a	3.9 ± 0.57 ^a	4.03 ± 0.72 ^b	25.2 ± 4.51 ^b	2.19 ± 0.58 ^a	93.57 ± 38.47 ^b	0.74 ± 0.52 ^a	2.92 ± 1.21 ^a	131 ± 44 ^b

Means with the same letter(s) in the same column are not significantly different at the 0.05 level

Fig. 2 Discriminant factor analysis of the three studied locations based on the pulps (A) and the seeds (B) physico-chemical parameters

flavonoids and saponins. This is confirmed by the factorial discriminant analysis (FDA), which shows an overlap between the three studied regions (Fig. 2). Our results are in agreement with those published by Avallone et al. [23] and Yousif and Alghzawi [24] for the water content, total fat, total protein and total sugars present in the pulp of the carob tree. The values of ashes determination were in general equal to values found by Calixto and Canellas [25], Avallone et al. [23] and Yousif and Alghzawi [24]. They found an ash content ranging between 1 and 6% [23–25]. The sugar content of the pulp of the three regions of Morocco is all in agreement with the content presented by Vekiaria et al. [26]. With respect to the content of the pulp proteins in the trees from the eight localities studies, the obtained results were all in agreement with those reported by Yousif and Alghzawi [24] and Santos et al. [27]. However, these results were lower than those found by Biner et al. [28].

4 Conclusion

Environmental factors seem to have an important effect on the morphometric characters of pods and seeds from the carob tree investigated in this study. The environment in the northern exposure and rain appear to improve the mass, length, width and thickness of the pods compared with the other regions such as the center and southwest. They also promote the mass of seeds per pod, mass of the pulp, peduncle length, width, length and thickness of the seeds.

The increase in mass of the pulp and the seeds of the carob tree in the localities with heavy rain could increase the commercial value of the fruit of the carob tree and may increase the income of land “bours” farmers.

The physicochemical studies showed that the pulps of the carob tree in southwestern Morocco have a higher content of flavonoids and saponins compared to the pulps of carob trees in the center and northern regions. These components have specific bioactivities, which could indicate a potential use of the southwest carob pulp in the pharmaceutical, food and medicinal plants.

The statistical analysis showed that the environmental conditions have acted the quality of carob pods. Carob trees are excellent for the population in the arid areas; indeed, these evergreen trees, widely cultivated for the edible fruit, could also be used against the desertification.

This research highlights the importance of carob tree domestication. This species does not require special cultural care and produces even during a drought year. Carob tree has a role in the sustainable development of rural people and is a real source of profit for arborists.

This kind of study rich the research on the carob trees and add novel data on this subject.

Acknowledgements The authors warmly thank Dr. Helle RAVN WEBER, Ph.D. (Silkeborg, Danemark), for the English proofreading of the manuscript.

Compliance with ethical standards

Conflict of interest All the authors declare that: this research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors, they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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