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Morphological and Biochemical Variability of Moroccan Carob (*Ceratonia siliqua* L.) Produced in Beni Mellal Region

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ABSTRACT

The carob is the fruit of an evergreen (Ceratonia Siliqua L.) *cultivated in the Mediterranean area. The carob tree is an important economic resource for Morocco's rural populations. This species is used in reforestation actions and its cultivation in modern orchards is being undertaken to valorise marginal lands and substitute for drought sensitive species. However, little data is available on its intra-specific variability and its adaptability. Morphological criteria (pod length, width, thickness, seed number, volume, weight and specific gravity) and biochemical composition (protein, sugar, fibre, ash and total phenol content) of carob varieties from three different regions of Beni Mellal in Morocco were investigated. The obtained results showed that significant differences were found between ecoregions for all characters which were examined, what indicates a high intra-diversity. Correlations studies between morphological criteria and biochemical characteristics were carried out and their variabilities are discussed in this paper.*

Key words: *Carob, Ceratonia Siliqua L., pulp, seed morphological characteristics, Biochemical characteristics, Morocco.*

INTRODUCTION

The carob tree (*Ceratonia siliqua* L.*)* is an angiosperm, dicotyledonous belonging to the order *Rosales*, family *Fabaceae*. It tolerates drought explaining its large distribution in the arid and semi-arid Mediterranean climate[7,13]. The carob tree is widely used in the Mediterranean regions cultivated for ornamental and industrial purposes[29].

World production is estimated at about 315 000 tons per year, produced from about 200 000 hectares with very variable yields depending on the cultivar, region, and farming practices[17] and the main producers for (pulp, seeds) respectively are Spain (36%, 28%), Morocco (24%, 38%), Italy (10%, 8%), Portugal (10%, 8%), Greece (8%, 6%), Turkey (4%, 6%) and Cyprus (3%, 2%) of the world production[1].

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Carob tree has an economic and environmental importance in Morocco. It is used in reforestation of arid and degraded areas and also as for ornamental purposes[14,28]. The pulp and the seeds have some interesting properties and are often used in food and pharmacological industry[5]. Chemical composition of the carob pod depends on cultivar, origin and harvesting time2. The two main carob pod constituents are (by weight): pulp (90%) and seed (10%)26.

The pulp of carob pods (fruit of Ceratonia siliqua L.) contains a high content of sugar (40–60% of total sugar), predominantly sucrose, which constitutes about 30%. It is low in protein (3–4%) and lipids (0.4–0.8%)23. The pods also contain a high amount of dietary fibre and polyphenols. The pulp of carob pods is used extensively as a raw material for the production of syrups21,22. The pulp and the seeds are valorised in different applications. Carob fruits can be made into syrup, honey, bean meal powder, and alcohol. Carob alcohol is used in the pharmaceutical industry and in wine; carob honey is used in cakes and pastries and as a sweetener for compotes and jams; and carob powder is used in baby foods and baked goods.

Moreover, biochemical composition of carob had been studied for different countries of the Mediterranean area. It had been observed that this composition is depending not only on technological factors such as the extraction and analytical methodologies, but also on the genotype of the plant, the geographical origin, the climate conditions and the harvesting and storage procedures5,6,19,20,24. However, in spite of the great interest to carob and their use in different applications, few studies are available on Moroccan carob especially in Beni Mellal region. So to select the best varieties, an intensive investigation on the morphological and biochemical composition for the different carob Moroccan varieties is needed. Based on the above considerations, the main objective of the current study was to assess morphological characteristics and biochemical composition of Moroccan carob produced in Beni Mellal region in order to propose the best characteristics cultivars that can be helpful for the development of new orchards with the best agro industrial profitability.

MATERIALS AND METHODS

Sampling and experimental method for morphological and biochemical analysis

Three Moroccan carob wild type populations were randomly collected from various geographic sites from Beni Mellal region (Figure 1). In every area, 10 trees were randomly chosen for collection of composite samples. 20 pods from each zone were randomly chosen to measure the different morphological parameters. The seeds were removed and the carob was ground to particles of 0.5 ≤ mm for biochemical analysis. Samples were analysed within 2 months.

Morphological analysis

For twenty pods of each zone, following parameters have been measured: weight, length, width, thickness, volume, number of seeds, specific gravity, size index, number of seeds /pod, seeds weight /pod and % seed /pod.

Length (cm) of pod was measured using a measuring tape, whereas width (cm) was assessed with the Vernier calliper (top, middle, and bottom of pod). Thickness (cm) was evaluated with Iwanson gauge (1110 mm) where three parts of the top, middle and bottom of the pod were covered. Weight (g) of pods (seeds and pulp) were taken using a top-loading balance. Volume (cm^3) was estimated by submerging carob pod in a known volume of water inside a graduated cylinder (1000 cm^3). Size index was determined as the ratio of length over width and specific gravity is the density (pod weight /pod volume) of carob pod over density of water[16].

Biochemical analysis

To determine the chemical composition of carob pulp (contents of total polyphenols, total sugars, fibre, protein and ash content), samples from seedless pods of morphological measurements were crashed, and then grounded into powder using a hammer mill (diameter less than 0.5 mm). Extracts were prepared as follows: 1 g of carob powder was mixed with 20 ml of water and 20 ml of acetone in a reactor at room temperature (20 – 22°C, 30 min).

a) Determination of total phenols contents

Total phenolic compounds were determined colorimetrically at 760 nm and expressed as Gallic acid equivalents, according to the method described by Singleton *et al*[25]. Samples were added to Folin-Cioccalteu reagent and sodium carbonate solution solution and placed in the dark for 15 min before spectrophotometric analysis.

b) Determination of sugar contents

Total sugars were determined colorimetrically at 480 nm according to the method described by Dubois *et al*[10]., Standards were prepared with glucose solutions at different concentrations.

c) Determination of protein contents

Total nitrogen of carob powder was determined according to the AOAC official method 955.04[4] using a Macro Kjeldahl digestion and distillation apparatus.

d) Determination of fibre contents

Four grams of carob powder were digested with 200 ml of 5% HCL for 30 minutes. The mixture was filtered and washed with hot water. Then, residue was digested with 200 ml of 5% NaOH under reflux for 30 min. The mixture was filtered and washed with distilled water until neutrality of pH. The material was washed with 20 ml of ethyl alcohol and 20 ml of ethyl ether. Finally, the residue was dried at 100°C for two hours and the residual mass was considered fibers[8].

e) Determination of fat contents

The percentage of fat is determined after successive extractions by an azeotropic mixture of toluene: ethanol (38: 62). The samples are subjected to continuous extraction for 24 hours using a soxhlet. The mass of extracted fat is determined by weighing after evaporation of the solvent[11].

f) Determination of ash and moisture contents

The ash content of the carob powders was determined according to the AOAC official method 972.15[3]. Moisture was determined according the procedure of AFNOR.

Statistical Analysis

The numerical values obtained from various parameters measured were analysed by SPSS software version 20 to calculate averages and standard deviations (±SD). All measurements were run in triplicates (n=3), unless elsewhere specified. Furthermore, correlation between morphological and biochemical parameters were evaluated using Pearson's correlation coefficient.

RESULTS AND DISCUSSION

Morphological characteristics

The mean and standard deviations of all measured values are assigned to variables in the table 1. At the outset, we note that each criterion pod analysed can be considered as a distinctive means of a shaft to another.

Among the morphological characteristics, different notions can be distinguished: the pod size (which gathers pod length, width, thickness and size index), the pod weight (mass, volume, specific gravity) and the seed yield in a pod (number, weight).

a) The pod size

The pod length varied from 12 ± 1.95 cm to 15 ± 1.87 cm. The longest were Ait Oum El Bakht, the shortest were Dir Elksiba. According to Tutin *et al*[27]., Tous *et al*[26]., and Batlle *et al*[5]., who reported that the average pod size may range from 10 to 30 cm and classified length pods into three categories: Slightly long cut (15 <L ≤20 cm), Mean size with (14 ≤ L ≤15 cm) and slightly short size with (10 ≤ L <14 cm), Dir Elksiba and Naour are considered slightly short size (12± 1.1 and 12.3 ± 1.95cm). However, Ait Oum El Bakht are considered mean size (15 ± 1.87cm). These results are consistent with those of Elbatal *et al*[11]., who obtained 11.83± 4.3 cm as the mean value of the carob tree fruit originating in the Beni Mellal region and show that there is considerable variability in fruit size of the Moroccan carob tree.

The width of the carob bean pods has an important agronomic indication. It is independent of pod size and can provide information not only on its compressed or expanded condition, but also on the volume of seeds and pulp. It varies from 1.5 to 2.5 cm according to Tutin *et al*[27]., and from 1.5 to 3.5 cm according to Batlle and Tous[5]. The pods of Ait Oum ElBakht are quite wide (2.1± 0.3 cm). While the smallest widths were found in those of the region of Naour (1.43 ± 0.13 cm) followed by Dir Elksiba (1.76± 0.23 cm). Moreover, Elbatal[12] reported that the Beni-Mellal region is characterized by carob trees whose pods are wider than 1.54cm. However, Ait Oum Elbakht had a wider pod then this average.

For the pod thickness, it is also highly variable from one region of origin to another and is a criterion for distinguishing compressed or bulky pods. It can reach 1 cm especially in the fleshy pods[5]. This variable allowed us to distinguish cultivars characterized by voluminous pods, namely those of Dir Elksiba (1.37cm), other cultivars with flattened or compressed pods: Ait Oum Elbakht (0.69cm) and Naour (0.4cm). In addition, Elbatal[12] (2015) reported 1.54cm as the mean value of the pods thickness from the Beni Mellal region. These results showed that Ait Oum Elbakht was the taller and the wilder but the less pulpy one. This result was also found by other authors; wild types are known for their non-fleshy pulp with higher seed production and higher seed to husk ratio[9,15].

b) The pod weight

For the pod mass and volume, classification was the same; all the volume is full of matter. Naour got the smallest mass and volume (4.3g; 5.32 cm^3) whereas Ait Oum Elbakht and Dir Elksiba got respectively (7.2g; 6.7g) in weight and (7.5 cm^3; 6.7 cm^3) in volume. Ait Oum Elbakht was the heaviest and has the highest volume. However, the ratio between mass and volume leads to a different classification. Ait Oum Elbakht has a high mass and volume has a specific gravity inferior to Dir Elksiba. Since Ait Oum Elbakht was the longest so for sure it occupies a larger space (volume) but it doesn't mean that it was the denser. Since density is the amount of matter crammed into a given space, obviously and statistically, Dir Elksiba is presented as the Jess juicy pulp among the other carob types. So pods of Dir Elksiba are fully denser than big pods of Ait Oum Elbakht.

c) Seed yield in a pod

According to results presented in table1, the seed number and weight give the same classification: Dir Elksiba had the highest seed number followed by Ait Oum Elbakht and Naour. (12.5; 11.8; 10.67 respectively). The parameter % seeds yield /pod gives different classification: Naour has the highest value then Dir Elksiba and Ait Oum Elbakht has the lowest value. This is explained by the fact that, the type Naour has small seeds weight and the small pod weight among all carob pod varieties.

Data obtained from this study showed a very significant diversity in the yield of pulp and seeds of carob in Beni Mellal region. Moreover, Moroccan crops are largely characterized by high seeds yield average [17.47-29.44%] content and medium pulp yield average [71.30-82.30%][12]. Spanish, Tunisian, Portuguese and Turkey crops produce low to medium seeds yield and medium to high pulp yield. Furthermore, the results show a great intra-specific variability between the populations of the Beni Mellal carob tree. This diversity seems to take place according to the geographical origin of the population.

d) Statistical analysis

The Pearson correlation coefficient was calculated to objectively interpret and compare the morphological data of the carob samples and evaluate the most important variables able to discriminate carob.

The correlations between the analysed agro-morphological traits are summarized in table 3. Pod length is correlated positively with the pod width but negatively with thickness and % seeds/ pod with respective linear regression coefficients of r = 0.822, -0.822 and -0.957 respectively. Furthermore, pod width was positively correlated with the pod weight and volume but also negatively correlate with % seeds/pod (r = 0.932, 0.987 and – 0.952). The pod weight is also correlated with the most criteria pod and seeds, except the pod length, thickness (r = 0.560, - 0.013). Moreover, Volume is also correlated with all criteria except the pod thickness (r = **-** 0.221).

Biochemical characteristics

Table 2 presents the seven variables describing biochemical characteristics of the carob pulp. as in the literature, we found that carob pulp is rich in polyphenol for the three regions (7.64 ± 0.1 mg/100g of dry matter for Dir Elksiba to 4.53 ± 0.8 mg/100g of dry matter for Ait Oum Elbakht, for Naour 3.7 ± 0.3 mg/100g of dry matter) with very significant differences. the rate of moisture in the pulps carob of the three different regions in Beni Mellal was estimated at 16.94% to 8.3%. the rate of fibre content is substantially the same for the pulp: Ait Oum Elbakht 10.3 g/100g of dry matter, Naour 10.2 g/100g of dry matter , Dir Elksiba 10.05 g/100g of dry matter , but no significant difference. the carob is rich in total sugar which earned him his very sweet flavour and its use as animal feed sugars fruit. the results of our study show that the rate of total sugars in the pulp of Ait Oum Elbakht (37.9±2.3 g/100g of dry matter) is slightly higher than Naour (31.6 ±0.1 g/100g of dry matter) and Dir Elksiba is the lowest (26.28±3.1 g/100g of dry matter) (table 2). moreover, the protein analysis showed insignificant differences: Dir Elksiba (4.3±0.21 g/100g of dry matter) ; Naour (4.3±1.91 g/100g of dry matter) ; Ait Oum Elbakht (4.1±0.3 g/100g of dry matter). more results revealed that the differences between the rate of fat seem to be relatively insignificant: Naour (0.46±1.3 g/100g of dry matter); Dir Elksiba (0.58±0.07 g/100g of dry matter); Ait Oum Elbakht (0.66±0.05 g/100g of dry matter).

Correlation between carob characteristics

The correlation matrix between morphological and biochemical parameters were summarized in table 3. Pearson’s coefficients were calculated and significant differences were found. The correlation analyses established by region provided a specific understanding about the way how fruit characteristics correlates within cultivar. Significant correlations were found between characteristics describing pod size, pod weight and seed yield and those describing biochemical composition. Thus, pods length, width, weight and volume have an important positive correlation with moisture values. The rate of ash correlates negatively with width, volume and weight of pods. Moreover, fibre and total sugar correlate positively with length and negatively with thickness and seeds/pods ratio (%). The protein values correlate negatively with both length and width. In addition, total phenol correlates positively with thickness, seeds number and weight, so high polyphenol content is established by a high seeds yield. However, fat value correlate with all pod size and pod weight except for thickness.

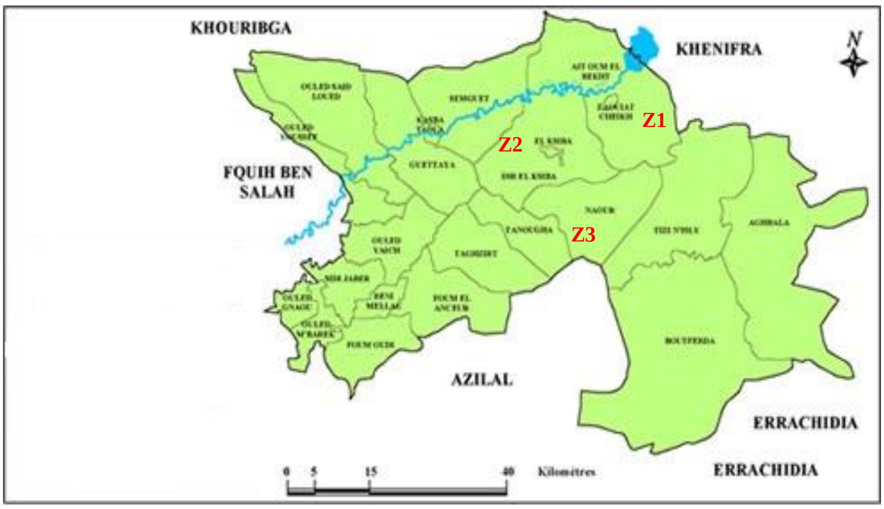

Fig. 1: Repartition map of selected Moroccan carob populations collected from Beni Mellal region - Zl: Ait Oum Elbakht carob population; Z2: Dir Elksiba carob population; Z3: Naour carob population (METLW, 2017)

Table 1: Mean values of the Physical measurements of carob pods populations

	Length (cm)	Width (cm)	Thickness (cm)	weight (g)	Volume (cm^3)	Specific gravity	Size index	Nb seeds/pod	Seeds weight (g)	% seeds/pod
Ait Oum El Bakht	15±1.5	2.1±0.3	0.3±0.25	7.2 ± 0.18	7.5 ±0.19	0.96 ±0.03	7.14 ±0.72	11.8 ±1.3	1.8 ±0.23	25 ± 2.82
Dir Elksiba	12± 1.1	1.76±0.23	0.57 ±0.12	6.7 ± 0.1	6.7 ± 0.24	1 ±0.02	6.81 ± 0.8	12.5 ± 1.5	2 ± 1.1	29.85 ± 1.45
Naour	12.30 ± 1.95	1.43 ± 0.13	0.4 ± 0.10	4.3 ± 1.36	5.32 ± 1.91	0.80 ± 0.10	8.6 ± 1.30	10.67 ± 1.86	1.34 ± 0.36	31.16 ± 3.21

Table 2: Mean values of the biochemical measurements of carob pods populations

	Moisture (%)	Ash (g/100g of dry matter)	Total Sugar (g/100g of dry matter)	Fibre (g/100g of dry matter)	Protein (g/100g of dry matter)	Total phenol (mg/100g of dry matter)	Fat (g/100g of dry matter)
Ait Oum ElBakht	16.94±1.8	3.13±0.1	37.9±2.3	10.3± 0.2	4.1±0.3	4.53±0.85	0.66±0.05
Dir Elksiba	11.4 ± 2.05	3 ± 0.5	26.28 ± 3.1	10.05±0.3	4.3±0.21	7.64 ± 0.1	0.58±0.07
Naour	8.30 ± 1.5	3.63 ± 0.3	31.6 ± 0.10	10.2±0.3	4.30 ± 1.91	3.7±0.30	0.46 ± 1.30

Table 4: Pearson's correlations between morphological and biochemical parameters

	Length (cm)	**Width (cm)**	**Thickness (cm)**	**weight (g)**	**Volume (Cm3)**	**Nb seeds/pod**	**Seeds weight (g)**	**% seeds/pod**	**Moisture**	**Ash**	**total sugar**	**Fiber**	**Protein**	**Total phenol**	**Fat**
Length (cm)	1.000	.822	-.836	.560	.720	.044	.132	-.957	,899	-,234	,927	,854	-,996	-.402	.746
Width (cm)	.822	1.000	-.374	.932	.987	.605	.673	-.952	.988	-.746	.549	.405	-.870	.191	.992
Thickness (cm)	-.836	-.374	1.000	-.013	-.221	.512	.434	.641	-.511	-.338	-.981	-.999	.782	.839	-.257
weight (g)	.560	.932	-.013	1.000	.978	.852	.895	-.776	.866	-.936	.209	.047	-.633	.534	.970
Volume (Cm3)	.720	.987	-.221	.978	1.000	.725	.783	-.890	.951	-.843	.408	.253	-.780	.346	.999
Nb seeds/pod	.044	.605	.512	.852	.725	1.000	.996	-.332	.477	-.982	-.334	-.483	-.134	.897	.699
Seeds weight (g)	.132	.673	.434	.895	.783	.996	1.000	-.414	.553	-.995	-.249	-.403	-.222	.855	.759
% seeds/pod	-.957	-.952	.641	-.776	-.890	-.332	-.414	1.000	-.987	.506	-.779	-.666	.979	.119	-.907
Moisture (g/100g)	.899	.988	-.511	.866	.951	.477	.553	-.987	1.000	-.636	.670	.540	-.935	.040	.962
Ash (g/100g)	-.234	-.746	-.338	-.936	-.843	-.982	-.995	.506	-.636	1.000	.147	.307	.321	-.796	-.822
total sugar (g/100g)	.927	.549	-.981	.209	.408	-.334	-.249	-.779	.670	.147	1.000	.987	-.889	-.716	.442
Fiber (g/100g)	.854	.405	-.999	.047	.253	-.483	-.403	-.666	.540	.307	.987	1.000	-.803	-.820	.289
Protein (g/100g)	-.996	-.870	.782	-.633	-.780	-.134	-.222	.979	-.935	.321	-.889	-.803	1.000	.317	-.803
Total phenol (mg/100g)	-.402	.191	.839	.534	.346	.897	.855	.119	.040	-.796	-.716	-.820	.317	1.000	.311
Fat (g/100g)	.746	.992	-.257	.970	.999	.699	.759	-.907	.962	-.822	.442	.289	-.803	.311	1.000

CONCLUSION

Knowledge of the genetic variability is essential in plant breeding. The identification of this genetic variability in certain morphological traits is the essential first step in the description of genetic resources. The present study revealed that analysis of diversity in agro-morphological local populations of carob tree (*Ceratonia siliqua* L.) in Morocco (Beni Mellal region) showed significant differences between the analysed traits. These data are in perfect agreement with the results of some work carried out in Morocco, which showed that significant differences were found between ecoregions for morphological and biochemical characters which were examined, what indicates a high phenotypic diversity.

Moreover, this study had brought supported information about Beni Mellal cultivars in order to help studying technological transformation with best performing varieties. Furthermore, studies are necessary to fully elucidate the relation between morphological, chemical and geographical characteristics to reveal the origin of this diversity and help for choosing appropriate agriculture conditions to plant carob tree with industrial interest

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