Evaluation of Pod and Seed Diversity and Characterization of Carob Tree (*Ceratonia siliqua* L.) Cultivars in Bouznika region Morocco

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Abstract
The carob tree (Ceratonia siliqua L.) 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 valorize marginal lands and substitute for drought sensitive species. Fifteen cultivars carob tree from Bouznika (Morocco) were studied to assess their genetic variation based on pods and seeds measures.

The mean of the main descriptive morphological values of pods was weight (8,34g), length (13,2cm), width (1,69cm), thickness(0,66cm), number of seeds/pod (12,92), pulp weight (6,27g), seed lenght (0,88cm), seed width(0,66cm), seed thickness(0,42cm), seeds weight (2,14g) and seed yield (25,89%).The relationship among these characters was analysed by principal component analysis (PCA) and hierarchical cluster resulting in the separation of these cultivars classed in three grouped and two ungrouped populations.

Keywords: *Ceratonia siliqua* L., Pods, Seeds and Morphological diversity.

Introduction
The carob tree has been grown since antiquity in most countries of Mediterranean basin, usually in mild and dry places with poor soils. The carob tree is an important component of the Mediterranean vegetation and its cultivation in marginal and prevailing calcareous soils of the Mediterranean region is important environmentally and economically.[9] The carob tree is an evergreen, dioecious plant species,[23,31] sometimes hermaphrodie and rarely monoecious.[9]

Moreover, male trees are sterile and unproductive.[37] This species plays a significant role in protecting soil against degradation and erosion[20,40,41] and in combating desertification, thanks to its deep roots and evergreen leaves. This is a multipurpose and industrial fruit trre spicies that has high capacity to resist drought.[1,33,37,44] All parts of the carob tree, i.e. foliage, flower, fruit wood, bark and root, possess amazing properties. This tree has ornamental and landscape values. The fruit of carob is a brown pod, consituted of pulp (90%) and seeds (10%), the pulp contain many bioactive substances including carbohydrates, proteins, dietary fibres that may be used in the pharmaceutical industry[42] because they have numerous positive effects o blood sugar levels, cholesterol level and cancer[19] and polyphenols. The roasted powder of this part (carob powder) can be substituted for cocoa.[22,39] The seeds consisted of three parts ; germ,endosperm and husk.[4,14]

The domestication of Ceratonia siliqua based on spontaeous populations, aiming at the production of large fruits with high sugar content for human and animal nutrition resulted in a limited number of cultivars.[30] More recently, domestication of some wild uncultivated trees has been developed with the purpose of increasing kernel yield and gum quality for industrial exploitation.[27]

In Morocco, the carob tree occupies an area of 30000 ha, this form of natural artificial plantations in the country is up to 1150m except very arid;[12] It is foound in the western and eastern Rif.[1] The main population spontaneous carob is located in the regions of Meknes, Agadir, Essaouira, Taza, El Hoceima and Khenifra, in association with olive (*Olea europea*), mastic (*Pistacia lentiscus*), cedar (*Junepurus phoenicea*) and argan tree (*Argania spinosa*).[2]

World carob production is estimated at 191355.64 tonnes[18]. It is mainly concentrated in Spain, Italy, Morocco, Portugal, Greece, Turkey, followed by Cyprus, Algeria, Lebanon and Tunisia last.[18] In Morocco, production has increased slightly, fromm 20000 tonnes in 2012 to 21983 tonnes in 2017.[18]

Morphological characters of pods and seeds are the most valuable and quantitative marker widely used to identify carob varieties.[16] Carob pods are characterized by a high sugar content (48-56%)[21,28] and are a good source of protein, fiber and minerals,[5,13] the different rates of carob chemical composition are depending on the geographical origin, climate conditions and mainly on the genotype.[9,35]

In selection of breeding studies, even method seems easy, application is very inconvenient and requires lots of attention. A vast number of characters according to breeding objective can be worked; in this situation, much time and workforce can be needed. Therefore, to know some relationships between characters will decrease workload by providing working on less character. For this purpose, some studies hase been conducted on carob genotypes.[6,7,11,15,16,25,32,34,35,38]

It is important to note that the different types of carob tress are not well known in Morocco. The present work proposes to etablish a morphological study of several cultivars fo carob pods and seeds of populations carob and determine the variablitity that exists in the species and also to select and recommend the most suitable plantation that is of interst to the agro-food industries.

Material and Methods

Plant material: Fifteen cultivars of cultivated carob were localized in region of Bouznika North-West in Morocco. The plant material consists of pods of carob tree. Thirty pods were taken randomly from each carob tree (*Ceratia siliqua* L**.**).

Morphological analysis of fruit: The selection of the fruit pods and seeds for characterization was done by adapting the International Plant Genetic Ressources Institue (IPGRI) descriptors.[9] Mature pods were collected of each cultivar. For pods each cultivar we have estimated the length (cm), width (cm), thickness (cm), pod weight (g), pupl weight (g), number of seed/pod, yield ((Seeds weight/pod weight) x 100). For ten randomly selected viable seeds from each cultivar, the weight (g), length (cm), width (cm) and thickness (cm) were measured.

Statistical analysis: Analysis of morphological and chemical variations was based on the totality of the character measurement related to pods and seeds. Variations were analyzed using ANOVA-one way after testing for normality and homogeneity of variance.

Correlation between morphological and chemical parametrs was evaluated using Pearson's correlation coefficient.[36] Ecoregions ordination and classification were perfomed using the principal component analysis (ACP) and the hierarchical cluster analysis respectively. The ACP was performed on the matrix of mean values of measured characters while the hiearchical cluster was based on Pearson's correlation matrix.

The statistical analysis of the data was carried out using the SPSS software windows version 22. Matrices generated for the different morphological traits are analyzed using software version 3.2.1 MVSP using the UPGMA method (Hierarchical clustering).

Results

Morphological analysis: Morphological traits related with pod's size seem to be the most variable. In fact, pods length varied among variaties from 10.6 cm for P4 to 15.48cm for P5, the mean seed number varied from 10.14 for P1 to 14 .4 for P9 and the seed yield showed the highest variation, since it varied from 21.2% for P2 to 34.56% for P7. In addition, seed characteristics also varied between variaties.

Mean values correcponding to seeds length, with thickness and weight varied respectively from 0.76 (P3) to 0.97 (P11) ; 0.36cm (P15) to 0.47 cm (P5, P9 and P10) and 1.41g (P3) to 2.86 g (P12). It is worth noting that the widest pods are 011 with a value of 2.01 cm and the thinnest are P3 with an average value of 1.34cm.

Note that the least thick po dis that of P14, its value is about 0.58cm. The pods from P5 are the heaviest with a weight of 10.31g. Note the weights of pulp from P2 and seeds from P12 as 9.53g and 2.86g respectively. On the other hand, the smallest weight was obtained with pods from P3 with an average value of 5.20g, the pulp weight is 3.61g and that of seeds 1.41g (table 1).

Correlation among all morphological traits is summarized in table. Pod width is correlated positively with pod weight, pulp weight, seed lenght, seed width and seed weight with respective linear regression coefficients of r=0.737, 0.659, 0.800 and 0.653. Further, pod lenght showed a positive correlation (r=0.664) with seed number/pod. Seed weight is also correlated positively with pod width, pod weight, pulp weight, seed lenght and seed width respectively r=0.653, 0.743, 0.520, 0.769 and 0.754. Pod weight is correlated highly positively with pulp weight, seed lenght, seed width and seed weight with respectively r=0.951, 0.793, 0.809 and 0.743 (table 2).

The plot PCA identified two principal component (PC) that explained 73.60% of the total variance. The first axis can be interpreted as an expression of pod characteristics and yield, it accounted for 48.40% of total variation as in fig. 1. The highest loadiong was pod weight, seed width, seed lenght, pulp weight and pod width (Table 2).

The second axis expained 25.20% of total variance and is related to quantity expressed in terms of seed number/ pod and pod length, with positive signs and pod thichness with negative signs (Table 2).

Principal component and cluster analysis discriminated the sample cultivars in three clusters (Fig.1) using the first two principal components and accounted for about 73.60% of the total variability among the carob cultivars, based on fruit and seed traits respectively.

Cluster I including P9, P7, P6, P8, P13 and P14 is plotted on the left-lower and right-lower quandrant. Cluster II consisted of cultivars P5 and P10. The cultivars P1, P2, P11, P12 and P15 of cluster III are placed on the upper left quandrant (Fig.1). The cultivar P3 was plotted ungrouped on the left-lower quadrant. The cultivar P4 were plotted ungrouped in the left part of the graph, they were positively correlated to PC2.

Table 1
Results of morphologic study of several cultivars of carob tree (*Ceratonia siliqua* L.)

	Length of pod (cm)	Width of pod (cm)	Thickness of pod (cm)	Seeds number/pod	Weight of pod	Weight of pulp	Yield (%)	Length of seed (cm)	Width of seed (cm)	Thickness of seed (cm)	Weight of seed (g)
P1	13,39±0,98	1,85±0,1	0,64±0,03	12,14±1,95	7,74±1,20	5,8±1,03	24,16	0,93±0,04	0,7±0,04	0,42±0,04	1,87±0,23
P2	13,5±2,08	1,81±0,12	0,78±0,04	12±2,68	9,53±2,6	7,95±1,7	21,3	0,9±0,04	0,68±0,04	0,38±0,04	2,03±0,48
P3	11,88±1,43	1,34±0,17	0,59±0,06	11,6±1,34	5,2±1,06	3,61±0,78	27,11	0,76±0,06	0,58±0,02	0,41±0,03	1,41±0,25
P4	10,6±0,3	1,61±0,2	0,79±0,07	11,6±1,7	7,98±1,1	6,08±0,9	22,93	0,89±0,06	0,62±0,03	0,45±0,02	1,86±0,27
P5	15,48±0,72	1,81±0,06	0,64±0,03	14,17±0,89	10,31±0,49	7,64±0,39	25,22	0,96±0,03	0,73±0,02	0,47±0,03	2,6±0,18
P6	13,84±1,85	1,54±0,12	0,66±0,06	12,8±2,28	7,41±1,32	5,53±1,01	24,83	0,79±0,07	0,61±0,04	0,41±0,03	1,84±0,38
P7	13,72±1,66	1,62±0,05	0,61±0,04	14±2,90	7,59±1,30	5,26±0,87	34,56	0,86±0,05	0,64±0,04	0,43±0,03	2,32±0,42
P8	14,06±0,83	1,62±0,10	0,67±0,04	14,4±1,82	8,8±1,08	6,78±0,75	28,72	0,9±0,05	0,66±0,02	0,42±0,02	2,18±0,50
P9	12±1,18	1,53±0,11	0,68±0,09	12,67±1,53	7,22±0,19	5,15±0,20	23,29	0,81±0,04	0,65±0,03	0,47±0,04	2,05±0,14
P10	14,95±0,79	1,73±0,13	0,64±0,05	13,5±1,0	9,39±1,37	6,93±0,92	26,2	0,95±0,04	0,73±0,04	0,47±0,04	2,46±0,30
P11	12,9±0,62	2,01±0,04	0,62±0,03	13,25±0,96	8,6±0,69	6,32±1,03	27,62	0,97±0,04	0,69±0,03	0,4±0,02	2,42±0,35
P12	12,08±0,45	1,86±0,10	0,74±0,06	12,2±2,10	9,33±1,60	6,45±1,10	30,65	0,96±0,03	0,7±0,01	0,44±0,02	2,86±0,62
P13	12,38±0,88	1,61±0,15	0,65±0,03	13,75±2,02	7,99±1,18	5,97±1,10	26,53	0,87±0,03	0,63±0,02	0,39±0,01	2,12±0,61
P14	15,42±1,03	1,62±0,12	0,58±0,05	13,4±1,90	8,62±1,23	7,1±1,08	22,97	0,84±0,03	0,65±0,01	0,4±0,03	1,98±0,39
P15	12,83±0,90	1,83±0,06	0,67±0,03	12,33±0,60	9,4±1,13	7,43±0,81	22,34	0,9±0,04	0,67±0,02	0,36±0,03	2,1±0,17
Mean values	13,27±1,38	1 ,69±0,17	0,66±0,06	12,92±0,93	8,34±1,25	6,27±1,12	25,89±3,51	0,88±0,06	0,66±0,04	0,42±0,03	2,14±0,35

Table 2
Factor loadings for each variable on the components of PCA analysis

Variable	PC1	PC2
Pod length (cm)	,537	**,655**
Pod width (cm)	**,847**	-,200
Pod thichness (cm)	,181	**- ,844**
Seed number/pod	,391	**,804**
Pod weight (g)	**,958**	-,089
Pulp weight (g)	**,890**	-,163
Yield (%)	,165	,569
Seed lenght (cm)	**,889**	-,113
Seed width (cm)	**,915**	,037

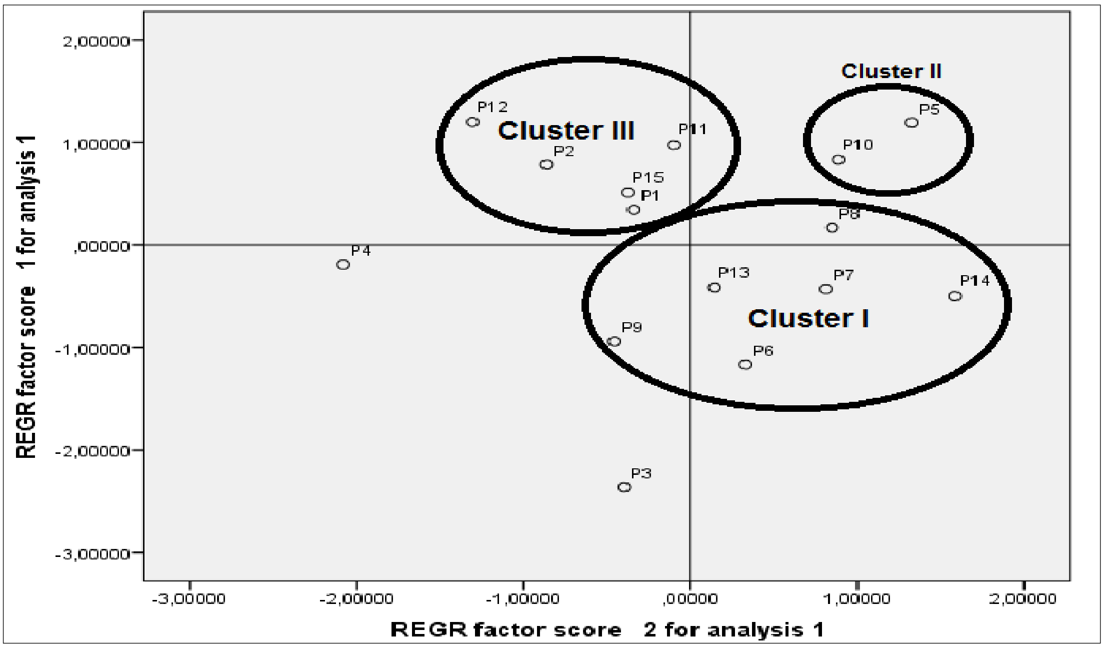

Figure 1: Principal component analysis of Moroccan cultivars of carob tree on the space formed by the first two axes performed on the basis of pods and seeds morphological characters

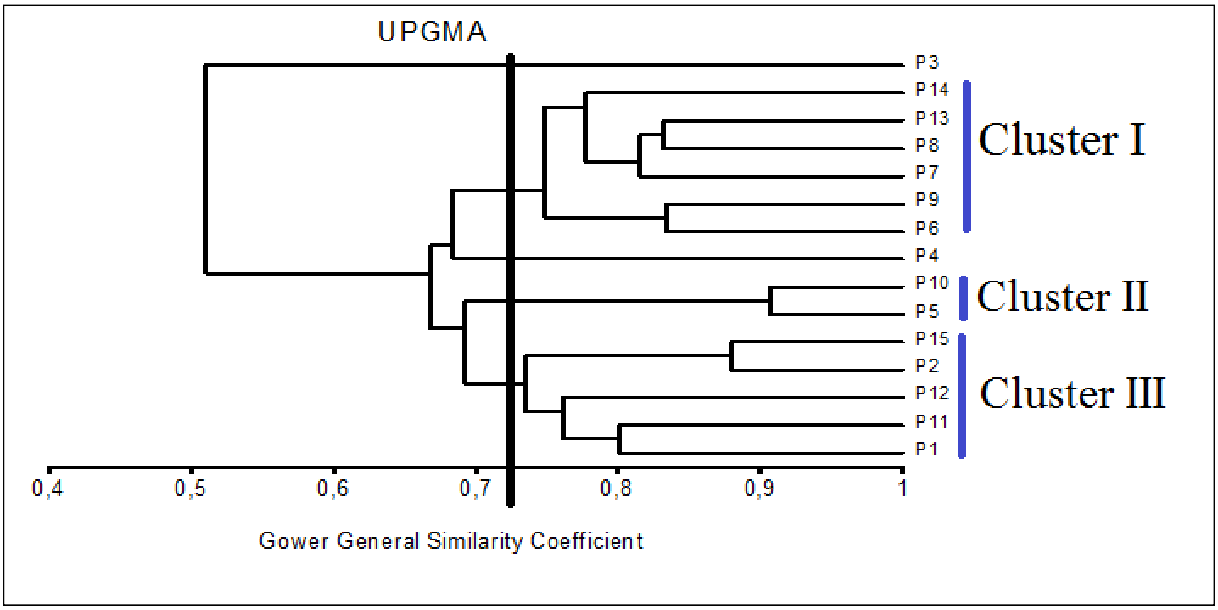

Figure 2: Dendrogram (hierarchical clustering) of 15 cultivars of Moraccan carob tree based on morphological traits

Hierarchical cluster analysis leads to identify three major groups (Fig.2) confirming the PCA results. The cultivars P6, P7, P8, P9, P13 and P14 were placed in cluster I. Cluster II consisted of P5 and P10. Cluster III included P1, P2, P11, P12 and P15. Cultivars P3 and P4 can be considered as rather singular.

Discussion

Morphological traits of pods and seeds constitute a quantitative marker largely used together with productivity, vigour and precocity as characters differentiating carob cultivars.[3,7,11,15-17,25,34,35,38,43] Using 12 fruit and seed phenotypic characters, Barracosa et al[7] reported a high Diversity of carob cultivars in Portugal.

In Algeria cultivars, Boublenza et al[11] showed a high diversity in the morphological parameters of pods and seeds. Morphological parameters of pods and seeds show a high diversity in Morocco carob population, type and geographical origin of trees being taken as the source of variation.[16,34] In the world, about 50 named cultivars are reported in the literature[9] within which about half were reported in the Mediterranean basin.

The values of morphological traits obtained by population in the present study were compared with those from other countries of the Mediterranean basin. Cultivars from Algeria[11], from Lebanon[17] and from Tunisia[32] prented high similarity in morphological characteristics of the fruit with Moroccan carobs.

The value obtained in the study can be compared with those found by Boublenza et al[11] in Algeria (lenght=15.77cm, width=2.23cm, thickness=0.62cm, seeds number=10.76), Haddarah et al[17] in Lebanon (length=17.84cm, width=2.48cm, thickness=0.70cm, seeds number=10.65), Naghmouchi et al[32] in Tunisia (length=17.33cm, width=2.48cm, thickness=1.01cm, seeds number=14.21). The weight of the seeds varied between 0.11 and 0.21g, with a mean value of 0.16g. This value is somewhat lower than the generally accepted mean of 0.20g and which has been used as a measure of weight 1 carob seed=0.20g=1carat.[12]

Data obtained from morphological studies have been used to provide total correlation coefficient between morphological fruit and seed characters.[7,11,15,16,25,32,34,35]

However, a wide range of values were observed for each correlation when cultivars were considered separately (Table 3). The correlation analyses establisched by cultivar provided a specific understanding about the way how fruit and seed characteristics correlate within each cultivar. This approach can be useful for development of a breeding programme aiming at the selection of cultivars in order to increase the seed yield, seed thickness, individual and total seed weight by fruit, characteristics that are determinant to improve the industrial exploitation. This must obviously be done in close agreement with agricultural pratices.

In Tunisain cultivars, Naghmouchi et al[32] observed a high correlation coefficient between fruit lenght and total seed weight (0.63). In the present study, all cultivars showed no correlation coefficient between lenght and seed yield.

The cultivar P7 showed seed yields higher by 34.56% than those of other cultivars. According to Albanell et al[3] and Barracosa et al[7], it is preferable to use thin narrow carobs which are not too beavy and carry a high number of seeds to obtain an incremental yield of seeds.

The pods from wild carob tress have superio seed and gum quality than the cultivated ones, they are in great demand by agri-food industries.[8,27] The results obtained in the present study are consistent with earlier reports.[7,11,15,16,25,32,34,35,38]

Table 3
Pearson coefficient correlation between morphological characters of pods and seeds of Moroccan carob tree.

	1	2	3	4	5	6	7	8	9	10	11
Pod Length (1)	1										
Pod Width (2)	0.197	1									
Pod Thichness (3)	-0.488	0.202	1								
Seed number/pod (4)	0.664**	0.083	0.463	1							
Pod Weight (5)	0.484	0.737**	0.317	0.361	1						
Pulp Weight (6)	0.502	0.659**	0.331	0.277	0.951**	1					
Yield (7)	0.018	0.035	-0.303	0.434	-0.148	-0.356	1				
Seed Lenght (8)	0.220	0.891**	0.244	0.234	0.793**	0.659**	0.110	1			
Seed Width (9)	0.497	0.800**	0.080	0 .299	0.809**	0.684**	-0.020	0.873**	1		
Seed Thichness (10)	0.074	-0.131	0.065	0.184	0.043	-0.147	0.207	0.163	0.305	1	
Seed Weight (11)	0.298	0.653**	0.128	0.479	0.743**	0.520*	0.425	0.769**	0.754**	0.343	1

*Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level

The carob tree (*Ceratonia siliqua* L.,), which is an endemic tree in Morocco, grows mainly all over the country. Research on this species has been increasingly growing over the past few years because this tree is economically and agromically intersting. The principal component analysis (PCA) and the hierarchical cluster were used to group the different cultivars into three groups (Fig. 1 and 2); this first group is for the cultivars P6, P7, P8, P9, P13 and P14, the second is for cultivars P5 and P10 and the third is for the cultivars P1, P2, P11 and P15, P3 and P4 are not grouped.

Conclusion

The variations in the morphological features are influenced by the environment and geographical locations ; these two parameters have an effect on the quality of the carob tree cultivars.

Furthermore, planting these carob cultivars, which are highly needed by the agro-food industries, can be an important economic resource for the rural population. The morphometric analysis is an essential and preliminary step in the genetic study of this species in order to identify the different varieties existing in Morocco.

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