Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6979

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **7 (1):** 22-34 (2019)

Research Article

International Journal of Pure & Applied Bioscience

Characterization of Carob Trees Suggests High Morphological Variability in Populations of the Middle Atlas Region, Morocco: Towards Use of Carob Germplasm in Breeding Program

El Bakkali Ahmed[1*], Essarioui Adil[2], Mekkaoui Abderahman[1], Hssaini Lahcen[1], Mamouni Ali[1]

[1]INRA, UR Amélioration des Plantes et Conservation des Ressources Phytogénétiques, Meknès, Morocco

[2]INRA, UR Système Oasiens, Errachidia, Morocco

*Corresponding Author E-mail: ahmed_elbakkali@yahoo.fr

Received: 19.10.2018 | Revised: 24.11.2018 | Accepted: 3.12.2018

ABSTRACT

Carob tree (Ceratonia siliqua L.) is a Mediterranean species known for its high tolerance to drought conditions. In Morocco, carob tree is predominantly encountered in most mountainous regions under agro-forestry systems. Despite its economic and environmental importance, carob tree is still to be neglected by research and development programs resulting in no commercial cultivars selected so far. Yet, carob populations can be a precious genetic resource to reach this objective. The aim of this study was to evaluate morphological variability among 70 female carob trees growing in 5 localities in the Middle Atlas region, Khenifra province. Morphological variability was assessed based on 14 characters related to leaves, leaflets, pods and kernels. Results showed a high variation among carob trees for almost all the investigated characters. Specifically, clustering analysis yielded 5 site-independent homogenous groups, indicating important morphological diversity in local carob germplasm. Correlation and regression analyses showed causality relationships among traits and therefore can be used as predictors in selection programs. Overall, Middle Atlas carob populations are characterized by short and narrow pods that are rather light, skinny and thin with high number of light seeds and high kernel yield. Taken together our results contribute significant insights into morphological variability on carob trees at regional scale and shed light on the potential for using carob populations to select promising genotypes that are adapted to local conditions and have high industrial and agronomical performances.

Key words: *Ceratonia siliqua L., Morphological diversity, Kernel yield, Middle Atlas, Morocco.*

INTRODUCTION

Carob tree (*Ceratonia siliqua* L.) is a leguminous subtropical species belonging to the Leguminosae family and Caesalpinoidea sub-family. It is a dioecious and diploid species (2n=24) with some hermaphrodic forms[7]. This tree is essentially grown for its sweet edible pods, but also used as ornamental tree because of its evergreen foliage. It has been suggested that carob tree has been domesticated in the Mediterranean basin.

Cite this article: El Bakkali, A., Essarioui, A., Mekkaoui, A., Hssaini, L., Mamouni, A., Characterization of Carob Trees Suggests High Morphological Variability in Populations of the Middle Atlas Region, Morocco: Towards Use of Carob Germplasm in Breeding Program, *Int. J. Pure App. Biosci.* **7(1):** 22-34 (2019). doi: http://dx.doi.org/10.18782/2320-7051.6979

Ever since, its cultivation for human and animal consumption has spread throughout many countries[33, 34] where it is particularly used in restoration of marginal arid and semi-arid areas due to its great adaptive plasticity and high drought tolerance[7,27].

Currently, world carob pod production is estimated at about 158,609 t in an area of almost 66,874 ha [15]; more than 73% (115,925 t) is cropped in the European Union, (mainly Portugal, Spain, Italy, and Greece), with Portugal being the largest producer (40,385 t). The other 27% is produced mainly in Morocco, Turkey, Algeria and Tunisia[15].

The most valued part of carob pods is the seeds that represent about 10% of fruit weight. Recent advances in food and pharmaceutical sciences have uncovered many therapeutic, dietetic, and cosmetic virtues of carob pods. For example, carob beans and seeds are used for making industrial products such as gums, sugar, citric acid, and alcohol[6,7,28]. Additionally, locust bean gum (LBG; additive E-410) extracted from seeds is used as stabilizer and thickening agents in food industry [7, 25] and also applied in pharmaceutical industry as drug delivery [29]. Such prospering industries have led to increased international demand and fostered interest in carob cultivation. Because chemical attributes of desirable carob pod extracts have been reported to depend on extraction methods, storage conditions, and the interaction between environment and tree-genotype, selecting promising genotypes that suit post-harvest processing requirements has long been the focus of a large body of breeding programs[32]. Specifically, much work has been carried out on the genetic diversity using molecular markers[2,5,21], on fruit and leave morphological[3, 4,8,19 25] and chemical traits characterizations[23, 24, 31]. As a result, many cultivars differing largely in their pulp productivity, seed yield, and LBG content, have been selected, and the best-performing ones in terms of kernel and pod production (e.g. Duraio) have been grown in orchards in many Mediterranean countries, mainly Spain[32].

In Morocco, carob tree is an ancient crop with important environmental and economic roles for local communities. With a cultivation area of about 10,421 ha and a production exceeding 22,000 t/year, Morocco contributes by 14% to the world annual production and ranks the 4th international producer behind Portugal, Spain and Italy[15]. This is despite the fact that carob trees in Morocco grow traditionally in the form of wild populations under agro-forestry systems that lack the practice of integrated modern cultivation techniques such as grafting, silvaculture treatments, mineral fertilization etc. In addition, carob strands grow widely in many regions such as the Rif Mountain in the north, high, Anti and Middle Atlas and south-west in association with other species like *Argania spinosa*, *Tetraclinis articulate*, *Olea europaea*, *Quercus rotundifolia* and/or *Q. suber* [17]. Environmental factors limiting its distribution in Morocco are mainly the absolute minimum temperature (<3°C) and altitude with its maximum reach at around 1,150m and exceptionally up to 1,600 m. Studies led on carob tree from Morocco distinguish between four carob types according to tree sex; unproductive “dkar” or spontaneous male, productive “dkar” or spontaneous female, “lanta” or grafted female and sterile[16, 26]. Despite its wide distribution, economic and environmental importance, carob tree is neglected by research and development programs and studies are limited to diversity [18, 30, 13, 20, 12]. Hence, characterization and identification of local genetic resources could yield information on outstanding accessions that could be used for the selection of carob cultivars with more useful agronomic and industrial traits, thus offering potential alternatives to forests exploitation.

The overarching goal of this work is to contribute to the development of modern carob cultivation in Morocco. Specifically, this study aims to characterize local carob trees from one of the main producer region in Morocco, the Middle Atlas[1], using morphological traits related to leaves, leaflets, pods and seeds. Understanding how morphological traits correlate and how variability is clustered at regional scale will bring new insights into: (i) selection process through decreasing workload by providing working on less characters and (ii) establishment of a local collection capturing the total diversity and includes agronomical and industrial promising genotypes as a first step of a long-term breeding program.

MATERIAL AND METHODS

Plant material

Plant samples were collected on 70 female carob trees growing in 5 different geographic locations in the region of Middle Atlas, Khenifra province-Morocco (Fig. 1). This region is characterized by an average annual precipitation between 300 and 750 mm with very low winter temperatures down to 2°C and Peak Mountains exceeding 1,000 m[30].

Measurement of plant morphological traits

For each sampled carob tree, leaves (n=20) and pods (n=25) were collected randomly during the summer for two successive years. Leaves, leaflets, pods and seeds were described based on 14 morphological characteristics as reported by Battle and Tous[7]. Specifically, five characters were measured on leaves; length (LL, cm), width (WL, cm), number of leaflets/leave (NLf), and length (LLf, cm) and width of leaflets (WLf, cm). Additionally, five characters related to pods were also determined; weight (WeP, g), length (LP, cm), width (WP, cm), thickness (TP, cm) and weight of pulp (WePu, g). Furthermore, three characters of the seeds/pod were described; number (NS), weight (WeS, g) and number of aborted seeds (NA). Finally, for each pod, seed yield (Y) was calculated as (seed weight /pod weight)*100.

Statistical analyses

Normality of data was tested using Shapiro-Wilk test. One-way ANOVA was conducted to test for significant differences among variables and Duncan's post hoc test was used for mean comparison. To analyze the relationships between seed yield (Y) and yield components, Pearson's correlation coefficient and simple regression analysis were computed using R environment.

In order to find the main variation trends between characters in carob trees, data were processed according to Principal Component Analysis (PCA) using FactoMineR package[22] in R environment. Finally, Hierarchical cluster analysis (HCA) was used to investigate similarities among sampled trees. For classification, the Ward's method and the Squared Euclidean distance were used. To define the number of classes (or clusters) to retain, the cutree function in FactoMineR package was used.

RESULTS

Sampled trees showed great variability for almost plant traits. All of the investigated variables; except length of leaves (LL), thickness of pods (TP), number of seeds (NS) and number of aborted seeds (NA); follow a normal distribution (Fig. 2; Shapiro-Wilk test, p-value > 0.05). Pod weight (WeP) ranged from 3.1 to 11.9 g and seed weight (WeS) from 0.8 to 3.2 g/pod. Similarly, seed number/pod varied from 6.0 to 14.3. This resulted in seed yield (Y) spanning from 18.1% to 45.4% with an average of 27.9% (Fig. 2). Overall, these results suggest high potential for significant morphological variability among carob trees in the Middle Atlas area.

Aside from trees in Aguelmame location that showed a significantly smaller mean number of leaflets/leaf than those in the other locations (Table 1), the other sites did not differ significantly in their foliar characteristics. However, sampling sites exhibited a more pronounced variability in pod and seed traits. With the exception of pod length (LP), the five sites differed significantly in the other traits (Table 2). Carob trees in Aguelmame locality showed the smallest values for WP, TP, WeP, WePu, NS, and WeS compared to the other sampling areas. In contrast, this site harbored trees with significantly the greatest NA/Pod and Y among all localities. Taken together, these findings imply probable site-dependent distribution of carob traits in the Middle Atlas region.

Correlation analyses revealed different patterns of association among various traits in carob trees (Table 3). There were strong and highly significant positive correlations between WeP and WePu (r=0.917), WL and LLf (r=0.806), WeP and WeS (r=0.730), and NS and WeS (r=0.629). In addition, WeS and NS were found to be significant positive correlated to Y, though these association were less important than expected (r=0.158 and r=0.193, respectively). In contrast, seed yield (Y) correlated negatively with WP (r=-0.4), TP (r=-0.28), and WeP (r=-0.52). With regards to leaves/leaflet and pods/seeds characters, NLf was positively and significantly correlated with WeP (r=0.33), TP (r=0.32) and WeS (r=0.288). Finally, no significant correlations were detected among other traits such as between Y and LP, NLf and NS, and WeP and TP.

Simple regression coefficients (b values) of seed yield on the different characters were computed. The significance of the coefficients obtained was tested by calculating t values (Table 4). Among the 13 traits used to explain the seed yield, six were shown significant among them four with negative b value; i.e weight of the pods (WeP); width of the pods (WP); thickness of the pods (TP) and the weight of pulp (WePu), and two traits with positive b value: weight (WeS) and number of seeds (NS). Weight of the pulp gives the highest regression coefficient (b value = -2.491) followed by weight of seeds (b value = 1.974). Regression coefficient for number of seeds and weight of seeds were positively significantly correlated with kernel yield indicating that increase in these two characters would increase the kernel yield. Other traits including weight of pods, width of the pods, thickness of the pods and weight of pulp showed significant and negative 'b' values suggesting that kernel yield would be decreased with the increase of these characters.

The multivariate analysis revealed that the three axes of PCA explained 61.7% of total variance of the original variables set (Table 5, Fig. 3a). The first component (PC1), accounting 27.4% of the total variance, is explained mainly by pods and seeds characters such as the thickness and the width of pods and the weights of pods, pulp and seeds. In the second component (PC2), it was explained by foliar characteristics such as the length and the width of leaves and leaflets whereas the third component explained mainly by the seed yield, number and weight of seeds. Low contributions of the number of aborted seeds, length of pods and number of leaflets were observed on the three first components (Table 5; Fig. 3b). The individual factor map (Fig. 3c) allows separating some accessions. In fact, accessions from Aguelmame site showed clearly distinct whereas those from the others sites showed as one group explained mainly by the similarity in morphological traits.

In order to better visualize pomological diversity within local carob trees, Hierarchical Ascendant Classification (HAC) was carried out on the basis of 14 quantitative parameters (Fig. 4). The analysis yielded 5 clusters of trees with distinct characteristics (Fig. 4 and Table 6). Six trees out of 11 from Aguelmame belong to cluster1 and 2 trees from the same site are contained in the closest group (cluster2), indicating greater similarities among carob trees within this locality than in others. Trees from the other locations are almost evenly scattered among the other clusters, suggesting the existence of heterogeneous carob tree populations with various traits in every site. Compared to other clusters, individuals in the cluster 1 are characterized by the lowest number of leaflets/leave and seeds/pod, the thinnest, skinniest, and lightest pods and seeds, but in contrast the greatest seed yields (Table 6).

DISCUSSION

Studying genetic variability on the basis of morphological traits and genetic resources description is fundamental to advance research in plant breeding. Phenotypic characteristics have been used worldwide for estimating diversity in carob species[4,5,18,20,30,32,8,1913,14]. For example, tremendous morphological variability among carob cultivars has been reported in Spain and Portugal [4, 5]. In Morocco, previous reports showed a high level of variation in carob morphological characters within and among multiple ecoregions[18,30,14] indicating significant potential for selecting promising genotypes that are suitable for industrial processing and adapted to local conditions. Our findings from underexplored areas contribute to a more comprehensive understanding of morphological diversity in Moroccan wild carob populations under agro-forestry contexts with predominant outbreeding system.

Most studies carried out on carob morphological diversity in Morocco focused on traits related to pods and kernels as the most important yield components. Here, in addition to beans and seeds, we were interested in a boarder range of characters including leaves and leaflets. Our results revealed significant positive correlations between the number of leaflets/leave and both the weight and thickness of pods, indicating probable effect of the number of leaflets on fruit characetristics. Additionally, as previously observed by El Kahkahi *et al.*[14], significantly high positive correlations between leaves and leaflets traits for both width and length, and the great number of leaflets observed in the trees we surveyed (up to 9 leaflets/leave) are in agreement with previous studies[7]. The relationship between leaf and leaflet characteristics and their causality relationship with pod and seed weights and pods thickness suggest that leaf and/or leaflet number can be used as a indicator for pod productivity to accelerate selection process during early and unproductive developmental stages of carob trees. Overall, this study highlights the importance of expanding the range of studied characters in setting up effective programs of selection for carob cultivars with desirable characteristics.

The best values of yield components found in our investigation are comparable or even greater than those of internationally known cultivars. For example some of our sampled trees exceeded the two major Spanish cultivars, Duraio and Rajol [32], in their performance in terms of number of seeds and length and width of pods; though overall pod weight in the Spanish cultivars is heavier. Additionally, a subset of promising genotypes identified here had similar traits with regard to number and weight of seeds/pod as carob trees from the Balearic Islands and near the Mediterranean Coast[3]. These results indicate a great similarity between local carob trees and those of other Mediterranean regions and the high performance of Moroccan carob germplasm to be selected to develop the modern cultivation of the species.

Compared to other regions in Morocco, particularly from the north[20, 30], the surveyed populations in the Middle Atlas region are characterized by relatively short, narrow and skinny pods with light pods and seeds and high seed yield. The high seed yield observed in the present study was almost similar to Sidina *et al.*[30] study recorded in Beni Mellal province (27% vs 25%, respectively). Taken together, these findings are in agreement with the correlation and simple regression analyses (Table 3 and 4). In fact, significant correlations were revealed between pods, seeds characters and yield that are congruent with previous studies[4 32, 19, 12]. Hence, the variation in the seed yield could be explained positively by: the number and weight of seeds, and negatively by: the pulp weight, the pod weight, and the thickness and width of pods. Thus, to achieve high kernel yield, narrow, thin, not too heavy pods with high number of seeds would be more interesting, although length of pods in not a reliable predictor of yield[3, 4].

Over all the five sampled localities, Aguelmame showed the prevalence of distinct genotypes compared to the four others sites that were quite similar in their population makeup. As the Aguelmame locality is far from the others localities (Fig. 1), this result highlights possible role of environmental conditions in the variation of phenotypic parameters, and perhaps genotypic variability, among regions. Additionally, geographic proximity among the other 4 locations could be in favor of important gene flow through wind or insect pollen transportation, or animal dissemination of seeds, resulting in a subset of sites with greater similarities. Overall, the five homogenous clusters identified by the HCA bring an important insight on the diversity in the Middle Atlas region and they could be a basis for further studies through selecting genotypes, within each cluster, to construct local core collections capturing total phenotypic variability with minimum number of accessions[9]. Moreover, further studies are required such as molecular and biochemical analyses to better understand the diversity in association to environmental conditions and geographic distribution. Finally, the surveyed trees will be useful for conducting field assessments and suitable for developing a long-term breeding program in carob.

Table 1. Mean values and standard deviations of leaves and leaflets measurements according to carob sampling sites.

	Leaves		leaflets		
Sampling localities	LL (cm)	WL (cm)	NLf	LLf (cm)	WLf (cm)
Ait Ishaq	11.12 ± 2.60^{a}	9.53 ± 1.29^{b}	7.4 ± 0.6^{a}	4.84 ± 0.70^{a}	3.24 ± 0.48^{a}
Aguelmame	10.54 ± 3.03^{a}	10.77 ± 2.15^{a}	6.5 ± 0.9^{b}	5.36 ± 0.98^{b}	3.36 ± 0.46^{a}
El Ksiba	11.45 ± 2.62^{a}	10.38 ± 1.49^{ab}	7.1 ± 1.1^{ab}	4.85 ± 0.85^{ab}	3.30 ± 0.45^{a}
Ouaoumana	10.19 ± 1.97^{a}	10.06 ± 1.19^{ab}	7.1 ± 0.6^{ab}	4.71 ± 0.54^{ab}	3.06 ± 0.46^{a}
Zaouit Cheikh	11.29 ± 1.87^{a}	10.32 ± 1.04^{ab}	7.5 ± 1.0^{a}	4.87 ± 0.47^{ab}	3.09 ± 0.37^{a}
Mean	**10.94 ± 2.37^{ns}**	**10.20 ± 1.43^{ns}**	**7.2 ± 0.9***	**4.90 ± 0.71^{ns}**	**3.18 ± 0.45^{ns}**

*p≤0.05; ** p≤0.01; *** p≤0.001; ns, non-significant

Table 2. Mean values and standard deviation of pods and seeds measurements according to carob sampling sites.

Sampling localities	Pods					Seed			Y (%)
	LP (cm)	WP (cm)	TP (cm)	WeP (g)	WePu (g)	NS /pod	WeS (g)	NA / pod	
Ait Ishaq	13.61 ± 2.05^{a}	1.65 ± 0.26^{b}	0.61 ± 0.15^{a}	6.9 ± 1.9^{a}	5.1 ± 1.6^{a}	12.3 ± 0.9^{a}	1.8 ± 0.3^{ab}	0.1 ± 0.1^{c}	26.2 ± 3.2^{c}
Aguelmame	13.18 ± 2.62^{a}	1.41 ± 0.25^{a}	0.36 ± 0.14^{b}	5.0 ± 1.9^{b}	3.4 ± 1.6^{b}	9.9 ± 1.3^{c}	1.6 ± 0.6^{b}	1.0 ± 0.8^{a}	33.5 ± 5.5^{b}
El Ksiba	12.18 ± 2.28^{a}	1.73 ± 0.14^{b}	0.57 ± 0.07^{a}	6.7 ± 1.4^{a}	4.5 ± 1.2^{ab}	12.5 ± 1.3^{a}	2.0 ± 0.3^{a}	0.2 ± 0.2^{bc}	30.3 ± 4.3^{a}
Ouaoumana	13.53 ± 2.02^{a}	1.60 ± 0.20^{b}	0.55 ± 0.11^{a}	7.2 ± 1.6^{a}	5.4 ± 1.5^{a}	10.7 ± 2.3^{bc}	1.7 ± 0.4^{ab}	0.5 ± 0.4^{b}	24.8 ± 5.5^{d}
Zaouit Cheikh	11.91 ± 2.00^{a}	1.64 ± 0.38^{b}	0.57 ± 0.18^{a}	7.2 ± 1.9^{a}	5.0 ± 1.3^{a}	11.7 ± 1.7^{ab}	1.9 ± 0.5^{ab}	0.5 ± 0.7^{b}	26.9 ± 5.6^{c}
Mean ± sd	**12.82 ± 2.24^{ns}**	**1.63 ± 0.23****	**0.54 ± 0.16*****	**6.7 ± 1.9***	**4.8 ± 1.5***	**11.5 ± 1.8*****	**1.8 ± 0.4***	**0.5 ± 0.6*****	**27.9 ± 5.7*****

*p≤0.05; ** p≤0.01; *** p≤0.001; ns, non-significant

Table 3. Pearson's correlations between 14 investigated traits.

	LL	WL	NLf	LLf	WLf	WeP	LP	WP	TP	WePu	NS	WeS	NA
WL	**0.673*****												
NLf	0.617***	0.171ns											
LLf	0.528***	0.806***	0.065ns										
WLf	0.499***	0.651***	-0.006ns	0.725***									
WeP	0.102ns	-0.018ns	0.330**	-0.053ns	-0.167ns								
LP	0.158ns	0.163ns	0.150ns	0.138ns	-0.017ns	0.298**							
WP	0.184ns	-0.052ns	0.208ns	-0.042ns	-0.063ns	0.486***	-0.086ns						
TP	0.106ns	-0.063ns	0.320**	-0.111ns	-0.063ns	0.203ns	-0.095ns	0.350***					
WePu	0.118ns	0.014ns	0.301**	0.011ns	-0.128ns	0.917***	0.390***	0.570***	0.296**				
NS	0.196ns	-0.003ns	0.172ns	-0.124ns	-0.001ns	0.411***	0.291*	0.275*	0.294*	0.336**			
WeS	0.155ns	0.060ns	0.288*	-0.063ns	-0.082ns	0.730***	0.262*	0.231*	0.063ns	0.506***	0.629***		
NA	0.037ns	0.223ns	-0.035ns	0.208ns	0.108ns	0.054ns	-0.061ns	-0.258*	-0.405***	-0.093ns	-0.500***	0.108ns	
Y	0.006ns	0.101ns	-0.180ns	0.004ns	0.179ns	-0.524***	-0.038ns	-0.399***	-0.277**	-0.673***	**0.193****	0.158**	0.039ns

ns, non-significant. *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.

Table 4. Regression coefficients (b values) of different component traits on seed yield in carob.

Variables	Regression values (b values)	p-value
Length of leaves (LL)	0.014	0.961
Width of leaves (WL)	0.397	0.406
Number of leaflets (NLf)	-1.109	0.135
Length of leaflets (LLf)	0.0321	0.973
Width of leaflets (WLf)	2.211	0.138
Weigth of pods (WeP)	-1.579	0.0001***
Length of pods (LP)	-0.009	0.751
Width of pods (WP)	-0.963	0.0001***
Thickness of pods (TP)	-1.001	0.020*
Weigth of pulp (WePu)	-2.491	0.0001***
Number of seeds (NS)	0.606	0.011*
Weight of seeds (WeS)	1.974	0.019*
Number of aborted seeds (NA)	0.395	0.745

*** = Significant at 1‰ level. ** = Significant at 1% level. * = Significant at 5% level

Table 5. Factor loadings for each variable on the components of PCA analysis.

	PC1	PC2	PC3
Length of leaves (LL)	2.73	19.36	0.10
Width of leaves (WL)	0.07	26.75	0.06
Number of leaflets (NLf)	7.25	2.36	0.02
Length of leaflets (LLf)	0.02	24.76	1.88
Width of leaflets (WLf)	0.36	20.96	0.00
Weigth of pods (WeP)	20.79	0.19	0.81
Length of pods (LP)	3.47	1.01	4.02
Width of pods (WP)	10.73	0.32	3.45
Thickness of pods (TP)	5.41	0.60	0.23
Weigth of pulp (WePu)	21.16	0.13	4.21
Number of seeds (NS)	9.17	0.03	29.47
Weight of seeds (WeS)	11.32	0.07	13.44
Number of aborted seeds (NA)	1.43	2.51	5.97
Yield (Y)	6.09	0.95	36.34
Eigen value	3.83	3.11	1.70
% of variance	27.39	22.24	12.11
Cumulative %	27.39	49.62	61.73

Table 6. Main characteristics of the different clusters formed by the Hierarchical clustering analysis (HCA)

Variables	Overall mean	Cluster 1		Cluster 2		Cluster 3		Cluster 4		Cluster 5	
		Mean	Value test	Mean	Value Test	Mean	Value test	Mean	Value test	Mean	Value test
LL (cm)	10.94			12.43	4.034	8.31	-3.576				
WL (cm)	10.20			11.04	3.762	7.97	-4.984				
NLf	7.18	6.20	-3.197								
LLf (cm)	4.90			5.36	4.062	4.00	-4.052				
WLf (cm)	3.19			3.61	4.612	2.50	-4.830				
WeP (g)	6.69	3.85	-4.538					9.90	2.440	8.02	4.409
LP (cm)	12.82			11.87	-2.732			9.63	-2.049	14.42	4.463
WP (cm)	1.62	1.29	-4.282					1.30	-2.013		
TP (cm)	0.53	0.29	-4.714	0.60	2.697			0.19	-3.131		
WePu (g)	4.76	2.57	-4.292							5.97	4.929
NS	11.46	9.53	-3.202			10.14	-2.345			12.7	4.261
WeS (g)	1.81	1.23	-3.843					3.00	3.730	2.06	3.368
NA	0.47							2.45	4.986		
Y (%)	27.98	33.01	2.663								
Number of trees		**8**		**17**		**9**		**2**		**34**	
Description of clusters		**Low number of leaflets, Light, narrow, thin, and skinny pods, Low number of seed, Light seeds, High seed yield**		**Long and wide leaves, Long and wide leaflets, Short and thick pods**		**Short and narrow leaves, Short and narrow leaflets, Low number of seeds**		**Heavy, short, narrow and thin pods, Heavy seeds with high number**		**Long, heavy, fleshy pods Heavy seeds with high number**	

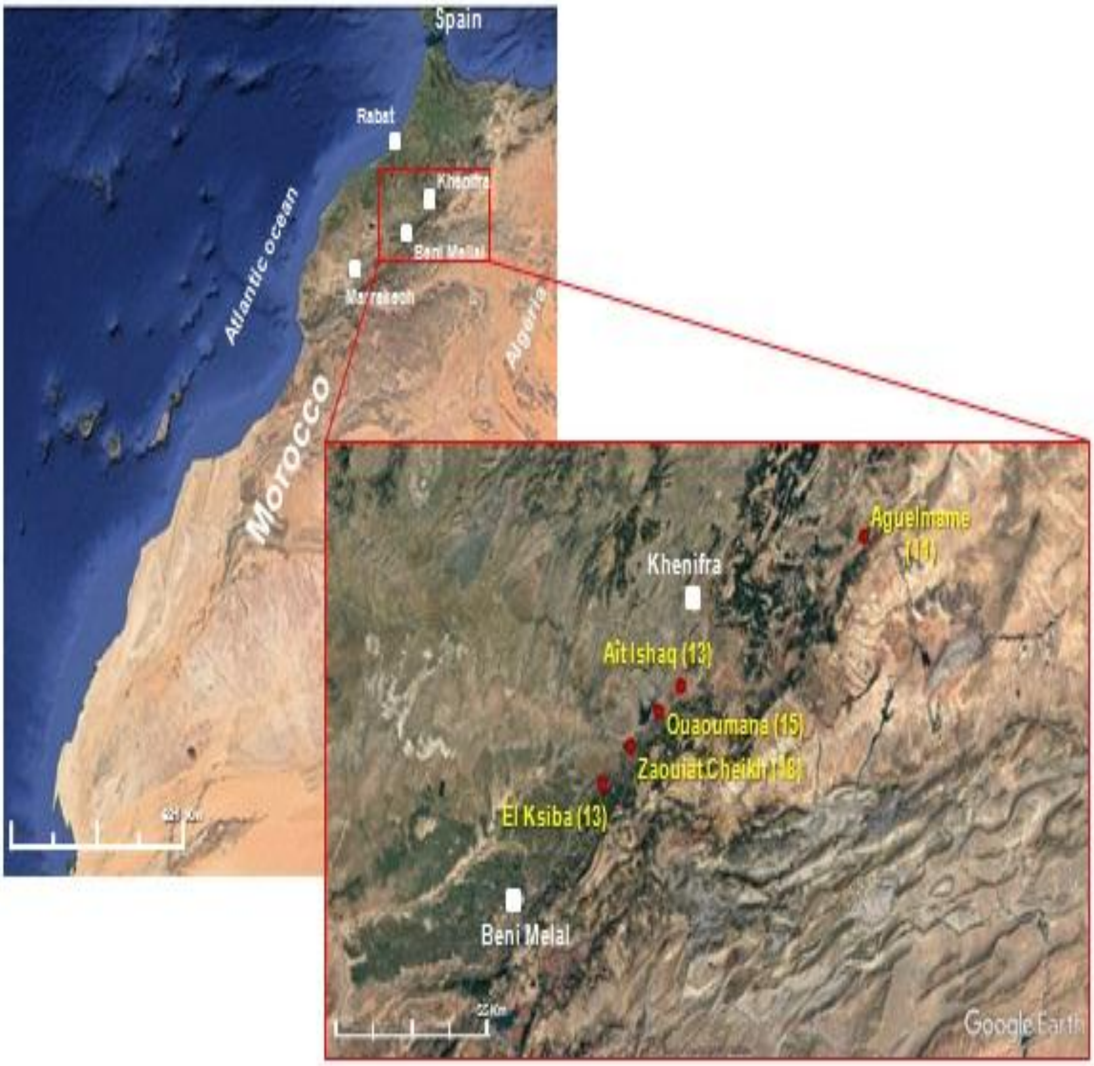

Fig. 1. Map indicating the sampling areas. Numbers in brackets indicate the number of sampling trees within the area.

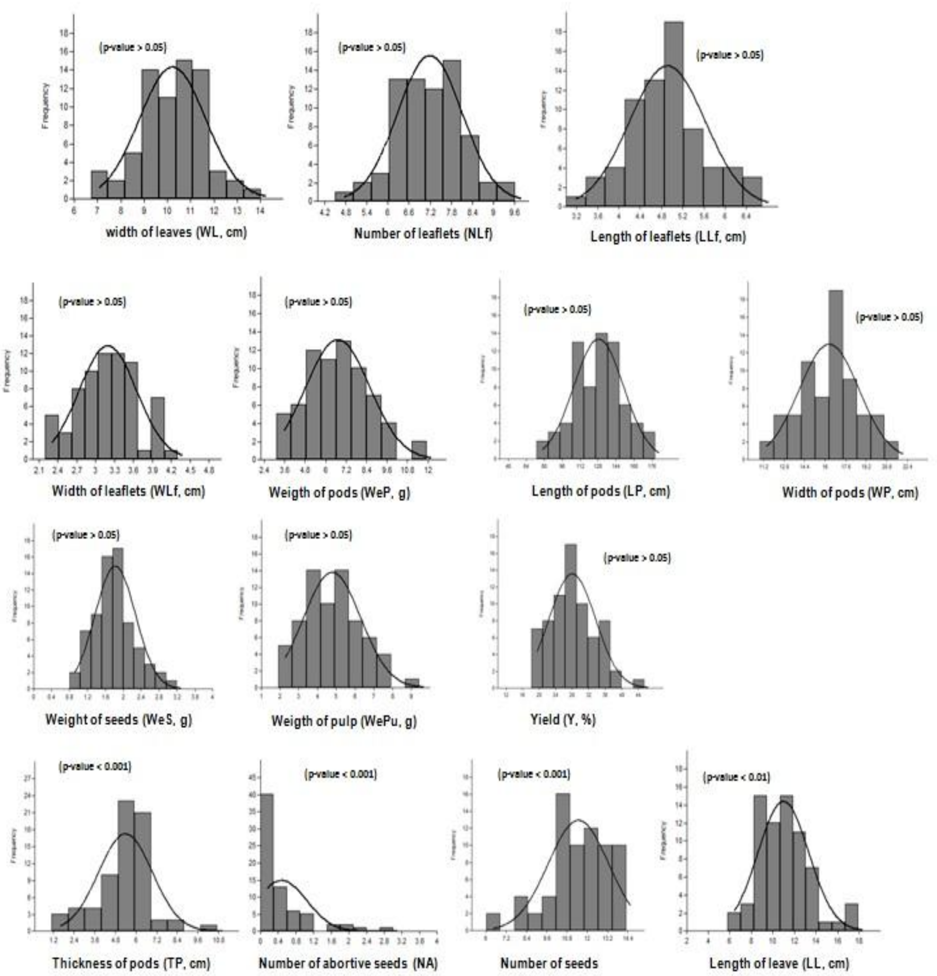

Fig. 2. Histogram of the distribution of morphological traits measured in the carob trees panel of 70 accessions. Shapiro-Wilk normality test is indicated.

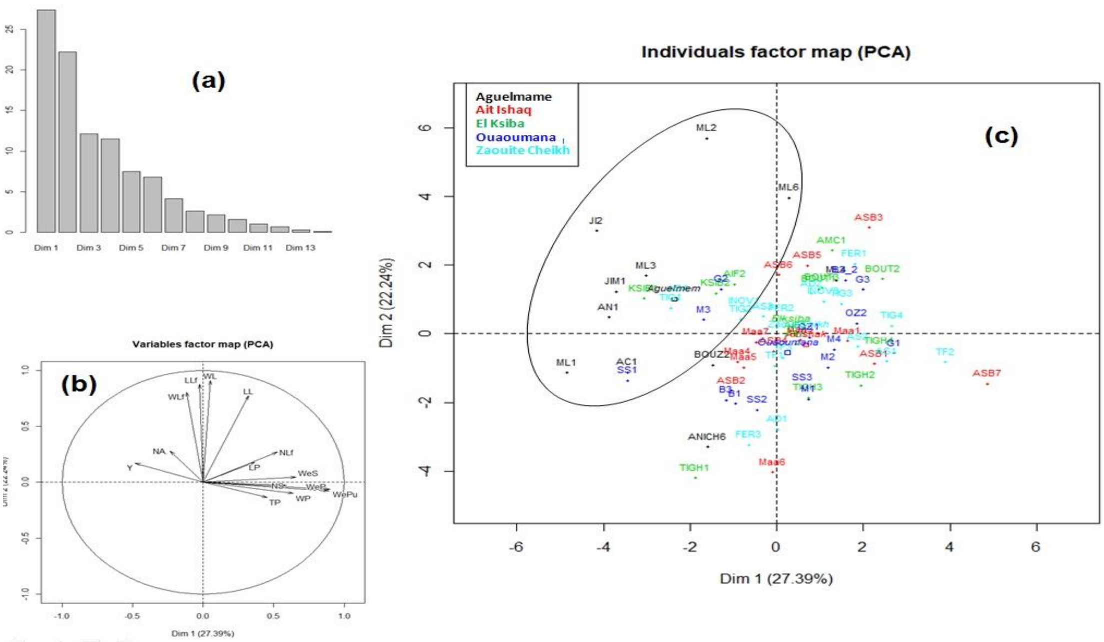

Fig. 3. Principal Components Analysis (PCA) results. Variations explained by each principal component (a), Projection of the 14 quantitative variables in the two axis 1 and 2 (b). Cumulative variation Projection of score of 70 carob trees onto the two axis 1 and 2 (c).

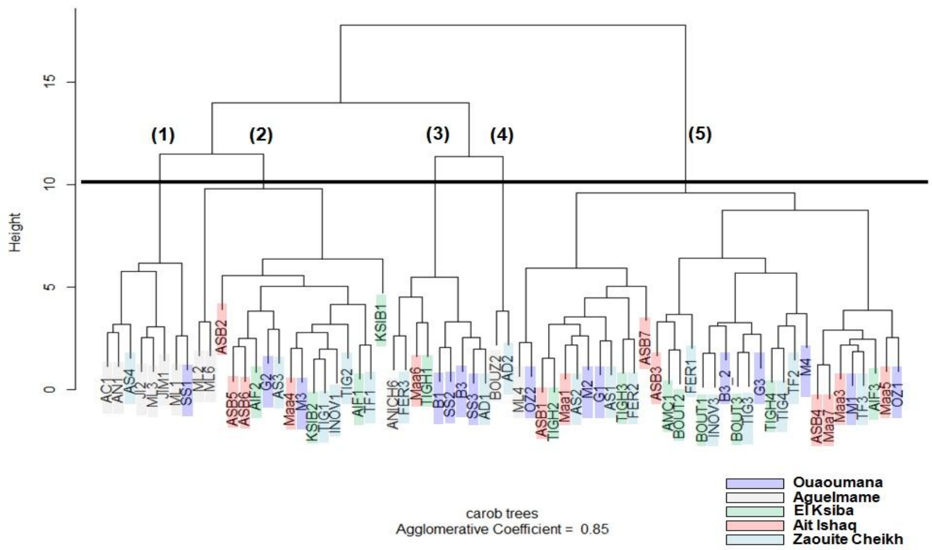

Fig. 4. Dendrogram of the 70 carob trees based on 14 morphological traits.

CONCLUSION

This study adds significant knowledge to our understanding of morphological variability in carob tree populations at local scale in Morocco (i.e. Middle Atlas). This emphasizes high potential of local carob germplasm in providing promising cultivars for development of industrial carob cultivation in prospect using locally adapted genotypes. Our results are an important stride towards achieving this goal. However, further investigations related to morphological characterization are still needed in various uninvestigated areas (i.e. north and south of Morocco). Such studies should also be completed by biochemical and molecular assessment for comprehensive understanding of genetic diversity in carob trees. Overall, our findings along with other results from across multiple previous studies provide clear indication that carob tree in agro-forestry systems is an as-yet-untapped genetic resource for the development of modern carob cultivation in Morocco.

Acknowledgments

The authors acknowledge the useful help of M. Lahlou and H. Outghouliast for grafting and maintaining the collected ecotypes in the experimental station of Ain Taoujdate, Meknes-Morocco. This study was carried out in the frame of the Midterm Research Program of INRA-Morocco through the use of its resources.

REFERENCES

1. Ait Chitt, A., Belmir, H. and Lazrak, A., Bulletin mensuel d'information et de liaison du *PNTTA MAPM/DERD* **153:** 1–4 (2007).
2. Afif, M., Messaoud, C., Boulila, A., Chograni, H., Bejaoui, A. and Nejib Rejeb, M. Genetic structure of Tunisian natural carob tree (*Ceratonia siliqua* L.) populations inferred from RAPD markers. *Ann. Forest Sci*. **65(7):** 710–710 (2008).
3. Albanell, E., Caja, G. and Plaixats J. Characterization of carob fruits (*Ceratonia siliqua* L.) cultivated in Spain for agro-industrial use. *Int. Tree Crops J*. **9 (1):** 1-9 (1996).
4. Barracosa, P., Osório, J. and Cravador, A. Evaluation of fruit and seed diversity and characterization of carob (*Ceratonia siliqua* L.) cultivars in Algarve region. *Scientia horti.,* **114(4):** 250-257 (2007).
5. Barracosa, P., Lima, M.B. and Cravador, A. Analysis of genetic diversity in Portuguese *Ceratonia siliqua* L. cultivars using RAPD and AFLP markers. *Scientia Horti*., **118:** 189-199 (2008).
6. Batista, M.T., Amaral, M.T. and Proenca Da Cunha, A., Carob fruits as source of

natural oxidant. In: Proceedings of the 3rd International carob Symposium, *Tavira, Portugal, June:* 19–23 (1996).

7. Batlle, I. and Tous, J. Promoting the Conservation and Use of Underutilised and Neglected Crops 17 Carob Tree *Ceratonia Siliqua* L. Institute of plant genetics and crop plant research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy, 92pp. (1997).
8. Bostan, S.Z. and Kasko Arici, Y. Evaluation of Some Carob (*Ceratonia siliqua* L.) Genotypes in Silifke (Mersin, Turkey) Province by Cluster Analysis. *Fruit Sci.* **2(1):** 35-41 (2015).
9. El Bakkali, A., Haouane, H., Moukhli, A., Costes, E., Van Damme, P. and Khadari, B. Construction of core collections suitable for association mapping to optimize use of Mediterranean Olive (*Olea europaea* L.) genetic resources. *PLoS ONE* **8(5):** e61265 (2013).
10. El Fazazi, K., Jbilou, M., Assaidi, A., Benbati, M. and Harrak, H. Morphological and Biochemical Variability of Moroccan Carob (*Ceratonia siliqua* L.) Produced in Beni Mellal Region. *Int. J. Pure App. Biosci*. **5 (4):** 14-21 (2017).
11. El Kahkahi, R., Zouhair, R., Ait Chitt, M., and Errakhi, R. Morocco carob (*Ceratonia siliqua* L.) populations: Morphological variability of pods and kernel. *Int. J. Pure App. Biosci*. **2(4):** 38–47 (2014).
12. El Kahkahi, R., Zouhair, R., Diouri, M., Ait Chitt, M. and Errakhi, R. Morphological and biochemical characterization of Morocco carob tree (*Ceratonia siliqua* L.). *Int. J. Biol. Med. Res.* **6(2):** 4946-4952 (2015).
13. FAOSTAT, the Statistical Database. Food and Agriculture Organization of the United Nations (FAO), Rome (2016).
14. Gharnit, N., El Mtili, N., Toubi Ennabili, A., Ennabili, A. Social characterisation and exploitation of carob tree (*Ceratonia siliqua* L.) from Mokrisset and Bab Taza (NW of Morocco). *Science Letters* **3 (2):** 10p (2001).
15. Gharnit, N., El Mtili, N., Ennabili, A., and Sayah, F. Floral charcaterisation of carob tree (*Ceratonia siliqua* L.) from the province of Chefchaouen (NW of Morocco). *Moroccan J. Biol.* **1:** 41–51 (2004).
16. Gharnit, N., El Mtili, N., Ennabili, A., and Sayah, F. Pomological characterization of carob tree (*Ceratonia siliqua* L.) from the province of Chefchaouen (NW of Morocco). *Moroccan J. Biol.* **2(3):** 1–11 (2006).
17. Haddarah, A., Ismail, A., Bassal, A., Hamieh, T., Ioannou, I. and Ghoul, M. Morphological and chemical variability of Lebanese carob varieties. *Eur. Scientific J*. **9(18):** 353-369 (2013).
18. Konate, I., FilaliI-Maltouf, A. and Berraho, E.B. Diversity analysis of Moroccan carob (*Ceratonia siliqua* L.) accessions using phenotypic traits and rapid markers. *Acta Botanica Malacitana* **32:** 79-90 (2007).
19. La Malfa, S., Currò, S., Douglas, A.B, Brugaletta, M, Caruso, M. and Gentile, A. Genetic diversity revealed by EST-SSR markers in carob tree (*Ceratonia siliqua* L.). *Bioch. Syst. Ecol.* **55:** 205-211 (2014).
20. Lê, S., Josse, J. and Husson F. FactoMineR: An R package for multivariate analysis. *J. of Statistical Software* **25:** 1–18 (2008).
21. Naghmouchi, S., Khouja, M.L., Khaldi, A., Rejeb, M.N., Zgoulli, S., Thonart, P. and Boussaid, M. Biochemical Diversity of Wild Carob Tree Populations and Its Economic Value. Topics in Conservation Biology, Dr. Tony Povilitis (Ed.*), InTech*. ISBN: 978-953-51-0540-4 (2012).
22. Nahla, A.A. Relationships among Phenotypic, Chemical and Genetic Characteristics of Some Selected and Evaluated Carob Strains (*Ceratonia siliqua*). *Int. J. Plant & Soil Sci*. **3(11):** 1415-1427 (2014).
23. Neukom, H. Galactomannans: Properties and applications. Lebensm. Wiss. Technol. 22: 41–45 (1989).

24. Ouchkif M, Etude sur le caroubier. Projet Oued Srou, Appendice n° 8, Direction Provinciale d'Agriculture de Khénifra, Maroc (1988).
25. Ozturk, M., Dogan, Y., Sakcali, M.S., Doulis, A., and Karam, F. Ecophysiological responses of some maquis (*Ceratonia siliqua* L., *Olea* oleaster Hoffm. & Link, *Pistacia lentiscus* and *Quercus coccifera* L.) plant species to drought in the east Mediterranean ecosystem. *J. Envir. Biol*. **31:** 233–245 (2010).
26. Petit, M.D. and Pinilla, J.M. Production and purification of a sugar syrup from carob pods, *LWT-Food Sci. Technol*. **28:** 145–152 (1995).
27. Sandolo, C., Coviello, T., Matricardi, P. and Alhaique, F. Characterization of polysaccharide hydrogels for modified drug delivery. *Eur. Biophys*. J. **36(7):** 693–700 (2007).
28. Sidina, M.M., Hansali, E.M., Wahid, N., Ouatmane, A., Boullı, A. and Haddiouı, A. Fruit and seed diversity of domesticated carob (*Ceratonia siliqua* L.) in Morocco. *Scientia Horticulturae* **123:** 110-116 (2009).
29. Tetik, N., Turhan, İ., Oziyci, H.R., Gübbük, H., Karhan, M., Ercişli, S. Physical and chemical characterization of *Ceratonia siliqua* L. germplasm in Turkey. *Scientia Horticulturae* **129:** 583-589 (2011).
30. Tous, J., Romero, A., Hermoso, J.F., Ninot, A., Plana, J. and Batlle I. Agronomic and commercial performance of four Spanish carob cultivars. *HortTechnology* **19(2):** 465-470 (2009).
31. Zohary, M. Geobotanical Foundations of the Middle East 2 Vols. *Stuttgart* (1973).
32. Zohary, M. Domestication of the carob (*Ceratonia siliqua* L.). *Israel J. Plant Sci*. **50:** 141–145 (2002).