BIO Web of Conferences **109**, 01040 (2024) https://doi.org/10.1051/bioconf/202410901040
WA2EN2023

Assessing Morphological Trait Variability in Moroccan Carob (*Ceratonia siliqua* L.) Ecotypes for Adaptive Breeding in Response to Climate Change and Sustainable Food Security

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Abstract. The study focused on the morphological diversity of pods and seeds from six ecotypes of carob tree (*Ceratonia siliqua* L.), originating from different regions of Morocco including Khemissat, Safi, Aït Attab, Berkane, Ouezzane, and Agadir. Thirty pods per ecotype were collected from 10 different trees. Measurements were taken to analyze the morphology of seeds and pods. The results revealed significant differences among ecotypes for most morphological characteristics such as pod length, pod width, pod thickness, total pod weight, pulp weight, total number of seeds in the pod, seed weight within the pod, seed length, seed width, seed thickness, single seed weight, and weight of 100 seeds. Correlation analysis revealed associations among different morphological measures, highlighting the influence of pod morphology on seed yield. Principal component analysis (PCA) and hierarchical clustering analysis confirmed the presence of two distinct groups of ecotypes: the first group comprising the ecotypes Agadir, Aït Attab, Safi, and Khemissat, while the second group included the ecotypes Berkane and Ouezzane. These results have significant implications for variety selection and improvement of carob tree cultivation, with significant potential to enhance food security in Mediterranean regions. By better understanding the impact of ecotype on morphological characteristics, selection efforts can be directed towards developing varieties better adapted to changing environmental conditions, thereby contributing to strengthening the resilience of agricultural systems face of climate change.

1 Introduction

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Food security, a major global issue, is exacerbated by the challenges of climate change, threatening agricultural production and the stability of food systems [1]. From this perspective, it is crucial to understand plant responses to environmental changes and to develop adaptation strategies to ensure sustainable agricultural production [2]. The carob tree (*Ceratonia siliqua* L.), commonly found in the Mediterranean region, offers a promising prospect for diversifying agriculture in coastal semi-arid areas. With great resilience to harsh conditions such as drought and alkaline soils, this evergreen tree can live up to 200 years, producing between 300 and 800 kg of carobs per tree [3]. The carob fruit, the pod, is rich in nutritious pulp, containing carbohydrates, dietary fiber and essential minerals, with polyphenolic properties beneficial to human health [4-5]. Carob flour also finds applications in food production, particularly in gluten-free baked goods [6]. Carob seeds are an important source of carob gum, used in various industries for its thickening and stabilizing properties [7-8]. Growing interest in the exploitation of carob seeds highlights its economic potential as a source of locust bean gum, a natural food additive used in the food, pharmaceutical and cosmetic industries [9-10].

In Morocco, the carob tree is commonly referred to as "kharroub", "slaghoua", and "tikida" in the Moroccan dialect [11]. It is distributed across the plains and medium mountains of the Rif, the Middle Atlas, the High Atlas, and the Anti-Atlas, encompassing humid, sub-humid, semi-arid, and coastal arid bioclimates with warm and temperate variations [12]. According to data from the Food and Agriculture Organization of the United Nations (FAO), Morocco ranks second globally in terms of the area dedicated to this cultivation, covering 10,224 hectares [13]. Presently, the Kingdom has initiated a strategy to cultivate this species resilient to challenging climatic conditions, aiming to expand the cultivation to 100,000 hectares by 2030 in private sectors [14]. Moreover, Morocco holds the third position among the primary producers of carob trees, following Portugal and Italy [13].

The objective of this study is to investigate how various ecotypes of the carob tree influence its morphological characteristics, considering their adaptation to diverse environments. By comparing multiple ecotypes, we aim to discern the notable effects they exert on these traits and elucidate any correlations with environmental factors. Furthermore, we seek to evaluate the implications of these findings for agricultural productivity and food security amidst the backdrop of climate change.

2 Materials and methods

2.1 Plant material

The study was carried out on six ecotypes of carob (*Ceratonia siliqua* L.) from various regions of Morocco (Table 1). Thirty pods were collected from 10 different trees for each ecotype. The selected pods were characterized by their optimal stage of maturity and the absence of visible defects.

Table 1. Geographic and meteorological conditions of the domestic carob ecoregions utilized in the study.

Ecotype	Geographic Region	Altitude (m)	Precipitation (mm)	Climate
Khemissat	Central Plateau	400-500	350	Continental Mediterranean
Safi	Atlantic Coast	70-100	450	Temperate Oceanic
Aït Attab	Middle Atlas Mountain	500-800	600	Mountainous
Berkane	Northeast	150-350	300	Semi-arid Mediterranean
Ouezzane	Rif Mountain	350-550	600	Mountain Mediterranean
Agadir	West Coast	150-350	200	Coastal Desertic

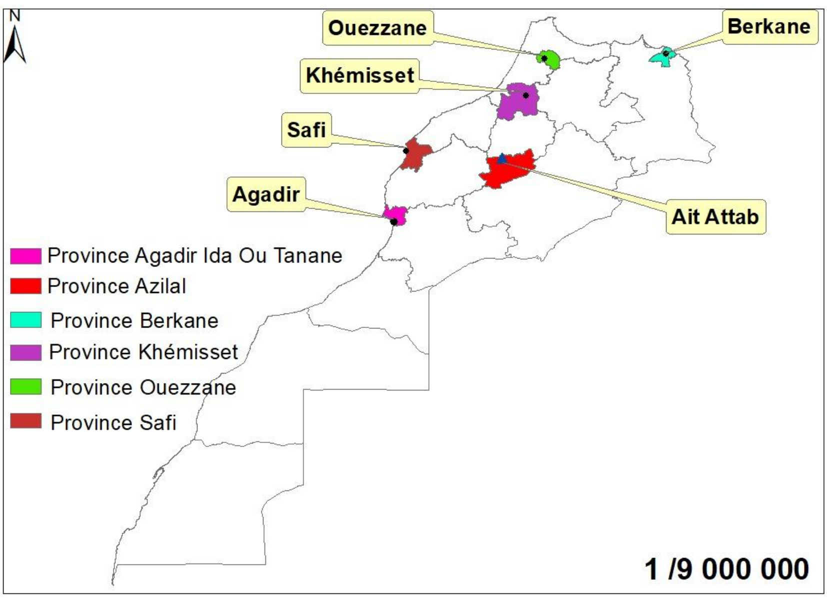

Fig. 1. Geographical distribution of Moroccan carob cultivars studied.

2.2 Morphological study of fruits

The morphological analysis of the fruits involved measuring several parameters on a representative sample of pods and seeds. Pod length (from peduncle to apex), width (at the widest point), and thickness were measured using a caliper. The total weight of the pods was determined using an analytical balance.

After separating the pulp from the seeds, the pulp was weighed with an analytical balance to obtain its weight. The total number of seeds per pod was determined by manual counting after pod opening. The seed weight per pod was measured using an analytical balance.

Seed dimensions (length, width, and thickness) were measured using a caliper. The weight of a single seed and the weight of 100 seeds were determined using an analytical balance.

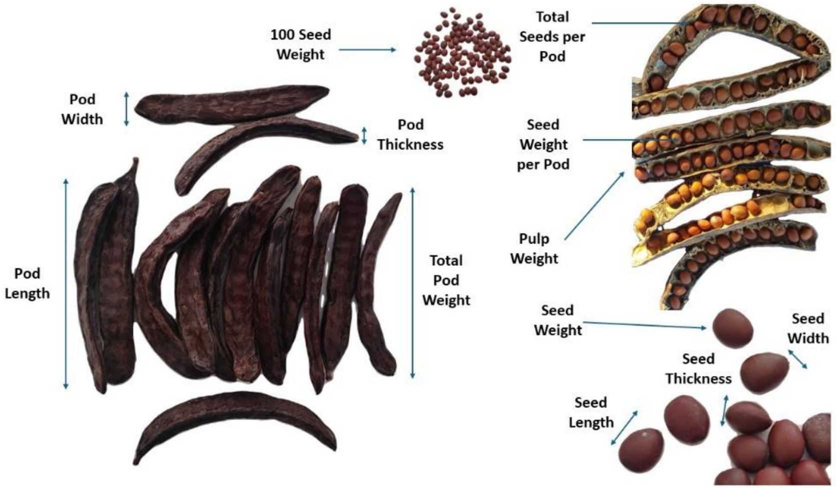

Fig. 2. Morphological parameters studied in carob fruits (seeds and pods).

2.3 Statistical Analysis

Data analysis was conducted using IBM SPSS Statistics 23 software. Analysis of variance (ANOVA) was performed for each morphological parameter to ascertain the significant effect of the ecotype. Tukey post-hoc tests were utilized to identify significant differences among ecotypes. Correlation analyses were conducted to assess the relationships between the various morphological parameters. Principal component analysis (PCA) was performed to visualize the data structure and identify the variables contributing most to observed variation. Finally, hierarchical analysis was conducted to classify the ecotypes based on their morphological characteristics.

3 Results

3.1 Morphological analysis

The results of the analysis of variance (ANOVA) reveal significant differences among the studied ecotypes across various morphological parameters of pods and seeds (Table 2). Pod length exhibited significant variability among ecotypes (F-value = 13.883, $p < 0.001$). Similarly, pod width (F-value = 40.893, $p < 0.001$) and pod thickness (F-value = 133.899, $p < 0.001$) varied significantly among ecotypes, indicating distinctive morphological characteristics. Total pod weight and pulp weight also exhibited considerable variability across ecotypes (F-value = 185.614 and 177.207, respectively, $p < 0.001$). Moreover, the total number of seeds per pod (F-value = 30.518, $p < 0.001$) and seed weight per pod (F-value = 95.050, $p < 0.001$) showed significant differences among ecotypes, emphasizing the diversity in reproductive traits. Seed dimensions, including length, width, and thickness, demonstrated significant variation among ecotypes, reflecting distinct seed morphologies (F-values ranging from 4.815 to 34.697, all $p < 0.001$). Additionally, seed weight (F-value = 106.934, $p < 0.001$) and the weight of 100 seeds (F-value = 239.328, $p < 0.001$) varied significantly among ecotypes, underscoring differences in seed quality and yield potential. Overall, these findings highlight the substantial morphological diversity among the studied carob tree ecotypes, which may have implications for agricultural practices and breeding strategies.

Table 2. Analysis of variance (ANOVA) of morphological characteristics of carob pods and seeds.

Morphological Parameter	Sum of Squares	df	Mean Square	F-value	P-value
Pod Length	337.393	5	67.479	13.883	0.000
Pod Width	11.731	5	2.346	40.893	0.000
Pod Thickness	8.478	5	1.696	133.899	0.000
Total Pod Weight	5734.84	5	1146.968	185.614	0.000
Pulp Weight	4614.009	5	922.802	177.207	0.000
Total Seeds per Pod	655.657	5	131.131	30.518	0.000
Seed Weight per Pod	82.986	5	16.597	95.050	0.000
Seed Length	0.598	5	0.120	23.300	0.000
Seed Width	1.571	5	0.314	4.815	0.000
Seed Thickness	0.701	5	0.140	34.697	0.000
Seed Weight	0.270	5	0.054	106.934	0.000
100 Seed Weight	2134.328	5	426.866	239.328	0.000

The six ecotypes investigated exhibit remarkable diversity in the morphological attributes of both pods and seeds (Table 3). Pod length ranges from 11.04 cm (Aït Attab) to 14.33 cm (Berkane), showcasing considerable variation among the studied ecotypes. Ouezzane stands out with the widest pods, measuring 1.95 cm, whereas Aït Attab displays the narrowest pods at 1.49 cm. Notably, pod thickness remains relatively consistent across ecotypes, hovering around 0.45 cm.

Significant variability is observed in total pod weight and pulp weight, with Berkane exhibiting the highest average values (18.23 g and 15.62 g, respectively) and Aït Attab recording the lowest (5.74 g and 4.39 g, respectively). Similarly, the total number of seeds per pod exhibits some disparity, with Berkane presenting the highest average count (12.34) and Khémissat displaying the lowest (7.44).

Seed weight demonstrates moderate variation, ranging from 1.01 g (Aït Attab) to 2.56 g (Berkane), reflecting the distinct characteristics of each ecotype. Conversely, seed dimensions, including length, width, and thickness, show relatively low variability compared to other parameters, with average measurements ranging around 0.8-0.9 cm.

The weight of 100 seeds mirrors the trends observed in total pod weight and pulp weight, with considerable diversity among ecotypes, ranging from 12.43 g for Aït Attab to 23.45 g for Berkane. These findings underscore the significant morphological disparities among the studied ecotypes, highlighting the need for comprehensive understanding to harness their full potential in agricultural practices.

Table 3. Average values of morphological characteristics of Moroccan carob pods and seeds.

Traits\Ecotype	Agadir	Ouezzane	Berkane	Aït Attab	Safi	Khemissat
Pod Length (cm)	12,82±2,75 b	13,98±2,06 a	14,32±1,32 a	11,03±1,81 c	12,65±2,62 b	12,84±2,32 b
Pod Width (cm)	1,5±0,29 c	1,95±0,36 a	1,9±0,1 ab	1,49±0,2 c	1,5±0,18 c	1,79±0,19 b
Pod Thickness (cm)	0,37±0,09 c	0,47±0,17 b	0,78±0,1 a	0,25±0,07 d	0,33±0,11 c	0,44±0,08 b
Total Pod Weight (g)	8,01±2,48 c	12,56±2,92 b	18,23±2,98 a	5,73±1,06 d	5,97±1,47 d	8,5±3,18 c
Pulp Weight (g)	6,18±2,19 c	10,37±2,64 b	15,62±2,84 a	4,39±0,84 d	4,58±1,22 d	7,23±3,01 c
Total Seeds per Pod	11,1±2,69 b	10,2±1,92 bc	12,3±1,36 a	9,7±2,19 c	9,9±1,92 bc	7,4±2,1 d
Seed Weight per Pod (g)	1,78±0,45 c	2,1±0,49 b	2,55±0,35 a	1,32±0,35 d	1,32±0,4 d	1,01±0,42 e
Seed Length (cm)	0,78±0,07 c	0,9±0,06 a	0,81±0,05 b	0,91±0,06 a	0,85±0,09 b	0,85±0,07 b
Seed Width (cm)	0,56±0,05 bc	0,79±0,61 a	0,62±0,04 b	0,6±0,07 b	0,63±0,06 b	0,61±0,05 b
Seed Thickness (cm)	0,32±0,06 a	0,31±0,06 a	0,34±0,06 a	0,24±0,05 bc	0,26±0,06 b	0,21±0,05 c
Seed Weight (g)	0,18±0,01 c	0,24±0,02 a	0,21±0,01 b	0,15±0,01 e	0,17±0,02 d	0,16±0,02 de
100 Seed Weight (g)	17,3±0,8 c	21,54±1,55 a	20,24±0,84 b	13,83±1,24 e	15,81±2,05 d	15,92±2,46 d

(Values sharing the same letters within each category are not significantly different at a 5% significance level).

3.2 Correlation analysis

The correlation matrix illustrates the relationships between various morphological parameters of carob pods and seeds (Table 4). Pod length is positively correlated with several measures, including total pod weight (r = 0.556), pulp weight (r = 0.528), total number of seeds (r = 0.599), weight of one seed (r = 0.308), and the weight of 100 seeds (r = 0.371). This suggests that, in general, longer pods tend to contain heavier seeds. Pod thickness is positively correlated with total pod weight (r = 0.818), pulp weight (r = 0.821), and seed weight (r = 0.471). This indicates that greater pod thickness is associated with higher pod and seed weight. Total pod weight is positively correlated with almost all other measures, including pulp weight (r = 0.992), total number of seeds (r = 0.511), single seed weight (r = 0.575), and the weight of 100 seeds (r = 0.654). As expected, heavier pods generally contain more material (pulp) and heavier seeds. Pulp weight was positively correlated with the total number of seeds (r = 0.455) and the weight of a seed (r = 0.557). This suggests that a greater amount of pod pulp is associated with a greater number of seeds and heavier seeds. The total number of seeds is positively correlated with the weight of one seed (r = 0.800) and the weight of 100 seeds (r = 0.291). This means that, in general, the more seeds in a pod, the heavier they are individually and collectively (weight of 100 seeds). The weight of a seed is positively correlated with the weight of 100 seeds (r = 0.589). As expected, individually heavier seeds contribute to a higher 100-seed weight. Additionally, seed length, seed width, and seed thickness do not show strong, significant correlations with many other measurements. Pod length did not show a strong correlation with seed length (r = -0.075) or seed width (r = 0.032). Furthermore, pod thickness has a weak negative correlation with seed length (r = -0.189). Also, pod width does not show strong correlations with most seed measurements.

Table 4. Pearson's correlation coefficient between the morphological parameters of the pods and seeds of the Moroccan carob tree.

Morphological Parameter	PL	PW	PT	TPW	PuW	TSPP	SWPP	SL	SW	ST	SW
PL	1										
PW	0.320**	1									
PT	0.327**	0.475**	1								
TPW	0.556**	0.637**	0.818**	1							
PuW	0.528**	0.642**	0.821**	0.992**	1						
TSPP	0.599**	0.104	0.277**	0.511**	0.455**	1					
SWPP	0.541**	0.381**	0.552**	0.767**	0.715**	0.800**	1				
SL	-0.075	0.098	-0.189**	-0.128*	-0.122*	-0.112	-0.117*	1			
SW	0.032	0.090	0.052	0.066	0.052	0.060	0.142*	0.053	1		
ST	0.177**	0.058	0.308**	0.356**	0.329**	0.350**	0.471**	-0.235**	0.014	1	
SW	0.308**	0.434**	0.462**	0.575**	0.557**	0.299**	0.589**	0.159**	0.119*	0.549**	1
100 SW	0.371**	0.434**	0.555**	0.654**	0.640**	0.291**	0.589**	-0.089	0.169**	0.435**	0.706**

PL: Pod Length; PW: Pod Width; PT: Pod Thickness; TPW: Total Pod Weight; Puw : Pulp Weight; TSPP: Total Seeds per Pod ; SWPP: Seed Weight per Pod; SL: Seed Length; SW: Seed Width; ST: Seed Thickness; SW: Seed Weight ; (** The correlation is significant at the 1%. * The correlation is significant at 5%).

3.3 Principal component analysis

The results of the Principal Component Analysis (PCA) provide insight into the underlying structure of the dataset regarding the morphological characteristics of carob pods and seeds. The PCA generated two principal components, F1 and F2, which collectively explain a significant portion (85.62%) of the variance in the data (Figure 3). The first principal component, F1 appears to be primarily associated with variables related to pod dimensions and weights. Ecotypes with higher positive scores on F1, such as Berkane and Ouezzane, are associated with larger pod and seed dimensions, suggesting they exhibit greater overall size and weight characteristics. Conversely, ecotypes with lower scores on F1, such as Aït Attab and Agadir, appear to have smaller pod and seed dimensions, indicating they may possess comparatively smaller overall size and weight characteristics. The second principal component, F2 captures the variability in the size and weight of carob seeds. Ecotypes with higher positive scores on F2, such as Ouezzane (2.450) and Khemissat (0.967), may exhibit distinctive seed traits or proportions compared to ecotypes with lower scores on F2, such as Berkane and Safi.

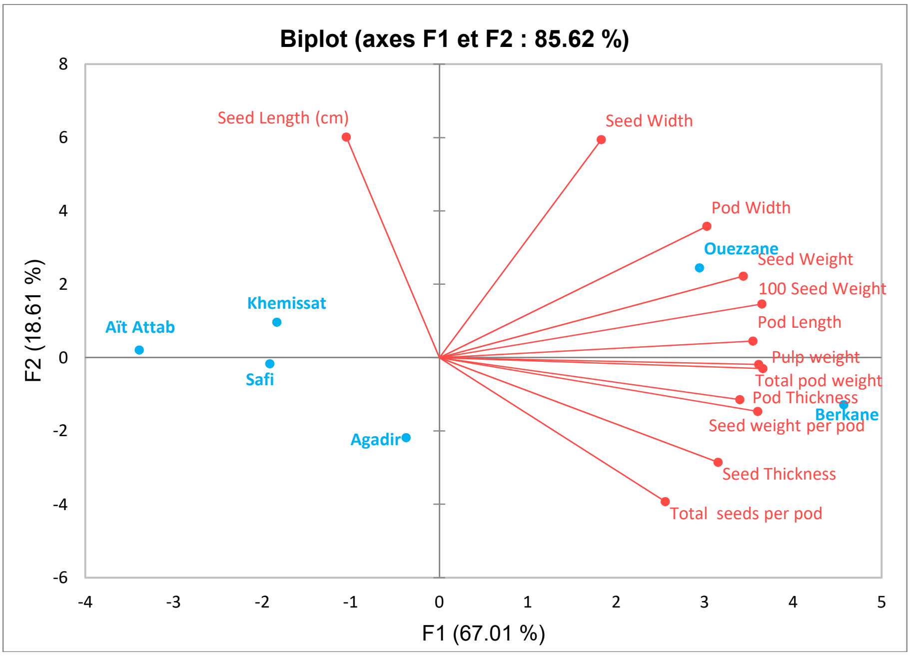

Fig. 3. Principal component analysis of morphological characters (pods and seeds) of 6 ecotypes of Moroccan carob trees.

3.4 Hierarchical analysis

The hierarchical analysis results, based on the proximity matrix (Euclidean distance) among the various carob ecotypes examined, delineate two distinct groupings (Figure 4). The first cluster comprises the Agadir and Safi ecotypes, showing the lowest Euclidean distance between them, indicating a notable similarity in morphology. These ecotypes stand out for their larger, wider, and thicker pods, along with higher seed yields, positioning them as promising candidates for breeding programs aimed at enhancing carob tree productivity and yields. In contrast, the remaining ecotypes form a second cluster, including Aït Attab, Khemissat, Agadir, and Safi, suggesting a degree of similarity in their morphological traits. Conversely, Berkane and Ouezzane exhibit significant Euclidean distances from most other ecotypes, signifying marked differences in their morphological features compared to the rest. These ecotypes are characterized by smaller pods and more moderate seed yields in contrast to Berkane and Ouezzane, thus highlighting their distinct morphological profiles.

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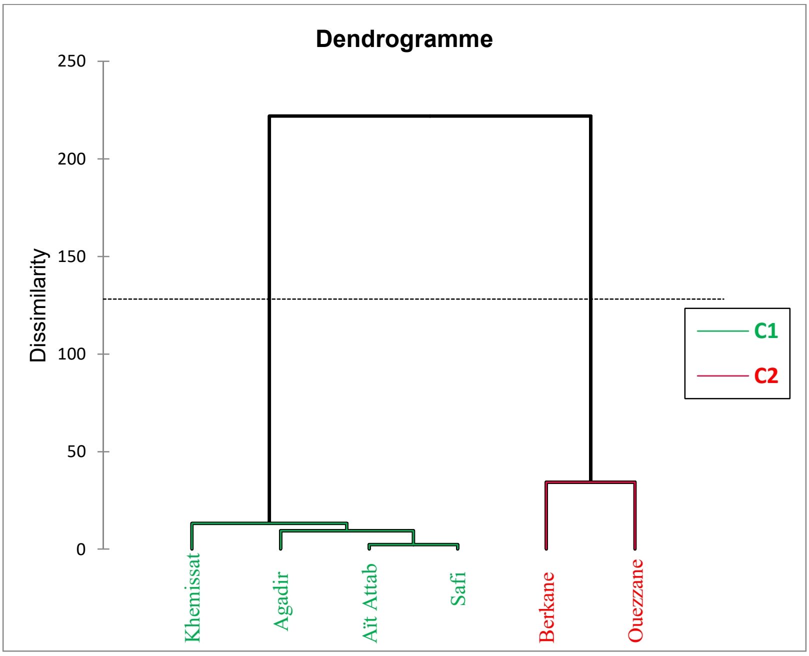

Fig. 4. Dendrogram illustrating the hierarchical grouping of 6 Moroccan carob ecotypes based on morphological traits (pods and seeds).

4 Discussion

The Mediterranean climate is subject to multiple pressures: decreased precipitation, rising temperatures, and intensified extreme weather events (Lionello & Scarascia, 2018). In response to these changes, Mediterranean plant species exhibit phenotypic plasticity that enables them to adapt [15]. This phenotypic adaptation capability is a significant asset for their survival [16]. Scientists have long been interested in characteristics that allow plants to adapt to their environment [17]. Among these characteristics, functional traits, which include plant morphology, physiology, and phenology, play a crucial role in understanding adaptation and evolution mechanisms [18]. Regarding the carob tree, its physical characteristics and functioning serve as valuable tools for variety classification [19]. The physical characteristics of pods and seeds help distinguish different cultivars and provide insights into tree vigor, productivity, disease resistance, sex, and precocity [20].

In Spain, researchers identified a strong correlation between fruit length and total seed weight in carob tree cultivars. The correlation coefficient of 0.60 indicates a positive and significant relationship between the two variables. Additionally, they observed a strong correlation between fruit length and total seed weight in Spanish cultivars. The number of seeds per pod was also very similar between Spanish and Cypriot carob trees, with a consistent ratio between the number of seeds and pod length. These observations suggest that the number of seeds is a stable characteristic of the species, while seed weight is influenced by fruit length [21].

A study by [22] examined the diversity of domesticated carob trees in Morocco across 13 ecoregions, finding significant differences in pod and seed characteristics. They identified two main groups: northern regions with larger pods and higher pulp weight, and central/southwestern regions with shorter pods but higher seed yield. Our research corroborates the findings of [22], indicating correlations among morphological traits, geographical parameters, and precipitation. Like their study, ours also identifies a geographical structure within carob diversity in Morocco. We note similarities in group composition, especially concerning ecotypes. Interestingly, certain ecotypes, such as Berkane and Agadir, consistently cluster together in both studies (Figure 5).

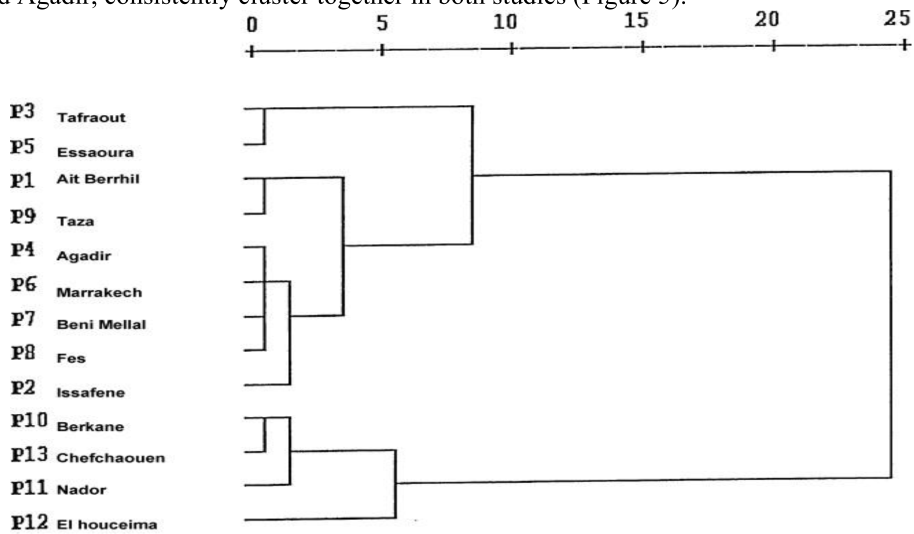

Fig. 5. Dendrogram (hierarchical clustering) of 13 Moroccan carob ecoregions based on morphological traits [22].

The study conducted by [23] examined the variability of pod and seed characteristics of carob among different populations. Significant differences were observed, with a wide variation in traits and a high coefficient of variation. Genetic diversity, environmental conditions, and geographical distribution are factors contributing to this variability. This diversity has significant implications for agriculture and the food industry, as it can affect seed yield, economic value, and nutritional composition of carob crops. Understanding this variability is thus essential for selecting high-yielding varieties and improving crops.

Several studies have shown that carobs have various morphological traits in their pods and seeds, which allow cultivars to be differentiated in terms of productivity [24, 25, 26]. The industrial applicability of the species is significantly impacted by the size of both the kernels and pods, as well as the number of kernels within each pod. These attributes may vary depending on the genotype and geographical region of origin [27]. Moreover, regional factors such as soil texture, plant age, cultivation techniques, and climate further contribute to the variability observed in carob pod traits. Understanding these interactions is crucial for optimizing cultivar selection, enhancing productivity, and ensuring sustainable food security.

5 Conclusion

This study highlights the importance of ecotype in determining the morphological characteristics of *Ceratonia siliqua* L. (carob tree) pods and seeds. The results demonstrate significant diversity among different ecotypes, with notable variations in pod length, width, thickness, and weight, as well as in the number and weight of seeds. Correlation analysis reveals interesting associations among various morphological measures, underscoring the influence of pod morphology on seed yield. The observed correlations suggest that longer and thicker pods tend to contain a greater number of seeds, as well as individually heavier seeds, which has direct implications for crop productivity and quality. Furthermore, principal component analysis (PCA) and hierarchical clustering analysis confirm the presence of distinct groups of ecotypes, thus reinforcing the robustness of the observed differences. This study underscores the importance of considering the genetic diversity of carob tree ecotypes in variety selection programs to maximize the potential of this crop to address future challenges in food security and adaptation to climate change.

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