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Image analysis of Moroccan carob seeds (*Ceratonia siliqua* L.) revealed substantial intraspecific variations depending on climate and geographic origin

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Abstract

Background: The carob tree (*Ceratonia siliqua* L.) is one of the most iconic tree species of the Mediterranean region, with valuable economic, ecological and cultural value. Carob has been exploited around the Mediterranean region since antiquity and has been regarded as an important component of natural habitats and traditional agroecosystems. Several studies have focused on its morphological, biochemical, and genetic diversity. However, less is known about the intraspecific variation of seed traits. In this regard, and as an overall objective, we intend to evaluate the amplitude and the expression of intraspecific variations of carob seed traits at different ecological scales ranging from individual trees to different geographical landscapes. In addition, we investigated how the climate along the study area affects the extent of carob seed variability. Using image analysis techniques, we measured seven traits related to the size and the shape of 1740 seeds collected from 18 populations of spontaneous *C. siliqua* distributed along a latitudinal transect in Morocco under different bioclimatic conditions.

Results: The morphometric analysis of carob seed showed the effectiveness of adopted approach to highlight the amount and the amplitude of intraspecific variation according to geographic and climatic factors. Seed trait analysis revealed high intraspecific variability, explained by differences between and among carob populations and geographic zones. Seed area, perimeter, length, and width showed the largest variability between geographic zones. However, circularity, aspect ratio, and seed roundness showed higher variability at the tree level. Finally, our results show that seed traits vary depending on altitude and climate condition.

Conclusions: Revealing the amount and the structure of intraspecific traits variability of carob seed provides interesting insights to understand the mechanisms underlying trees adaptation to various environmental and ecological conditions. Therefore, intraspecific variation of seed traits should be integrated into trait-based functional ecology to assess plant species responses to environmental changes.

Keywords: Carob tree, Image analysis, Seed traits, Intraspecific variation, Morocco

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Introduction

The carob tree (*Ceratonia siliqua* L., Leguminosae) is an evergreen, thermophilous, and dioecious Mediterranean fruit tree (Quézel and Médail 2003), with some rare hermaphrodite and monoecious cases (Batlle and Tous

1997). The carob is a slow-growing and long-lived tree with high resistance to drought and limited resistance to extreme cold (Benmahioul et al. 2011; Tous et al. 2013). It has been exploited around the Mediterranean region since antiquity as a food and forage source (Zohary 2002). In fact, several authors have reported that the carob tree represents an important component of natural habitats of the Mediterranean region and its traditional agroecosystems (Ramón-Laca and Mabberley 2004; Hmimsa 2009; Viruel et al. 2019). Previous archeological and historical studies (de Candolle 1883; Hillcoat et al. 1980; Zohary 2002) suggested an Eastern domestication center of the carob tree followed by human-driven dissemination to the Western parts of the Mediterranean region. However, recent phylogeographic evidence suggested a strong west–east genetic structuring and the presence of multiple domestication centers from native populations all over the Mediterranean basin. Consequently, four main genetic groups of *C. siliqua* are identified and correspond to South Morocco, the Iberian Peninsula, the Central Mediterranean, and the Eastern Mediterranean (Baumel et al. 2021). In addition, the increasing commercial value of carob has triggered more attention to this multipurpose tree. Carobs have been broadly grown for their fruits (commonly known as pods), which are nowadays highly used in the agro-food industry, in which the pulp represents the major part and is mainly used to produce syrups and powder (Bengoechea et al. 2008; El Batal et al. 2011; Papaefstathiou et al. 2018; Brassesco et al. 2021). The seeds are the most valuable components of carob pods, accounting for 10 to 20% of their weight (Sidina et al. 2009; Boublenza et al. 2019). The gum extracted from the endosperm of carob seeds is extremely sought for pharmaceuticals and cosmetics products (Ayaz et al. 2007; Stavrou et al. 2018).

Carob tree has been the subject of many studies concerning its ecology and distribution (Tous et al. 2013; Baumel et al. 2018), phylogeny and evolution (Barracosa et al. 2008; Viruel et al. 2019), morphology (Sidina et al. 2009; Naghmouchi et al. 2009), and biochemistry of pods and seeds (Haddarah et al. 2014; Boublenza et al. 2017). Despite the plethora of research on various aspects of carob, this tree needs further attention for its value as a genetic resource and its highly appreciated ecologic and economic benefits. However, this situation is threatened by the current global changes, including climate change and the increasing anthropogenic pressures on natural habitats. More importantly, the understanding of its seed trait variability according to environmental factors could bring new insights on carob responses to changes in environmental conditions (Saatkamp et al. 2019). Thus, it will contribute to the consolidation of trait-based models taking into account the intraspecific variations (Albert et al. 2010a, b), which represent a large fraction of the total trait variation (Kassout et al. 2019; Kuppler et al. 2020).

Seeds are an essential element determining the success of flowering plants in their environment (Venable and Browns 1988; Saatkamp et al. 2014). Therefore, seed-related traits have been recognized as important factors in the persistence, dispersal, and expansion of plant species (Fricke et al. 2019; Beckman et al. 2020). Additionally, it was found that they are associated with germination capacity and, therefore, the success of growth and development (Gholami et al. 2009; Baskin and Baskin 2014; Xu et al. 2019). Hence, seed traits may determine plant strategies for adapting and colonizing different habitats (Lamberti-Raverot et al. 2019). In this regard, it has been shown that variations in seed size, mass, and morphology are induced by changes in environmental conditions, and therefore, they exhibit different phenotypes as a response to their surrounding environments (Pluess et al. 2005; Moles and Westoby 2006; Fenner and Thompson 2005). For instance, seed size increases with increasing rainfall or higher temperatures in low latitudes (Molina-Montenegro et al. 2018; Wu et al. 2018). Yet, on a global scale, the interspecific seed size decreases by two or three orders of magnitude with latitude, from the equator to 60° N (Moles et al. 2007). In addition, previous findings show that seed size and shape respond to changes in climatic variables (De Frenne et al. 2010; Soper Gorden et al. 2016), geographic gradients (Murray et al. 2004; Fenollosa et al. 2021), and changes in local factors such as water availability and soil pH (Fenner 1992; Tautenhahn et al. 2008). Consequently, variations in seed-related traits can occur both within and between species (Moles and Westoby 2006; Wiwart et al. 2019), which seems to be the result of genetic differences and heterogeneous environmental conditions (Westoby et al. 1992; Fenner and Thompson 2005; Moles et al. 2005). Therefore, seed morphological traits have been used to understand the taxonomic position of plant species (Minuto et al. 2006; Bacchetta et al. 2008, 2011; Pinna et al. 2014; Cervantes et al. 2019; Martín-Gómez et al. 2019) and to determine the impact of environmental factors on their phenotypic variability (Grime et al. 1987; Fenner 1992; Cochrane et al. 2015). Furthermore, they have been used to infer domestication events, geographic origin, and varietal diversity of crop species (Terral et al. 2004, 2010, 2012; Wallace et al. 2019; Wiwart et al. 2019; Bonhomme et al. 2021). However, the complexity of the ecological mechanisms underlying the variation in seed traits makes it even more difficult to establish general rules of their variability according to environmental gradients (Fenner and Thompson 2005; Saatkamp et al. 2019). Moreover, even though the well-known importance of within-species variation in the ecological and evolutionary processes

(Laforest-Lapointe et al. 2014), the intraspecific variation of seed traits is still a major concern in trait-based ecology (Saatkamp et al. 2019; Kuppler et al. 2020).

Despite the increasing number of available publications on carob morphological traits (Naghmouchi et al. 2009; Sidina et al. 2009; Boublenza et al. 2017), there is still a lack of information on the seed shape and size-related traits and their variations according to environmental factors. In this context, the aims of our study are to (1) characterize the intraspecific variability of seed traits along the geographic distribution of the carob tree in Morocco; (2) understand how trait variability is structured between and within carob populations, and (3) explore how seed traits vary depending on environmental factors, such as climate and altitude.

Materials and methods

Study area and field sampling

Sampling in the field requires no authorization; the study populations are located in natural public areas outside protected natural areas. The species studied (*Ceratonia siliqua* L.) is not rare or protected.

Eighteen populations of spontaneous carob trees growing under natural conditions and distributed along a latitudinal gradient from the North to South region of Morocco were investigated (Additional file 1: Appendix S1). The field context includes a wide range of habitats and vegetation types across different bioclimatic stages, from sub-humid to semi-arid. At each population, five healthy and mature trees were randomly selected with a distance of at least 20 to 30 m from each other, to capture a range of genetic variations. Then from each tree, 1 kg of healthy pods was harvested from the four exposures (north, south, east, and west), in which 25 pods were randomly taken, and then 20 seeds were randomly selected to undergo image analysis. It is noteworthy to mention that instead of five, only three and four trees were selected, respectively, from Imi N'Tlit and Ouaouizeght populations (Additional file 1: Appendix S1). A total of 1740 seeds were sampled and measured. The sampling procedure allows for a four-level hierarchical model, each level representing an organizational scale: (1) 'the geographic zone' between different geographic sampling zone (e.g., North, Center, and South); (2) 'the population level' between different populations; (3) 'the tree level' between different trees in the same population, and (4) 'the seed level' between different seeds of the same tree.

The climate of each population was defined using five variables: mean annual temperature (MAT, °C), mean temperature of the coldest month (MTCM, °C), mean temperature of the warmest month (MTWM, °C) and the mean annual precipitation (MAP, mm), all extracted from the Worldclim database (Fick and Hijmans 2017), at a resolution of 30 arc-second (~1 km^2). Altitude was recorded on each population as an environmental variable, using a GPS (Garmin, GPSmap62).

Seed image analysis

Digital images of 20 seeds, collected from each tree, were captured with a flatbed scanner (Canon LIDE 120) at 600 dpi resolution. Image analysis was performed using ImageJ (v. 1.53e) program (Rasband 2018). The measurements were performed on each image containing 20 seeds, which were placed in the scanner without touching one another (Additional file 2: Fig. S1). To enhance the contrast between carob seeds and the background, a blue-dark paper background was used. In the first step of image segmentation, 8-bit gray images were obtained. A thresholding procedure was then adopted to generate binary images, which were used for forwarding measurement (Additional file 2: Fig. S1). Seven shape descriptors were measured using ImageJ and each one captures a ratio describing the variation in seed shape (Cope et al. 2012). These measurements are as follows: area (mm^2), perimeter (mm), length (mm), width (mm), circularity, roundness, and aspect ratio (Table 1). Circularity is a

Table 1 Summary statistics of the corresponding seed traits

Variable (unit)	*N*	Mean	S.D.	Min–Max	CV (%)	$F_{18, 1740}$
Area (mm^2)	1740	55.28	10.25	30.32–89.39	18.54	125.67***
Perimeter (mm)	1740	29.31	2.86	21.86–41.21	9.74	129.75***
Width (mm)	1740	7.27	0.79	5.56–10.75	10.91	80.74***
Length (mm)	1740	9.83	1.03	6.05–12.48	10.43	85.42***
Circularity	1740	0.803	0.03	0.686–0.878	3.70	16.64***
Aspect ratio	1740	1.396	0.13	1.045–1.938	9.38	25.15***
Roundness	1740	0.722	0.07	0.516–0.957	9.20	25.01***

One-way ANOVA tests result and coefficient of variation (CV%) per seed trait are given

Values labeled with "***" are statistically significant at $P < 0.001$; *N*, number of seeds used in the analysis; S.D., standard deviation; Min, minimum value; Max, maximum value ratio of area to perimeter measured as: $\text{Cir.} = 4\pi * \left(\frac{\text{area}}{\text{perimeter}^2}\right)$, with values increasing from 0.00 to 1.00 and, respectively, moving from an elongated shape to a perfect circle shape. Aspect ratio is measured as: $\text{AR} = \frac{(\text{Major axis})}{(\text{Minor axis})}$, reflecting the overall length-to-width ratio of a seed. Roundness is measured as: $\text{Round.} = \frac{(4*\text{area})}{(\pi*(\text{major axis})^2)}$, of which the value 1 indicates a completely round seed.

Statistical analysis

The output of digital image analysis was analyzed using R software v. 4.0.5 (R Core Team 2020). Descriptive analyses (e.g., mean, standard deviation) were used to highlight general trends in carob seed traits. To assess the amplitude of trait variability, the coefficient of variation was calculated as CV (%) = standard deviation$_{\text{trait}}$/mean$_{\text{trait}}$ × 100 (Sokal and Braumann 1980). To evaluate the population's and geographic zone's differentiation in individual seed traits, a one-way ANOVA was conducted, and the mean values were compared using the Tukey HSD post hoc tests with a significance level of $P = 0.05$ using the *multicomp* (Hothorn et al. 2008) and *agricolae* (Mendiburu and Yaseen 2020) R packages. ANOVA assumptions were tested with Shapiro–Wilk and Levene tests for normality and homoscedasticity. To determine the relationship between the geographic distance and seed traits variation among carob populations, Mantel tests and 999 permutations were undertaken using the *Mantel()* function in the *vegan* package (Oksanen et al. 2019). Trait distances were calculated as Euclidean distance using *dis()* function. Principal component analysis (PCA) was performed for inter-population comparisons of trait values, using *FactoMiner* R package (Lê et al. 2008). To quantify the trait variance across the studied hierarchical levels of geographic zones, populations, trees, and seeds, a variance decomposition procedure was used (Albert et al. 2010b; Kassout et al. 2019). The *lme()* function in the *nlme* package (Pinheiro et al. 2021) was used to fit a general linear model using the restricted maximum likelihood method (RMEL) across the studied levels, and then the *varcomp()* function in the *ape* package (Paradis et al. 2004) was used to extract the variance expressed at each level. To examine the relationships between seed traits, climate variables, and altitude, linear regression models were used and R^2 allowed to evaluate the explanatory power of the regression models using *lm()* function.

Results

Variability in seed trait depending on geographic zone

Seed traits of *Ceratonia siliqua* showed substantial variation across the 18-studied populations (Fig. 1, Table 1). Among the seed traits, area is the most variable trait with a coefficient of variation of 18.54%, while circularity is the least variable with a CV of 3.70%. Thus, width and length show an important variation with a CV of 10.91% and 10.43%, respectively. Perimeter, aspect ratio, and roundness show less variation compared to other traits (Table 1). Except for perimeter, CV values vary in the same direction as the variance *F* ratio. For example, the area has a high CV value (18.54%) and a high *F* value (Table 1).

Considering the inter-population comparisons, all the studied seed traits show significant differences between carob populations, with *F* values ranging from 125.67 for area to 25.01 for roundness (Table 1). Considering the geographic zone, populations located in the North region exhibit bigger seeds with high area, perimeter length, and width values (Fig. 1). However, populations located in the South region exhibited a high aspect ratio (AR > 1.39) and are wider compared to seeds from North (except Toughza and Afertane populations) and Center regions. Thus, seeds from populations located in the Center region show higher circularity values compared to most populations from the North and South regions (Fig. 1). Moreover, roundness values are higher in populations located in the Center and North regions (except for Toughza and Afertane populations) compared to populations in the South region (Fig. 1). Taking into consideration mean values per geographic zone, significant differences were found between the North, Center, and South regions (Additional file 3: Fig. S2; Additional file 4: Appendix S2). However, Tukey's HSD post hoc tests show that seeds width in the Center and South regions are not statistically different, thus for circularity between the North and South regions (Additional file 2: Fig. S1; Additional file 4: Appendix S2). Mantel tests show that the correlation of geographic with trait's distances across the studied carob populations resulted in significant correlations for all the studied traits, except for circularity (Additional file 5: Appendix S3).

Structure and amount of intraspecific variation between and within geographic zones

Concerning the estimates of variance percentage across the studied hierarchical level (geographic zone, population, tree, and seed), area, perimeter, width, and length had a higher portion of variance ratio at the geographic zone level. They also contained 43 to 56% of the total variance between geographic zones (Table 2). Moreover, they express a considerable amount of variance at the tree level compared to the population level, ranging from 28% for width to 34% for length (Table 2). Thus, circularity, aspect ratio, and roundness had higher values at the tree level, and express 24 to 43% of the total variance

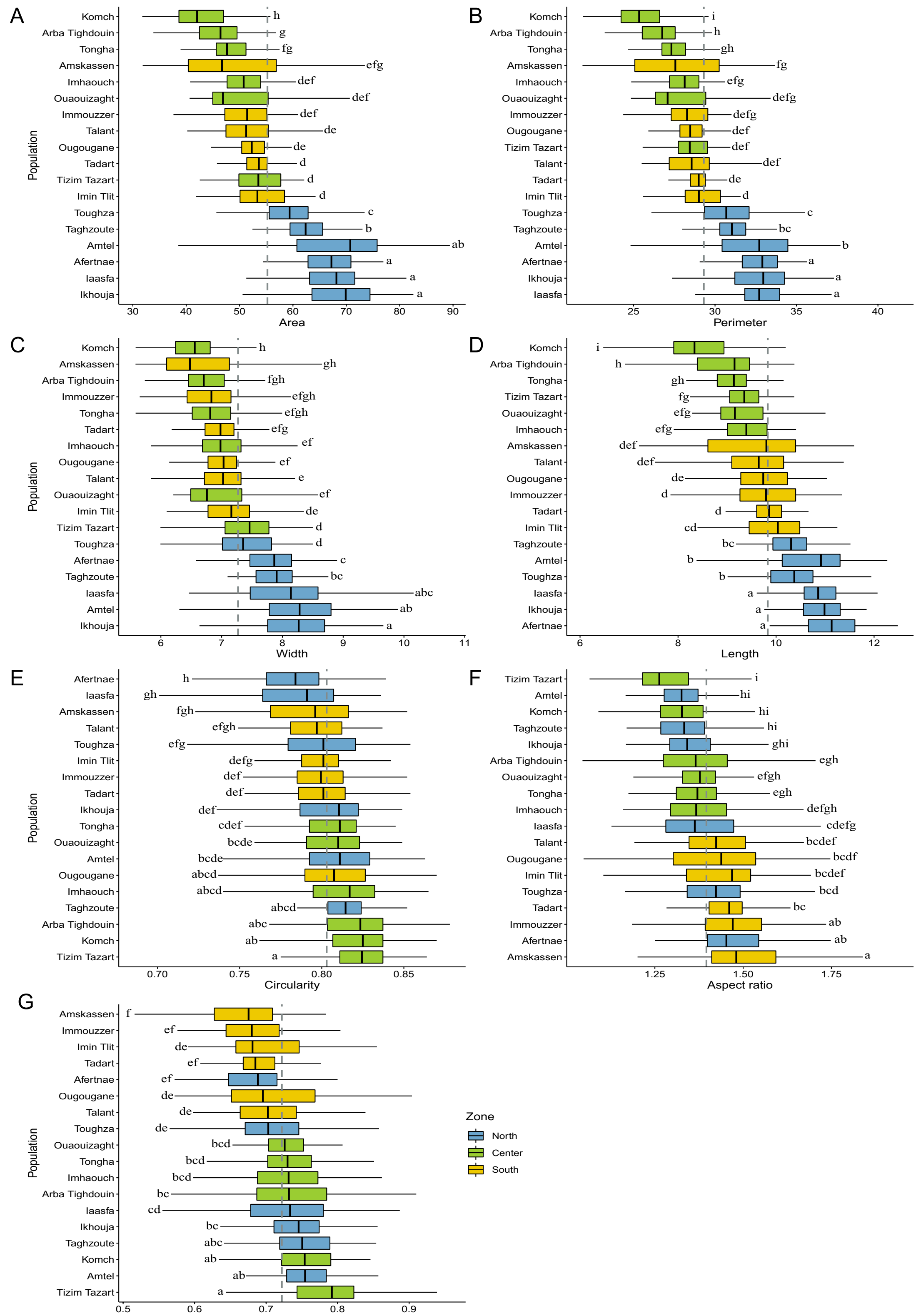

Fig. 1 Boxplot of mean values of carob seed traits and their population and geographic provenances. **A** area, **B** perimeter, **C** width, **D** length, **E** circularity, **F** aspect ratio, **G** roundness. Levels connected by the same letter are not statistically different at $P < 0.05$ in Tukey HSD post hoc tests. Dashed lines represent the general mean value of the traits

Table 2 Estimated percentage variance across hierarchical levels (zone:population:tree:seed) and seed traits

Trait	Geographic zone	Population	Tree	Seed	Residual
Area	**56.62**	2.20	29.95	4.40	6.83
Perimeter	**56.32**	2.28	29.72	5.37	6.30
Width	**43.49**	3.06	28.27	0.00	25.17
Length	**47.58**	0.94	34.00	0.00	17.48
Circularity	8.13	3.06	**24.86**	0.00	63.95
Aspect ratio	13.81	1.40	**43.29**	0.00	41.51
Roundness	13.47	1.87	**40.74**	0.00	43.92

The maximum values of variance per hierarchical level are in bold

(Table 2). A small fraction of the variance was observed between seeds of the same tree (e.g., 'the seed level') for area and perimeter (4.4 and 5.37%, respectively). Generally, traits expressing the highest value in their percentage variances at the highest hierarchical level (geographic zone) also exhibited the highest value in *F* ratio (Table 1). Therefore, traits with a low *F* ratio express a high variance percentage at the tree hierarchical level (Table 1).

In the PCA analysis of seed traits, the first three principal components captured 99.83% of the variation in traits (Fig. 2, Additional file 6: Appendix S4). Therefore, the first two axes explained 60.07 and 38.02% of the total variation, respectively (Fig. 2). The first PCA axis was found positively correlated with area, perimeter, width, length, and circularity, thus it discriminates between populations from North compared to populations from the Center and South regions (Fig. 2). The second axis was positively correlated with the aspect ratio and negatively with roundness and circularity, thus it represents a discriminant axis between populations from the South region compared to populations from the Center and most populations from the North region (Fig. 2, Additional file 6: Appendix S4). The results of PCA analysis supported the discrimination of all carob populations based on shape descriptors and geographic origin.

Seed trait variations and environmental variables

The variation observed in seed shape traits appears to be driven by variation in altitude and climate variables (Fig. 3). With increasing altitude, area, perimeter, width, and length decreased (Fig. 3A), however, they show the opposite trends with increasing temperatures (MAT and MTCM). Hence, with increasing rainfall (MAP), width and roundness increases, contrary to the aspect ratio, which seems to decrease with increasing MAP and MTWM (Fig. 3C, D). Nonetheless, circularity

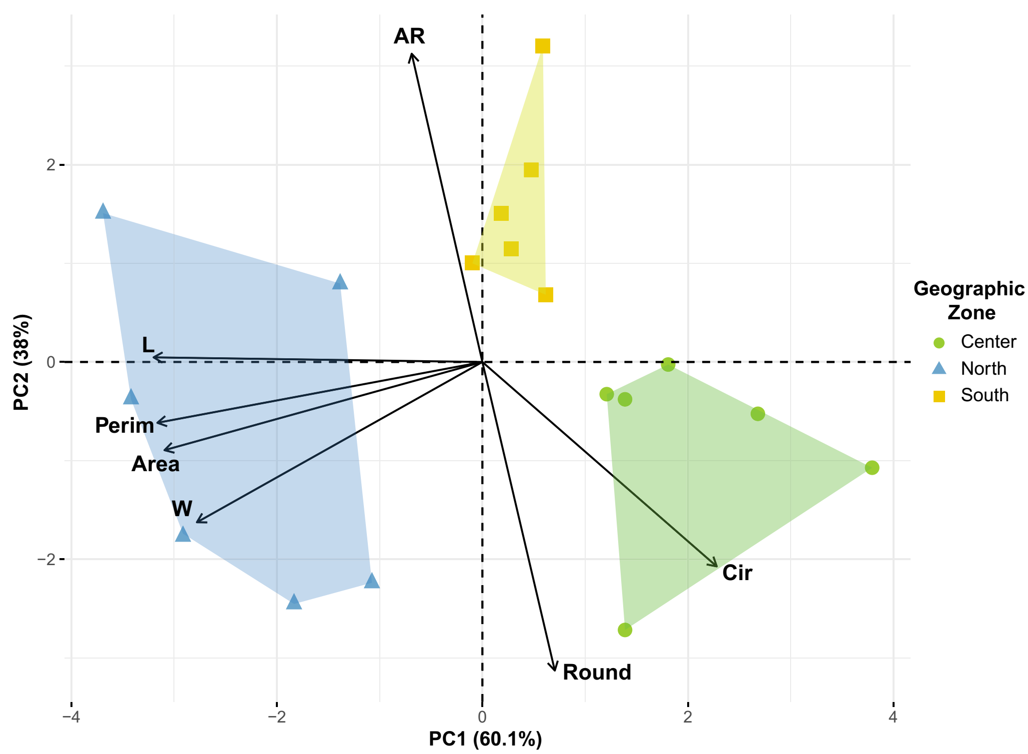

Fig. 2 Principal component analysis biplot of axes 1 and 2 (98.08% of variability) of carob seed traits belonging to different geographic zone. *L*: length, *W*: width, Perim: perimeter, AR: aspect ratio, Cir: circularity, Round: roundness

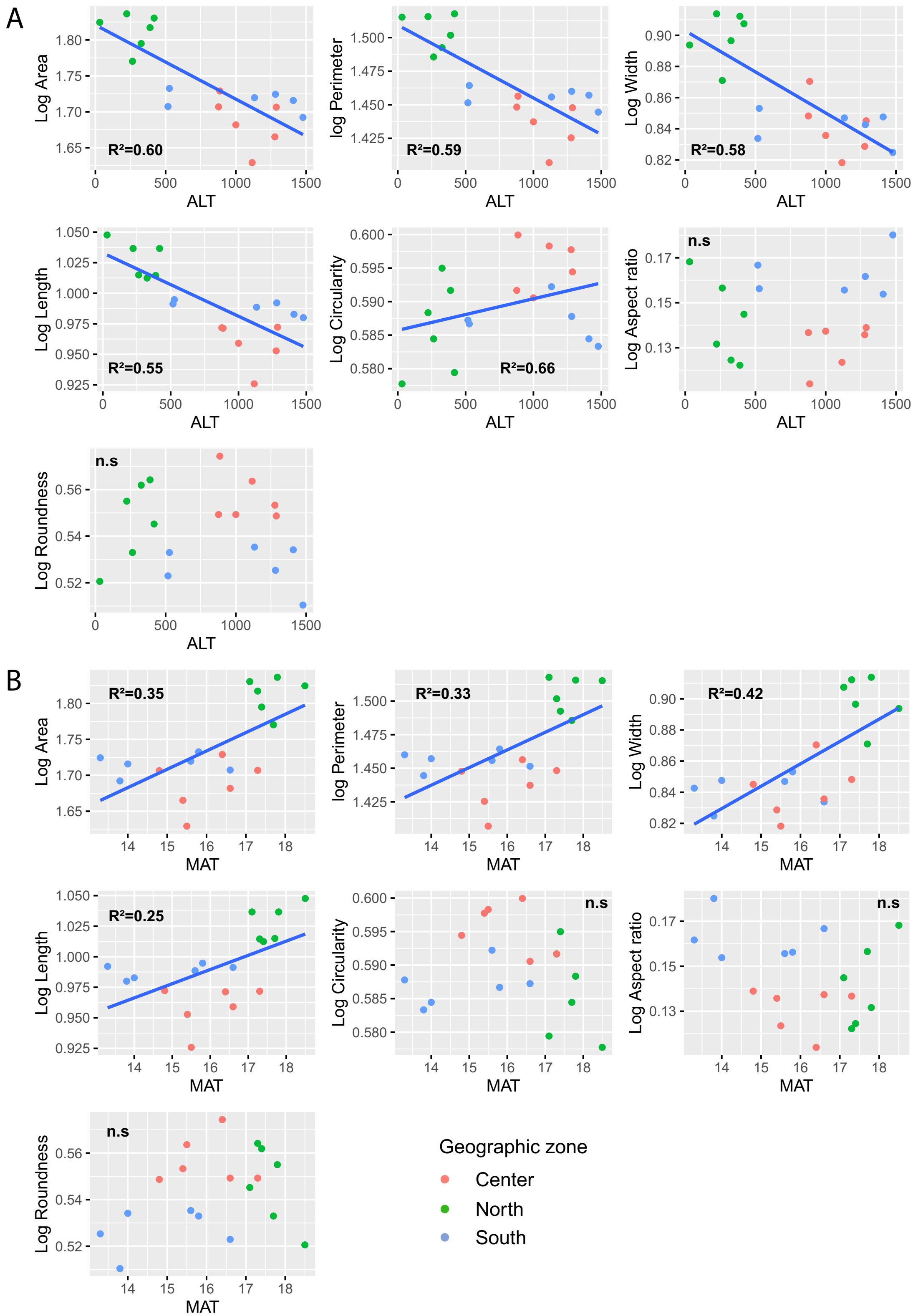

Fig. 3 Relationship between carob seed traits and **A** altitude, **B** MAT, **C** MAP, **D** MTCM, and **E** MTWM. n.s. non-significant

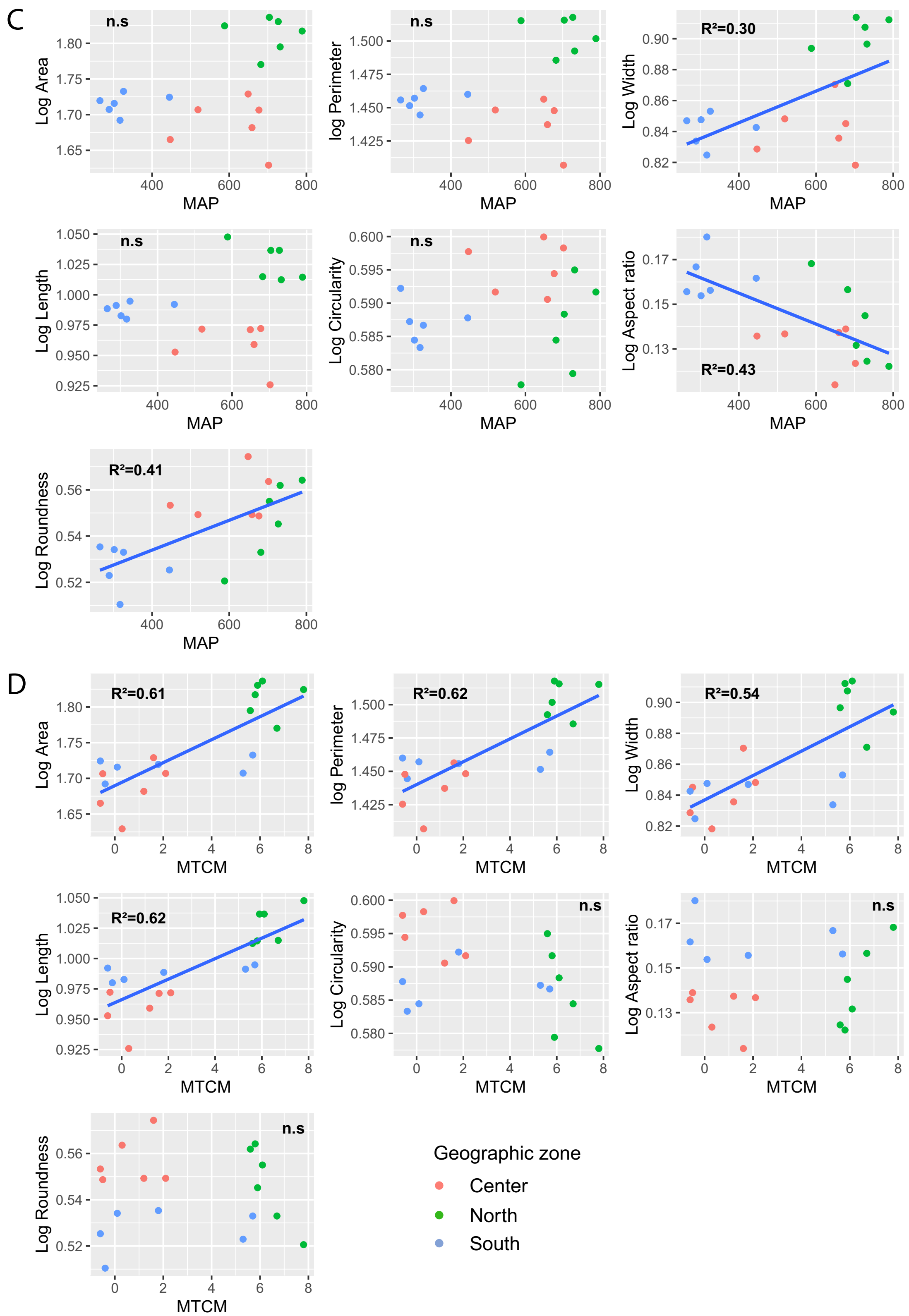

Fig. 3 continued

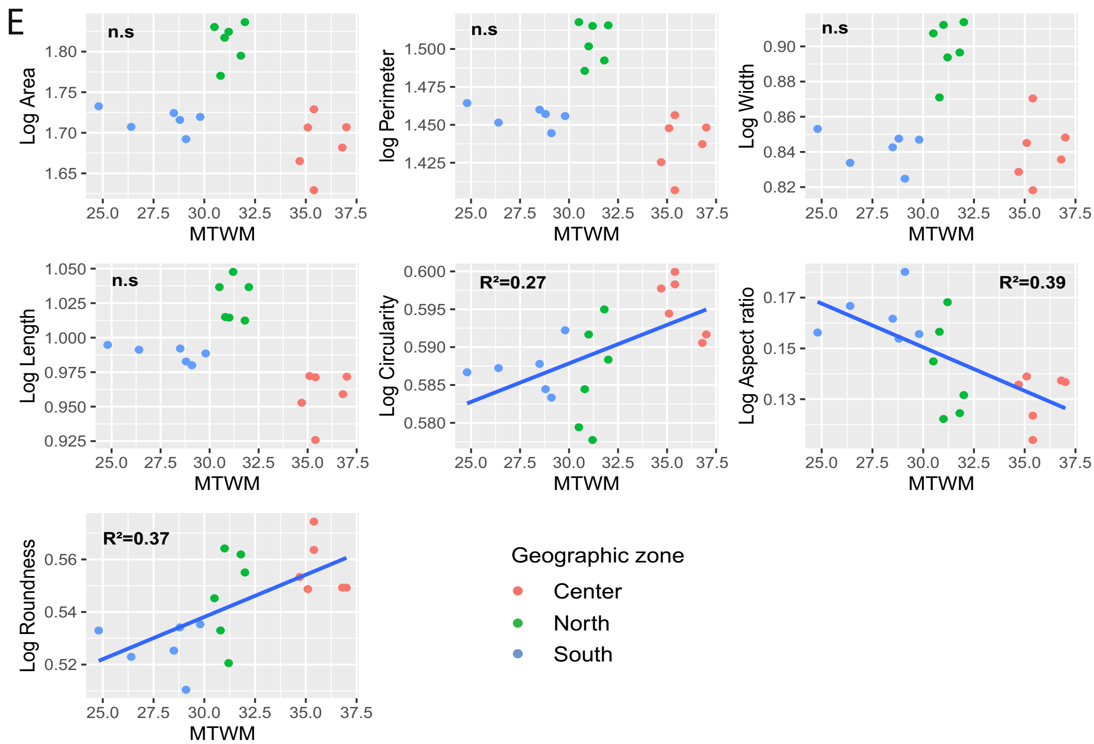

Fig. 3 continued

shows a positive and significant relationship with altitude and mean annual temperature of the warmest month (MTWM, Fig. 3E). Roundness seems to be sensitive to variation in MTWM, with a positive and significant relationship (Fig. 3E).

Discussion

The diversity of seed traits reflects a series of plant strategies to deal with stressful environmental conditions (Fenner and Thompson 2005). Therefore, seed-trait functional ecology provides a new opening forward to the establishment of an integrative and comprehensive framework in trait-based ecology (Saatkamp et al. 2019). Accordingly, taking into consideration intraspecific seed trait variation in trait-based studies could greatly consolidate the understanding of individual plant species responses to environmental gradients (Saatkamp et al. 2019) and, therefore, the effects of individuals on ecological processes (Kuppler et al. 2020). In this study, using non-destructive and reputable image analysis approaches, our results revealed a substantial intraspecific variation in seed-related traits of the remarkable Mediterranean tree *Ceratonia siliqua*. Moreover, seed traits showed a differential extent of variation across the studied ecological scales, from individual trees to geographic zone, suggesting that *C. siliqua* has adopted different functional strategies in response to heterogeneous environmental conditions found from local to regional scale. Thus, along its distribution area in Morocco, our results highlighted the effects of climate and altitude on carob seed variability. Our results provide new evidence of seed trait variability between and within carob populations along environmental gradients and, therefore, contributes to the ongoing concomitant efforts of the seed-trait functional ecology (Saatkamp et al. 2019).

Considering the results obtained from analyzing seed traits, the significant differences found between carob populations and the important magnitude of variation (e.g., CV%) strongly suggest a high level of phenotypic variability and, therefore, a substantial intraspecific variation (Albert et al. 2010a; Kassout et al. 2019). Overall, carob seeds coming from the North region are bigger and larger compared to seeds from the Center and South regions. Differently, seeds from the South show higher aspect ratios and lower roundness values than other populations from the North and Center. Thus, circularity values are higher in carob populations from the Center region compared to the North and South regions (Fig. 1, Table 1). These results confirmed with a clear discrimination between carob populations from different geographic regions (Fig. 2). The phenotypic variation found in seeds suggests that carob populations respond differently to biotic and/or abiotic factors within their environments (Sultan 2000). The heterogeneous and changing environmental conditions found along the sampling gradient may have affected the expression and the magnitude of the observed variation (Vázquez et al. 2017; Kassout et al. 2019). Hence, the significant relationship between trait variability and geographic distance (Additional file 5: Appendix S3) reflects the effects of contrasting environmental conditions found among carob populations on seed trait variability. Therefore, the population genetic structure of Moroccan carob trees highlights clear discrimination between populations from the South and those from the North region (Baumel et al. 2021), which could also explain the observed pattern of seed variation and differentiation. Baumel et al. (2021) identified four main regions as the ancestral area of the carob evolutionary history, in which South Morocco and North Morocco with the Iberian Peninsula are two major areas. These findings, together with the strong climatic gradient alongside the North–Center–South transect, are likely contributing to shape the variability of carob seeds. In addition, the clear discrimination of carob populations according to their geographic origins is consistent with the Mediterranean biogeographic refugia of plants described in Morocco (Medail and Diadema 2009), which has probably played a crucial role in the radiation of plants including thermophilous species such as the carob and the olive tree.

Variance partitioning shows a substantial amount of intraspecific trait variation (ITV) of carob seeds across the studied ecological levels (Table 2). These findings contribute to increasing evidence showing that intraspecific trait variation is consistent within species (Fajardo and Piper 2011; Kassout et al. 2019; Kuppler et al. 2020) and sustain its relevant contribution to the overall trait variation (Albert et al. 2010a; Violle et al. 2012). The observed intraspecific variation can arise from genetic variability or phenotypic plasticity affected by differences in environmental conditions (Violle et al. 2012). The carob tree is a slow-growing species (Batlle and Tous 1997) and will experience different sorts of stressful environmental conditions. Thus, it shows substantial genetic variability (Viruel et al. 2019) that may contribute to the observed seed trait variation. Therefore, the variation expressed in each of the studied ecological scales will have been affected by genotype, phenotypic plasticity (Geber and Griffen 2003; Albert et al. 2010a), and potential differences in phenology, land use, and history of the study area. However, several studies pointed to the predominance of environmental factors in the expression of intraspecific variability (Jung et al. 2014; Siefert et al. 2015; Kassout et al. 2019, 2021; Kuppler et al. 2020). Partitioning of the variance in carob seeds shows that area, length, width, and perimeter expressed an important amount of variation between geographic zones. Differently, the aspect ratio, roundness, and circularity indicate higher variation at the tree level (Table 2). These results indicate that intraspecific variations may be observed and expressed at multiple levels, from the geographical to the individual level (Evangelista et al. 2019). Moreover, these findings suggest that some seed traits (e.g., area and width) are more variable depending on large-scale variations in environmental factors, such as climate; however, other traits (e.g., aspect ratio) are more sensitive to variations in local-scale conditions found at the individual tree level (Messier et al. 2010). For instance, in the widespread wild olive trees in Morocco, Kassout et al. (2019) showed that ecophysiological leaf-related traits express important variability depending on the large-scale gradient of aridity. In contrast, hydraulic conductance traits (e.g., vessel lumen size) were largely controlled by variations in local conditions, such as water availability and vegetation type (Kassout et al. 2021). Fenollosa et al. (2021) revealed high inter-population variability in seed traits of *Carpobrotus edulis* according to both geographic and local conditions. Furthermore, within-individual tree variation in seed traits may be found to be relevant (Fenner and Thompson 2005), and the spatial and temporal environmental changes could have a significant effect on the expression of such variability within and among carob populations (Herrera 2017). In our study, variation expressed at the tree level, which is larger than the variation expressed at the population level (Table 2), could be interpreted by the importance of genetic diversity found among carob trees (Baumel et al. 2018).

Concerning the environment–traits relationship, our results demonstrated that variations in altitude and climate variables within our sampling area have a significant effect on the variation in carob seed traits. We found that seed size-related traits (e.g., area, width, and length) decreased with increasing altitude and temperatures (MAT, MTCM). In other words, at lower altitudes and higher mean annual temperatures, seeds are bigger and wider. These findings are in agreement with the 'energy constraints" hypothesis (Qi et al. 2014) assuming that morphological traits can be negatively correlated with elevation, as a result of low seeds development in high elevation and low temperature. Thus, previous findings showed that variation in seed traits could be driven by geographical or environmental variables (Jesús et al. 2017; Mojzes et al. 2018). On a large geographic scale, Soper Gorden et al. (2016) showed that climate is the dominant factor explaining between-species variability in seeds size. Likewise, the aridity gradient found in our study could play an important role in trait variation patterns (Kassout et al. 2021). Indeed, we found that increasing aridity (low MAP and high MTWM) has a significant effect on seeds' shape (e.g., circularity, roundness). It is clear that carob seeds produced under arid conditions have different phenotypes (e.g., high AR) compared to those found under humid conditions. Consequently, the observed variability in seed traits related to environmental conditions could be explained by the conjoint effect of climate variability, water, and nutrient availability (Baraloto and Forget 2007; Souza et al. 2010).

Conclusions

Digital image analysis has made it possible to reveal substantial intraspecific variations of seed traits in *C. siliqua* along a geographic latitudinal gradient in Morocco. The variation in environmental variables could explain the observed trends of seed trait variation along with the studied ecological scales. Our results show that seed traits of carob populations exhibit different phenotypes according to their geographic origin. Therefore, the present study represents a step forward in the understanding of intraspecific variation in seed traits along the environmental conditions. This will help in implementing more comprehensive and mechanistic trait-based models to understand plants' potential distribution and their responses to global change.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13717-022-00378-w.

Additional file 1: Table S1. Geographic locations and climate of the 18 studied populations of *Ceratonia siliqua* L.

Additional file 2: Figure S1. Protocol used in digital image analysis of carob seeds using ImageJ V1.53. A: Original image, B: 8-bit grayscale, C: Binary image. The scale bar represents 10 mm.

Additional file 3: Figure S2. Kernel density plot of carob seed traits depending on their geographic zone. A: Area, B: Perimeter, C: Width, D: Length, E: Circularity, F: Aspect ratio, G: Roundness. Different letters indicate significant differences (one-way ANOVA with Tukey post hoc method, $P < 0.05$). Dashed lines represent mean values per geographic zone.

Additional file 4: Table S2. One-way ANOVA test and Tukey HSD post hoc results of the studied carob populations according to their geographic origin.

Additional file 5: Table S3. Mantel test results for the correlation between geographic variation with trait variation.

Additional file 6: Table S4. Trait loading, eigenvalues, and percentage of trait variation explained by the first three principal components (PCs).

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Author contributions
JK and MA designed the study. JK performed analysis and led the writing. JK, MA, YH and SEF collected the data. All authors contributed substantially to the final manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets of the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no conflicts of interest associated with this manuscript. All the authors agreed to submit this manuscript.

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