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Morphological evaluation by principle component analysis of oleaster accessions (*Olea europaea* L., var. *Sylvestris*) in Al-Jabal Al-Akhdar, Libya

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Abstract

Wild olive or oleaster (*Olea europaea* L. var. *Sylvestris*) is considered one of the common plant species contributing to the biodiversity of Al-Jabal Al-Akhdar, Libya. The objective of the present study is to evaluate phenotypic variability and hence the genetic variation of wild olive (oleaster) in Al-Jabal Al-Akhdar. Principal component analysis (PCA) was used to compare the fruit and seed characteristics of 12 wild populations growing and naturally spreading in the study area. The results reported that the first three PCs recorded eigen values more than 1 and explained the 94.61% of variation. PC1 had 48.09%, PC2 explained 28.66% and PC3 had 17.86% of the total variation. Based on morphological traits analysis, it can be concluded that oleaster exhibits a higher genetic diversity. The dendrogram derived from the morphological data serves as a valuable tool for elucidating the phylogenetic relationships among oleaster populations in Al-Jabal Al-Akhdar.

Keywords: Morphological analysis, Oleaster, Principal component analysis, Cluster analysis, Al-Jabal Al-Akhdar.

التقييم المورفولوجي باستخدام تحليل المكونات الأساسية لعشائر الزيتون البري (*Olea europaea* L, var. *Sylvestris*) في الجبل الأخضر، ليبيا

ليبييا

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الملخص:

الزيتون البري (*Olea europaea* L., var. *Sylvestris*) يوجد بشكل طبيعي في منطقة الجبل الأخضر في ليبيا، وهو أحد الأنواع النباتية الرئيسية المكونة للتنوع البيولوجي في إقليم برقة. الهدف من هذه الدراسة هو تقييم التباين المورفولوجي وبالتالي التباين الوراثي للعشائر الطبيعية للزيتون البري في الجبل الأخضر. تم استخدام تحليل المكونات الرئيسية (PCA) لمقارنة خصائص الثمرة والبذرة في 12 عشيرة برية تنمو وتنتشر طبيعياً في منطقة الدراسة. اشارت النتائج أن أول ثلاث مكونات رئيسية (PCA) سجلت قيماً ذاتية أكبر من 1 وفسرت 94.61% من التباين. سجل المكون الأول (PC1) 48.09% والمكون الثاني (PC2) 28.66%، وفسر المكون الثالث (PC3) 17.86% من التباين الإجمالي. وبناءً على نتائجنا، يمكننا أن نستنتج أن الزيتون البري يتميز بدرجة عالية من التنوع الوراثي والذي يشاهد أثره في التباين الكبير في الصفات المورفولوجية. الكلمات المفتاحية: تحليل مورفولوجي، الزيتون البري، تحليل المكونات الرئيسية، التحليل العنقودي

Introduction

The olive tree (*Olea europaea* L.) stands as a quintessential symbol of the Mediterranean Basin, holding considerable relevance in social, economic, and ecological contexts (Carrión *et al.*, 2010). Subsp. *europaea* exhibits a consistent distribution throughout the entire Mediterranean Basin. It is categorized into two distinct botanical varieties: the cultivated olive (var. *europaea*) and the wild olive (var. *Sylvestris*) also known as oleaster (Green, 2002; Fanelli *et al.*, 2022). Both the wild olive (*Olea europaea* L., var. *sylvestris*) and its domesticated counterparts (*Olea europaea* L., var. *europaea*) are diploid organisms ($2n = 2x = 46$), primarily exhibiting allogamous reproduction, and are found throughout the Mediterranean region (Green, 2002; Ali, 2008).

Morphological evaluation serves as the basic and useful tool for wild olive characterization. The primary morphometric traits examined were related to the fruit and pit morphology. The majority of morphological investigations have focused on the oleaster, aiming to uncover the diversity present within isolated populations (Ismaili *et al*, 2018; Rodrigues *et al*, 2020) and make a comparison with cultivated olive (Hannachi *et al*, 2008; Khouatmianiet *al.*, 2021) . Overall, oleaster exhibited a greater degree of variability in comparison to var. *europaea* (Baldoni and Belaj, 2009; Belajet *al.*, 2010), characterized by smaller fruits and pits, as well as a reduced flesh percentage compared to cultivated olives (Boucheffaet *al.*, 2019).

According to a number of previous studies, olester forests are indigenous to Al-Jabal Al-Akhdar (Abuseifet *al.*, 2024; Ali *et al*, 2024). Wild olive trees are naturally found in the plains and plateaus of Al-Jabal Al-Akhdar, where they coexist with Phoenician juniper forests as companion species. They may be appeared either as individual trees or in aggregations, particularly in the vicinity of the village of Al-Gharib (Ali, 2008). In order to establish a database for the local genetic resources of oleaster (*Olea europaea* L. subsp. *europaea* var. *sylvestris* through the analysis morphological variability, the present work specifically seeks to characterize the wild olive accessions distributed throughout Al-Jabal Al-Akhdar, Libya, employing morphological parameters of both the fruit and the pit.

Material and Methods

Study location: Al Jabal Al-Akhdar, located in the Cyrenaica region of northeastern Libya, represents the largest IPA in the country. The distinctive physiographic and climatic characteristics that separate the Cyrenaican mountains from the rest of Libya have led to Al Jabal Al-Akhdar housing approximately 75 to 80% of Libya's flora, along with a considerable number of the endemic plant species, despite occupying merely 1% of Libya's total land area (Radfordet *al.*, 2011). The mean annual precipitation in Al Jabal Al-Akhdar ranges from 250 to 650 mm and the average minimum and maximum temperatures recorded are 59°F in January and 83°F in August (Ali *et al.*, 2024).

Plant material: Samples of fruits from 12 accessions of oleaster (*Olea europaea* L., var. *sylvestris*), were collected from various locations in the Al-Jabal Al-Akhdar region. The physical

characteristics examined for the fruits and pits from various oleaster accessions encompassed several parameters: volume, weight, dimensions (length and diameter), shape index and flesh ratio. A sample of ten fruits from each tree, with four trees per accession, was randomly collected. The morphological measurements of both fruit and pit were assessed according to standard methods (Iqbal *et al.*, 2019; Ali *et al.*, 2024)

Data analysis: Morphological parameters were analyzed using Minitab software (version 18), to calculate means and standard deviations. This statistical approach allowed a comparative evaluation of olive accessions on each characteristic. Statistics for each morphological trait represent the mean derived from 40 drupes or pits collected from each accession. Significance of quantitative traits was tested by ANOVA at a significance level of P 0.05. Quantitative morphological parameters were standardized and subjected to principal component analysis (PCA) using XLSTAT software (2024). The phylogenetic tree of the oleaster accessions was established by the cluster analysis based on Ward's method.

Results

Descriptive analysis: The results of the descriptive analysis of 11 quantitative traits in 12 natural accessions of oleaster (*Olea europaea* L., var. *sylvestris*) which scattering naturally in Al-Jabal Al-Akhdar, are summarized in Table (1).

Table (1): Summary statistics of 11 traits for 12 accessions of wild olive in Al-Jabal Al-Akhdar.

Trait	Minimum	Maximum	Mean	St. deviation	CV%
Fruit volume	6.23	11.70	8.52	1.56	18.31
Fruit weight	0.62	1.18	0.88	0.17	19.89
Fruit length	11.96	15.90	13.55	1.23	9.07
Fruit diameter	6.49	10.92	9.31	1.20	12.92
Fruit shape	1.27	2.47	1.49	0.31	21.41
Pit volume	1.90	3.00	2.51	0.31	12.59
Pit weight	0.20	0.35	0.27	0.04	16.91
Pit length	9.91	14.63	11.43	1.23	10.80
Pit diameter	5.42	6.69	6.10	0.37	6.15
Pit shape	1.63	2.51	1.88	0.24	13.03
Flesh/Fruit	0.57	0.74	0.67	0.05	6.71

All analyzed traits among 12 accessions of wild olive showed highly significant differences at a level of P=0.05. The coefficient of

variation (CV) pointed out a high variation for all morphological characteristics among oleaster accessions. The highest values of CV were recorded for fruit shape (21.41%), fruit weight (19.89%), fruit volume (18.34%) and pit weight (16.91%) while pit diameter (6.15%) and flesh/fruit (6.71) documented the lowest ones.

Correlation analysis: The correlation coefficient was applied for all morphological characters in order to reduce the number of principle components. Table (2) shows the correlation matrix for 11 morphological traits in 12 accessions of wild olive. The results documented that a highly positive correlation was observed between fruit volume and each of the following: fruit weight ($r=0.743$), fruit length ($r=0.610$) and flesh/fruit ratio ($r=0.579$). Fruit weight recorded a positive correlation coefficient with fruit length ($r=0.809$), pit volume ($r=0.717$) and pit weight ($r=0.657$). Values of 0.621, 0.847, 0.673, 0.869 and 0.695 were documented as correlation coefficient for fruit length with the following: fruit shape index, pit volume, pit weight, pit length and pit shape index, respectively.

Table (2): Correlation matrix of the 11 variables used in PCA of 12 accessions which scattering naturally in Al-Jabal Al-Akhdar.

Variables	Fruit volume	Fruit weight	Fruit length	Fruit diameter	Fruit shape index	Pit volume	Pit Weight	Pit length	Pit diameter	Pit shape index	Flesh/fruit ratio
Fruit Volume	1	0.743	0.610	0.364	0.104	0.461	0.279	0.385	0.043	0.333	0.579
Fruit weight	0.743	1	0.809	0.431	0.177	0.717	0.657	0.493	0.425	0.216	0.560
Fruit length	0.610	0.809	1	-0.046	0.621	0.847	0.673	0.869	0.132	0.695	0.308
Fruit diameter	0.364	0.431	-0.046	1	-0.799	-0.003	0.017	-0.441	0.538	-0.663	0.496
Fruit shape index	0.104	0.177	0.621	-0.799	1	0.500	0.417	0.857	-0.303	0.911	-0.197
Pit volume	0.461	0.717	0.847	-0.003	0.500	1	0.893	0.818	0.476	0.488	-0.080
Pit Weight	0.279	0.657	0.673	0.017	0.417	0.893	1	0.698	0.694	0.275	-0.236
Pit length	0.385	0.493	0.869	-0.441	0.857	0.818	0.698	1	0.038	0.868	-0.127
Pit diameter	0.043	0.425	0.132	0.538	-0.303	0.476	0.694	0.038	1	-0.458	-0.226
Pit shape index	0.333	0.216	0.695	-0.663	0.911	0.488	0.275	0.868	-0.458	1	-0.021
Flesh/fruit ratio	0.579	0.560	0.308	0.496	-0.197	-0.080	-0.236	-0.127	-0.226	-0.021	1

Values in bold are significant at $P=0.05$

A negative correlation was found between fruit diameter with the fruit shape index ($r= -0.799$) and the pit shape index ($r= -0.663$). Fruit shape index recorded the values of 0.857 and 0.911 as a correlation coefficient with the pit length and pit shape index.

Values 0.893 and 0.818 were recorded as correlation coefficient between pit volume and each of pit length and pit diameter, respectively. A highly positive correlation was observed between pit weight and each of the following; pit length ($r=0.698$) and pit diameter ($r=0.694$). Finally, the correlation coefficient between pit length and pit shape index was highly positive, recording 0.868.

Principle Component Analysis: In order to extract the important information about the variability of oleaster in Al-Jabal Al-Akhdar, Principle Component Analysis (PCA) was applied to study 11 morphological traits in 12 natural populations. All the total variation has been derived from 11 PCs which are expressed as eigenvalues, variability (%) and cumulative values (%), shown in Figure (1). The results indicated that the effect of variables was reduced to 3 PCs which explained most of the observed variance. The eigenvalues were 5.29, 3.15 and 1.96 for PC1, PC2 and PC3 respectively. The values 48.08%, 28.65% and 17.86% were recorded for PC1, PC2 and PC3 respectively, accumulating 94.61% of variability% (Table 3).

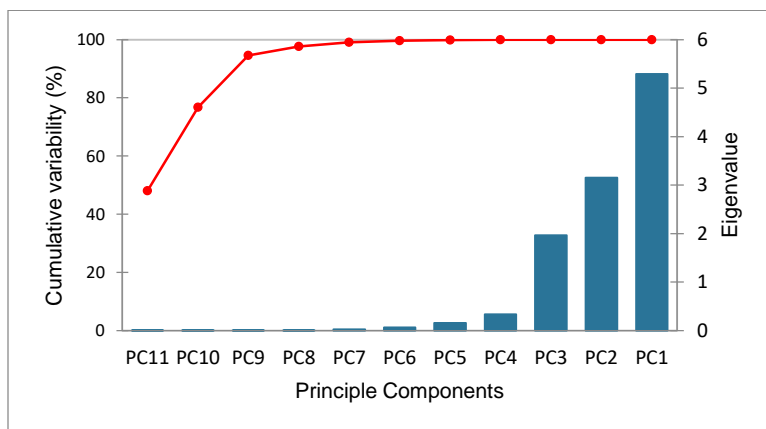


Figure (1): Graph of principle components for 11 Morphological variables in 12 accessions of wild olive in Al-Jabal Al-Akhdar.

Table (3): Eigenvalues, Variability% and Cumulative% for 11 Principle Component Analysis in wild olive

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
Eigenvalue	5.290	3.153	1.965	0.337	0.158	0.062	0.022	0.006	0.005	0.002	0.000
Variability%	48.08	28.66	17.86	3.059	1.441	0.56	0.20	0.05	0.04	0.017	0.003
Cumulative%	48.08	76.74	94.61	97.66	99.11	99.67	99.87	99.93	99.98	99.99	100.00

Table (4) shows a summary of the principal component analysis of morphological data of wild olives. Three of the 11 PCs have eigenvalues greater than 1 and are retained in the analysis. PC1 exhibits positive loadings for all variables, with the exception of fruit diameter, which shows a slight negative correlation of -0.088 with this component. Additionally, pit diameter and flesh/fruit ratio present weak positive correlations of 0.079 and 0.036, respectively, with PC1. Meanwhile, PC2 recorded three negative correlations: fruit shape index (-0.365), pit length (-0.157) and pit seed shape index (-0.326). The third component recorded negative correlation values with pit variables: pit volume (-0.206), pit weight (-0.388), pit length (-0.045) and pit diameter (-0.512).

Table (4): Eigenvectors of the three principal components for 11 morphological traits in 12 oleaster accessions.

Trait	PC1	PC2	PC3
Fruit volume	0.247	0.261	0.362
Fruit weight	0.320	0.351	0.124
Fruit length	0.415	0.069	0.121
Fruit diameter	-0.088	0.540	0.047
Fruit shape index	0.319	-0.365	0.027
Pit volume	0.394	0.109	-0.206
Pit weight	0.341	0.137	-0.388
Pit length	0.412	-0.157	-0.045
Pit diameter	0.079	0.370	-0.512
Pit shape index	0.325	-0.326	0.209
Flesh/Fruit	0.036	0.284	0.580

The correlation circle (variable chart) shows a projection of the initial variables into the PC space (Figure. 2). Variables which are close to each other indicate that they are strongly positively correlated. In other words, there is a strong positive association between the narrow-angle variables. The first component accounted for 48.09% while the second component *explained* 28.66% of variability among accessions.

According to Table (5), PC1 is highly correlated with morphometric parameters of fruit (volume, weight, length, shape index) and also with pit morphological measurements (volume, weight, length, shape index). Fruit diameter is the only variable that contributes in the PC2 (Table 5). PC3 had two variables with high contribution (pit diameter and Flesh/fruit ratio).

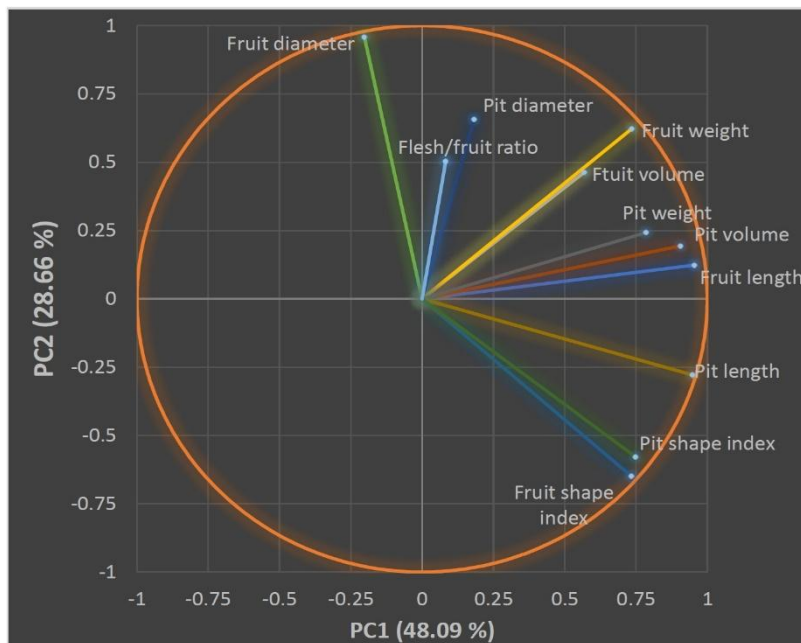


Figure (2): Variable plot illustrating the distribution of 11 morphological traits evaluated by PCA of 12 oleaster accessions.

Table (5): The squared cosines of the 11 variables of 12 oleaster accessions.

	PC1	PC2	PC3
Fruit Volume	0.323	0.215	0.257
Fruit weight	0.541	0.388	0.030
Fruit length	0.911	0.015	0.029
Fruit diameter	0.041	0.919	0.004
Fruit shape index	0.538	0.421	0.001
Pit volume	0.821	0.038	0.083
Pit Weight	0.617	0.059	0.295
Pit length	0.898	0.078	0.004
Pit diameter	0.033	0.432	0.515
Pit shape index	0.560	0.334	0.086
Flesh/fruit ratio	0.007	0.254	0.660

Values in bold correspond for each variable to the factor for which the squared cosine is the largest.

Figure (3) illustrates a projection of 12 oleaster accessions based on the first three PCs. The Acc3, Acc4 and Acc12 were positioned at the right of the graph, indicating a significant degree of similarity

and forming a relatively similar group. Meanwhile, the Acc5, Acc6 and Acc8 were located at the left, forming a set. Furthermore, the Acc2, Acc7 and Acc11 were concentrated around the center of the graph in one collection. It is worth noting that the Acc1 was greatly separated from the rest of the wild olive accessions in Al-Jabal Al-Akhdar, while Acc9 and Acc10 occupied intermediate positions on the plot.

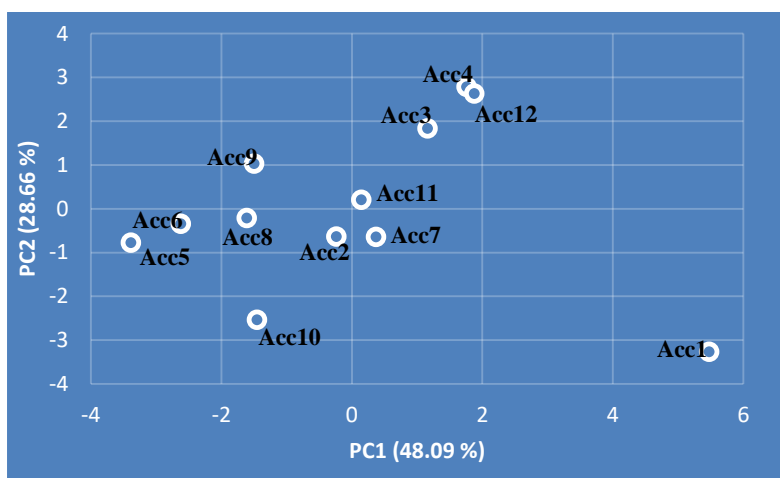


Figure (3): A diagram illustrating the interrelations among 12 wild olive accessions evaluated based on 11 morphological characteristics.

Cluster analysis: The results of the hierarchical cluster analysis were obtained in the form of a dendrogram using the Ward analysis method performed on eleven fruit and pit morphological parameters related to 12 wild olive accessions under study (Fig. 4). It can be deduced that the wild olive accessions (oleaster) were divided into two main groups containing four clusters. The first cluster included three accessions (Acc3, Acc4 and Acc12) while the second cluster contains only one accession (Acc1) that has been isolated early from the rest of the oleaster populations. The third cluster, which is considered the largest, consists of Acc10, Acc7, Acc5, Acc6 and Acc8. Finally, the fourth cluster comprised of three accessions (Acc2, Acc9 and Acc11). The dimensions and weight of fruits and pits, as well as the shape index of fruits and pits, played an important role in the hierarchical analysis, while the volume of fruits and pits had a minor role in the classification of oleaster. For instance, the hierarchical analysis separates Acc1 from the rest of the wild olive accessions of Al-Jabal Al-Akhdar. This accession is characterized by the highest values of fruit and pit length, as well as the fruit and

pit shape index. On the other hand, Acc3, Acc4 and Acc12 were grouped into one cluster due to the values of fruit and pit weight, fruit and pit dimensions and fruit and pit shape index.

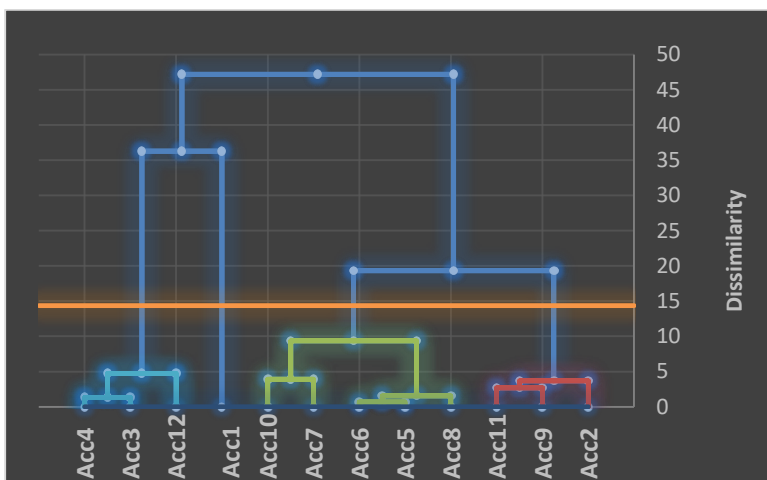


Figure (4): Dendrogram generated by Ward's method based on 11 morphological traits illustrating the phylogenetic relationship among 12 oleaster accessions in Al-Jabal Al-Akhdar, Libya.

Discussion

Descriptive statistics: The results of the present study showed that morphological characters, especially fruit and pit measurements, were an effective means to distinguish and classify in wild olive accessions. These results are consistent with the results of previous studies on wild olive (Falek *et al.*, 2022) and cultivated olive (Laaribiet *et al.*, 2014; Mnasriet *et al.*, 2017).

Furthermore, the arithmetic means and coefficients of variation of the morphological characters of the natural population of oleaster in Al-Jabal Al-Akhdar showed highly significant differences. These results indicate the existence of a high variability among the accessions investigated in the present study, which is consistent with the achieved by (Falek *et al.* 2022) who confirmed the existence of a high variation in the morphological characters of wild olive collections in Algeria.

Correlation Coefficient Analysis: The current results reported that some morphological characters have significant positive correlations and others do not have significant correlations for all accessions. Most of the quantitative traits, especially fruit and pit measurements, showed significant positive correlations. On the contrary, some of them (fruit diameter with fruit or pit shape index)

showed significant negative correlations. This means that quantitative traits that showed strong significant correlation are effective in distinguishing between natural populations of wild olives. It also indicates the effectiveness of correlation coefficients in Principle Component Analysis. These results are similar to those of numerous studies carried out on both cultivated or wild olives (Cantini *et al.*, 1999; Hanachi *et al.*, 2008; Iqbal *et al.*, 2019; Falek *et al.*, 2022) which confirmed the importance of morphometric fruit data in the discriminating of olives.

Principle Component Analysis: The first component explains 48.09% of the variance observed in 11 morphological traits in 12 natural population of wild olive accessions with a high contribution from fruits and pits traits. Because most of morphological variables are highly positively correlated with the first component (PC1), it can be interpreted as being a general morphometric factor in oleaster classification. These results are similar to several previous studies (Cantini *et al.*, 1999; Laaribiet *al.*, 2014; Mnasriet *al.*, 2017; Iqbal *et al.*, 2019; Falek *et al.*, 2022), which indicated the importance of using fruit morphological characteristics of principle component analysis in order to distinguish between different groups of olives, whether wild or cultivated.

The variable plot technique was an effective and capable means of identifying relationships between different variables, which facilitated the presentation and documentation of data on the contribution of variables to the principle component analysis. This was clearly evidenced by the results of the present study and by the results of some previous studies (Falek *et al.*, 2022). Morphological characteristics of fruits and pits (weight, volume, length, diameter and flesh/fruit ratio) are the most important traits using to classify oleaster populations.

A glance at the results of the present study regarding the distribution of the morphological traits on the principle components shows that flesh/fruit ratio, despite its importance, did not contribute to PC1 (general morphometric factor). The reason, in fact, is that the flesh/fruit ratio (mesocarp) in wild olive in Al-Jabal Al-Akhdar is very small. Some previous reports confirmed that a wild olive tree uses its resources in its vegetative structures than in its fruit. Therefore the fruits of oleaster have very thin mesocarp (ESAO, 2024). Since wild olive reproduces by seed and cross-pollination, the variation in the morphological characteristics of wild olive is large due to genetic segregation. This means that wild olive

(oleaster) has a high degree of heterogeneity and therefore the genetic variability is high within and between populations.

Cluster analysis: The quantitative morphological traits of the fruit and pit were a useful tool to clarify the relationships among the oleaster accessions in Al-Jabal Al-Akhdar, Libya. Moreover, the dendrogram obtained from the morphological data can be considered as a good key to understanding the phylogenetic relationship between wild olive populations. This was in agreement with those of (Hanachi *et al.* 2008; Mnasriet *al.* 2013; Iqbal *et al.* 2019; Falek *et al.* 2022) who classified olives into phylogenetic trees according to morphological characterization. Furthermore, the current study confirmed that morphometric analysis of olive morphological traits can be used as a reliable method for the classification of oleaster accessions. It allows obtaining clear information for genetic comparison and diversity within the species. The similarity among certain accessions grouped within the same cluster is a result of gene flow dynamics. Gene flow can be observed when several populations of the same species in a region show similar values for a number of traits related to the same vegetative structure (e.g. fruit morphological traits). Gene flow occurs through the transfer of pollen between different natural populations, since wild olives reproduce sexually and are open-pollinated. Also, morphological traits are quantitative genetic traits that are affected by environmental conditions, and therefore the variance of these traits are due to complex genetic and environmental criteria and the interaction between them.

Conclusion

As far as we are aware, the current research represents the first comprehensive reference study utilizing principal component analysis to investigate the morphological variability of oleaster populations in Al-Jabal Al-Akhdar. This region is considered as an isolated geographical area (biological island) due to the fact that it is surrounded by the Mediterranean Sea on three sides and the Sahara on the fourth. Natural Populations in this region may have their own evolutionary form as a result of isolation, making it useful to study them to determine the variation between and within those populations.

In conclusion, this research has demonstrated the presence of wild olive accessions with interesting morphological characteristics that deserve to be explored in greater depth. The conservation of these

genetic resources must be implemented through measures that minimize the risk of losing the genetic variation existing in such endemic plant species.

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