



Article Morphological and Eco-Geographic Variation in Algerian Wild Olives

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Abstract: Algerian wild olives can represent an important resource for cultivated olive breeding, since they are characterized by great morphological variability. Moreover, they grow in different bioclimatic environments, including dry and hot climates, making the collections of wild olives a good source of abiotic stress resistance traits. Our study aimed to investigate the morphological diversity of 175 wild olive trees collected in North Algeria along with a wide range of different bioclimatic habitats for studying traits of olive accessions in relation to their different ecogeographical parameters. Wild olive trees were found in five different bioclimates areas spanning from humid to Saharan areas. They showed high variation in all traits, in particular fruit and stone weight, which expressed the highest coefficient of variation, and a high positive correlation between fruit weight/width. Cluster analysis separated the samples into two groups mostly based on fruit and stone size, while no relationship was observed with the area of sampling. Only the Saharan samples showed significantly different foliar and fruit characteristics compared to samples from other bioclimatic areas.

Keywords: *Olea* var. *sylvestris*; oleaster; biodiversity; bioclimatic evaluation; pluviometric variation; Algeria

1. Introduction

The olive tree (*Olea europaea* subsp. *aeuropea* L.) is an evergreen tree belonging to the *Oleaceae* family. In the Mediterranean Basin, it has high social and economic importance, being a fundamental nutritional source due to olive oil's high levels of monosaturated fatty acids and phenolic compounds [1]. Six subspecies are known: *cuspidata, guanchica, cerasiformis, laperrinei, maroccana,* and *europaea*. In particular, wild olive trees, or oleaster (var. *sylvestris*) and cultivated olive trees (var. *europaea*) constitute the two botanical varieties of *O. europaea* [2]. Several morphological, ecological, and cytogenetic studies have shown that *O. europaea* subsp. *europaea* var. *sylvestris* is the wild relative of the cultivated olive [3].

Wild and domesticated olives grow in the same climatic area, but the wild type shows some morphological differences, such as smaller fruit size and lower oil content in the mesocarp [4]. The different pedoclimatic growing environments of wild olive include areas characterized by extreme levels of drought, high temperatures, and high salinity [5,6]. Wild olive exhibits a higher genetic diversity than the cultivated subspecies [7], holding agronomic traits, such as a shorter juvenile period and resistance to biotic and/or abiotic stress [8–10], which could be useful to transfer into the cultivated olive. Moreover, wild olive can be useful to improve the olive oil's health value and taste, being richer than the cultivated olive, in antioxidants and oleic acid [11–13], which are components increasingly appreciated by the pharmaceutical sector and consumers.



Citation: Falek, W.; Mascio, I.; Gadaleta, S.; Fanelli, V.; Bechkri, S.; Khelifi, D.; Miazzi, M.M.; Montemurro, C. Morphological and Eco-Geographic Variation in Algerian Wild Olives. *Plants* **2022**, *11*, 1803. https://doi.org/10.3390/ plants11141803

Academic Editor: Georgios Koubouris

Received: 5 May 2022 Accepted: 6 July 2022 Published: 8 July 2022

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Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). Algeria is a large Mediterranean country that stretches over 2.4 million square kilometers, with the Atlas Mountains dividing the territory into two parts. In Northern Algeria, the coasts are generally very close to the mountains and are the most populated and cultivated areas. To the south, the main part is occupied by the Sahara desert, where other olive subspecies such as *O. europaea* subsp. *laperrinei* have been discovered and studied [14,15]. At the beginning of the 2000s, olive groves covered only 2.3% of the total cultivated area, but in recent years, the Algerian Agriculture Ministry has allocated resources for the development and support of the olive-growing sector, which has allowed increasing the area dedicated to olive growing, from 165,600 hectares in 1999 to 439,000 in 2020, and the olive production from 363,381 to 1,079,508 tonnes [16].

While only 36 cultivars are described and listed in the catalog of Algerian olive varieties [17] Algeria still preserves important reserves of wild olives, which have captured attention as a resource to diversify and enrich the country's olive heritage [18,19]. The awareness that the availability and conservation of the wild olive germplasm suitable for breeding programs are crucially important has prompted intensifying research and conservation projects for new germplasm in all olive-producing countries [20–23]. An efficient collection of natural diversity to mine for specific traits can be obtained by focusing on natural areas characterized by extreme climates. Therefore, populations of wild olive that have remained isolated in remote areas far from the cultivated fields, could represent a good source of traits useful in olive breeding programs.

The morphological and ecogeographic characterization of the wild olive genetic resources in Algeria would provide solid information on the current state of the species and would help to define adequate conservation strategies. For this aim, other studies have already been conducted, but they involved a few samples and were collected in limited areas [18,24,25]. Today, molecular techniques are preferred for genetic diversity evaluation [26–28], and advances in high-throughput sequencing have made the genomes of many crops available, including wild olive [29]. Nonetheless, morphological characterization is the first step in biodiversity description and classification, and it is required to understand genotype-phenotype relationships for the development of crop breeding [30,31].

The aim of this study was the morphological characterization of a wide collection of wild olive samples growing in different ecoclimatic zones of Northern Algeria (i) to explore the variation of samples in relation to their ecogeographic origin, and (ii) to investigate the possibility of identifying, in specific genotypes, traits of adaptation worthy of being further studied for use in breeding programs.

2. Materials and Methods

2.1. Field Surveys and Sampling

Prospecting and sampling were conducted during the harvest period of 2017 in Northern Algeria in a wide range of natural habitats characterized by different bioclimatic conditions (Figure 1). Thirty-four provinces located along the coast and in the inner province of Laghouat, at an altitude ranging from 18 m asl (Tipaza_1) to 1270 m asl (Batna_8), were inspected, and sampling sites were geo-referenced using a Global Positioning System (GPSGARMIN Trex[®] model 30) (Supplementary Table S1). From each of the 175 wild olive samples, 30 leaves and 30 fruits were randomly collected in different parts of the tree and stored in refrigerated boxes until their analysis. Samples were collected from shrubs often dense, twiggy, and spiny with ovate-oblong to elliptic leaves and small drupes (<1 cm long) with fleshy but thin mesocarp and low oil content, following the methodology established by the International Union For The Protection Of New Varieties Of Plants (UPOV) for primary characterization of olive [32] (Table 1).



Figure 1. Map of Northern Algeria showing the sampling sites of wild trees. The average precipitation and temperature are also shown. The picture refers to the sample Constantine_11—Constantine, Djbel El Ouehch. Source: https://earlywarning.usgs.gov (accessed on 4 May 2022).

2.2. Ecogeographic Data

For each sampling site, the altitude and the principal climate parameters, annual rainfall, average of the maximum temperature of the hottest month, and average of the minimum temperature of the coldest month, were recorded by the National Office of Meteorology of Algeria (ONM), except for thirteen locations for which the data were not available. For these locations, the data were recorded from CLIMATE-DATA.ORG (http://fr.climate-data.org/ (accessed on 1 November 2017) (Supplementary Table S1). Climate parameters were used to calculate the pluviothermic Emberger coefficient (Q2), whose values correlate with humidity levels (differential dryness) characterizing the Mediterranean climate [33]. Moreover, the pluviothermic coefficient along with the minimum temperature of the coldest month was used to obtain the Emberger's climogram [34].

2.3. Morphological Quantitative and Qualitative Traits

The morphological characterization of wild olive samples was carried out by using 32 olive descriptors indicated by UPOV and modified for wild olives, including 5 traits related to the tree, 4 descriptors for leaf, 11 for the fruit, and 12 for the stone, considering up to 4 classes in qualitative traits [6,32] (Supplementary Table S1, Supplementary Figure S1). Twenty traits were considered exclusively qualitative, 4 were considered exclusively quantitative, and 8 morphological traits were considered from both a qualitative and quantitative point of view (Table 1).

Organs	Trait	Trait Code	Classes							
	Tree Vigor	TV ¹	1 = Weak	2 = Medium	3 = Strong					
TREE	Tree Habit	TH ¹	1 = Falling down	2 = Spread out	3 = Erected					
	Canopy Density	CD ¹	1 = Loose	2 = Medium	3 = Compact					
ARCHITECTURE	Tree Production fruit	TP ¹	1 = Weak	2 = Medium	3 = Strong					
	Maturation	M 1	1 = Early	2 = Medium	3 = Late					
	Shape	LSH ³	1 = Elliptic (Length/Width < 4)	2 = Elliptic-lanceolate (Length/Width 4–6)	3 = Lanceolate (Length/Width > 6)					
LEAF SHAPE	Blade Length (cm)	LBL ³	1 = Short (<5 cm)	2 = Medium (5-7 cm)	3 = Long (>7 cm)					
AND SIZE	Blade Width (cm)	LBW ³	1 = Narrow (< 1 cm)	2 = Medium (1-1.5 cm)	3 = Wide (>1.50 cm)					
	Longitudinal Curvature Blade	LCB ¹	1 = Epinastic	2 = Flat	3 = Hyponastic	4 = Helicoidal				
	Weight (g)	FW ³	1 = Low (<0.5 g)	2 = Medium (0.5–1 g)	3 = High (>1 g)					
FRUIT SHAPE AND SIZE	Shape	FSH ³	1 = Spherical (L/A < 1.25)	2 = Oval (Length/Width 1.25–1.45)	3 = Longer (Length/Width > 1.45)					
	Symmetry (position A)	FSA ¹	1 = Symmetrical	2 = Slightly Asymmetrical	3 = Asymmetrical					
	Maximal Diameter	FMD ¹	1 = Toward base	2 = Central	3 = Toward apex					
	Apex Shape (position A)	FASH ¹	1 = Pointed	2 = Rounded	-					
	Base Shape (position A)	FBSH ¹	1 = Rounded	2 = Truncate	3 = Pointed					
	Nipple	NI ¹	1 = Absent	2 = Tenuous						
	Lenticels	LE ¹	1 = Few	2 = Many						
	Dimension Lenticels	DLE ¹	1 = Small	2 = Large						
	Length (cm)	FL ²	/	/	/					
	Width (cm)	FWI ²	/	/	/					
	Weight (g)	SW ³	1 = Low (<0.15 g)	2 = Medium (0.15–0.30 g)	3 = High(>0.3 g)					
	Shape (position A)SSH 3 1 = Spherical (Length/Width < 1.4)		1 = Spherical (Length/Width < 1.4)	2 = Oval (Length/Width 1.4–1.8)	3 = Elliptic (Length/Width 1.8–2.2)	4 = Longer (Length/Width > 2.2)				
	Symmetry (position A)	SSA ¹	1 = Symmetrical	2 = Slightly Asymmetrical	3 = Asymmetrical					
	Maximal Diameter	SMD ¹	1 = Toward base	2 = Central	3 = Toward apex					
STONE SHAPE	Groves distribution	SDG ¹	1 = Regular	2 = Irregular						
AND SIZE	Apex Shape	SASH ¹	1 = Pointed	2 = Rounded						
AIND SIZE	Base Shape	SBSH ¹	1 = Rounded	2 = Truncate	3 = Pointed					
	Surface	SS ¹	1 = Smooth	2 = Rugose	3 = Scabrous					
	Number of Grooves	SNG ³	1 = Reduced (<6)	2 = Medium (6–7)	3 = High (>7)					
	Mucron	SM ¹	1 = Without mucron	2 = With mucron						
	Length (cm)	SL ²	/	/	/					
	Width (cm)	SWI ²	/	/	1					

Table 1. Qualitative and quantitative descriptors were used for the characterization of Algerian wild olive samples, following the UPOV methodology. Eight traits were considered as both quantitative and qualitative descriptors.

¹ qualitative trait; ² quantitative trait; ³ trait considered as both qualitative and quantitative.

2.4. Data Analysis

Quantitative traits were checked for normality of frequency distributions and variance heterogeneity and used for descriptive statistical analysis which included range, average, standard deviation, and coefficient of variation (CV%) to evaluate the amplitude of trait variability [35]. Correlation analysis among the observed variables [36] and one-way analysis of variance (ANOVA) at the significant level of p < 0.05 were performed using XLSTAT software (https://www.xlstat.com, accessed on 4 May 2022).

Qualitative traits data were converted into a discrete data matrix and used in the software GENALEX v.6.5 (accessed on 4 May 2022) [37]. Diversity across wild olive trees and the efficiency of descriptors in distinguishing the individuals were evaluated by calculating the Shannon information index (Hj), and the discrimination power (Dj) [38].

A principal component analysis (PCA) was also performed to identify the patterns of variation within the samples in order to determine the relative importance of the classification variables [39] and to select them for further hierarchical cluster analysis. Cluster analysis was carried out by using the software DARWIN v. 6.0.010 (http://darwin.cirad.fr, accessed on 4 May 2022) based on Ward's minimum variance method [40], and the tree was visualized by using the software FigTree 2016-10-04-v1.4.4 (http://tree.bio.ed.ac.uk/ software/figtree, accessed on 4 May 2022).

3. Results

3.1. Ecogeographic Data Analysis

The bioclimatic data and the pluviothermic Emberger quotients (Q2) confirmed a wide variability of growing conditions for the 175 collected wild olive samples. These were found to belong to five bioclimatic groups as follows: 61 samples to the humid and temperate/warm winter area, 37 samples to the sub-humid/temperate winter area, 49 samples to the semi-arid/cool-temperate winter area, 24 samples to the arid/temperate winter area, and 4 samples to the Saharan area (Figure 2).



Figure 2. Climogram showing the distribution of the collected wild olive samples according to the Emberger's coefficient.

3.2. Quantitative Traits Analysis

The results of the descriptive statistics for the 175 samples on the 12 analyzed quantitative variables are summarized in Table 2. The coefficient of variation (CV) indicated a high variability for all descriptors among the wild olive samples, with the highest values observed for the traits "fruit weight" and "stone weight", and the lowest observed for "stone width".

Table 2. Results for descriptive statistics obtained for the 175 samples based on the 12 analyzed quantitative traits.

Trait	Range	Mean	Standard Deviation	CV (%)
Leaf Blade Length (cm)	2.97-8.99	5.38	0.96	17.87%
Leaf Blade Width (cm)	0.56-2.05	1.11	0.29	26.19%
Leaf Shape (L/I)	2.87-9.31	5.14	1.24	24.09%
Fruit Weight (g)	0.11 - 1.87	0.61	0.35	57.82%
Fruit Length (cm)	0.64-2.19	1.22	0.24	19.81%
Fruit Width (cm)	0.48-1.36	0.84	0.16	19.52%
Fruit Shape (L/I)	1.09-2.13	1.48	0.18	11.86%
Stone Weight (g)	0.04-0.98	0.19	0.10	51.57%
Stone Length (cm)	0.56 - 1.64	1.04	0.18	17.16%
Stone Width (cm)	0.38-0.81	0.56	0.07	11.70%
Stone Shape (L/I)	1.32-2.93	1.87	0.26	13.93%
Stone Number of Grooves (SNG)	4.18 - 14.65	9.57	1.89	19.73%

A highly positive correlation was observed between fruit and stone traits. In particular, the highest values were recorded between fruit weight/fruit width (r = 0.90), fruit weight/fruit length (r = 0.87), and stone length/fruit length (r = 0.89). On the contrary, very weak correlations were found among all the leaf traits and with the other analyzed traits. (Table 3).

Table 3. Correlation between 12 morphological quantitative traits based on individual tree averages. The values higher than 0.7 indicate a strong correlation and are reported in bold.

Descriptors	LBW	LSH	FW	FL	FWI	FSH	SW	SL	SWI	SSH	SNG
Leaf Blade Length (cm) (LBL)	0.57 ***	0.15	0.41 ***	0.45 ***	0.42 ***	0.08	0.32 ***	0.35 ***	0.22 **	0.25 ***	0.00
Leaf Blade Width (cm) (LBW)		-0.67 ***	0.36 ***	0.37 ***	0.45 ***	-0.14	0.33 ***	0.18 *	0.26 ***	0.01	-0.10
Leaf Shape (L/I) (LSH)			-0.10	-0.07	-0.20 **	0.27 ***	-0.11	0.08	-0.13	0.21 **	0.13
Fruit Weight (g) (FW)				0.87 ***	0.90 ***	-0.03	0.72 ***	0.69 ***	0.71 ***	0.26 ***	0.05
Fruit Length (cm) (FL)					0.86 ***	0.30 ***	0.72 ***	0.89 ***	0.69 ***	0.55 ***	0.08
Fruit Width (cm) (FWI)						-0.22 **	0.71 ***	0.60 ***	0.76 ***	0.12	0.03
Fruit Shape (L/I) (FSH)							0.03	0.56 ***	-0.11	0.85 ***	0.11
Stone Weight (g) (SW)								0.64 ***	0.75 ***	0.17	0.14
Stone Length (cm) (SL)									0.66 ***	0.74 ***	0.09
Stone Width (cm) (SW)										0.00	0.04
Stone Shape (L/I) (SSH)											0.07

*** significant at p < 0.001; ** significant at p < 0.01; * significant at p < 0.05.

ANOVA along with the pairwise post-hoc Tukey test performed on quantitative traits showed that only the Saharan samples were significantly different (p < 0.05) for traits "leaf blade length" and "width", and for "fruit width" (Table 4).

	Trait											
Bioclimate	Leaf Blade Length (cm)	Leaf Blade Width (cm)	Leaf Shape (L/I)	Fruit Weight (g)	Fruit Length (cm)	Fruit Width (cm)	Fruit Shape (L/I)	Stone Weight (g)	Stone Length (cm)	Stone Width (cm)	Stone Shape (L/I)	Stone Number of Grooves
Sub-humid	$5.25\pm0.83~\mathrm{a}$	$1.09\pm0.26~\mathrm{a}$	5.13 ± 1.37 a,b	$0.59\pm0.31~\mathrm{a}$	1.22 ± 0.21 a	$0.85\pm0.16~\mathrm{a}$	1.46 ± 0.17 a,b	0.22 ± 0.16 a	$1.05\pm0.17~\mathrm{a}$	$0.58\pm0.07~\mathrm{a}$	1.84 ± 0.23 a	$9.71\pm2.08~\mathrm{a}$
Humid	5.26 ± 0.87 a	$1.09\pm0.30~\mathrm{a}$	5.12 ± 1.38 a,b	0.61 ± 0.34 a	1.20 ± 0.22 a	$0.83\pm0.16~\mathrm{a}$	1.47 ± 0.17 a,b	$0.18\pm0.07~\mathrm{a}$	1.01 ± 0.16 a	$0.54\pm0.06~\mathrm{a}$	$1.87\pm0.25~\mathrm{a}$	$9.55 \pm 1.71 \mathrm{~a}$
Semi-arid	$5.56\pm1.10~\mathrm{a}$	$1.16\pm0.27~\mathrm{a}$	4.93 ± 0.99 a,b	$0.57\pm0.35~\mathrm{a}$	0.57 ± 0.26 a	$0.81\pm0.15~\mathrm{a}$	1.48 ± 0.17 a,b	$0.18\pm0.08~\mathrm{a}$	$1.04\pm0.20~\mathrm{a}$	$0.56\pm0.07~\mathrm{a}$	$1.86\pm0.30~\mathrm{a}$	9.42 ± 2.16 a
Arid	5.34 ± 0.91 a	$0.99\pm0.28~\mathrm{a}$	5.74 ± 1.06 a	0.69 ± 0.45 a	$1.30\pm0.30~\mathrm{a}$	$0.85\pm0.20~\mathrm{a}$	1.55 ± 0.20 a	$0.19\pm0.07~\mathrm{a}$	$1.10\pm0.18~\mathrm{a}$	0.55 ± 0.06 a	1.98 ± 0.26 a	$9.77\pm1.59~\mathrm{a}$
Saharan	$6.64\pm1.25b$	$1.56\pm0.27b$	$4.49\pm0.48\mathrm{b}$	$0.89\pm0.28~\mathrm{a}$	$1.41\pm0.17~\mathrm{a}$	$1.04\pm0.12b$	$1.37\pm0.19\mathrm{b}$	$0.25\pm0.09~\mathrm{a}$	$1.05\pm0.21~\mathrm{a}$	$0.60\pm0.05~\mathrm{a}$	1.74 ± 0.21 a	$9.11\pm1.38~\mathrm{a}$

Table 4. Descriptive statistics with average \pm standard deviation for each of the 12 quantitative morphological traits in each bioclimatic area. Different letters indicate significant difference (p < 0.05).

3.3. Qualitative Traits

All qualitative traits were shown to be highly polymorphic except Nipple (NI), Lenticels (LE), and Dimension of Lenticels (DLE) which were monomorphic and were therefore excluded from further analyses. Shannon information index (Hj) was >0.6 for all traits except for the stone number of grooves (Hj = 0.37), reaching the maximum value of 1.28 for the longitudinal curvature of the blade (LCB) which also expressed the highest value of discriminating power (Dj = 0.70, average = 0.54) (Table 5, Supplementary Figure S2).

Table 5. Results obtained for diversity indices on qualitative traits analysis: number of total classes (KT), number of observed classes (Kj), Shannon's information index (Hj) and Discriminating power (Dj) are reported.

Trait	Trait Code	KT	Kj	Hj	Dj
Tree Vigor	TV	3	3	0.979	0.582
Tree Habit	TH	3	3	1.071	0.649
Canopy Density	CD	3	3	0.682	0.390
Tree Production fruit	TP	3	3	0.881	0.511
Maturation	Μ	3	3	0.635	0.346
Leaf Shape	LSH	3	3	0.868	0.501
Leaf Blade Length (cm)	LBL	3	3	0.841	0.513
Leaf Blade Width (cm)	LBW	3	3	0.920	0.566
Longitudinal Curvature Blade	LCB	4	4	1.281	0.701
Fruit Weight (g)	FW	3	3	0.988	0.601
Fruit Shape	FSH	3	3	0.910	0.566
Fruit Symmetry (position A)	FSA	3	3	1.066	0.646
Fruit Maximal Diameter	FMD	3	3	0.956	0.565
Fruit Apex Shape (position A)	FASH	2	2	0.693	0.500
Fruit Base Shape (position A)	FBSH	3	3	1.021	0.614
Nipple	NI	2	1	-	-
Lenticels	LE	2	1	-	-
Dimension Lenticels	DLE	2	1	-	-
Stone Weight (g)	SW	3	3	0.979	0.594
Stone Shape (position A)	SSH	4	4	1.046	0.609
Stone Symmetry (position A)	SSA	3	3	1.063	0.645
Stone Maximal Diameter	SMD	3	3	1.034	0.627
Stone Distribution Grooves	SDG	2	2	0.682	0.489
Stone Apex Shape	SASH	2	2	0.680	0.487
Stone Base Shape	SBSH	3	3	0.795	0.448
Stone Surface	SS	3	3	1.081	0.655
Stone Number of Grooves	SNG	3	3	0.366	0.175
Stone Mucron	SM	2	2	0.691	0.498
Average				0.89	0.54
Min				0.37	0.18
Max				1.28	0.70

According to the 4 classes considered for qualitative descriptors, wild olive samples were classified as illustrated in Figure 3. Most of the samples were characterized by strong vigor (class 3), spread or erect branches (class 2), compact canopy density (class 3), and high production (class 3) with late maturation (class 3). Leaves were typically elliptic-lanceolate (class 2), with medium or narrow blade width (class 2), medium blade length (class 2), and epinastic (class 1)/hyponastic (class 3) longitudinal curvature blade (Figure 3). Most samples bore fruits of low weight (class 1), oval or long shape (classes 2 and 3), and both symmetrical and asymmetrical (classes 1, 3). They were also characterized by a central maximum diameter (class 2), apex shape rounded or pointed (classes 1, 2), and a fruit base shape usually truncate or pointed (classes 2, 3). The stones were characterized by low or medium weight (classes 1, 2), elliptic shape (class 3), slightly asymmetrical/asymmetrical shape (classes 2, 3), central or toward apex maximal diameter, high number and irregular



distribution of grooves, pointed apex and base, a surface scabrous or smooth, and with or without mucron (Figure 3).

Figure 3. Histograms represent the distribution of the qualitative traits relative to tree habit (**A**), leaf (**B**), fruit (**C**), and stone (**D**) among the analyzed wild olive samples. For each trait (bar), the % of samples falling into each of the four classes considered for descriptors is indicated.

The PCA performed on qualitative traits showed that the first two principal components explained 26.97% of the total variance (Figure 4). The first component, PC1, accounted for 15.43%, with the highest contribution from the "stone and fruit weight", the "stone apex shape", the "stone maximal diameter", and the leaf traits "blade width", "blade length", and "Longitudinal Curvature Blade". The second component, PC2, accounted for the 11.54 variance among samples, with the highest contribution from the "fruit and stone shape" and "symmetry" (Figure 4).



Figure 4. Plot illustrating the relationships among the 25 polymorphic qualitative morphological traits assessed via PCA analysis of 175 wild olive trees (Nipple, Lenticels, and Dimension Lenticels were excluded because they showed to be monomorphic among samples).

3.4. Cluster Analysis

The hierarchical cluster analysis performed on all 32 traits highlighted the relationships between the analyzed wild olive samples, dividing the samples into two clusters, which comprised 99 samples (CI) and 76 samples (C II), respectively (Figure 5).



0.2

Figure 5. Dendrogram generated by Ward's method based on morphological markers, illustrating the similarity among the 175 wild olive Algerian samples. The colors red and blue were used to distinguish between the two obtained clusters, Cluster 1 and Cluster 2.

The clustering appeared to be based on fruit and stone traits, with Cluster I including mostly the samples with fruit weight > 0.5 g and medium/large stone (>0.15), and Cluster II comprising mostly the samples with fruit weight < 0.5 and medium/small stone weight (<0.15 g) (Figure 6). On the contrary, no relationship was found with the ecogeographic origin of samples, as Cluster I included samples collected from all the different climatic areas considered, from Saharan to humid, while Cluster II grouped prevalently the samples collected in sub-humid/humid areas, characterized by temperate/warm winters (Figure 6).



Figure 6. Histogram illustrating the % of olive samples included in clusters I and cluster II of the dendrogram, considering the traits fruit (**A**) and stone (**B**), and the climatic factors (**C**,**D**).

4. Discussion

In recent years, the loss of genetic variability due to the spread of intensive agriculture and as a result of climate change, has triggered greater attention to the recovery and conservation of wild germplasm. This can be a source of genetic variability useful for overcoming new challenges, such as the recent outbreak of the devastating uncontrolled epidemic of Olive Quick Decline Syndrome (OQDS) caused by the bacterium *Xylella fastidiosa* spp. *pauca* [41]. Mining the available genetic resources of wild olive can allow finding interesting traits to be used in projects for favorable alleles introgression into cultivated olive to face biotic and abiotic stresses [42,43].

Due to its peculiar ecogeographic characteristics, Algeria has vast territories still untouched by anthropization, where wild olive trees still survive in extreme ecoclimatic conditions [24,44]. In recent years, the variability in the Algerian wild olive trees has aroused growing interest, prompting the exploration of the genetic diversity of the species, even in comparison with domestic olive [24,45,46].

The present study enabled the collection of 175 wild olive samples from 34 Algerian provinces located in the north of the country. The specimens were found in five different bioclimatic areas, classified according to the Emberger's quotients, ranging from the humid areas near the coasts to inland regions in the Saharan Region, confirming the great adaptability of this species to very different climatic environments, including extremes.

The morphological characterization confirmed a high variability between samples for all observed traits except for Nipple (NI), Lenticels (LE), and Dimension of Lenticels (DLE), which were shown to be monomorphic. The greatest variation was found for the weight of fruit and stone, which reached values higher than those already reported [35]. Fruit and stone descriptors showed a strong correlation and were very effective in discriminating between olive genotypes. On the contrary, leaf traits exhibited little variation and discrimination power, confirming previous reports for both wild olive [35] and the cultivated

one [24,47]. In particular, the endocarp traits proved to be very stable and highly discriminating compared to other traits more influenced by environmental conditions [48,49]. In fact, they are frequently used to catalog olive cultivars because they can be effectively kept for a long time and are easily exchangeable [50,51].

The mean fruit and stone weight for the wild olive samples was 0.61 g and 0.19 g, respectively, reflecting values consistent with those previously reported for the wild olive [24,35,52–54]. However, several samples showed a medium-large fruit size, more similar to that of the cultivated olive tree. One explanation for this could be that these samples are not true wild olives but, more likely, feral forms derived from a hybridization event with cultivated olives or cultivars that have escaped cultivation.

In addition, fruit and stone size were at the base of the results of the clustering analysis, which included the samples with larger fruits and seeds in Cluster I, and the samples with smaller fruits and seeds in Cluster II. On the contrary, the dendrogram did not show any link with the ecoclimatic origin of samples, as both clusters included samples from all climatic areas, although Cluster II collected more samples from sub-humid/humid areas. This is in contrast to previous observations obtained on the same Algerian wild olive trees through a genetic analysis with 16 microsatellite markers, which grouped the samples according to the growing climatic conditions [25]. This result could be due to the physical isolation of trees, which restricts gene flow between populations, leading to a marked genetic differentiation which, instead, does not correspond to a clear morphological variation. Only the Saharan samples resulted in significantly different leaf traits and fruit, indicating the potential of the wild trees belonging to this area and the necessity to deeply characterize them.

Overall, this study allowed us to collect and morphologically characterize a very diverse heritage of wild olive growing in Algeria. This germplasm showed valuable variability for many traits, and the Saharan samples, in particular, appeared to be significantly different for leaf traits, from samples growing in other bioclimatic areas. Deeper studies are needed, using more descriptors and integrating the morphological data with genetic studies, to better understand the impact the bioclimatic variables have on wild olive diversity. This will allow us to effectively exploit the potential of the wild olive, whose variation deserves to be adequately cataloged and preserved for future exploitation in breeding programs.

Supplementary Materials: The following supporting information can be downloaded at: https:// www.mdpi.com/article/10.3390/plants11141803/s1, Supplementary Table S1: List of the 175 samples analyzed in this study; Supplementary Figure S1: An example of some descriptors indicated by UPOV for morphological characterization of olive; Supplementary Figure S2: Pictures representing some of the qualitative traits of the samples analyzed in this study.

Author Contributions: Conceptualization, W.F., D.K., S.B. and C.M.; investigation, W.F., I.M., S.G. and M.M.M.; writing—original draft preparation, W.F., M.M.M. and I.M.; review and editing, M.M.M., I.M., C.M. and V.F.; supervision, I.M., M.M.M. and C.M.; funding acquisition, D.K., S.B. and C.M. All authors have read and agreed to the published version of the manuscript.

Funding: The APC was funded by MIUR-PON Ricerca e Innovazione 2014–2020 (project AIM1809249attività 2, linea 1). Valentina Fanelli was supported by MIUR-PON Ricerca e Innovazione 2014–2020 (project AIM1809249-attività 2, linea 1).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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