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Stone diversity in wild and cultivated olive trees (*Olea europaea* L.)

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Abstract: The olive tree is represented in Tunisia by two varieties: var. *sylvestris* (wild olive trees) and var. *europaea* (cultivated olive trees including diverse cultivars). Seed (stone) size and shape analysis may provide the basis for relationships between varieties and cultivars as well as to study the responses to environmental conditions. A semi-automated method of image analysis allows to obtain data related with magnitudes descriptive of stone size and shape and to compare between wild and cultivated olives. Also, the effect of bioclimate on size and shape was analyzed in some cultivars. Stone size and shape presents high variability. In cultivated trees stones are larger. Mean seed image area is 0.38 and 0.75 cm² for wild and cultivated plants respectively. Roundness and circularity were compared as to their potential to define seed shape. Mean values were higher for circularity, but roundness is more variable reaching higher values in some individuals and varieties. Roundness is more useful to compare seed shape variations. In addition, climate factors affect the stone characteristics in cultivars; those of sub-humid region having larger stones. Phenotypic parameters of stone are discriminating parameters for the analysis of intra-specific, intra-varieties and intra-cultivars variability in *Olea europaea*.

Keywords: *Olea europaea*, stone, diversity, roundness, circularity, size, Tunisia

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Introduction

Olive (*Olea europaea*, L.) is one of the most important crops in Mediterranean countries. The olives are used for table consumption as well as an important source of oil. The olive tree has a long tradition of historic and cultural significance (Fig. 1).

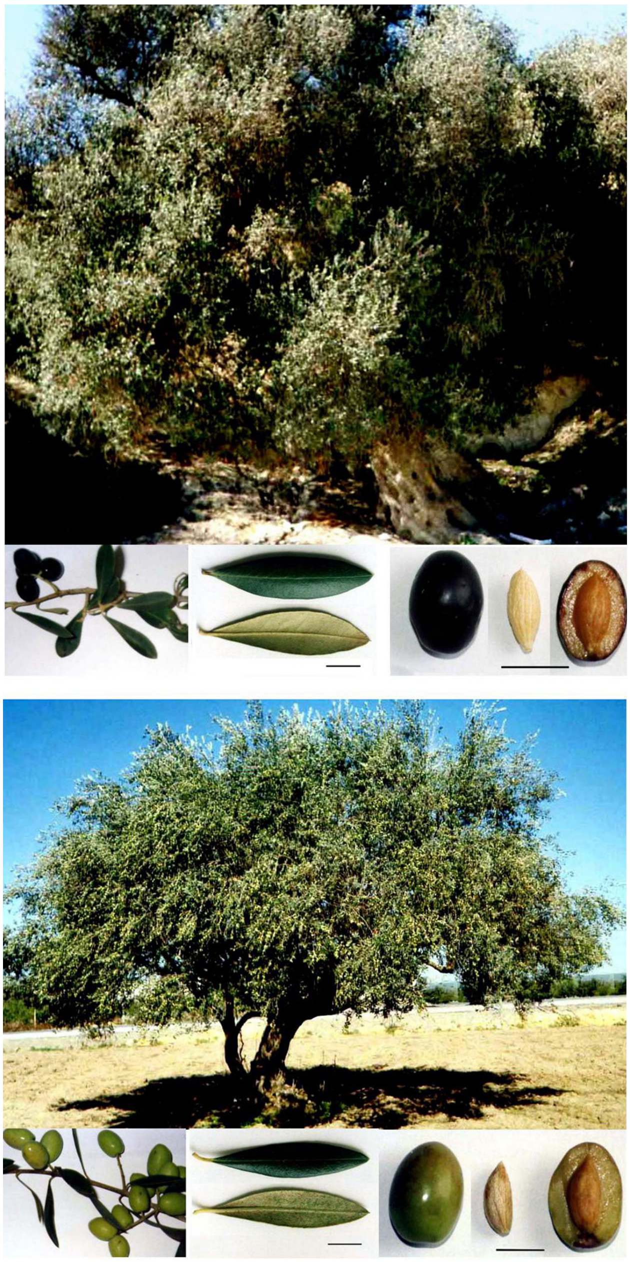


Fig. 1. Olive trees. Wild (above) and cultivated (below). Branch with fruits, leaves, fruits and seeds. Bar represents 1 cm

Olea europaea trees spread in the Mediterranean Basin where they are indigenous as well as in other regions with a Mediterranean climate where they have been introduced (Costa, 1998). All cultivated and wild olive trees belong to two botanical varieties: The wild olive trees or oleaster correspond to *Olea europaea* subsp. *europaea* L. var. *sylvestris*. The cultivated olive trees correspond to *Olea europaea* subsp. *europaea* L. var. *europaea* (Green, 2002) and comprise olive cultivars grown for oil or table olives. In addition to these two olive forms, a third form resembling oleasters by their physiognomy, has escaped from cultivation, and has been called feral form (Besnard et al., 2001; Mekuria et al., 2002; Breton et al., 2006a).

The description of oleaster and olive cultivars and/or the relationships between them were conducted using several markers as morphological traits (Bartolini et al., 1998; Rotondi et al., 2003; Idrissi & Ouazzani, 2003; Hannachi et al., 2008a,b; Belaj et al., 2011), oil criteria (Baccouri et al., 2011; Dabbou et al., 2011; Krichene et al., 2007; Hannachi et al., 2013) and molecular markers (Belaj et al., 2007; Belaj et al., 2010; Hannachi et al., 2010; Erre et al., 2010; Belaj et al., 2011; Noormohammadi et al., 2014).

Several historical reports pointed out that Oleaster forests are native in Tunisia (Camps-Fabrer, 1997). Based on nuclear and cytoplasmic SSR markers, the indigenous and feral types have been noted in Tunisia both in natural and agro-ecosystems (Hannachi et al., 2008b, Hannachi et al., 2010). The modern Tunisian oleasters seem to issue from one refuge area (Breton et al., 2006a). In addition, gene flow continued between oleaster and cultivated olive trees (Lumaret & Ouazzani, 2001) because several widespread olive cultivars are male sterile (Besnard et al., 2000; Hannachi & Marzouk, 2012). In the case of olive cultivars in orchards, they may be pollinated by surrounding oleasters as reported in several countries (France, Algeria, Morocco, Tunisia and Spain). However, this crossing has no consequence on the oil quality which is determined by the maternal tissue of the fruit. Therefore such hybrid fruits may lead again to feral trees (Breton et al., 2005). Therefore, the use of wild olive trees in breeding programs has been proposed as an alternative approach to increase the diversity of genetic resources such as for example in Spain (Belaj et al., 2010). To attempt this aim, *in situ* and *ex situ* characterization of olive genotypes is crucial using several descriptors. In addition, the characterization of wild and cultivated olive trees is important to preserve the genetic diversity, to select parent for genetic crossing.

Seed morphology is an interesting tool in the description of plant families, species and varieties. Morphological analysis based in the comparison with geometric objects is useful to characterize the seeds in plant families (Cervantes et al., 2016) and for the determination of taxonomic relationships of species and subspecies. The shape of seeds in model species was quantified by comparison with a cardioid (Cervantes et al., 2010; Cervantes et al., 2012) thus allowing to accurately measure changes in seed shape in mutants, during development or in response to environmental conditions or stress situations (Martín et al., 2014). In addition comparison of seed images with geometric figures allows the identification of features between subspecies and varieties (Saadaoui et al., 2013), changes to growth conditions (Saadaoui et al., 2015) or particular climatic adaptations (Martín Gómez et al., 2016). In general, seed shape has less variation than size, but both magnitudes are independent and valuable in phenotyping.

In Tunisia, the olive, existing in a wild and cultivated state, is characterized by a high genetic variability. This work aims to find new phenotypic descriptors, which allowed us to better discriminate the varieties and cultivars. Stone size and shape were analyzed for multiple sites, in different bioclimatic zones of the country.

Material and methods

Plant material

Stones (seeds) were collected from 36 cultivated trees belonging to a total of 19 cultivars and from 55 wild olive trees, making a total of 91 trees (Table 1). Main characteristics of geographical sites are shown in Tables 1 and 2, geographical locations in Fig. 2. Samples comprise ten stones of each olive tree.

Seed scanning

Seeds were scanned with a CanoScanLiDE 110 with resolution set at 300 ppp horizontal and 300 ppp vertical. Each image contains ten seeds of each tree, thus the analysis includes 91 images of ten seeds each (Fig. 3).

Image analysis

Image analysis was done with ImageJ (Ferreira & Wayne, 2010) recording automatically the values of seed length (cm), seed width (cm), perimeter (cm), area (cm^2), circularity index and roundness.

Circularity Index (Cox, 1927; Riley, 1941; Schwartz, 1980) or form factor (Rovner & Gyulai, 2007) is defined as:

$$I = \frac{4\pi \text{ x area}}{\text{perimeter}^2}$$

Roundness (Rovner & Gyulai, 2007) is defined as:

$$R = \frac{4 \text{ x area}}{\pi \text{ [Major axis]}^2}$$

Circularity Index depends on the perimeter, while Roundness depends on the major axis. Thus, in objects of uneven or rough surface, the values of the former decrease very rapidly with the oscillations in the surface. The ratio between Circularity Index and Roundness is given by:

$$I/R = \frac{\pi^2 \text{ [Major axis]}^2}{\text{perimeter}^2}$$

In consequence increases in the perimeter of a figure, maintaining the major axis constant or under low change, results in reduced circularity values relative to roundness.

Statistical analysis

The program SPSS v. 21 was used for the ANOVA with Scheffé test (different sample number between populations and bioclimatic regions), T Student test and Pearson correlation.

Principal Component Analysis (PCA) was conducted on wild and cultivated plants based on stone size and shape data. The hierarchical classification was also done using Euclidean Distance and Ward as the aggregation method. Analyses were computed using the software Statistica (Sta Soft Inc., Johannesburg, ZA).

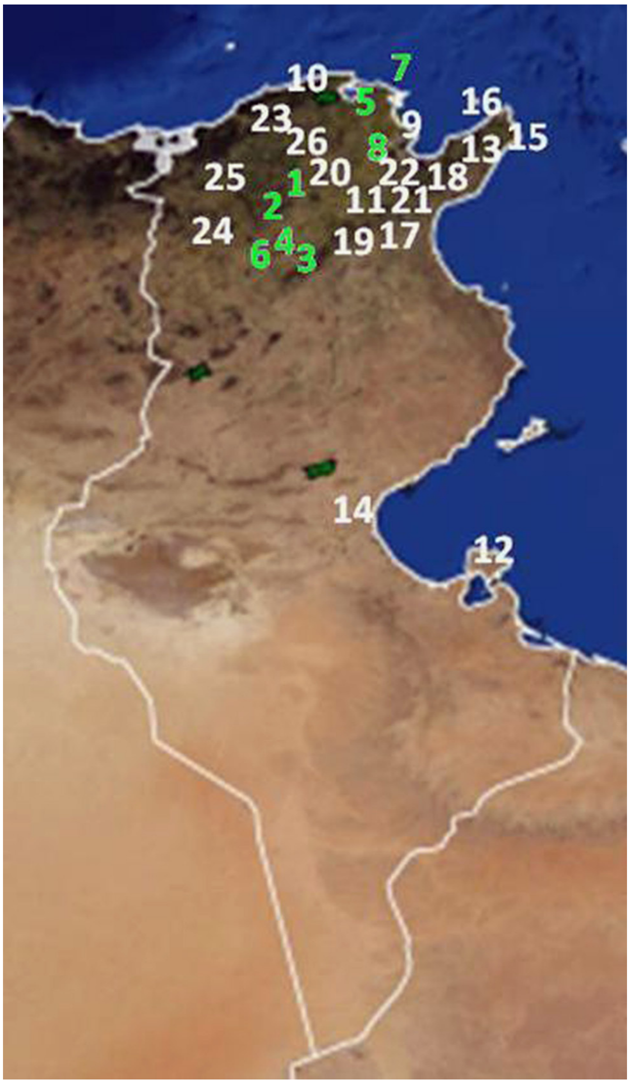


Fig. 2. Geographical sites of studied cultivated and wild olive trees. Green numbers (1 to 8) represent localities that have wild and cultivated populations. In white numbers (9 to 26), localities with only cultivated populations

Table 1. Codes and localities of wild (oleaster) and cultivated olive trees

Olive Tree cultivar	Code	Locality	Province	Olive Tree	Code	Locality	Province
Chétoui	C1	Slouguia	Béja	Oleaster	O10	Zaghouan	Zaghouan
Chétoui	C2	Laaroussa	Siliana	Oleaster	O11	Zaghouan	Zaghouan
Chétoui	C3	Téboursouk	Béja	Oleaster	O12	Zaghouan	Zaghouan
Chétoui	C4	El-Alia	Bizerte	Oleaster	O13	Zaghouan	Zaghouan
Marsaline	C5	Slouguia	Béja	Oleaster	O14	Djerba	Medenine
Marsaline	C6	Laaroussa	Siliana	Oleaster	O15	Azmour	Haouaria
Chaïbi	C7	Testour	Béja	Oleaster	O16	Zaghouan	Zaghouan
Chaïbi	C8	Téboursouk	Béja	Oleaster	O17	Zaghouan	Zaghouan
Chaïbi	C9	Laaroussa	Siliana	Oleaster	O18	Azmour	Kélibia
Chaïbi	C10	Tébousouk	Béja	Oleaster	O19	Azmour	Kélibia
Chemlali	C11	Slouguia	Béja	Oleaster	O20	Chenenni	Gabès
Meski	C12	EL-Alia	Bizerte	Oleaster	O21	Azmour	Kélibia
Meski	C13	Dougga	Béja	Oleaster	O22	Azmour	Kélibia
Meski	C14	Slouguia	Béja	Oleaster	O23	Testour	Béja
Meski	C15	El-Alia	Bizerte	Oleaster	O24	Testour	Béja
Besbessi	C16	Laaroussa	Siliana	Oleaster	O25	Belvedère	Tunis
Besbessi	C17	Testour	Béja	Oleaster	O26	Ras-Jbel	Bizerte
Besbessi	C18	Slouguia	Béja	Oleaster	O27	Slouguia	Béja
Berboui	C19	Laaroussa	Siliana	Oleaster	O28	Téboursouk	Béja
Gerboui	C20	Téboursouk	Béja	Oleaster	O29	Ichkeul	Bizerte
Gerboui	C21	Téboursouk	Béja	Oleaster	O30	Ichkeul	Bizerte
Gerboui	C22	Laaroussa	Siliana	Oleaster	O31	Ichkeul	Bizerte
Gerboui	C23	Dougga	Béja	Oleaster	O32	Ichkeul	Bizerte
Gerboui	C24	Téboursouk	Béja	Oleaster	O33	Kélibia	Kélibia
Oueslati	C25	Téboursouk	Béja	Oleaster	O34	Ech-Raf	Haouaria
Rajou	C26	Ras-Jbel	Bizerte	Oleaster	O35	Ech-Raf	Haouaria
Zayati	C27	Laaroussa	Siliana	Oleaster	O36	Ech-Raf	Haouaria
Nib	C28	Ras-Jbel	Bizerte	Oleaster	O37	El-Alia	Bizerte
Zarras	C29	Téboursouk	Béja	Oleaster	O38	Saouaf	Zaghouan
Sayali	C30	Slouguia	Béja	Oleaster	O39	Jbel-Abderahmen	Nabeul
Beldi	C31	Téboursouk	Béja	Oleaster	O40	Kélibia	Kélibia
Neb Jmel	C32	Testour	Béja	Oleaster	O41	Dhra-Joudar	Zagouah
Awam	C33	Dougga	Béja	Oleaster	O42	Mjez El Bab	Béja
Chemlali	C34	Tunis	Tunis	Oleaster	O43	Jbel-Abderahmen	Nabeul
Roumi	C35	Téboursouk	Béja	Oleaster	O44	Saouaf	Zaghouan
Limi	C36	Teboursouk	Béja	Oleaster	O45	Zriba	Zaghouan
Oleaster	O1	Ras-Jbel	Bizerte	Oleaster	O46	Zaghouan	Zaghouan
Oleaster	O2	Dougga	Béja	Oleaster	O47	Jbel West	Zaghouan
Oleaster	O3	Belvedère	Tunis	Oleaster	O48	Tunis	Tunis
Oleaster	O4	Belvedère	Tunis	Oleaster	O49	Sejnan	Bizerte
Oleaster	O5	Testour	Béja	Oleaster	O50	Borj-Messaoudi	El-Kef
Oleaster	O6	Slouguia	Béja	Oleaster	O51	Tbaba	Jendouba
Oleaster	O7	Ichkeul	Bizerte	Oleaster	O52	Mateur	Bizerte
Oleaster	O8	Ichkeul	Bizerte	Oleaster	O53	Tunis	Tunis
Oleaster	O9	Ichkeul	Bizerte	Oleaster	O54	Laaroussa	Siliana
				Oleaster	O55	Laaroussa	Siliana

C: Cultivated olive tree; O: Oleaster (wild olive tree).

Results

Size and shape comparison between stones obtained from the wild plants

Stone size and shape showed high variability both in wild and cultivated olive trees. In wild trees seed image area oscillated between 0.12 and 0.74 cm^2 with mean values of 0.38 cm^2 and standard deviation of 0.11 cm^2 (Table 3). Roundness oscillated between 0.37 and 0.93 with mean values of 0.53 and standard deviation of 0.08. Circularity oscillated between 0.51 and 0.84 with mean values of 0.69 and standard deviation of 0.05. The Pearson correlation values

Table 2. Main characteristics of localities of studied wild and cultivated olive trees

Locality	Code	Latitude	Longitude	Altitude (m)	Bioclimate
Slouguia	1	36°36’37.81’’N	09°31’53.39’’E	68	Sub-humid
Testour	2	36°33’06,92’’N	09°26’58,96’’E	93	Sub-humid
Laaroussa	3	36°22’51,94’’N	09°27’16,37’’E	197	Semi-arid
Téboursouk	4	36°27’40.60’’N	09°14’47.48’’E	472	Sub-humid
El-Alia	5	37°10’38.70’’N	10°02’05.21’’E	142	Sub-humid
Dougga	6	36°24’02.75’’N	09°14’14.75’’E	380	Sub-humid
Ras-Jbel	7	37°11’59.56’’N	10°07’28.84’’E	82	Sub-humid
Tunis	8	36°49’12.99’’N	10°10’13.97’’E	25	Upper semi-arid
Belvedère	9	36°49’21.27’’N	10°10’23.83’’E	43	Upper Semi-arid
Ichkeul	10	37°07’15.22’’N	09°38’55.91’’E	204	Sub-humid
Zaghouan	11	36°24’00.81’’N	10°09’29.77’’E	177	Upper Semi-arid
Djerba	12	33°49’25.53’’N	10°50’46.72’’E	19	Arid
Azmour	13	36°55’28.18’’N	11°00’24.88’’E	56	Sub-humid
Chenenni	14	33°54’05.05’’N	10°05’20.62’’E	13	Arid
Kélibia	15	36°51’49.67’’N	11°05’58.48’’E	12	Sub-humid
Ech-Raf	16	36°59’57.92’’N	11°02’12.69’’E	32	Sub-humid
Saouaf	17	36°11’47.54’’N	10°11’33.63’’E	199	Upper Semi-arid
Jbel-Abderahmen	18	36°44’21.79’’N	10°41’26.58’’E	340	Upper Semi-arid
Dhra-Joudar	19	36°16’25.20’’N	10°01’14.77’’E	358	Upper Semi-arid
Mjez El Bab	20	36°38’44.87’’N	09°36’15.34’’E	55	Upper Semi-arid
Zriba	21	36°20’19.66’’N	10°11’33.71’’E	186	Upper Semi-arid
Jbel West	22	36°32’08.64’’N	10°04’26.91’’E	136	Upper Semi-arid
Sejnan	23	37°03’07.55’’N	09°14’31.94’’E	195	Sub-humid
Borj-Messaoudi	24	36°12’00.64’’N	08°44’41.48’’E	720	Sub-humid
Tbaba	25	36°30’28.66’’N	08°39’53.02’’E	501	Sub-humid
Mateur	26	37°02’32.83’’N	09°40’10.48’’E	32	Sub-humid

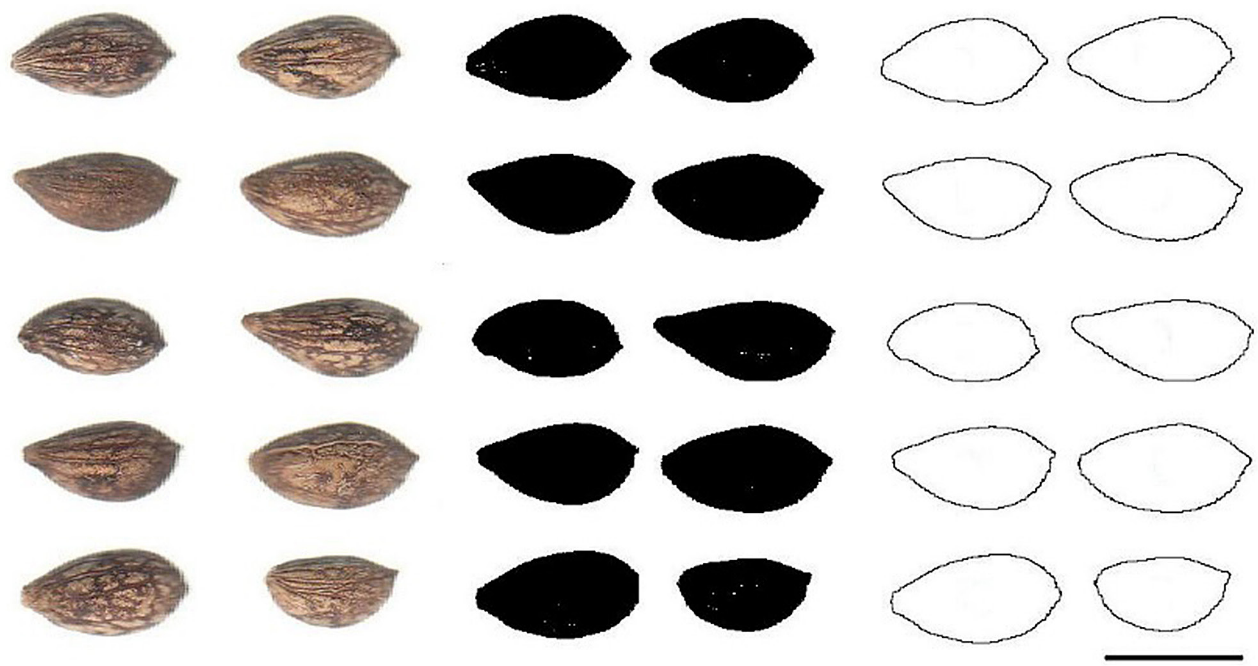


Fig. 3. A summary of the method used to quantify stone size and shape. Left: Stone images obtained by scan. Center: Profiles corresponding to each image. Right: Silhouettes. The program calculates: length, perimeter, area, circularity index and roundness index from the silhouettes

Table 3. Values of magnitudes related with size and shape in *Olea europea* seeds from wild and cultivated plants and results of T test comparing both groups

		N	Mean	Min	Max	Standard Deviation	Standard error mean	Bilateral sig.
Area	wild	550	0.38	0.12	0.74	0.11	0.00	0.000
	cultivated	359	0.75	0.40	1.47	0.21	0.01	
Roundness	wild	550	0.53	0.37	0.93	0.08	0.00	0.003
	cultivated	359	0.55	0.30	0.85	0.11	0.01	
Circularity	wild	550	0.69	0.51	0.84	0.05	0.00	0.000
	cultivated	359	0.66	0.46	0.79	0.05	0.00	
Length	wild	550	1.01	0.52	1.52	0.20	0.01	0.000
	cultivated	359	1.39	0.87	2.12	0.23	0.01	
Width	wild	550	0.52	0.31	0.73	0.07	0.00	0.000
	cultivated	359	0.73	0.48	1.12	0.12	0.01	
Length/Width	wild	550	1.92	1.08	2.72	0.28	0.01	0.047
	cultivated	359	1.88	1.18	3.32	0.35	0.02	

between seed area and roundness or seed area and circularity were negative, i.e., larger seeds had lower values of roundness and circularity (Table 4).

Size and shape comparison between stones obtained from the cultivated olive trees

In the cultivated plants, the seed image area oscillated between 0.40 cm^2 and 1.47 cm^2 with mean values of 0.75 cm^2 and standard deviation of 0.21 cm^2 (Table 3). Roundness oscillated between 0.30 and 0.85 with mean values of 0.55 and standard deviation of 0.11, and circularity oscillated between 0.46 and 0.79 with mean values of 0.66 and standard deviation of 0.05. The Pearson correlation values between seed area and circularity were negative. The Pearson correlation values between seed area and roundness were not significant. Larger seeds had lower values of circularity, but not of roundness (Table 4).

Climatic effect in stone size and shape and local differences in wild plants

Larger seed size in arid regions and smaller in sub-humid, upper semi-arid and semi-arid regions (Table 5a), may be the result of larger seeds in the two trees used in the arid region and not specific effect of climate on stone size. Roundness was higher in the semi-arid region and lower in the arid region with intermediate values in the other two regions (sub-humid and upper semi-arid). Similar results were obtained for circularity index. Relationships between roundness and circularity values in the climatic regions were conserved, although with lower values in roundness.

Table 4. Values of Person Correlation. **:Significant at level 0,01 (bilateral) ; *:Significant at level 0,05 (bilateral)

		wild stones					
		Area	Roundness	Circularity	Length	Width	Length/Width
cultivated stones	Area	1.000	–.441** sig. bilateral (0.000)	–.446** sig. bilateral (0.000)	.916** sig. bilateral (0.000)	.862** sig. bilateral (0.000)	.459** sig. bilateral (0.000)
	roundness	–0.038 sig. bilateral (0.474)	1.000	.766** sig. bilateral (0.000)	–.736** sig. bilateral (0.000)	–0.009 sig. bilateral (0.832)	–.979** sig. bilateral (0.000)
	circularity	–.404** sig. bilateral (0.000)	.633** sig. bilateral (0.000	1.000	–.676** sig. bilateral (0.000)	–.134** sig. bilateral (0.002)	–.792** sig. bilateral (0.000)
	Length	.763** sig. bilateral (0.000)	–.635** sig. bilateral (0.000)	–.741** sig. bilateral (0.000)	1.000	.632** sig. bilateral (0.000)	.756** sig. bilateral (0.000)
	Width	.843** sig. bilateral (0.00	.476** sig. bilateral (0.000)	–0.036 sig. bilateral (0.502)	.335** sig. bilateral (0.000	1.000	0.022 sig. bilateral (0.604)
	length /width	0.010 sig. bilateral (0.848)	–.967** sig. bilateral (0.000)	–.702** sig. bilateral (0.000	.629** sig. bilateral (0.000)	–.490** sig. bilateral (0.000)	1.000

Table 5. Comparison of size and shape in wild *Olea europaea* stones by climatic regions (a) and by localities in the climatic regions (b). N is the number of seeds analyzed in each case. The same letter (a, b, c) in the superindex for a given column indicates no difference between bioclimates or localities in the results from one factor Anova

a)

BioClimate	N	Area	Roundness	Circularity	Length	Width	Length/Width
Sub-humid	312	0.39 ^{a}	0.53 ^{b}	0.68 ^{a,b}	1.03 ^{b}	0.52 ^{a}	1.97 ^{b}
Semi-arid superior	198	0.35 ^{a}	0.54 ^{b}	0.70 ^{b}	0.97 ^{a,b}	0.50 ^{a}	1.93 ^{b}
Semi-arid	20	0.35 ^{a}	0.62 ^{c}	0.73 ^{c}	0.90 ^{a}	0.54 ^{a}	1.67 ^{a}
Arid	20	0.49 ^{b}	0.46 ^{a}	0.66 ^{a}	1.22 ^{c}	0.54 ^{a}	2.25 ^{c}

b)

Locality	N	Sub-Humid		
		Area	Roundness	Circularity
Azmour	50	0.39 ^{a,b,c}	0.54 ^{a,b,c}	0.66 ^{a,b}
Borj-Messaoudi	10	0.33 ^{a,b}	0.58 ^{b,c}	0.72 ^{a,b}
Dougga	10	0.51 ^{c,d}	0.63 ^{c}	0.70 ^{a,b}
Ech-Raf	32	0.45 ^{b,c,d}	0.48 ^{a,b}	0.68 ^{a,b}
El-Alia	10	0.31 ^{a}	0.63 ^{c}	0.71 ^{a,b}
Ichkeul	70	0.31 ^{a}	0.52 ^{a,b,c}	0.69 ^{a,b}
Kélibia	20	0.57 ^{d}	0.50 ^{a,b}	0.67 ^{a,b}
Mateur	10	0.43 ^{a,b,c}	0.56 ^{a,b,c}	0.71 ^{a,b}
Ras-Jbel	20	0.44 ^{a,b,c,d}	0.45 ^{a}	0.64 ^{a}
Sejnan	10	0.30 ^{a}	0.50 ^{a,b}	0.67 ^{a,b}
Slouguia	20	0.38 ^{a,b,c}	0.50 ^{a,b}	0.70 ^{a,b}
Tbaba	10	0.33 ^{a,b}	0.56 ^{a,b,c}	0.70 ^{a,b}
Téboursouk	10	0.32 ^{a,b}	0.58 ^{b,c}	0.73 ^{b}
Testour	30	0.43 ^{a,b,c}	0.53 ^{a,b,c}	0.70 ^{a,b}
Mean	**312**	**0.39**	**0.53**	**0.68** ^{a}
		Upper semi-arid		
Belvedère	30	0.32 ^{a,b}	0.60 ^{b,c}	0.73 ^{b,c}
Dhra-Joudar	10	0.24 ^{a}	0.61 ^{b,c}	0.77 ^{c}
Jbel West	10	0.30 ^{a,b}	0.51 ^{a}	0.67 ^{a}
Jbel-Abderahmen	20	0.31 ^{a,b}	0.49 ^{a}	0.69 ^{a,b}
Mjez El Bab	10	0.36 ^{a,b}	0.66 ^{c}	0.76 ^{c}
Saouaf	20	0.33 ^{a,b}	0.53 ^{a,b}	0.67 ^{a}
Tunis	20	0.28 ^{a}	0.63 ^{c}	0.77 ^{c}
Zaghouan	68	0.4 ^{b}	0.50 ^{a}	0.67 ^{a}
Zriba	10	0.54 ^{c}	0.45 ^{a}	0.64 ^{a}
Mean	198	0.35	0.541	0.70
		Semi-arid		
Laaroussa (O54)	10	0.34 ^{a}	0.75 ^{b}	0.77 ^{b}
Laaroussa (O55)	10	0.37 ^{b}	0.5 ^{a}	0.69 ^{a}
Mean	**20**	**0.35**	**0.63**	**0.73**
		Arid		
Djerba	10	0.36 ^{a}	0.50 ^{b}	0.68 ^{b}
Chenenni	10	0.61 ^{b}	0.43 ^{a}	0.63 ^{a}
Mean	**20**	0.46	0.46	0.66

Differences between localities in the sub-humid region were found for size (area) and shape (roundness and circularity, Table 5b). Area values oscillated between 0.30 (Sejnan) and 0.57 cm^2 (Kélibia). Roundness values oscillated between 0.45 (Ras-Jbel) and 0.63 (Dougga and El-Alia) and circularity between 0.64 (Ras-Jbel) and 0.73 (Téboursouk).

In the upper semi-arid region, differences between localities were found for size (area) and shape (roundness and circularity; Table 5b). Area values oscillated between 0.24 (Dhra-Joudar) and 0.54 cm^2 (Zriba). Roundness values oscillated between 0.45 (Zriba) and 0.66 (Mjez El Bab) and circularity between 0.64 (Zriba) and 0.77 (Dhra-Joudar and Tunis).

Comparison of size and shape between stones of two wild individuals from the same population in the semi-arid region revealed differences in seed size and shape (Table 5b).

Differences between seeds from plants of diverse cultivars grown in different regions

The cultivation of a variety in two different regions allows to investigate the size and shape of the seed in response to the climatic and geographic effect.

Seeds from cultivars Besbessi, Chaïbi, Chétoui, Gerboui and Marsaline where obtained both in sub-humid and semi-arid climates. Seeds from cultivar Chemlali where obtained from Sub-humid and Semi-arid superior climates. Although some of the cultivars presented a trend to increased seed area in the subhumid climate (Chaïbi, Chétoui, Marsaline, Chemlali; Table 6), this was not conserved in all cultivars. Roundness values were higher in the semi-arid regions in four of six varieties (Table 6).

Size and shape comparison between stones obtained from wild and cultivated plants: mutivariate analysis

Principal components analysis (PCA) reduced stone size and shape data to two principal components which accounted for 98.88% of the total variation (Table 7). The first component (PC1) accounted for 70.34% of the total variation and was mainly correlated negatively with area, perimeter and positively with circularity, length/width, and length^2/perimeter^2. The second component PC2 explained 28.54% of the total variation and is defined positively with roundness and perimeter and negatively with length (Table 7). The dispersion of studied plants on the plan defined by PC1 and PC2 showed that wild plants were grouped at the upper right dial defined by circularity, length/width, lengh^2/perimeter^2, roundness, length and width. The cultivated plants were placed at the left dial (Fig. 4). The stones obtained from wild and cultivated plants revealed differences both in size and shape (Table 3). Stones from cultivated plants are larger and present lower values of circularity index and higher values of roundness than the stones from wild plants. However, the centre of PCA plot contains a mixed group of wild and cultivated olive trees (Fig. 4).

The hierarchical analysis was conducted based on stone data of studied plants (Fig. 5). The dendrogram obtained showed two cluster CL1 and CL2; each one was divided on two sub-clusters. In all four sub-clusters were identified (CL1/1, CL1/2, CL2/1 and CL2/2). Sub-cluster CL1/1 comprises only wild plants which were well separated from cultivated ones. Sub-cluster CL1/2 comprises 19 wild and three cultivated olive trees, C24 (Gerboui), C19 (Gerboui)

Table 6. Comparison of size and shape between seeds from plants of diverse cultivars grown in different regions

Olive tree Cultivar	Bioclimate	N	Area	Roundness	Circularity	Length	Width	Length/ Perimeter
Chemlali	Sub-humid	10	0.51 ^{b}	0.51 ^{b}	.67 ^{a}	1.23 ^{a}	0.59 ^{b}	2.1 ^{a}
	Semi-arid superior	10	0.47 ^{a}	0.45 ^{a}	.66 ^{a}	1.20 ^{a}	0.53 ^{a}	2.3 ^{b}
Besbessi	Sub-humid	20	1.02 ^{a}	0.54 ^{a}	0.64 ^{a}	1.62 ^{b}	0.85 ^{a}	1.97 ^{b}
	Semi-arid	10	1.08 ^{a}	0.71 ^{b}	0.65 ^{a}	1.42 ^{a}	0.99 ^{b}	1.44 ^{a}
Chaïbi	Sub-humid	30	0.54 ^{b}	0.54 ^{a}	0.68 ^{a}	1.20 ^{a}	0.62 ^{b}	1.95 ^{a}
	Semi-arid	10	0.47 ^{a}	0.53 ^{a}	0.70 ^{a}	1.13 ^{a}	0.57 ^{a}	1.97 ^{a}
Chétoui	Sub-humid	30	0.67 ^{b}	0.46 ^{a}	0.63 ^{a}	1.46 ^{b}	0.64 ^{a}	2.27 ^{b}
	Semi-arid	10	0.62 ^{a}	0.49 ^{b}	0.64 ^{a}	1.36 ^{a}	0.64 ^{a}	2.15 ^{a}
Gerboui	Sub-humid	40	0.68 ^{a}	0.68 ^{a}	0.71 ^{b}	1.17 ^{a}	0.78 ^{a}	1.50 ^{b}
	Semi-arid	10	0.74 ^{a}	0.79 ^{b}	0.68 ^{a}	1.11 ^{a}	0.87 ^{b}	1.28 ^{a}
Marsaline	Sub-humid	9	0.80 ^{b}	0.46 ^{a}	0.64 ^{a}	1.56 ^{b}	0.69 ^{a}	2.26 ^{b}
	Semi-arid	10	0.66 ^{a}	0.69 ^{b}	0.70 ^{b}	1.13 ^{a}	0.76 ^{b}	1.49 ^{a}

Table 7. Variation percentage and correlated variables with principal components (PC) on stone size and shape of wild and cultivated plants

	PC 1	PC 2
Variation percentage	70.341	28.541
Variables		
Area	–0.282	0.012
Perimeter	–0.084	0.0004
Roundness	0.145	0.216
Circularity	0.181	0.112
Length	–0.056	–0.058
Width	–0.070	0.098
length/width	0.147	–0.095
Length²/perimeter²	0.186	–0.053

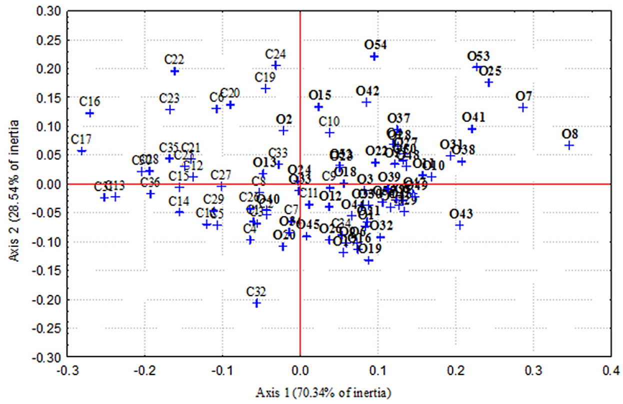
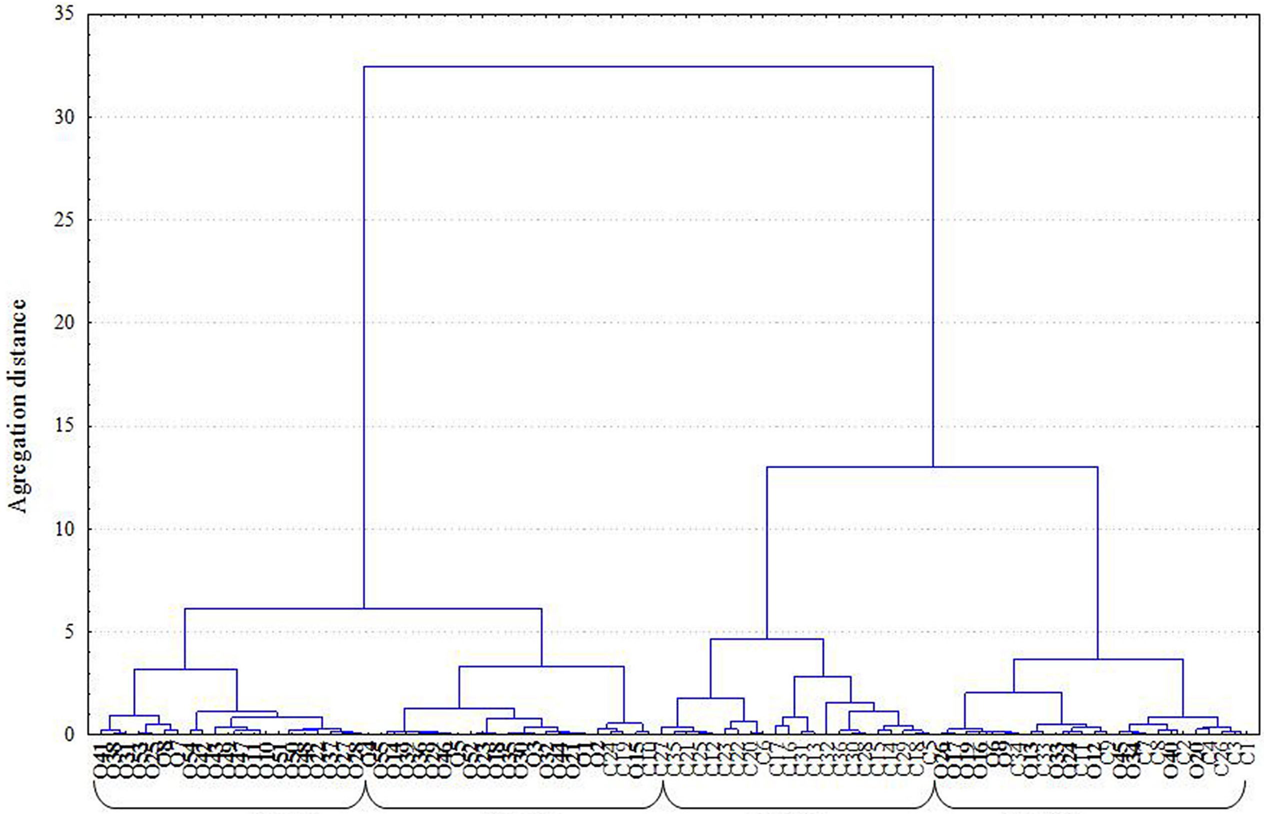


Fig. 4. Principal Component Analysis on stone size and shape of wild and cultivated olive trees, O: Oleaster (wild plants) and C: Cultivar (cultivated plants)



	CL1/1	CL1/2	CL2/1	CL/2/2
Area	0.286	0.414	0.867	0.536
Perimeter	2.219	2.739	4.086	3.206
Roundness	0.590	0.540	0.560	0.479
Circularity	0.724	0.690	0.651	0.651
length	0.833	1.043	1.480	1.265
Width	0.477	0.550	0.800	0.587
Length / Width	1.755	1.930	1.881	2.169
Length2 / Perimeter2	0.141	0.146	0.132	0.156

Fig. 5. Hierarchical analysis of wild and cultivated plants based on stone size and shape using the Euclidean distance and the Ward algorithm, O: Oleaster (wild plants) and C: Cultivar (cultivated plants)

and C10 (Chaïbi). Sub-cluster CL2/1 contains only cultivated plants. The CL2/2 sub-cluster comprises wild (14 oleasters) and cultivated plants (10 cultivars) sharing closely some stone characters, and thus confirming the PCA plot.

The oleaster stones in CL1/1 were characterized by smaller area (0.286 cm^2), perimeter (2.21 cm), length (0.83 cm) and width (0.47 cm) and were well separated from cultivars in CL2/1 characterized by higher area (0.867 cm^2), perimeter (4.08 cm), length (1.48 cm) and width (0.80 cm).

The range of variation is much larger for seed size than shape. Figure 6 presents the range of variation in stone size for seeds of wild and cultivated plants. Figure 7 shows the range of variation of shape in stones of wild and cultivated plants.

Discussion

Quantification of seed shape requires the comparison of the seed image with a geometric figure (Cervantes et al., 2016). The method can be applied manually or in a semi-automated way based on automated image analysis programs. ImageJ program (Ferreira & Wayne, 2010) allows to record automatically the values of circularity index and roundness in a high number of seed samples, thus being useful in the first approximation of seed shape quantification in a new species.

A semi-automated method for size and shape quantification in stone of *Olea europaea*, wild and cultivated, has been applied. As described before in the analysis of size and shape in *Ricinus communis* (Martín Gómez et al., 2016), the range of variation in size is much larger than for shape. Stones of the wild olive trees (oleaster) present a broad range of values and are smaller than the stones of the cultivated plants. Increased size in the cultivated varieties is expected due to: 1) Selection for higher size 2) Improved growth conditions in the cultivated varieties in comparison with the wild.

The PCA and cluster did not reveal a clear difference in shape between the two olive types wild and cultivated. Seed shape was not a target of selective pressures in during grapevine cultivation (Terral et al., 2010) and there is no shape difference between mature and immature stones (Terral et al., 2004).

The semi-automated method of image analysis gives higher values for circularity than for roundness but a large amount of variation is observed both for the wild and cultivated seeds with both magnitudes. Shape analysis may be done using different models for the wild and the cultivated stones, and it could be useful to apply particular geometric models to the groups obtained by cluster analysis.

Based on stone size and shape data segregate in two clusters that predominantly contain the wild and the cultivated olive trees (Figure 4, 5) in a similar result as revealed by molecular markers (Hannachi et al., 2010; Belaj et al., 2011). An intermediate group comprised some oleasters and cultivars with closely related stones traits (CL1/2 and CL2/2). The two olive cultivars Gerboui (C19 and C24) were closely related with oleaster trees in CL1/2 independently to their bioclimatic location. The sub-cluster CL2/2 contains 8 oleasters and 14 olive cultivars. A similar

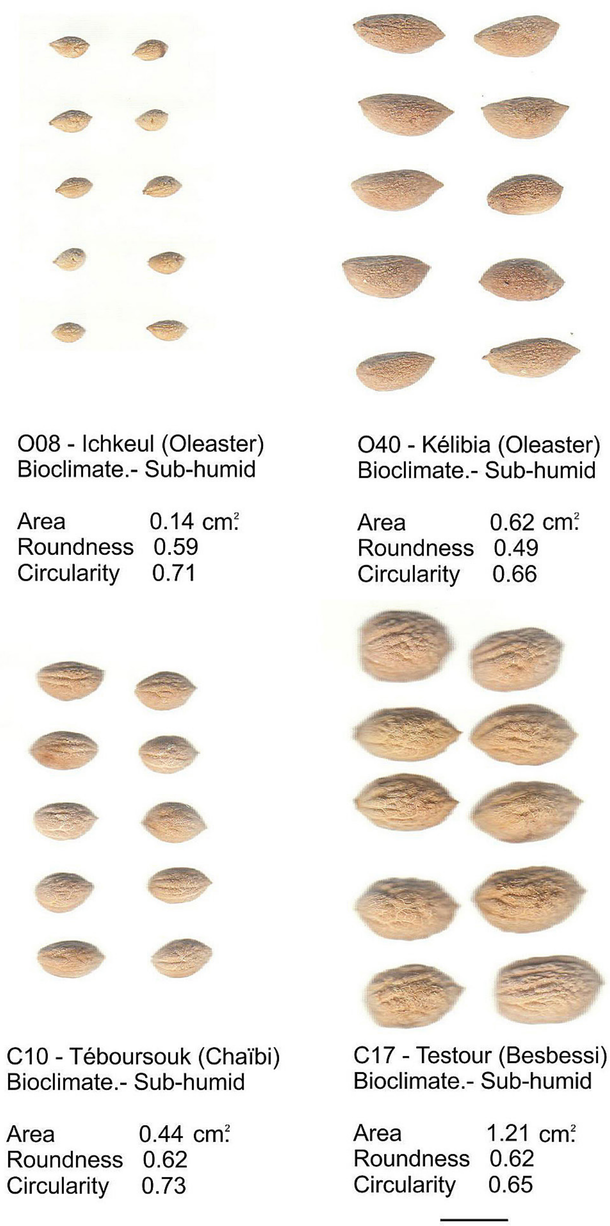


Fig. 6. Images of stones with minimum and maximum values of area

Top: Stones from wild populations O08 and O40. Seeds from O08 are the smaller and O40 larger among the wild populations.

Bottom: Stones from cultivated populations C10 and C17. Seeds from C10 are the smaller and C17 larger among the cultivated.

continuous variation between cultivated and wild olive trees has been noted in studies based on morphological trait of tree, leaf, fruit and stone (Hannachi et al., 2008a, b) or based on molecular markers applied on Tunisian (Hannachi et al., 2010) and Spanish wild and cultivated olive trees (Belaj et al., 2010).

This close relationship may be explained by: i) the intermediate cultivars would be the results of local domestication. As indicated based on assignation and admixture analyses few of Tunisian cultivars are issued from the Tunisian oleaster (Breton et al., 2006a; Hannachi et al., 2008a). It is documented that several independent domestication events occurred in different regions of the Mediterranean basin (Terral et al., 2004; Terral & Arnold-Simard, 1996); probably concomitantly with domestication in the Near East (Lanfranchi & Bui, 1995; Terral, 2000). Therefore, the olive cultivars may display diversity according to domestication origins (Besnard et al., 2001; Breton et al., 2006b). ii) The phylogeography of the oleaster and cultivars trees is due to gene flow. The gene flow has continued between cultivated and wild

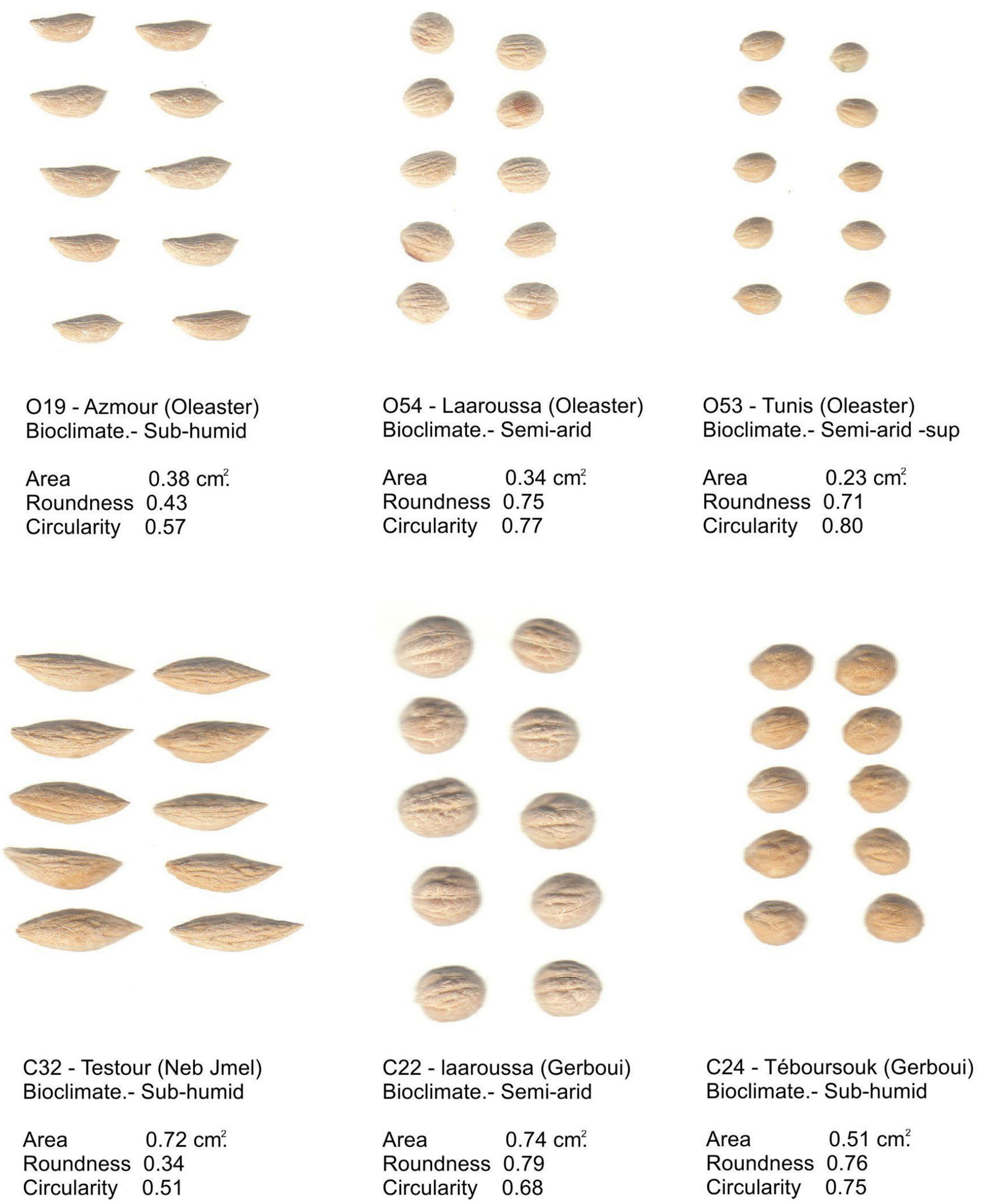


Fig. 7. Images of stones with minimum and maximum values of circularity and roundness

Top: Seeds from wild populations O19, O54 and O53. Seeds from O19have the lowest values of circularity and roundness; O54 and O53 the highest values of roundness and circularity respectively.

Bottom: Stones from cultivated populations C32, C22 and C24. Stones from C32 have the lowest values of circularity and roundness; C22 and C24the highest values of roundness and circularity respectively.

olives (Lumaret & Ouazzani, 2001) and is locally detected between introduced and local oleasters and, in this case, they do not carry the same cytoplasm within oleasters (Breton et al., 2005); iii) the feral form of olive (Besnard et al., 2001; Mekuria et al., 2002; Breton et al., 2006a) escaping to cultivation practices and therefore losing some characters of stone. Feral olive forms were placed in intermediate position based on morphological trait of tree, leaf, fruit and stone (Hannachi et al., 2010). Genetically, feral olives can be shown to be progeny of nearby cultivated olive trees (Mekuria et al., 2002). Some endocarp criteria such as perimeter and area with others were found to be useful for taxonomic analysis applied of *Prunus* (Depypere et al., 2007).

In the wild populations, an increase in size is observed in some trees grown in the arid region. This is unexpected and may be due either to particular micro-environmental climatic factors concerning these trees or it may be related to the fact that these stones have also reduced values of circularity and roundness. Thus, larger sizes may be associated/ or required with elongated seeds, and this later character may be useful in the arid conditions, for example helping to seed transport or survival under occasional flows or in response to water. Differences in size and shape were observed among localities in the four climatic regions.

In general there are climatic and geographic factors that determine changes in seed size and shape. For example a trend has been observed in seeds of the same species to decrease in size with latitude (Soper Gorden et al., 2016). However, it has been also noted the genetic primarily additive effect on seed size and shape; indeed a minimum of three or four genetic factors or blocks of genes appears to control seed heritability of seed size of *Sorghum vulgare* of the order of 60% (Voigt et al., 1966).

Also, seeds of plants grown in the desert have been reported to be smaller in some instances. For example, a relationship between seed size and soil persistence has been described such that persistent seeds are smaller (Peco et al., 2003). The accurate quantification of seed diversity is important from an ecological perspective. Seed size is embedded of attributes defining the life history of a plant species (Westoby, 1998). According to the geographical localities, the wild olive populations are found in a high diversity of environments with different altitudes and soils and were reported to contain more variability than the cultivated one (Belaj et al., 2010).

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