Study of morphological differences of wild olive(*Olea europaea* L., var. Sylvestris) in the Jabal Akhdar region in northern Libya

∗.Ezzudin S. Ali1 ∗.Mazik M. Khalid ∗.Abaidalah A. Saleh

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Abstract: This study was conducted on wild olives (*Olea europaea* L., var. sylvestris) with the aim of studying the morphological differences of wild types spread in the Jabal al-Akhdar region in northern Libya. The study area was divided into twelve regions separated by 10 km at different altitudes from sea level, and four trees were randomly selected from each region. The study's results were subjected to variance and standard deviation analysis, which showed phenotypic variation between the types present in the study area at all the studied characteristics in terms of Fruit volume, Fruit weight, Fruit length, Fruit diameter, Fruit shape, flesh weight, flesh /fruit miss, Pit volume, Pit weight, Pit length, Pit diameter, Pit shapes index, and Flesh pit mass ratio that shows the presence of genetic diversity between the wild olive types spread in the study area. This allows these types to be used as a source of germplasm that is used to increase the desired characteristics such as fruit size, oil content, production, and resistance to environmental and biological conditions to improve and breed olives.

Key words: Olea europaea, morphological, genetic diversity

دراسة الاختلافات المورفولوجية للزيتون البري *Olea europaea* L., var. sylvestris في منطقة الجبل الأخضر شمال ليبيا

عز الدين شعيب علي، أستاذ، قسم البستنة، كلية الزراعة، جامعة عمر المختار، البيضاء، ليبيا

خالد مسعود مازق محاضر، قسم البستنة، كلية الزراعة جامعة عمر المختار، البيضاء، ليبيا

علي صالح عبيدالله، محاضر، قسم البستنة، كلية الزراعة جامعة عمر المختار، البيضاء، ليبيا

المستخلص: أجريت هذه الدراسة على الزيتون البري (*Olea europaea* L., var. sylvestris) بهدف دراسة الاختلافات المورفولوجية للأنواع البرية المنتشرة في منطقة الجبل الأخضر شمال ليبيا. تم تقسيم منطقة الدراسة إلى اثني عشر منطقة تفصل بينها 10 كيلومترات مربعة على ارتفاعات مختلفة عن سطح البحر، وتم اختيار أربع أشجار عشوائياً من كل منطقة. وخضعت نتائج الدراسة لتحليل التباين والانحراف المعياري، والتي أظهرت وجود التباين المظهري بين الأصناف الموجودة في منطقة الدراسة في جميع الصفات المدروسة من حيث حجم الثمرة، وزن الثمرة، طول الثمرة، قطر الثمرة، شكل الثمرة، وزن اللحم، نسبة اللحم/الثمرة، حجم البذرة، وزن البذرة، طول البذرة، قطر البذرة ومؤشر شكل البذرة ونسبة البذرة / الثمرة. مما يدل على وجود تنوع وراثي بين أصناف الزيتون البري المنتشرة في منطقة الدراسة مما يسمح باستخدام هذه الطرز كمصدر للتنوع الجيني التي تستخدم في زيادة الخصائص المرغوبة مثل حجم الثمرة ومحتوى الزيت والإنتاج ومقاومتها للظروف البيئية والبيولوجية لغرض تحسين وتربية الزيتون.

الكلمات الدالة: الزيتون البري، الاختلافات المورفولوجية، للتنوع الجيني

Introduction:

The genus *Olea* (*Oleaceae*) is considered to have about 40 species, subspecies and varieties and is distributed in Africa, Europe, Asia and Oceania (Green 2002). The genus *Olea*

∗ **Department of Horticulture, Faculty Agriculture, Omar Al-Mukhtar University, Al -Bayda, Libya**

∗ **Department of Horticulture, Faculty Agriculture, Omar Al-Mukhtar University, Al -Bayda, Libya nagebmazig@gmail.com**

∗ **Department of Horticulture, Faculty Agriculture, Omar Al-Mukhtar University, Al -Bayda, Libya a.saleh74@yahoo.com**

has been divided into 3subgenera including *Olea europaea* subsp. *europaea* which is commonly known as olive. The olive plant (*Olea europaea* L.) is widely distributed in the Mediterranean region occurring in both natural ecosystems, var. *sylvestris* Mill., and agricultural landscapes ,var. *europaea* (Green 2002; Green and Wickens, 1989). Both wild olive (*Olea europaea L., var. sylvestris*) and its cultivated relatives (*Olea europaea L., var. europaea*) are diploid (2n = 2x = 46), predominantly allogamous and distributed along the Mediterranean basin (Green, 2002; Ali, 2008a; Ali, 2008b). It is suggested that the cultivated form of olive originated from wild olive (Zohary 1994). Mediterranean wild olive (*oleaster*) contains smaller fruit size and lower oil content compared to that of the cultivated olive.

Wild olive trees (also named oleaster) shrubs with multi-stems that are evergreen, drought-tolerant, and very adaptable to different environments (Médail *et a*l., 2001; Green, 2002; Kassa *et al*., 2019). The wild olive grows in various altitudes and soil types, including those with severe water deficits, salinity, and low temperatures. (Cantos *et al*., 2002; Baldoni *et al*., 2006; Klepo *et al*., 2013; Belaj *et al*., 2016; Chiappetta *et al*., 2017). .(Cantos *et al*., 2002; Baldoni *et al*., 2006; Klepo *et al*., 2013; Belaj *et al*., 2016; Chiappetta *et al*., 2017). Adaptability to adverse environmental conditions makes wild olive trees suitable for growing in marginal soil, colonizing deforested habitats, or restoring devastated areas (Bekele, 2005; Kassa *et al*., 2019). Wild olives (*oleasteers*) reproduce sexually by wind pollination and their seeds are mainly dispersed by birds (Herrera, 1995). They are important components of the Mediterranean scrublands (Zohary and Hopf, 2000). It is generally accepted that the cultivated olive (*Olea europaea subsp. europaea var. europaea*) originated from the wild Mediterranean olive, oleaster, (*Olea europaea subsp. europaea var. sylvestris*) by artificial selection from wild populations (Zohary and Spiegel-Roy, 1975). Moreover, it was shown through genetic analysis that the wild types descended mainly from the wild Mediterranean olive (Besnard *et al*., 2007). At present another analysis of 1,263 samples from 108 locations shows that the north Levant (i.e., the area close to the Syrian/Turkish border) was the center of the domestication of olive (Besnard *et al*., 2013). Olive domestication from wild oleaster populations has involved selecting from small numbers of acceptable genotypes with eligible characteristics such as the size of fruits or quantity of oil. Those genotypes were spread through vegetative propagation especially cutting. The practices of selection and vegetative propagation lead to the reduction of the genetic diversity of cultivated olives, whence, Loss or decrease of the gene pool of olives. (Rugini *et al.,* 2011), however, during the domestication process, there was hybridization with local olives (Besnard *et al*., 2007, 2013; Chiappetta *et a*l., 2017; Kassa *et al*., 2019). So the wild types of olives are considered the main sources of desirable characteristics for the improvement of resistance to abiotic stresses in olives such as Lack of water in the soil or low moisture content,(Cantos *et al*., 2002), and the quantity and quality of the crop (Hannachi *et al*., 2008; Baccouri *et al*., 2011; León *et al*., 2018), as well as resistance to tolerance to diseases .(Colella *et al*., 2008; Arias-Calderon *et al*., 2015; Trapero *et al*., 2015; Jimenez- Fernandez *et al*., 2016). Both wild olives and cultivated olives are found commonly in Libya. Wild olives or oleasters (*Olea europaea* subsp. *europaea* var. *sylvestris*) are found naturally in Al Jabal Al Akhdar region while cultivated olives (*Olea europaea* subsp. *europaea* var. *europaea*) are found along Mediterranean shores of Libya (Ali, 2008 a; b). Wild types of olives have a reputation for being resistant to hard conditions compared with cultivated olives. The nature of growth is different between cultivated and wild olives. The wild types are characterized by bush form whereas the cultivated olives grow generally in a tree form. Determinations of the genetic variation of olives are the main key to understanding genetic improvement. Therefore, studies of genetic similarities and differences in wild types are important for identifying genetic variability. The purpose of present research was to consider the morphometric study of the wild olives of Libya, with the aim to reveal morphological and genetic diversity available in Libyan wild olives.

Material and methods:

The present study was carried out during October and November, 2022 in the Laboratory of Horticulture Department, Faculty of Agriculture, Omar Al-Mukhtar University (OAU), Libya. Twelve accessions (populations) of wild olive, Oleaster, (*Olea europaea* L., var. sylvestris) were collected from different sites of El-Jabal El-Akhdar region. The Mean annual rainfall of the studied area varies between 600 and 250mm, during the Autumn-Winter period. The mean minimum and maximum temperature are 59oF (January) and 83 Fo (August), respectively (SWECO, 1986 a; b).

Plant material:

The investigated physical properties for fruits and pits of different wild olive accessions included fruit volume, fruit weight, fruit dimensions (length and diameter) , fruit shape index, flesh/fruit mass ratio, pit volume, pit weight, pit dimensions (length and diameter) and flesh/pit mass ratio.

Sample of 10 fruits from each tree for each accession were randomly collected (4 trees per accession) from different locations in El-Jabal El-Akhdar area, Libya (Table 1). Fruit and pit morphological properties were determined as following:

Volumes of fruits and their pits were determined from the volume of displaced water, after that the volume averages of fruits and pits were calculated.

Fruit and pit weights were determined by weighting the sample, and then the weighting average of fruit and its pit were calculated.

The dimensions of olive fruit and its pit (length and diameter) were measured by a digital vernier caliper with an accuracy of 0.01 mm, and then the averages were calculated.

Fruit Shape index of the measured samples were calculated according to the typical equation (Buyanor and Voronyuk, 1985) as follows:

FSI = Lf / Df

Where:

FSI = fruit shape index

Lf = length of fruit, mm

Df = diameter of fruit at the middle of its length, mm

Flesh/fruit mass and flesh/pit mass ratio were calculated for the individual fruit and its pit using the weight of fruit and its pit as follows:

Flesh/fruit mass ratio = (Wfr – Wpit)/Wfr

Flesh/pit mass ratio = (Wfr – Wpit)/Wpit

Where:

Wfr = the mass of single fruit, g

Wpit = the mass of pit for the same fruit, g.

Data analysis:

The values of all various morphological parameters were analyzed by Minitab software (version 17) to calculate averages and standard deviations, which facilitated the comparison of olive accessions together along each character. Statistics for each morphological trait is the mean of 40 drupes or pits which were collected from each accession.

Table (1). Accessions and sites of wild olive growing in El-Jabal El-Akhdar, Libya.

Accession	Region	Latitude	Longitude	Altitude (m)
P1	Al-Harera الحريرة	32°54'19.92''N	21°45'13.86''E	230
P2	Sambar صمبر	32°50'33.97''N	21°53'1.00''E	345
P3	Senyara سنيارة	32°52'34.37''N	21°55'9.47''E	287
P4	Ras Al-Helal راس الهلال	32°53'57.57''N	22°10'10. 3''E	12
P5	Blekhna بلخنة	32°48'1.49''N	21°57'45.07''E	650

P6	Boshmaro بوشمارو	32°43'14.37''N	21°49'56.66''E	660
P7	Satlona الستلونة	32°42'3.69''N	21°45'58.87''E	681
P8	Wadi El-Kuf وادي الكوف	32°42'25.91''N	21°34'17.83''E	453
P9	Salion سليون	32°46'28.29''N	21°35'47.94''E	335
P10	Wseta وسيطة	32°47'59.89''N	21°37'40.84''E	313
P11	Al-Hamam الحمامة	32°53'40.79''N	21°37'48.19''E	165
P12	Werdama وردامة	32°48'54.23''N	21°46'53.08''E	424

Results:

The results of physical properties for fruit and its pit of Libyan wild olive (*Olea europaea* L., var. *sylvestris*) accessions growing in El-Jabel El-Akhdar region are shown in Table (2 and 3). The analysis of variance revealed highly significant differences among all accessions for all the examined physical properties which including fruit volume, fruit weight, fruit dimensions, fruit shape index, flesh/fruit mass ratio, pit volume, pit weight, pit dimensions and flesh/pit mass ratio.

The fruit volume of Libyan wild olive is variable from one accession to another. P4 (Ras Al-Helal accession) recorded the highest value (11.70±2.38mm^3) while the lowest value (6.23±1.52mm^3) was recorded in P11 (Al-Hamama accession). For fruit weight, Table (2) shows that P4 (Blekhna accession) recorded the highest values (1.18±0.33g) while P10 (Westa accession) showed the lowest value (0.62±0.18g).

Table (2): Mean values of physical properties of Fruit Libyan wild olive

Accession	Fruit volume cm^3	Fruit weightg	Fruit length mm	Fruit diameter mm	uit shape index	Flesh weightg	Flesh/fruit mass ratio
P1	9.00±2.15	1.02±0.23	15.90±2.69	6.49±0.58	2.47±0.51	0.67±0.17	0.64±0.04
P2	7.50±1.02	0.75±0.21	12.73±1.78	8.71±1.05	1.46±0.17	0.43±0.16	0.57±0.09
P3	9.63±0.77	1.09±0.28	14.69±2.88	10.28±0.98	1.43±0.28	0.81±0.25	0.73±0.07
P4	11.70±2.38	1.18±0.33	14.81±1.49	10.92±1.40	1.36±0.11	0.88±0.28	0.74±0.05
P5	7.00±2.31	0.71±0.21	11.96±2.07	8.98±0.77	1.32±0.14	0.48±0.15	0.67±0.07
P6	7.25±2.62	0.73±0.27	12.11±2.97	9.12±1.08	1.32±0.26	0.49±0.17	0.66±0.04
P7	8.95±1.89	0.85±0.18	14.09±1.15	9.32±1.12	1.52±0.17	0.58±0.17	0.67±0.07
P8	8.00±3.39	0.86±0.54	12.59±2.48	9.18±2.44	1.42±0.28	0.60±0.39	0.69±0.07
P9	7.73±0.76	0.83±0.20	12.75±1.29	10.11±1.41	1.27±0.15	0.54±0.18	0.64±0.09
P10	8.75±4.30	0.62±0.18	12.75±1.99	8.27±0.80	1.54±0.18	0.41±0.14	0.65±0.06
P11	6.23±1.52	0.88±0.40	14.00±3.40	9.63±1.32	1.43±0.18	0.57±0.27	0.65±0.04
P12	10.50±3.31	1.10±0.33	14.23±1.75	10.76±1.54	1.34±0.20	0.75±0.28	0.67±0.06
LSD	0.03	0.12	1.12	0.67	0.13	0.10	0.04

Table (2) showed significant variations in the fruit dimensions for the 12 different studied accessions of Libyan wild olive. As shown in the table (2), the fruit length was ranged from 11.96±2.07mm for P5 (Satlona accession) to 15.90±2.69mm for P1 (Al-Harera accession). On other hand the fruit diameter was ranged from 6.49±0.58mm for P1 (Al-Harera accession) to 10.92±1.40mm for P4 (Ras Al-Helal accession). Moreover, The obtained values for fruit shape could be sorted gradually from the maximum value for P1 (2.47±0.51) to the minimum value for P9 (1.27±0.15). Table (2) shows that P3 (Senyara accession) and P4 (Ras Al-Helal accession) have the largest value for flesh / fruit mass ratio (0.74±0.05) while P2 (Sambar accession) has the smallest value (0.57±0.09).

According to Table (3), P1 (Al-Harera accession) recorded the biggest volume (3.00mm^3) and heaviest weight pit (0.35g+0.07). On other hand P5 (Blekhna accession) recorded the smallest volume (1.90$mm^{3+- 0.73}$) and P10 (Wseta accession) recorded the lowest weight (0.20g).

Table(3): Mean values of physical properties of Pit ,seed Libyan wild olive

Accession	Pit volume cm^3	Pit weightg	Pit length mm	Pit diameter mm	Pit shape index	Flesh/pit mass ratio
P1	3.00±0.17	0.35±0.07	14.63±2.7	5.90±0.63	2.51±0.56	1.86±0.31
P2	2.75±0.28	0.32±0.08	11.42±1.5	6.49±0.60	1.77±0.30	1.35±0.36
P3	2.68±0.51	0.29±0.08	11.40±1.1	6.15±0.71	1.86±0.18	2.96±1.00
P4	2.75±0.95	0.28±0.07	11.67±1.2	6.19±0.62	1.89±0.21	3.13±0.82
P5	1.90±0.73	0.22±0.08	9.91±2.0	5.86±0.57	1.68±0.25	2.26±0.66
P6	2.23±0.51	0.24±0.10	10.10±2.0	6.02±0.61	1.66±0.20	2.12±0.48
P7	2.50±0.58	0.26±0.04	11.84±1.2	5.78±0.41	2.05±0.21	2.26±0.62
P8	2.28±1.42	0.25±0.05	10.36±2.2	5.83±1.12	1.78±0.29	2.39±0.74
P9	2.38±0.70	0.28±0.04	10.69±1.2	6.56±0.45	1.63±0.16	2.02±0.95
P10	2.22±0.43	0.20±0.04	11.37±1.8	5.42±0.45	2.11±0.43	2.02±0.51
P11	2.70±1.00	0.30±0.13	11.75±3.1	6.40±0.69	1.81±0.33	1.88±0.29
P12	2.80±0.84	0.35±0.09	12.10±2.2	6.69±0.50	1.81±0.32	2.16±0.73
LSD	0.01	0.03	0.80	0.30	0.14	0.37

Analysis of the morphological data pertaining to the twelve wild olive accessions showed significant variations for pit dimensions (Table 3). Longest pit-length was recorded by P1 (14.63±2.7mm) but the maximum pit-diameter was seen in P12 (6.69±0.50mm). Meanwhile, shortest pit-length was recorded by P5 (9.91±2.0mm) and smallest pit-diameter was recorded by P10 (5.42±0.45mm). Pit length/diameter ratio (pit shape) was significantly higher in P1 (2.51±0.56) and lower in P9 (1.63±0.16), indicating a higher level of variance in the pit shape.

As shown in Table (3), there were significant variations in the flesh/ pit mass ratio ranging from 3.13±0.82 (P4) to 1.35±0.36 (P2). Furthermore, P4 (Ras Al-Helal accession) has the largest value for flesh weight (0.88±0.28) while P10 (Wseta accession) has the smallest value (0.41±0.14).

Discussion:

The difference in one or a few traits of the organism is referred to as variability or diversity. Because of that variability (diversity) can be described as the degree of the differentiation (variation) between or within species. In common parlance, variability and diversity are considered synonym to each other. Variability in natural plant populations is due to environmental conditions or genetic factors. Genetic variability which due to genetic factors is the core of the evolutionary process in the biology. In other words, evolution of natural plants is primarily based on existing genetic variability in the population. Genetic variability is the variation in alleles of genes or variation in DNA sequences in the gene pool of a species or population (Bhandari, 2017). The present study indicated that the choice of physical properties of fruits and pits to assess Libyan wild olive diversity is a useful and powerful tool. Several authors had resorted to the use of the physical properties of fruits and pits to identify different olive populations, collections, or germplasm (Ali, 1999; Ali et. al.,). Moreover, the physical properties of fruits and pits are widely used as quantitative markers to identify populations of wild olives. The present study indicated that differences between the physical properties of fruits and pits were significant for all wild Libyan olive accessions (Tables 2 and 3). The means and standard deviations of the physical properties of fruits and pits measured in Libyan wild olive showed highly significant differences among the studied accessions for all the examined characters. In agreement with our results, many authors have confirmed that olive accessions showed a high diversity in physical properties of fruits and pits (Fouad *et. al*.; 1992; Blahovec *et.al*.; 1994; Matouk *et. al.* ; 2005; Ozturk *et. al*., 2009).

The cultivar, environmental conditions, soil, and fertilizer are considered the main factors that impact the biological characteristics of olive fruit (Youssef *et al*., 2010; Hbaieb *et al*., 2017). The study by Bartolini *et al* found that light plays a substantial role in fruit growth and they concluded that shaded fruits developed at a slow rate compared with others (Bartolini *et al*., 2014). Moreover, the chemical composition of oil olive has the most attention in most recent studies on the other hand fruit quality has little attention. The cultivar and genotype were the main factors influencing the weight and shape of olive fruit meanwhile environmental factors such as soil, fertilization, and geographical location were the main influences on the quality of olive fruit (Youssef *et al*., 2010; Hbaieb *et al*., 2017). Genetic variability is the base for the survival of plants in nature and for plant improvement. Diversity in plant genetic resources provides an opportunity for plant breeders to develop new and improved cultivars with desirable characteristics. (Bhandari,*et al*,2017, Salgotraand Chauhan, 2023).

Sexual recombination is the core function of genetic variability. During meiosis, homologous chromosomes undergo crossing over which results in new recombination. Different factors affect the genetic variability in plants. Evolutionary forces like selection, mutation, migration, and genetic drift act continuously and result in continuous changes in allelic frequency in a population and affect genetic variability.(De Storme and Mason, 2014: Salgotraand Chauhan, 2023). Domestication is primarily artificial selection which favours few alleles at the cost of others resulting in increased frequency of selected alleles. Consequently, domestication reduces the genetic variability when compared to the variability in wild populations. (Gregory, 2009, Salgotraand Chauhan, 2023). In addition to that, natural selection also affects genetic diversity considerably. Directional and stabilizing selection decreases while disruptive selection increases genetic diversity. The mutation is also reported to increase genetic diversity (Gregory, 2009). Moreover, genetic diversity is the main for evolutionary change in all organisms. It is generally acknowledged that populations with less variation of genes are unable to adapt to new environmental conditions (e.g., climate change) and therefore may increase risk of extinction of these species (Chung, *et al*; 2023).

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