

Morphological, Agronomical and Genetic Characterization of Egyptian Olive Clones Compared with the International Cultivars

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EGYPTIAN olive clones 'Sewia', 'Maraki' and 'E52' were compared with two international cultivars 'Coratina' and 'Koroneiki' during two seasons (2011 and 2012) at olive collection farm located at Cairo- Alexandria desert Road . Results showed that, time of flowering varied according to cultivars and season. 'Coratina', 'Maraki' and 'Sewia' were earlier than 'Koroneiki', but it was late for E52. 'Coratina' had longer inflorescence (>3.5 cm) than the other cultivars which were medium (2.5-3.5 cm). The highest number of flowers per inflorescence (> 25) was recorded for 'Maraki' and the lowest number (<18) was recorded for 'Koroneiki'. Perfect flower percentage varied according to cultivars and to climate. It was the highest in 'Coratina' (>90%) and the lowest in 'Sewia' (75%). Fertility of pollen was highest in 'Sewia' and the lowest in 'E52'. All of the studied cultivars were self-incompatible. Under open pollination 'Coratina' produced the highest yield in the two seasons; 'E52' was alternate bearing cultivar. Fruit and stone weight were the lowest in 'Koroneiki', medium in 'E52', high in 'Coratina' and 'Maraki', and very high in 'Sewia'. Fruit shape was elongated in 'Koroneiki', 'ovoid-elongated' in 'E52' and 'Coratina', and ovoid in 'Sewia'. Stone shape was elongated in 'Koroneiki', 'E52' and 'Coratina'; ovoid in 'Maraki' and 'elliptic' in 'Sewia'. Oil content (%) was the highest in 'Maraki' and 'E52', followed by 'Coratina', 'Sewia' then 'Koroneiki' in decreasing order. Based on the quality parameters of the oil (acid value, peroxide value, UV absorption at 232 and 270 nm, polyphenols and tocopherol) provided that it ranked extra virgin. The ratio of total unsaturated to saturated fatty acids was the highest in 'Maraki', followed by 'Sewia' then 'Coratina', but the lowest ratio was found in 'E52' and 'Koroneiki'. Based on RAPD and ISSR-PCR genetic markers, the genetic similarity was between 'Sewia' and 'Maraki' and the least between 'Coratina' and 'Koroneiki'.

Keywords: Olive, Characterization, Sewia, 'Maraki', E52, Coratina, Koroneiki.

Olive cultivation is associated with several countries of the Mediterranean Sea basin and plays an important role in the diets, economies and cultures of the region (Zamora *et al.*, 2001). Over the last three decades the Egyptian olive agro sub-sector has seen unprecedented development, the total average reached 202.743 feddan in 2012 according to the statistics of the Ministry of Agriculture.

The olive tree has been cultivated for thousands of years and includes many varieties and strains, creating problems in their classification. Identification of olive cultivars is mainly performed through the analysis of bio morphological traits. The morphological characteristics used to distinguish olive cultivars carried out by Barranco and Trujillo (2000), based on those described by International Olive Council (RESGEN-CT96/97). It includes inflorescence dimension, number of flowers, fruit weight, shape, symmetry and color; endocarp weight, shape and symmetry. Several studies were carried out for identification of olive varieties grown in Egypt (Laz & Abd El-Razik, 2005, El-Said *et al.*, 2006, Hegazi, 2012 and Sayed, 2013).

The biological behavior of olive cultivars against self-and cross compatability is a vexed question in the scientific literature. However, the effects of climatic conditions on self- and cross-pollination and so the different degree of self- and cross-incompatibility hypothesized by some authors could be checked (Camposeo *et al.*, 2012).

Virgin olive oils are the oils obtained from the fruit of the olive tree solely by mechanical or other physical means under conditions, particularly thermal conditions, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration. The International Olive Council (2013) has defined the quality of olive oil based on parameters that include free acidity, peroxide value (PV), UV absorption (K232nm, K270nm) and sensory analysis. Fatty acids composition which is a purity parameter for olive oil is strongly affected by environmental conditions (Mousa *et al.*, 1996). It is worth to mention that Arbequina cv. under Egypt conditions showed low oleic acid content (44.00%) so it considered out of the limit (55-83%) establishment by the International Olive Council (Benincasa *et al.*, 2011).

In the present study, we report on flowering, fruit characters and olive oil chemical parameters of three distinct Egyptian olive cultivars (Maraki, Sewia (place of origin Siwa Oasis) and E52 which produced from a selection program of the Horticulture Research Institute) compared with Coratina (Italian) and Koroneiki (Greek) olive cultivars suitable to Egypt environmental conditions. Also, genetic diversity of these cultivars was carried out using RAPD and ISSR-PCR molecular based markers.

Material and Methods

The present investigation was conducted throughout two growing seasons (2011 and 2012) to evaluate three Egyptian olive cultivars Maraki, Sewia and E52 in comparison with two olive cvs. Coratina and Koroneiki. Six uniform trees of each cultivar were chosen for this study.

The selected olive trees were about 6 year-old, propagated by leafy cutting growing in the olive collection farm located at Cairo- Alexandria desert road

(about 64- kilometers far from Cairo). Trees were planted at 4×5m apart in sandy loam soil, under drip irrigation system, received regularly the recommended cultural practices and they were free from pathogens and physiological disorders.

Soil chemical and physical characteristics and water chemical characteristics were determined at the laboratory of the Soil, Water and Environmental Res. Inst. according to the methods described by Jakson (1973) and the results are summarize in Tables (1, 2, and 3).

TABLE 1. Chemical characteristics of the tested soil sample collected from the experimental area.

PH 1:2.5	E.C. ds/M (1:5)	Soluble cations (meq/ 100g soil)				Soluble anions (meq/ 100g soil)			
		Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻
8	10.5	7.4	4.6	5.00	0.36	-	0.6	7	9.03

TABLE 2. Physical characteristics of the tested soil sample collected from the experimental area.

Coarse sand %	Fine sand %	Silt %	Clay %	Texture class
21.16	50.4	13.2	14.8	Sandy loam

TABLE 3. Chemical characteristics of irrigation water used in the experiment.

PH 2.5:1	E.C. ds/M (1:5)	E.C ppm	Soluble cations (meq/L)				Soluble anions (meq/ L)				S.A.R
			Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	Co ₃ ⁻	Hco ₃ ⁻	Cl ⁻	So ₄ ⁻	
7.68	4.4	2816	10	8	27.1	0.34	-	2.4	32.5	10.65	9.03

The following characters were recorded according to the methodology for primary characterization of olive varieties, according Brancoo *et al.* (2000) and Cimato and Attilio (2008).

Flowering and fruit set

Date of Inflorescence emergence was recorded as soon as the first sign of inflorescence parts were noticed. Also dates of beginning and full bloom were recorded when 10% of total flowers were opened and 80% of total flowers were opened respectively. The end of blooming was recorded at the date in which all flowers were completely opened. Flowering periods for each cultivar was calculated by the days between beginning of flowering and end of blooming.

Sample of twenty inflorescences at balloon stage (the stage in which blooms are completely swollen, white and near to open) from each tree were randomly taken from the middle portion of the shoots to measure the following inflorescence characteristics:

- Length of inflorescence (cm): short <2.5, medium 2.5-3.5, long >3.5.
- Number of flowers per inflorescence: low <18 , medium 18-25, high >25
- Perfect flower percentage was determined according to Rallo and Fernández-Escobar (1985).

$$\text{Perfect flower percentage} = \frac{\text{No. of perfect flowers}}{\text{No. of total flowers}} \times 100$$

- Flowering density: Twenty shoots per each tree were used to determine average shoot length, number of inflorescence and the average number of inflorescences per one meter was calculated according to Mofeed (2009).

$$\text{Flowering density} = \text{No. of inflorescence} \times \frac{100}{\text{shoot length (cm)}}$$

- Pollen germination: Olive flowers collected at the balloon stage and left overnight under room temperature until anther dehiscence. Pollen germination were estimated by the hanging drop test in liquid media contains 10% sucrose and .01% H₃BO₃ (Fernandez-Escobar *et al.*, 1983). Germination percentage was determined using five fields of view under light microscope.
- Fruit set and index of self –incompatibility: Before flowers would open at balloon stage , ten flowering shoots were selected and labeled on each side of replicated trees. Average number of flowers per inflorescence was calculated from counting number of total flowers per inflorescence on each of the ten selected flowering shoots on each studied cultivars. Inflorescences of half previous flowering shoots were covered by paper bags as self-pollination. While the rest labeled flowering shoots were left without covering as an open pollination. In self-pollination and open-pollination percentage of final fruit set was determined at 60 days after full bloom on each of labeled shoots. Number of fruits was recorded on each of the selected shoots; the percentage of fruit-set was transformed to angle values before statistical analysis.

Fruit set % : Fruit set were calculated after 60 days from full bloom

$$\text{Fruit set \%} = \frac{\text{No. of fruits}}{\text{No. of total flowers}} \times 100$$

Self-incompatibility index: The degree of self-incompatibility (SI-Index) of the studied cultivars was calculated as the ratio between the number of fruits in self and open pollination, using the following formula (Moutier, 2002).

$$\text{SI - index} = \frac{\text{Number of fruit per cluster in case of self pollination}}{\text{Number of fruit per cluster in case of open pollination}}$$

The percentages of obtained SI-index are divided into the following categories:

0.3-1 = self –compatible

0.15-0.3 = partially self –incompatible

0- 0.15 =high self –incompatible

0 = completely self incompatible

Low value close to zero would be an indication of self-incompatibility (Androulakis and Loupassaki, 1990).

Yield (Kg/tree)

Fruit of each experimented tree was harvested during ripe stage (olive with superficial pigmentation on more than 50% of the skin) and the average yield was calculated.

Fruit characteristics

Fruit quantitative characters: 40 fruits from each studied tree at ripe stage were randomly collected to determine

- Fruit weight: The following categories have been established (Del Río & Caballero, 1994 and Barranco *et al.*, 2000) low < 2g medium (2-4g), high 4-6g, very high > 6g
- Fruit length and width
- Fresh weight of the stone: Low (<0.3g), Medium (0.3-0.45g) High (0.45-g). Very high (> 0.7 g)
- Stone length and width

Fruit qualitative characters

Qualitative Fruit characters: Based on observation of the same samples which chosen for the quantitative fruit characters were measured for qualitative characteristics, some characters refer to two positions. "Position A" is the position in which the fruit generally displays the greatest asymmetry when held by either end between the index finger and thumb. "Position B" is reached by turning 90° from position "A" in such a way as to present the most developed part to the observer.

- Fruit shape at Position A: Spherical "L/W < 1.25", Ovoid "L/W 1.25- 1.45" and Elongated "L/W > 1.45".
- Symmetry (in Position A): it was divided into three categories symmetric, slightly asymmetric and asymmetric.
- Apex shape : it was divided into two categories pointed and rounded.
- Base shape: it was divided into two categories truncate and rounded.
- Position of maximum transverse diameter "Position B": it was divided into two categories central and towards apex.
- Presence of lenticels: few or many.
- Size of lenticels: small or large.
- Position of starting color change: From the base uniformly, across the whole epidermis, from the apex.
- Stone shape at Position A: Spherical "L/W <1.4", Ovoid "L/W 1.4 - 1.8"; Elliptic "L/W 1.8- 2.2" and Elongated "L/W > 2.2".
- symmetry at Position A: it was divided into three categories symmetric, slightly asymmetric and asymmetric .
- Apex shape of stone : it was divided into two categories pointed and rounded
- Base shape of stone : it was divided into three categories truncate ,pointed and rounded.
- Position of maximum transverse diameter "Position B": central ,towards apex and towards base.

- Surface at position B :smooth ,rugose and scabrous.
- Number of grooves: Low < 7, Medium 7-10, High> 10).
- Termination of the apex at position A.: with mucro without mucro.

Oil characteristics

The percentage of oil based on fruit dry weight is the parameter used for comparing cultivar collection and virtual trials because it is not dependent on fruit moisture content (Del Rio & Caballero, 1994 and Tous & Romero, 1994). The fruit dry weight oil percentage was measured in ripe fruit by Soxhlet extraction apparatus as described in the A.O.A.C (2000). Also, olive oil was extracted from ripe fruit by mechanical method under conditions that do not lead to alteration in oil as adopted in International Olive Council (2013) to determine physiochemical quality parameters. Acid value % (as oleic acid), peroxide value (meq.O₂/kg oil) were determined according to the analytical methods described in A.O.A.C (2000). State of oxidation of the oil was determined spectrophotometer at maximum absorption K232nm and K270nm (I.O.C., 2013). Total tocopherol as a measurement of nutritional value the olive oil was determined according to the method of Wong *et al.* (1988). Total polyphenols which are responsible for stability of olive oil against oxidation were measured colorimetrically at 725nm after the Folin-Ciocalteu reagent to the extract (A.O.A.C, 2000). Fatty acid composition was determined in methyle esters of fatty acids of olive oil using gas chromatography with GC-Capillary column according to the method reported by International Olive Council (2013).

Molecular Characterization

Young and fresh leave samples were collected separately from all studied olive cultivars. Plant tissues were ground using liquid nitrogen to a fine powder, then bulked DNA extraction was performed using DNeasy plant Mini Kit (QIAGEN).

Randomly amplified polymorphic DNA (RAPD)

In this study, RAPD was used for the identification of the five olive cultivars according to Lu *et al.* (1996). PCR reactions were conducted using 28 arbitrary 7-mer primers. Their names and sequences are shown in Table (4). PCR was performed in 30-µl volume tubes according to Williams *et al.* (1990)

The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94° C for 4 min followed by 45 cycles of 1 min at 94° C, 1 min at 36° C, and 2 min at 72° C, then the reaction was finally stored at 72° C for 10 min. Amplified products were size-fractionated (using 1 kbp ladder marker) by electrophoresis in 1.5% agarose gels in TBE buffer at 120 V for 1 hr. The bands were visualized by ethidium bromide under UV florescence and photographed.

TABLE 4. List of the primers names and their nucleotide sequences used in the study for RAPD procedure.

NO	Name	Sequence	NO	Name	Sequence
1	OP-A01	5'-CAG GCC CTT C-3'	5	OP-B01	5'- GTT TCG CTC C-3'
2	OP-A02	5'-TGC CGA GCT G-3'	6	OP-C12	5'-TGT CAT CCC C -3'
3	OP-A07	5'- GAA ACG GGT G-3'	7	OP-C19	5'-GTT GCC AGC C- 3'
4	OP-A09	5'-GGG TAA CGC C-3'			

Inter simple sequence repeats (ISSRs) procedure

PCR reaction was conducted using 5 primers, their names and sequences are shown in Table 5. PCR was performed according to (Wang *et al.*, 2002). PCR was programmed for one cycle at 94° C for 4 min followed by 45 cycles of 1 min at 94 °C, 1 min at 57 °C, and 2 min at 72 °C. The reaction was finally stored at 72 °C for 10 min.

TABLE 5. List of the primers names and their nucleotide sequences used in the study for ISSRs procedure.

NO	Name	Sequence	NO	Name	Sequence
1	844B	5'CTCTCTCTCTCTCTCTGC3'	4	HB-13	5' GAG GAGGAG GC 3
2	HB-09	5' GTG TGT GTG TGT GG 3'	5	HB-15	5' GTG TGT GTG TGT GC 3'
3	HB-12	5' CAC CACCAC GC 3'			

Statistical analysis

The experimental treatments were arranged in a randomized complete design and the analysis of variance for the obtained data in both seasons was performed according to Snedecor and Cochran (1980) and mean separation was analyzed using Duncan's multiple range test at 5% level of probability (Duncan, 1955).

The similarity matrices were done using Gel works ID advanced software UVP-England Program. The relationships among genotypes as revealed by dendrograms were done using SPSS windows (Version 10) program. DICE computer package was used to calculate the pair wise difference matrix and plot the phenogram among cultivars (Yang and Quiros, 1993).

Results and Discussion*Flowering**Inflorescence and flowering span*

As showed in Fig.1 inflorescence emergence occurred during the period from February 9th to Feb 19th in the first season and from Feb 4th to Feb 18th in the second season. In general inflorescence emergence stated earliest in the second season than in the first one, that may due to the temperature prevailing in each season. These results agree with, Hartmann and Wisher (1975) and Fouad *et al.* (1992a).

Blooming dates and blooming periods of the studied cultivars are illustrated in Table 6. Dates of beginning of flowering full bloom and end of flowering were earliest in the first season then that at the second season. At the first season flowering started in April 8th-16th, Full bloom in April 13th-20th. The corresponding dates in the second season were April 11th-18th, April 17th-23rd and April 24th-30th.

Fig. 1. Inflorescence emergence during 2011 and 2012 seasons.

In the first season blooming started early in Maraki and Koroneiki (April 8th), followed by Sewia and Coratina (April 10th), while the latest one was E52 (April 16th). Full bloom was early in Koroneiki (April 13th) followed by

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Sewia, Coratina and Maraki (April 15th and 16th), while it was late in E52 (April 20th). Blooming was ended on (April 22nd) in Koroneiki, (April 24th) in Sewia, Maraki and Coratina, but it was late in E52 (April 28th).

In the second season start of blooming was earliest in Coratina (April 11th) and the latest in E52 (April 18th), in others genotypes it was on (April 13th) in Koroneiki and Maraki and on (April 15th) in Sewia. Full bloom displayed between (April 17th) to (April 23rd), while the end of flowering occurred between (April 24th) to (April 30th) according to cultivars. Coratina was the earliest and E52 was the latest cultivar. In both seasons blooming initiated about 7-9 weeks after inflorescence emergence.

These results are in agreement with Abo-El-Ez and Hassein (2009) who reported that, the dates of flower beginning, full and end of blooming differ according to cultivars.

Inflorescence characteristics

Data in Table 7 clearly showed that all cultivars had the medium inflorescence length (2.5-3.5 cm) except Coratina in both seasons as well as Maraki and E52 in the second season, they had long inflorescence length (> 3.5 cm) according to Barranco *et al.* (2000). Similarity, Abo-Shanab *et al.* (2010) found variation in inflorescence length in 10 cultivars, also Sayed, (2013) found variation in length of inflorescence in 10 imported olive cultivars.

Number of flowers per inflorescence ranged from 17.37 to 27.73 in the first season and from 18.66 to 27.29 in the second season. The highest number of flowers (<25) recorded in Maraki genotype (27.73 & 27.29), in contrast the lowest number (>18) recorded in Koroneiki (17.37 & 18.66) in the first and the second seasons respectively. The rest cultivars had medium number of flower (18-25). These results are in agreement with Fouad *et al.* (1992a), El-Said *et al.* (2006), Abd-Allatif (2007), Abo-Shanab *et al.* (2010) and Sayed (2013) who found that the average number of flowers per inflorescence ranged between 15 to 31. Variation in the number of flowers was dependent on the cultivars and annual variations can be reached up to 21% (Lavee *et al.*, 1996).

Results in Table 7 showed significant difference in percent of perfect flowers among the studied cultivars within each season. In the first season Coratina gave the highest value (93.90%), followed by E52, Koroneiki, Maraki and Sewia (90.95, 87.13, 85.09 and 74.17 %, respectively). Whereas in the second season Maraki recorded the highest percentage (98.13%) followed by Coratina, Koroneiki, E52 and Sewia (91.87, 90.17, 86.70, and 76.70%, respectively). It is worth to mention that Maraki showed noticeable variation in percentage of perfect flowers at the two seasons. These results are in line with Hartmann *et al.*

(1980), Fouad *et al.* (1992a) and Sayed (2013) who reported that the relative proportion of perfect flowers varied according to varieties and seasons. Sex ratio also varied according to genetic factors (Di Marco *et al.*, 1990), climate and alternate bearing (Lavee, 1996 and Al-Shdiefat & Qrunfleh, 2008).

The present study revealed the differences in flowering density between the cultivars in the two seasons. As shown in Table 7, indicated that Maraki had the highest flowering density (83.00 & 99.48) in the two seasons and Koroneiki at the second season (100.2), Coratina in the first season and E52 in the second season gave the lowest flowering density (55.60 & 32.02 respectively). Similarly, Fouad *et al.*, (1992a) and Moffed (2009) referred to flowering density per meter in olive ranged from 11.7 to 115 according to cultivars and seasons. Also, Laz (1993) cited that the variation between years can be attributed to the alternate bearing behavior of the olive tree.

Pollen fertility and pollination

Pollen grain germination percent as illustrated in Table 8 showed the highest germination percentage for Sewia pollen (46.45 & 46.20%); meanwhile pollen of E52 had the least fertility (17.18 & 20.42%) in both seasons. Pollen germination percentage for Coratina, Maraki and Koroneiki were moderate in this concern. The obtained values are in agreement with Fernandez-Escobar *et al.* (1983) who demonstrate that the percentage of olive pollen germination ranged from 10.6 to 41.5%. Also, Lavee and Datt (1978) reported that olive pollen germination after 12 hours ranged from 3 to 38%. Furthermore, Abd-Allatif (2007) reported that pollen germination percentage in nine olive cultivars ranged from 10.92 to 54.00 %.

At open pollination, Koroneiki produced the highest fruit set (9.2 and 7.93%) in the two seasons, followed with significant differences by E52 and Coratina. Sewia and Maraki had the least fruit set percentage at open pollination in the two seasons (Table 8). This results indicating that the latter cultivars could have a degree of cross-incompatible.

On the other side, fruit set under self pollination indicated that, all of the studies cultivars were self-incompatible, as the percent of fruit set ranged between 0.03% to 0.037 % (Table 8). The highest self-incompatible was recorded in Sewia and E52, but the lowest one was found in Koroneiki. Also self-incompatible index also proved that, all of the cultivars under study were self-incompatible as the obtained value were close to zero as previously detected by (Androulakis and Loupassaki, 1990).

In this concern, Camposeo *et al.* (2012) mentioned that the horticultural interest of self-compatible fruit tree cultivars depends on their ability to reach the optimal fruit set by self-pollination. They proved that Coratina olive is self-incompatible. Also, Abd-Allatif (2007) found variation in 7 olive cultivars and classified them in two groups: Partially self- incompatible with SI-index ranged from 0.15 to 0.3 (Arbequine, Dermilali and Manzanillo cvs.) and high self-

incompatible with Si index less than 0.15 (Koroneiki, Croila, Blanquette, Souri, Picual and Mostazal).

TABLE 6. Flowering of five olive cultivars during 2011 and 2012 seasons.

Cultivars	Start of flowering		Full bloom		End of flowering	
	2011	2012	2011	2012	2011	2012
Sewia	April 10 th	April 15 th	April 15 th	April 21 st	April 24 th	April 29 th
Maraki	April 8 th	April 13 th	April 16 th	April 19 th	April 24 th	April 26 th
E52	April 16 th	April 18 th	April 20 th	April 23 rd	April 28 th	April 30 th
Koroneiki	April 8 th	April 13 th	April 13 th	April 20 th	April 22 nd	April 25 th
Coratina	April 10 th	April 11 th	April 15 th	April 17 th	April 24 th	April 24 th

TABLE 7. Inflorescence characteristics of five olive cultivars during 2011 and 2012 seasons.

Cultivars	Length of inflorescence (cm)		Flower No. / inflorescence		Perfect flower %		Flowering density/m	
	2011	2012	2011	2012	2011	2012	2011	2012
Sewia	2.94 C	2.86B	23.63B	26.40A	74.17D	76.70D	73.96B	86.60B
Maraki	3.07 BC	3.64 A	27.73A	27.29A	85.09C	98.13A	83.00A	99.48A
E52	3.43AB	3.77 A	18.93C	24.17AB	90.95AB	86.70C	79.05A	32.02D
Koroneiki	2.97 BC	2.90 B	17.37C	18.66 B	87.13BC	90.17B	68.16C	100.2A
Coratina	3.67 A	3.82 A	19.33C	22.46AB	93.90 A	91.87B	55.60D	75.06C

TABLE 8. Pollen germination percentage, fruit set percentage at self or open pollination and self-incompatibility index of five olive cultivars during 2011 and 2012 seasons.

Cultivars	Pollen germination percentage		Fruit set %				Self-incompatibility index	
			Self-pollination		Open-pollination			
	2011	2012	2011	2012	2011	2012	2011	2012
Sewia	46.45A	46.2A	0.06B	0.03C	1.05C	1.27D	0.067 B	0.030 BC
Maraki	36.39C	38.91B	0.19AB	0.19B	1.33C	1.41D	0.15 A	0.14 A
E52	17.18E	20.42E	0.05B	0.07C	3.89B	4.97B	0.087AB	0.043B
Koroneiki	36.21D	29.2D	0.37A	0.28A	9.20A	7.93A	0.040 B	0.037 BC
Coratina	38.84B	32.11C	0.32A	0.14B	3.65B	3.24C	0.017B	0.013 C

Yield per tree (Kg)

As shown in Table 9 there were significant differences in fruit yield per tree according to the studied cultivars in the two seasons, indicating the significant of the environmental factors. The highest yield (Kg/tree) in the two seasons was recorded in Coratina (46.67 & 60.00), Maraki (41.67 & 55.73) then Sewia (45.00 & 46.33). Koroneiki came in the second (36.00 & 39.70 Kg/tree) in this concern. On the other hand, yield of E52 cultivar decreased greatly from the first season (36.67kg/tree) to only 7.50 Kg/tree in the second season. The net decreasing value was about 80% that may be due to criterion of alternate bearing in this cultivar.

Similarly, Fouad *et al.* (1992 a) and Abo-El-Ez & Hassnein (2009) detected variation in yield of olive cultivars at different location. Moreover, Caruso *et al.* (1995) during their studies on inflorescence structure and fertility of several olive cultivars, recorded variation between the years due to climate effect.

TABLE 9. Yield (Kg /tree) of five olive cultivars during 2011 and 2012 seasons.

Cultivars	Yield	
	2011	2012
Sewia	45.00 A	46.33 B
Maraki	41.67 AB	55.73 A
E52	36.67 B	7.50 C
Koroneiki	36.00 B	39.70 B
Coratina	46.67 A	60.00 A

Fruit characteristics

Table 10 shows morphological characterization of fresh ripe fruits of the five studied cultivars in both seasons. The data indicated that the fruit weight varied significantly and ranged from low in Koroneiki (1.44 & 1.42 g) to very high (> 6 g) in Sewia (7.16 & 6.63 g) in 2011 and 2012 seasons, respectively. High fruit weight (4-6 g) was showed in Coratina (4.38 & 4.37 g) in both seasons and Maraki (5.63 g) only in the first season, but it recorded the very high fruit weight in the second one (6.86 g). Whereas, E52 recorded the medium fruit weight (2-4 g) in both season (1.98 & 3.23 g).

The length/ width ratio for fruits conformed to the fruit shape in both seasons which recorded 1.18 & 1.31 in Maraki (spherical), 1.35 & 1.42 in Sewia (ovoid), 1.45 in Coratina and E52 (ovoid- elongated) and more than 1.45 (1.54 & 1.61) in Koroneiki (elongated) (Table 10).

Concerning symmetry (position A), fruits showed slightly asymmetric except E52 showed asymmetric section, with a round apex and base in Sewia, Maraki and Coratina, whereas, E52 and Koroneiki showed pointed apex and truncate base. Position of maximum transverse diameter (Position B) noticed in central in all cultivars except Maraki which was towards apex. Fruits of Sewia, Maraki, Coratina, E52 and Koroneiki start colors from the apex. As for presence of lenticels, all the studied genotypes had many lenticels on the fruits. However lenticels on Sewia and Maraki fruits were large, but they were so small in Coratina, E52 and Koroneiki (Table 10).

Table 11 shows characterization of stone weight of ripe fruits for the five studied genotypes in both seasons. Data indicated that the stone weight varied significantly and ranged from 0.02 & 0.027 g (low < 0.3 g) in Koroneiki to 1.07 & 1.20 g (very high > 0.7g) in Sewia in 2011 and 2012 seasons, respectively. Stone weight of the other studied genotypes were in between 1.07 & 0.96 in Maraki, 0.75 & 0.78 in Coratina and 0.43 & 0.33 in E52 in the two seasons, respectively.

Length/width ratio for stone conformed to the stone shape in both seasons, appeared elliptic shape in Sewia (2.11 & 2.19), Ovoid shape in Maraki (1.81 & 1.76) and elongated shape in Coratina, E52 and Koroneiki where L/W > 2.2.

Concerning symmetry (position A), all seeds showed slightly asymmetric except Maraki which showed asymmetric section, with a round apex and base in Sewia and Maraki, while that of Coratina and Koroneiki were pointed ones, E52 showed pointed apex and rounded base. Sewia and Maraki and Coratina genotypes appeared with mucro, rugose surface and the maximum transverse diameter was towards apex whereas, E52 and Koroneiki without mucro, at the maximum transverse diameter in center with smooth surface (Table 11).

Such differences in fruit and seed of olive cultivars were also reported by Hartmann & Papaioannou (1971), Fouad *et al.* (1992 b) and Del Rio & Caballero (2008). As well as Poljuha (2008) indicated that some fruit and stone characteristics can vary due to exogenous factors (environment, cultivation technology, etc.).

Oil content and its chemical parameters

As shown in Table 12 Maraki and E52 produced the highest fruit oil percent as a dry weight in the two seasons of study (52.82 & 51.23% in the 1st season and 53.45 & 54.54% in the 2nd season, respectively). Meanwhile Koroneiki recorded the lowest value (44.32 & 44.54 %) in the two seasons. Coratina and Sewia in decreasing order were in between in the two seasons. The highest oil yield per tree (kg) produced by Maraki and Coratina in both seasons, that was due to the heaviest yield recorded and the high oil percentage in these cultivars. On the contrary, E52 which recorded the highest oil content% in both seasons, it recorded the least value of oil yield per tree (1.80kg) in the second season as a result of the least fruit yield per tree (7.50 kg/tree) in this season.

Data of olive oil acid value showed no significant difference between the five studied cultivars in the second season (Table 12). In the first season, Sewia, Maraki and E52 cultivars recorded the lowest acid value, while Koroneiki and Coratina cvs had some increase in this value. However, the percent of acidity ranged from 0.10 to 0.38 % during both seasons, indicating that it greatly lower than the limit of the extra virgin olive oil (≤ 0.8) as indicated in the international standard of the IOC (2013).

The peroxide value of virgin olive oil ranged from 6.90 to 14.98 (meq.o₂/kg oil). E52 and Sewia oil had the highest peroxide value in the first season (13.98 and 14.98 respectively), while in the second season E52 only produced the highest value (10.94). The other cultivars came in the second place. All of the indicating peroxide values were less than the limits of international standard of extra virgin olive oil (≤ 20) according to IOC (2013).

Oil stability parameters

During both seasons, data in Table 13 showed that UV absorption at 232nm and 270nm of the olive oil under study were in the limits of extra virgin olive oil (≤ 2.5 and ≤ 0.22 , respectively) according to IOC (2013). Coratina and E52 had the highest value at K232nm in both season, while Koroneiki, Sewia and Maraki appeared to have low absorption values. As for UV absorption at 270nm Coratina, Koroneiki and E52 generally had the highest values, but Sewia had the least value in both seasons (Table 13).

These results indicated that the degree of olive oil oxidation of all samples under investigation was completely lower than the permitted limits. Moreover, oil of the three olive cultivars could be classified as extra virgin olive oil according to classification parameters cited by Boskou, (2006).

Among the main components that are related to oil stability is the total polyphenols content in the olive oil of the five cultivars under study. Table 13 shows the highest polyphenols value for Koroneiki ($192.0 \pm 180 \text{ mgKg}^{-1}$) and Coratina ($184 \pm 190 \text{ mgKg}^{-1}$) in the two seasons respectively, besides Sewia (180 mgKg^{-1}) and E52 (182 mgKg^{-1}) in the second season. The least value recorded in Sewia $145.0 \text{ (mg Kg}^{-1}\text{)}$ and E52 (162 mgKg^{-1}) in the first season and Maraki in the two season ($156 \pm 162 \text{ mgKg}^{-1}$).

In this concern, Del Carlo *et al.* (2004) pointed out that virgin olive oil contains Phenolic substance responsible for their stability against oxidation. Phenols components are transferred into the oil during the olive processing, but their concentration is dramatically reduced during storage (Okogeri and Tasioula- Margari, 2002 and Servili *et al.*, 2004).

Fatty acids composition

Gas chromatography analysis of fatty acids in the olive oils of the cultivars under investigation was presented in Table 14. They were significantly affected by olive cultivars. The main saturated fatty acids were Palmitic then Stearic acid, but the main unsaturated fatty acids were oleic, linoleic, linolenic acids in descending order. The highest content of Palmitic acids was found in E52 at the first season (13.5%), but it was obtained in Koroneiki and Coratina (14.52 & 14.45%). In the second season, Maraki, on the opposite had the lowest level of Palmitic acid. Stearic acid found at the highest level in Maraki in both seasons, but Coratina had the lowest level of this fatty acid.

Oleic acid was the monounsaturated fatty acid found in all olive oils under study. It was at the highest level in Maraki then Sewia, Koroneiki and Coratina, but the least content was detected in E52. On the other side, Linoleic and Linolenic acids were at highest levels in E52, but the lowest level was detected in Maraki.

The ratio of total unsaturated to saturated fatty acids was highest in Maraki followed by Sewia then Coratina in the two seasons, but the lowest ratio was found in E52 in the first season and Koroneiki in the second season.

These results are in agreement with the finding of Talantikite and Aitamas *et al.* (1998) and Fayek *et al.* (2001) on different olive cultivars, who mentioned that palmitic and oleic acids were the most abundant saturated and monounsaturated fatty acids respectively, and olive oil characterized by higher monounsaturated to saturated fatty acids.

TABLE 10. Comparison of fruit morphological characteristics of five olive cultivars in 2011 and 2012 according to the International Olive Council methodology.

Cultivars	2011				2012				Shape	Symmetry	Apex	Base	Size of lentils	Presence of lentils	Position of Maximum transverse Diameter (Position B)	Location of start of color change
	Weight g	Length (L)	Width (W)	L/W	Weight g	Length (L)	Width (W)	L/W								
Sewia	7.16 A	2.81 A	2.08 A	1.35 C	6.63 B	2.92 A	2.05 A	1.43 B C	Ovoid	Slightly asymmetric	Rounded	Rounded	Large	Many	Central	From the apex
Maraki	5.63 B	2.41 C	2.04 A	1.18 D	6.86 A	2.76 B	2.06 A	1.34 D	Spherical to ovoid	S asymmetric	Rounded	Rounded	Large	Many	Towards apex	From the apex
ES2	1.98 D	2.15 D	1.32 C	1.64 A	3.23 D	2.13 D	1.56 C	1.38 C D	Elongated	asymmetric	Pointed	Truncate	Small	Many	Central	From the apex
Koroneiki	1.44 E	1.85 E	1.20 C	1.55 A B	1.40 E	1.67 E	1.04 D	1.60 A	Elongated	S asymmetric	Pointed	Truncate	Small	Many	Central	From the apex
Coratina	4.38 C	2.56 B	1.77 B	1.45 B C	4.37 C	2.57 C	1.73 B	1.49 B	Ovoid to elongated	S asymmetric	Rounded	Rounded	Small	Many	Central	From the apex

TABLE 11. Comparison of seed morphological characteristics of five olive cultivars in 2011 and 2012 according to the International Olive Council methodology.

Cultivars	2011				2012				Shape	Symmetry	Apex	Base	Position of Maximum transverse Diameter (Position B)	Surface (position B)	Number of grooves	Termination of the apex (position A)
	Weight g	Length (L)	Width (W)	L/W	Weight g	Length (L)	Width (W)	L/W								
Sewia	1.07 A	1.97 A	0.92 A	2.14 B	1.20 A	1.93 A	0.88 B	2.19 B	Elliptic	Slightly asymmetric	Rounded	Rounded	Towards apex	Rugose	Medium	With macro
Maraki	1.07 A	1.71 C	0.97 A	1.76 C	0.96 B	1.72 B	0.98 A	1.75 C	ovoid	asymmetric	Rounded	Rounded	Towards apex	Rugose	Medium	With macro
ES2	0.43 C	1.90 A C	0.66 C	2.88 A	0.33 D	1.91 A	0.65 C	2.94 A	Elongated	asymmetric	Pointed	Rounded	Central	Smooth	Medium	Without macro
Koroneiki	0.20 D	1.20 D	0.52 D	2.31 B	0.27 D	1.10 C	0.41 D	2.68 A	Elongated	S asymmetric	Pointed	Pointed	Central	Smooth	Medium	Without macro
Coratina	0.75 B	1.80 B C	0.80 B	2.25 B	0.78 C	1.88 A B	0.84 B	2.24 B	Ovoid to elongated	S asymmetric	Pointed	Pointed	Towards apex	Rugose	Medium	With macro

TABLE 12. Oil content% (as dry weight), oil yield per tree (kg) , and some quality parameters of five olive cultivars during 2011 and 2012 seasons.

Cultivars	Oil content (%)		Oil yield per tree (Kg)		Acid value as (% C18:1)		Peroxide value (meq.o ₂ /kg oil)	
	2011	2012	2011	2012	2011	2012	2011	2012
Sewia	46.22C	50.86B	8.83B	10.24B	0.10B	0.34n.s	14.98A	7.37B
Maraki	52.82A	53.45A	9.65AB	13.39A	0.11B	0.32n.s	12.25BC	8.40B
E52	51.23A	54.54A	8.78B	1.80D	0.12B	0.27n.s	13.98AB	10.94A
Koroneiki	44.32D	44.54C	6.67C	7.05C	0.29A	0.26n.s	10.78C	6.90B
Coratina	48.65B	51.45B	10.42A	13.72A	0.21AB	0.38n.s	11.44C	7.33B

TABLE 13. Stability parameters evaluated in olive oil samples from five cultivars during 2011 and 2012 seasons.

Cultivars	K232nm		K272nm		Total Polyphenols (mg Kg ⁻¹)		Tocopherol (mg Kg ⁻¹)	
	2011	2012	2011	2012	2011	2012	2011	2012
Sewia	0.92E	1.11B	0.13D	0.15C	145.0E	180.0C	250.0C	260.0D
Maraki	1.11C	1.08C	0.17B	0.15C	156.0D	162.0D	243.0D	255.0D
E52	1.42B	1.49A	0.15C	0.22A	162.0C	182.7B	251.0C	270.8C
Koroneiki	1.10D	0.64E	0.13D	0.22A	192.0A	180.0C	276.0A	295.0A
Coratina	1.67A	1.00D	0.20A	0.17B	184.0B	190.0A	271.0B	280.0B

TABLE 14. Fatty acids (%) composition evaluated in olive oil samples from five cultivars during 2011 and 2012 seasons.

Cultivars	Palmitic acid C16:0		Stearic acid C18:0		Oleic acids C18:1		Linoleic acid C18:2		Linolenic acids C18:3		Unsaturated/saturated	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Sewia	11.27 C	12.69 CD	2.66 B	2.43 C	74.53 B	74.00 B	9.16 C	7.92 C	0.81 B	0.90 C	5.92 B	5.32 B
Maraki	10.04 C	11.82 D	2.94 A	2.70 A	77.20 A	76.07 A	6.90 E	7.44 C	0.66 C	0.84 D	6.36 A	5.55 A
E52	13.5 A	13.20 BC	2.40 D	2.02 E	68.40 D	62.08 D	13.23 A	18.22 A	0.84 B	1.30 A	5.04 E	4.79 D
Koroneiki	12.49 B	14.52 A	2.56 C	2.60 B	74.95 B	71.73 C	8.02 D	7.24 C	0.85 B	0.87 B	5.34 D	4.51 E
Coratina	12.48 B	13.45 AB	2.22 E	2.13 D	71.35 C	71.57 C	11.07 B	9.65 B	0.88 A	1.01 C	5.49 C	5.12 C

Molecular characterization as revealed by RAPD and ISSR-PCR markers.

Similarity matrix and cluster analysis as revealed by RAPD markers

To determine the genetic relationships among the five olive cultivars under study, seven tested primers of RAPD was used for scoring data (1 for presence and 0 for absence). Results of similarity index are shown in Table 15 and matrices similarity are shown in Figure 2). The genetic similarity ranged from 0.76 to 0.88, the highest genetic similarity revealed by the RAPD analysis (0.88) was between Maraki and Coratina followed by 0.85 between Maraki and Sewia, 0.82 between

Maraki and E52 then 0.8 between E52 and either of Coratina & Koroneiki. The lowest percentage in similarity (0.76) was between Coratina and Sewia and also between E52 and Sewia.

TABLE 15. Similarity index among five olive cultivars based on RAPD analysis.

	Sewia	Maraki	Coratina	E52	Koroneiki
Sewia	1				
Maraki	0.85	1			
Coratina	0.76	0.88	1		
E52	0.76	0.82	0.8	1	
Koroneiki	0.78	0.79	0.77	0.8	1

The RAPD dendrogram obtained by UPGMA analysis grouped the five cultivars into one main cluster and three minor clusters. The jaccard's coefficient ranged from 0.80 to 1.00 (Fig. 2). The highest similarity coefficient observed between Maraki and Coratina (0.88) while the least similarity coefficient were observed between Coratina, E52 and Sewia (0.76).

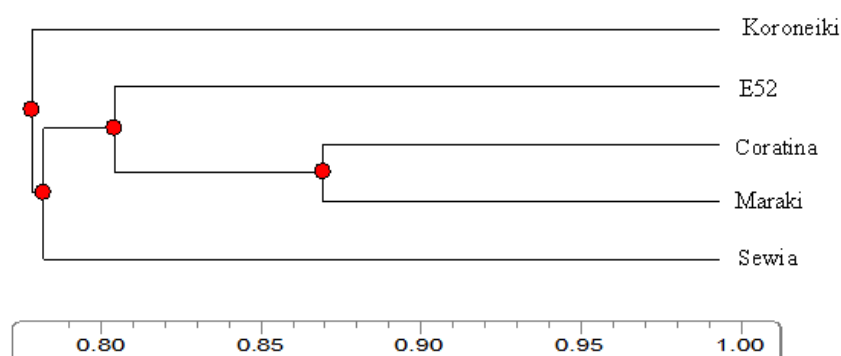


Fig. 2. UPGMA dendrogram based on the proportion of shared RAPD fragments obtained by using seven primers in the total DNA of five olive cultivars .

Similarity matrix and cluster analysis as revealed by ISSR-PCR Markers

The five tested primers of ISSR are illustrated in Table 16. Also, it was used to compute the similarity matrices as shown in Fig 3. The genetic similarity among the five cultivars ranged from 0.40 to 0.75, the highest genetic similarity revealed by the ISSR analysis (0.75) was between Sewia and Maraki, but the least percentage in similarity (0.40) was between Coratina and Koroneiki.

The ISSR dendrogram obtained by UPGMA analysis grouped the five genotypes into one main cluster and three minor clusters. The jaccard's coefficient ranged from 0.50 to 1.00 (Fig 3). The highest similarity coefficient observed between Sewia and Maraki (0.75) while the least similarity coefficient were observed between Koroneiki and Coratina (0.44).

Similarity matrix and cluster analysis as revealed by RAPD and ISSR-PCR markers

A result from the tested primers of RAPD and ISSR-PCR was used to compute the genetic similarity (Table 17) and phylogenetic dendrogram (Figure 5) among the five olive genotypes under study. The highest genetic similarity (0.81) was between Siwie and Maraki, but the least percentage in similarity (0.63) was between Coratina and Siwie and also between Coratina and Koroneiki.

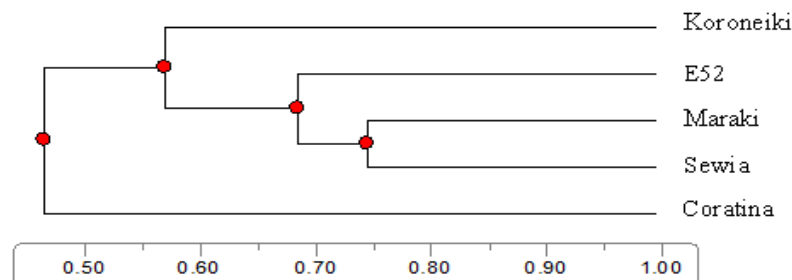


Fig. 3. UPGMA dendrogram based on the proportion of shared ISSR fragments obtained by using seven primers in the total DNA of five olive cultivars .

TABLE 16. Similarity index among five olive oil cultivars based on ISSR analysis.

	Sewia	Maraki	Coratina	E52	Koroneiki
Sewia	1				
Maraki	0.75	1			
Coratina	0.44	0.5	1		
E52	0.71	0.67	0.54	1	
Koroneiki	0.64	0.53	0.4	0.55	1

TABLE 17. Similarity index among the five olive cultivars based on two RAPD and ISSR-PCR markers.

	Sewia	Maraki	Coratina	E52	Koroneiki
Sewia	1				
Maraki	0.81	1			
Coratina	0.63	0.71	1		
E52	0.74	0.76	0.7	1	
Koroneiki	0.73	0.69	0.63	0.71	1

The dendrogram based on the recent overall markers (RAPD and ISSR) grouped the five cultivars into two clusters. The first included Coratina and the second divided into two sub-clusters, the first included Maraki and Sewia and the second contained Koroneiki and E52 (Fig. 4). The highest similarity coefficient

observed between Sewia and Maraki (0.81) while the least similarity coefficient were observed between Koroneiki and Coratina.

The high level of polymorphism observed in this study was between the two Egyptian genotypes Maraki and Sewia indicated that they are highly polymorphic cultivars. However the high diversity found between Egyptian and foreign genotypes under study is probably due to a diverse germplasm origin that presumably results from crosses between wild and cultivated olive resulting in new cultivars in different parts of the Mediterranean, and low breeding pressures (Besnard *et al.*, 2001a , Contento *et al.*, 2002 and Belaj *et al.*, 2003 c).

In conclusion, ISSR markers are more powerful of these two techniques for fingerprinting closely related cultivars such as those of *Olea europea L.* cultivars. Although most ISSR alleles are dominant, rather than co-dominant, ISSR amplification of *Olea europea L.* ISSR markers offers several advantages over RAPDs , the major one being rapid production of a large number of markers in a cost-effective manner. ISSR amplification has great potential in plant breeding and germplasm evaluation, especially for fingerprinting narrow-based germplasm like those present in *Olea europea L.*

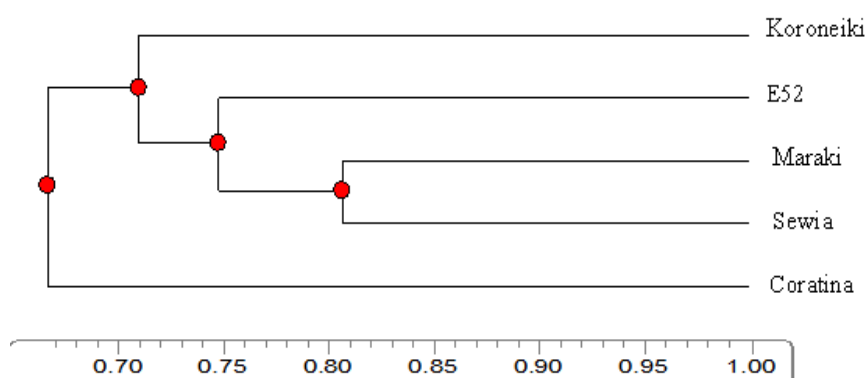


Fig. 4. Dendrogram for the genetic relationships among five olive cultivars based on two markers (RAPD and ISSR) .

Conclusion

Based on the quality parameters of the oil of the evaluated Egyptian oil clones provided that it have high yield and ranked extra virgin. The ratio of total unsaturated to saturated fatty acids was the highest in Maraki, followed by Sewia compared with the international cultivars Coratina and Koroneiki.

Based on the recent overall markers (RAPD and ISSR), the highest similarity coefficient observed between Sewia and Maraki while the least similarity were between Koroneiki and Coratina.

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الخصائص المورفولوجية المحصولية والوراثية لأصناف الزيتون المحلية مقارنة بالأصناف العالمية

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تم مقارنة ثلاثة أصناف زيتون محلية هي "مراقى وسيوى وE52 مع أصناف زيتون عالمية هي كوراتينا وكورناكى، وذلك خلال موسمي ٢٠١١، ٢٠١٢. في مزرعه على طريق مصر-الأسكندرية الصحراوي (الكيلو ٦٤). أوضحت النتائج الاتي. إختلف ميعاد التزهير طبقاً للصنف والموسم، كانت أصناف الكوراتينا والمراقى والسيوى أبكر تزهيراً من الكورناكى وأخرهم كان E52. سجل صنف الكوراتينا أطول نورة زهرية (<٣,٥سم) ، ولكن الأصناف الأخرى كانت متوسطة من (٢,٥-٣,٥). سجل المراقى أكبر عدد من الازهار فى النورة الزهرية (<٢٥)، وأقل عدد للازهار داخل النورة سجل فى الكورناكى (>١٨) . نسبة الازهار الخنثى اختلفت طبقاً للعوامل الوراثية والمناخ ، كانت اعلى نسبة فى الكوراتينا (<٩٠٪) وأقل نسبة سجلت فى السيوى (٧٥٪). كانت خصوبة وجودة حبوب اللقاح مرتفعة فى السيوى ومنخفضة فى E52. كانت جميع الأصناف تحت الدراسة عديمة التوافق الذاتى. سجل الكوراتينا أعلى نسبة محصول تحت التلقيح المفتوح في الموسمين، بينما الصنف E52 فأظهر وجود تبادل حمل. سجل الكورناكى أقل وزن للثمرة والبذرة، E52 كان متوسط الوزن، كبيرة الوزن فى الكوراتينا، بينما كانت الأصناف المراقى والسيوى ذات وزن كبير جداً وذلك طبقاً لوزن الثمرة والبذرة. كان شكل الثمرة متطاول فى الكورناكى، بيضاوي الى متطاول فى E52 والكوراتينا، وبيضاوي فى السيوى. كان شكل البذرة متطاول فى الكورناكى و E52 والكوراتينا، بيضاوي الشكل فى المراقى ومستدق فى السيوى . كان محتوى الزيت(٪) مرتفعاً فى المراقى و E52 ويليهم الكوراتينا والسيوى ثم أخيراً الكورناكى حسب الترتيب التنازلي. طبقاً لمعايير الجودة فى الزيت (نسبة الحموضة، رقم البيروكسيد، إمتصاص الاشعة فوق بنفسجية ٢٧٠ و٢٣٢ نانومتر، البوليفينول، والتوكوفيرول) كان تصنيف زيت الزيتون الناتج طبقاً للشروط السابقة على انه زيت زيتون بكر ممتاز. كانت النسبة بين الاحماض الدهنية الغير مشبعة الى الاحماض الدهنية المشبعة مرتفعة فى المراقى يليه السيوى ثم الكوراتينا ولكن أقل نسبة كانت فى E52 والكورناكى. استناداً الى الواسمات الوراثية ال RAPD-PCR و ISSR-PCR كان هناك تشابه وراثي بين كل من السيوى والمراقى وأقل تشابه كان بين كل من الكوراتينا والكورناكى .

الكلمات الدالة: الزيتون - الخصائص - سيوى - مراقى - E52 - كوراتينا - كورناكى .