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Distribution of Some Phenotypical Characters Within an Olive Population in Djebel Ouslet (Tunisia)

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ABSTRACT

This research was conducted with the main purpose to evaluate the level of genetic similarities among an olive population situated in Djebel Ouslet (central east of Tunisia). Morphological characters and phenological growth stages of four olive cultivars Ousleti, Brahmi, Chaibi and Jeli were described during 2004-2006. Morphological classification used, was based on tree, leaf, inflorescence, fruit and endocarp characteristics. Technological characters of the fruits concerned the oil content and their chemical composition. Floral phenology, flowering and pistil abortion rates of the cultivars were recorded at full bloom. Also, a pollen monitoring survey for each cultivar was investigated. This study shows the relative differences between cultivars in flowering, fruiting patterns, oil yield and fatty acid contents of the fruits. Under the conditions of Djebel Ouslet, the flowering period of the cultivars was similar and covered each other but little differences were recorded in the duration and the onset of flowering. The evaluation of self-fertility showed that all cultivars can be considered self-compatible and fruiting rates were higher under free-pollination than under self-pollination. The results obtained are consistent with the high pollen capacity of the cultivars, suggesting their use as pollinizers in the cross-pollination assays to improve productivity. Large differences in oil content and in fatty acid profiles were observed in the cultivars examined. But their olive oil are conform to international standards. The ranges of fatty acid for all the cultivars fall within the accepted limits for fatty acid composition of Virgin olive oil. This database of the most representative cultivars grown in Djebel Ouslet concerning their agronomic and chemical characteristics could be exploited for screening synonyms within this olive population and could provide information for cultural purposes and breeding programs.

Key words: Olive trees, floral biology, classification of olive, oil quality

INTRODUCTION

In Tunisia, olive cultivation is of great importance with 1.68 millions hectares and 65.9 millions of olive trees, producing over ten crop years (1996-2005) an average 149.000 tons olive oil. There is an enormous potential for olive oil (65%) and table olive (0.9%) production as an important export industry (yield for 2005 put production at 130.000 tons from which 109.400 tons of olive oil were exported). This olive oil must be competitive against other vegetables oils with similar chemical characteristics and which were cheaper (importation of 320.000 tons vegetable oil in 2005) (DGEDA, 2006).

This olive population presents multiple phenotypic expressions and manifests a diversity of morphologic and physiologic characteristics corresponding to different aptitudes and qualities. It comprised many of local cultivars which were selected for their qualitative and quantitative traits and for their adaptability to various microclimates such as cv. Gerboui (Mehri and Mehri-Kamoun, 2007), cv. Meski (Mehri *et al*., 2003).

To be sustainable and to be competitive on the international market, Tunisian olive industry has to adopt high quality techniques in management and production technology. For the important issues are to use best cultivars suitable for extreme conditions such as high salinity and drought between local olive cultivars. Unfortunately, there is limited reliable performance data for local olive cultivar under the Tunisian conditions. For this purpose, a local olive cultivar assessment program has been established in Tunisia to evaluate the performance and to describe local olive cultivars in order to resolve the confusion in cultivar identity and to assist olive producers in choosing best cultivars (Mehri and Hellali, 1995; Mehri *et al*., 1997; Mehri and Mehri-Kamoun, 2007; on Gerboui cultivar).

Different techniques have been used to evaluate olive diversity, to analyze germplasm variability and to differentiate between cultivars: morphological characters (Barranco and Rallo, 1984; Mehri and Hellali, 1995; Nieddu *et al*., 1995; Mehri *et al*., 1997; Cantini *et al*., 1999; Pinheiro and da Silva, 2005; Mehri and Mehri-Kamoun, 2007), isozyme analysis (Ouazzani *et al*., 1993; Hilali *et al*., 1995; Trujillo *et al*., 1995) and molecular markers (Fabbri *et al*., 1995; Angiolillo *et al*., 1999; Nikoloudakis *et al*., 2003). The latest technique was used to assess the genetic diversity of olive cultivars (Sheidai *et al*., 2007; Peyvandi *et al*., 2009) and to provide a good discriminatory system, independent of environmental conditions while identification based on quantitative and qualitative morphological characters give variability due to environmental fluctuation effect on expression of most morphological traits.

Many studies were conducted on olive oil which is used throughout the world and is believed to have an important role in human health and nutrition. Leaves of wild olive trees were used as green tea (Shah *et al*., 2002).

Olive oil can be used as good sources of natural antioxidant for medical and commercial uses. It has the highest antioxidant activity (Hajimahmoodi *et al*., 2008; Mudgal *et al*., 2010) and a high phenolic content (Gomez-Caravaca *et al*., 2007). Also, by-products of olive tree such as leaves and shoots were proved to be technically and economically an efficient horticultural substrate (Latigui *et al*., 2011).

In Tunisia, morphological and phenological traits have been used to identify olive cultivars following (Mehri and Hellali, 1995) in a survey of northern, central and coastal regions. This previous study allows discriminating 219 accessions out of 79 local denominations from which 43 are of unknown origin (Mehri *et al*., 1997). They also detected slight differences in three olive samples of the same cultivar Zarrazi from different geographic locations: Kalaa Kebira (coastal region), Siliana (Northern) and Gafsa (Southern area of Tunisia).

The objectives of the present study were to identify four olive cultivars that are the most representative from the central part of Tunisia (Djebel Ouslet), to evaluate the level of genetic variation between cultivars and within this olive population. The cultivars chosen were "Ousleti, Chaibi, Jeli and Brahmi" and the morphological descriptors concerned tree, leaf, inflorescence, fruit and endocarp. The olive fruits were examined for their morphology and oil composition (oil content and quality) and for endocarp characteristics. For phenological growth stages, floral biology and a pollen monitoring survey for each cultivar were investigated concerning average number of flowers, of hermaphrodite flowers/inflorescence, pistil abortion rate, floral phenology and flowering rate. Another objective was to compare self and free-pollination of the four cultivars by pollination assays in the field.

MATERIALS AND METHODS

Study site: The olive population used in this study was located at Djebel Ouslet in the central-east of Tunisia which occupied large parts of mountains and hills areas with a gradient of around 45% (Fig. 1). It has about 2300 ha of olive orchards located in agricultural region of the “Governorate of Kairouan”. This site has a typical Mediterranean climate (with a rainy mild winter, dry and warm summer) and a history of good olive oil quality. The mean annual rainfall of 300-400 mm year^{-1} is irregularly distributed in space and time. The textural class is sandy-clay and the main land degradation types in this site are predominant rocky areas and soil erosion. Recently, stone wall are constructed and olive and carob trees planted in trips running across the slope to reduce the erosion by reducing runoff and breaking up its trajectory over sloping lands.

Material: Local information on agronomic characteristics indicates that this olive material is very ancient and sourced from old colonial groves. Many of the trees have been overgrown by trees originating from seeds or by suckers. This material, where records are incomplete and unreliable leading to confusion about the identity of trees, comprised mainly four olive cultivars Ousleti, Brahmi, Chaibi and Jeli. The names given to those cultivars have arisen through the site or home locality (cv. Ousleti from Djebel Ouslet and Ousletia regions) or according the fruit maturation period (Chaibi), the oiling potential or the origin (grafted on wild trees such as cv. Jeli) or from seedlings such as cv. El-Horr). But all the cultivars are of unknown origin.

In this olive population, there is no set pattern to pruning, the trees received hardly any pruning or were pruned at long intervals which accentuate alternate bearing in the olive trees. It is characterized by a predominance of small growers using traditional management techniques.

Biometric characterization

Morphological characterization: Morphological characterization has been carried out of the four cultivars and standard morphological descriptors established for olive (IOOC methodology, IOOC, 1997) were used in this study concerning tree, leaf, inflorescence and fruit-endocarp. Samples from fruits, leaves and inflorescences were recorded for each cultivar for their subsequent description.

To evaluate foliage vigour and tree size, the canopy density, trunk circumference (at 0.20 m from the graft point or at 0.45 m from the ground) and the tree height of each cultivar were

Fig. 1: Traditional olive farming in Djebel Ouslet area

measured. Shoot growth: length, number of leaves and internodes per shoot were also recorded. The leaf characters measured were shape, apical and basal angle, leaf length and breadth ratio (L/W). Fruit characteristics included shape, colour and size, position of diameter maximum, shape of the base, apex and middle of the fruit, presence or absence of the mucro as well as depth of the peduncular cavity. Some important agronomic features of the cultivars were also recorded. The characteristics of the inflorescences observed concerned length and number of flowers per inflorescence.

Fresh fruit weight was determined by weighing 10 olives and calculating the average weigh per olive. The endocarps were cleaned of all residual flesh, weighed and flesh to pit ratio calculated and was determined by expressing the weight of the flesh (whole olive weight minus the stone weight) divided by the weight of the stone.

Olive quality characteristics: Olive samples of each cultivar were harvested from the grove in Djebel Ouslet at full colour development (December-January) black stage. They were analyzed for oil content and fatty acid composition.

Oil content: Oil content was obtained using a hammer mill. The crushed fruits were mixed for 30 min at 25°C and then the oil was separated by centrifugation.

Fatty acid analysis: The analyses were performed according to the method described in a previous work on cv. Gerboui (Mehri and Mehri-Kamoun, 2007). Fatty acid profiles of the oils were determined by gas chromatographic analysis of the Fatty Acid Methyl Esters (FAME), using a DB 225 capillary column operating isothermally at 220°C with a run time of 15 min. Nitrogen (0.6 bar) was the carrier gas and injector and flame ionisation detector temperatures of 220 and 250°C, respectively, were used. Peaks corresponding to different methyl esters fatty acids were identified.

Floral biology and phenological growth stages: In order to study the compatibility between the four cultivars studied, a morphological characterization of inflorescences, flowering phenology, a pollen monitory survey and fertility behaviour by pollination assays were conducted inside the experimental olive grove.

Flower quality and morphology: The purpose was to assess flower quality during the bloom period of each cultivar which refers to the relative development of the pistil (pistil abortion) and the resulting ability to set fruit. Branches about 40 cm long with full floral differentiation (90-95% of the buds) were chosen for each cultivar from a minimum of three trees. Flower morphology as well as the percent of staminate flowers were determined annually on at least 100 flowers of each cultivar collected at random around the tree at the beginning of bloom. The bloom period of the four cultivars was evaluated according to the phenological stages: first flowers opened; start of blooming (when 10% of flowers were opened); full blooming (when 50% of flowers were opened) and the end of flowers (when petals start to fall).

Pollen compatibility: Pollen compatibility for each cultivar was evaluated by estimating pollen germination and tube length into a solid germination medium containing 0.7% agar, 20% sucrose, 100 ppm H_3BO_3 (Mehri *et al.*, 2003) at pH 5. Cultures were held at 25°C for 72 h. Counted pollen grains were at least 200 per petri dish. A pollen grain was considered germinated when the length of its pollen tube was equal to or exceeded its diameter (Stanley and Linkens, 1974). Pollen germination and tube growth were determined after 3, 6, 12, 24, 48 and 72 h on 50 pollen grains chosen at random from various locations in the pollen sample.

Fertility behaviour: Research on the self fertility behaviour and free pollination requirements of each olive cultivar was carried out at Djebel Ouslet during 2004, 2005 and 2006. For each cultivar, five trees were used and before flower opening, ten shoots bearing about 150 inflorescences were tagged. Five shoots served as controls. Before anthesis, a suitable number of inflorescences and flowers were counted per shoot and were divided into two parts. One part was isolated in paper bags to determine fruit set under self pollination. When flowers start to open the enclosed branches were hand-shaken to insure pollination. The second part of inflorescences was left intact to evaluate fruit set under free pollination. After petal fall, about 30-40 days after full bloom and after loss of stigma reception, paper bags were removed and the fruit counted. Fruit set was recorded based on total number of flowers. To evaluate the self-fertility, an R1 index was calculated as the ratio between the fruit set in self and free-pollination.

Statistical analysis: All the experimental results obtained in this study are reported as mean values of experiments performed in two growing seasons 2004 and 2006, except for pollination assays elaborated in three consecutive years. The values were statistically elaborated to compare means, using Student's test. This test was performed for each character to identify significant differences.

RESULTS AND DISCUSSIONS

Floral biology and phenological growth stages of olive trees cultivars Chaibi, Brahmi, Jeli and Ousleti in Djebel Ouslet olive grove

Evaluation of flowering phenology: This study was conducted at Djebel Ouslet, during 2004 and 2006 by investigating floral biology of each cultivar: the average number of total flowers and of hermaphrodite flowers/inflorescence, pistil abortion rate, floral phenology and flowering rate. Flower quality refers to the relative development of the pistil and the resulting ability to fruit set. As shown in Fig. 2, the mean percentage of flower buds on the shoot varied among the cultivars

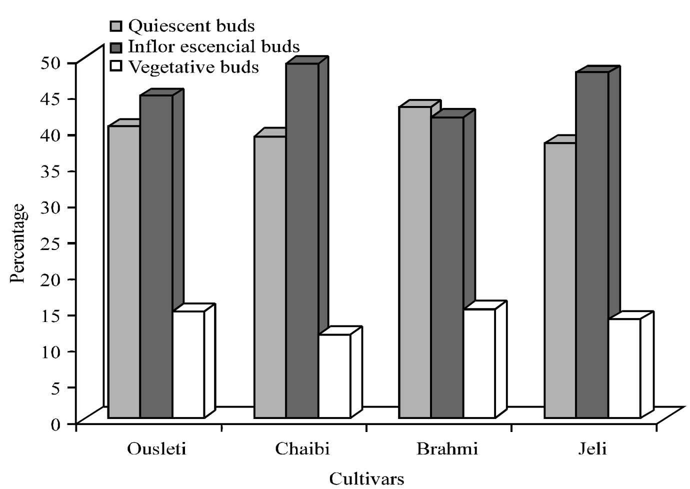

Fig. 2: Mean percentage of vegetative, floral and quiescent buds of four olive cultivars grown in Djebel Ouslet

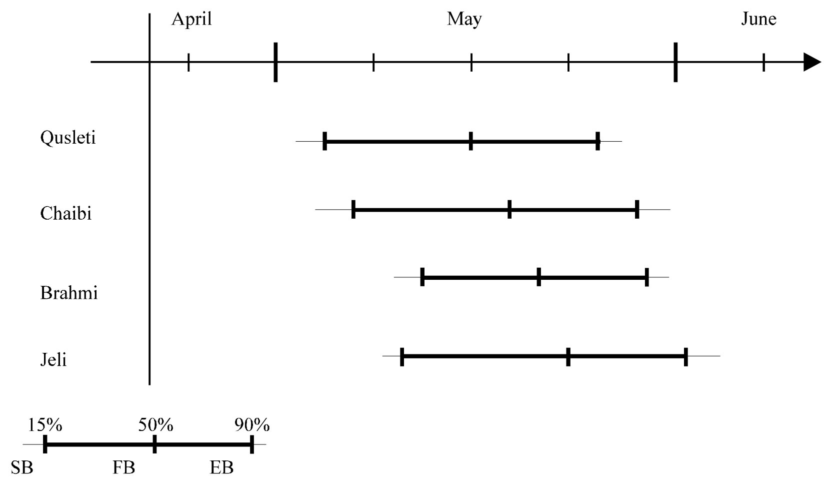

Fig. 3: Average blooming dates of 3 olive cultivars (Brahmi, Jeli and Chaibi) as compared to Ousleti cultivar grown in Djebel Ouslet during 2004-2006. The percentages indicate the proportion of flowers opened, SB: Start blooming, FB: Full blooming, EB: End blooming

with no significant difference. For all the cultivars, 39 to 43.1% of lateral buds are quiescent, 41.6 to 49.2% develop inflorescences and 11.8 to 15.3% develop shoots. It is important to consider these parameters under orchard conditions because the yield in “off” year is positively correlated with the number of reproductive buds (Cuevas *et al*., 1994). Also, a relation between shoot vigour, level of flowering and fruit set potential was found in olive (Lavee *et al*., 1999).

The average blooming dates of Chaibi, Brahmi and Jeli cultivars compared to Ousleti, are given in Fig. 3. There is a slight time difference between the dates of start blooming; it was by 2 days between Ousleti and Chaibi cultivars and by 9-10 days between Brahmi and Jeli cultivars. However, the period of full blooming coincided in all cultivars with difference of 2 days according to the cultivar. Chaibi, Brahmi and Jeli bloom later than the main cultivar Ousleti. The latest was the earliest to bloom with the others blooming at the same time or later with a maximum of 7 days. Cultivars Chaibi, Brahmi bloom 1 or 2 days later but cv. Jeli bloom 7 days later. The late blooming cultivar (Jeli) had longer blooming period but the flowers are predominantly staminate and their development retarded.

The onset of the bloom season varied between 17 and 21 days for the cultivars. Brahmi cultivar showed the shortest bloom. We noted during the experiments when winter was cold, late blooming followed. A clear influence of spring temperature on flower development was recorded. It’s in agreement with Bernad *et al*. (1995) on almond. Low spring temperatures increase the length of the bloom period while higher temperatures shorten it.

We have also observed a relationship between date of flower opening and the percentage of perfect flowers of the four cultivars studied. The first-opening flowers were predominantly perfect. Sterile flowers tend to open later suggesting that the critical for crop setting is early flowering in olive and insist on the onset of flowering. This difference may be attributed in flower quality to physiological and nutritional effects and reflect also genetic differences as signalled by Bernad *et al*. (1995) on almond.

All these results indicate that the flowering behaviour of the four cultivars in Djebel Ouslet area is very interesting; it allows a good pollination between the cultivars. Also, the flowering periods

Table 1: Pollen viability, germination rates and tube growth of four olive cultivars grown in Djebel Ouslet

Cultivars	Pollen viability (%)	Pollen germination (%)	Tube length (μm)
Ousleti	86.2±3.9	60.6±2.1	642±6.9
Chaibi	83.7±1.9	53.2±2.9	411±8.1
Brahmi	81.9±0.9	51.9±1.3	431±9.2
Jeli	88.8±2.8	61.7±3.5	664.2±7.01

of all cultivars cover each other. Griggs (1975) reported that a difference of maximum 7 days between bloom initiations could insure an efficient pollination in olive (Griggs, 1975).

Pollen viability and germination: The data in Table 1 show that all the cultivars had pollen grains with a very high degree of viability never below 80%. No statistical differences were found in mean pollen viability and a positive correlation between fresh pollen viability and germination was recorded in all cultivars. The maximum obtained after 72 h incubation were 60.6, 53.2, 51.9 and 61.7% for Ousleti, Chaibi, Brahmi and Jeli cultivars, respectively. The longest length of pollen tubes achieved was 642 and 664 μm for Ousleti and Jeli cultivars and 411 and 431 μm for Chaibi, Brahmi, respectively.

The fasted rate of germination is represented by Jeli cultivar (61.7%) and pollen tubes reached 664.2 μm after 72 h incubation. A significant difference was observed for Brahmi and Chaibi cultivars which exhibited both slower germination and pollen tube growth and required at least 72 h to reach 50% germination and 400 μm tube length. Their pollen germination proceeded very slowly compared with Ousleti.

Many studies on olive pollen signalled the potential the usefulness of pollen germination on fruit setting and the effectiveness of insecticides and bioinsecticides sprayed on trees during flowering on pollen capacity (viability, germination and tube growth) (Mehri *et al.*, 2007a, b).

Pollination and fertility behaviour: The objective was to compare self and free pollination in these four cultivars, from 2004 to 2006, to study their fertility behaviour. The level of fruit set based on total number of flowers was significantly different between the four cultivars. The amount of fruit set was high in cultivars Ousleti (2.2%) and Jeli (2.15%) and low in cultivars Brahmi (0.82%) and Chaibi (1.03%). A good commercial yields of olives can be achieved with fruit set as low as 1-2% of the flowers (Martin, 1990).

Fruiting rates were higher under free-pollination than obtained following self-pollination in all cultivars (Table 2) and produced more than a 3 fold increase in fruit set over that of self-pollination in cultivars Chaibi and Brahmi and two times in cultivars Jeli and Ousleti. But fruit set under free-pollination conditions were not significantly different between cultivars; they were ranged from 2.8 to 3.75%. Differences between treatments (self and free pollination) were high but differences between cultivars for each treatment were not significative. These results were fully confirmed with the values of R1 index calculated and defined as the ratio of fruit set following self-pollination to that following free-pollination. It varied greatly in all cultivars from self and free pollination assays but all indicate that the four cultivars were compatible at different level (R1>0.5) from 0.52 and 0.59 for cultivars Ousleti and Brahmi to 0.71 for cv. Jeli. Values of R1 low and close to zero, has been suggested to be an indication of self-incompatibility.

We consider that all the cultivars were self-compatible in Djebel Ouslet environment but cross-pollination was necessary to improve fruit-set. The percentages obtained after free-pollination

Table 2: Percentage of fruit set in self and free-pollinated flowers (based on total number of flowers) and fertility index (R1) (as the ratio of fruit set in self and free pollination) of four olive cultivars in the area of Djebel Ouslet

Cultivars	Self-pollinated fruit set (%)	Free-pollinated fruit set	Fertility index (R1)
Ousleti	2.20±0.9	3.75±1.08	0.523±0.08
Brahmi	0.82±0.07	3.2±0.8	0.598±0.11
Jeli	2.15±1.02	3.6±1.1	0.716±0.09
Chaibi	1.03±0.2	2.86±1.06	0.647±0.17

indicated clearly the need for cross-pollination to ensure and enhance fruit set in the four cultivars studied as suggested on other olive cultivars by Cuevas *et al.* (2001), Lavee and Datt (1978) and Lavee *et al.* (2002). It is urgent to study and to investigate the cross-pollination effect on pollination and fruit set under these conditions. Differences and variations in fruit set were found within and between the cultivars, this can be attributed to varying degree of self-fertility in olive as suggested by Cuevas and Rallo (1990) and Lavee *et al.* (2002). Such results are in agreement with other experiments conducted on other Tunisian cultivars in other environments, cv. Gerboui from the northern part of Tunisia (Mehri and Mehri-Kamoun, 2007).

Pollination results and the high pollen capacity of all cultivars (high pollen viability and “in vitro” germination rates) recorded in all cultivars studied, can suggest the existence of intercompatibility between those cultivars and showed their good performance as pollinizer for olive cultivars which are self-incompatible such as Meski, a Tunisian table olive (Mehri and Kamoun-Mehri, 1995; Mehri *et al.*, 2003).

Regarding to the high pollen capacity obtained, we can suggest a relationship between fertility behaviour of the cultivars studied and pollen performance. In fact fruit set following self and free pollination was correlated with high performance of self pollen tubes grown “in vitro” recorded in this study. Also, these findings of high pollen capacity suggested that all cultivars can be used as pollinizers in the cross-pollination to improve productivity. Also, the high pollen germination and high tube growth are consistent with the self-compatibility degree of the four cultivars studied.

Olive quality parameters: Olive quality parameters have been studied for all the cultivars (including oil content, fruit weight flesh to pit ratio and fatty acid composition) because they present major parameters of interest to growers. Comparison between all cultivars showed variability in quality parameters (oil and fatty acid contents of the fruit) based on IOOC standards and the ranges of fatty acid composition for all cultivars studied and listed in Table 3 and 4, fall within the accepted limits for fatty acid composition of Virgin Olive Oil (IOOC, 1997).

Oil content: From the results of oil yield shown in Table 3, the best performing cultivar in this site in terms of olive oil is Ousleti. There are, however large differences in oil content observed across the cultivars examined. Oil yields ranged from 5 for cv. Jeli to 25% for Ousleti fruits. Ousleti and Chaibi cultivars produced more than triple the amount of oil produced by cv. Jeli. Other factors must be considered since the characterization of olive oil is considered by many researchers as difficult due to variation in composition caused by extraction methods, environmental and storage conditions (Sweeney, 2003). Earlier findings (Lavee and Wodner, 2004), showed that the final oil content in the fruits is dependant on the interaction between the growing conditions and the genetic potential of the variety.

Jeli, Brahmi and Chaibi cultivars have low fruit weight, while Ousleti has medium sized olives. The average of olive weight and flesh to pit ratio were lower mainly in Chaibi and Jeli cultivars.

Table 3: The average and respective standard deviations of fruit weight, oil content and flesh to pit ratio of four olive cultivars in the survey of Djebel Ouslet

Cultivars	Average fruit weight	Oil content (%)	Flesh to pit ratio (%)
Jeli	2 g<w	5±1.2300	4.93±1.26
Brahmi	2 g<w	16.4±2.63	5.47±0.92
Ousleti	2 g<w<4 g	25±2.140	6.57±1.4
Chaibi	2 g<w	13.5±1.61	3.89±0.75

Table 4: Oil and fatty acid contents of four olive cultivars at Djebel Ouslet (Tunisia)

	Composition (%)						
Cultivars	Palmitic acid C16:0	Palmitoleic acid C16:1	Stearic acid C18:0	Oleic acid C18:1	Linoleic acid C18:2	Linolenic acid C18:3	Unsat/sat ratio
Accepted limits	7.5-20%	0.3-3.5%	0.5-5%	55-83%	3.5-21%	<1.0%	
Jeli	15.1±1.47	1.21±0.63	2.2±0.78	66.8±1.4	13.1±1.92	0.73±0.19	4.72
Brahmi	9.6±0.93	0.6±0.25	2.6±0.9	80.4±2.8	5.3±0.97	0.43±0.09	7.05
Ousleti	12.2±1.03	0.58±0.10	1.9±0.6	71.8±2.4	12.2±1.25	0.48±0.08	6.01
Chaibi	9.1±0.87	0.6±0.12	2.1±0.6	74.5±1.3	7.2±1.13	0.48±1.03	7.3

±Standard error of the difference, the accepted limits for fatty acid composition of Virgin Olive Oil (IOOC, 1997) are shown in the first row

Ousleti was the only medium sized olives with highest oil content. Fruit weight is one of the main variables to be considered when assessing the suitability of an olive cultivar for mechanical shakes at harvest (Civantos, 1996). The cultivars of smaller fruit such as Jeli have lower oil content than Ousleti with medium sized olives. It is generally accepted in olive tree that cultivars with smaller fruit have at maturation higher oil content than cultivars with large fruit (Morettini, 1972; Patumi *et al*., 2002; on cv. Kalamata).

Pit ratio of fruit was measured for all the cultivars because it is an important value for the table olive cultivars while oil yield is most important for the oil producing cultivars. Flesh to pit ratio greater than 5:1 is an indicator of suitability of olives for table fruit (Rahmani *et al*., 1997). Table 3 shows that the cultivars Ousleti and Brahmi have a ratio greater than 5:1 (6.57 and 5.45, respectively) indicating their suitability for dual purpose (oil and table olive production) while cultivars Chaibi and Jeli have lower, with 3.89 and 4.93, respectively. There are other factors such as shape, size and colour of fruit that are also of great importance and that are considered in this study.

Fatty acids: Table 4 shows the means of six fatty acid concentrations of the four olive cultivars. Comparison cultivars indicated that oils of the four olive cultivars have acceptable levels of oleic acid varying from 66.8 to 80.45%. Palmitic acid (C16:1) which is a saturated fatty acid and undesirable varied from high (15.1% in cv. Jeli) to low in cultivars Brahmi and Chaibi (9.68 and 9.13%, respectively). Oleic acid was the predominant fatty acid while linoleic acid was detected in very low rate, lower than the 1% limit set for virgin olive oil (Burr, 1998). The oleic acid content which is a monounsaturated fatty acid, was high in cultivars Brahmi, Ousleti and Chaibi, while the saturated fatty acid content was lower according to IOOC standards. A high level of oleic acid is considered favourable in olive oil due to enhanced oxidative stability (Smouse, 1996) and superior nutritional quality (Kritchevsky, 1996). The proportions of these 3 fatty acids were significantly different for each of the cultivar and are important as they have been used to indicate the best time for harvesting olives (Rahmani *et al*., 1997; Mailer *et al*., 2005; Sweeney, 2003). Linolenic acid (C18:3), a minor fatty acid in olives, present a level below 1% with a maximum in Jeli cultivar (0.73%).

The unsaturated/saturated and monounsaturated/polyunsaturated acid ratios of oil varied among cultivars from low in Jeli to high in Brahmi, We have determined in this study these two parameters which are of great interest and influence the quality of oil. The unsaturated/saturated influences the organoleptic characteristics of the oil; as oil with a high content of saturated fatty acids is more viscous. The monounsaturated /polyunsaturated acid ratio is important to the intrinsic oxidative stability of the oil and is associated to high stability and low rancidity of olive oil (Tous and Romero, 1993).

Because of lack of pruning and irrigation and poor health, the cultivars are difficult to compare for olive yield. But we consider that this olive population is a valuable genetic resource for olive germplasm and will provide a valuable gene pool for the future. The fatty acid profiles of these four cultivars can help in future selection of nutritionally olive oil. These results have revealed much about the performance of the four olive cultivars. However more data is required on total yields, health and vigour to gain a fuller picture on cultivar suitability for Djebel Ouslet area.

We noted a relationship between fruit size, oil content and fatty acid composition in all cultivars. The final oil content in olive fruits is dependent on the interaction between the growing conditions and the genetic potential of the cultivar (Lavee and Wodner, 2004) and seasonal climatic variations influence the oil content in olive fruit and fatty acid composition (Robards and Mailer, 2001). A correlation between fruit weight and flesh to pit ratio indicates that a greater fruit weight implies a proportionally higher increase in flesh than endocarp weight. Leon *et al*. (2004) have also reported a positive correlation between flesh oil content and flesh to pit ratio and between oil yield components and fatty acid composition.

Factors such as olive cultivars, cultivation area, seasonal climatic variations and agronomic practices of Djebel Ouslet can influence floral biology and oil quality parameters of cultivars studied. This also can be due to an alternate bearing phenomenon accentuated by the dry climate conditions.

Kaskoos *et al*. (2009) determined the fatty acid composition of the olive oil of cv Iraqi and noted that the unsaturated/saturated ratio was 3,25. The high unsaturated fatty acid content signified its potential as a health promoter. It can be expected to offer considerable resistance to oxidative rancidity during storage.

Biometric characteristics

Trees and shoot development: Table 5 includes, for each cultivar, shoot growth during vegetative period (length, number of leaves and internodes per shoot), trunk circumference at 0.45 cm from the ground and the height of every cultivar. The result of vegetative performance

Table 5: Average tree height and diameter, shoot growth, number of leaves/shoot and internodes/shoot of four olive cultivars grown in Djebel Ouslet

Cultivars	Shoot length (cm)	No. of leaves $shoot^{-1}$	No. of inter nodes $shoot^{-1}$	Height (cm)	Trunk circumference (cm)
Ousleti	12.58	14.25	8.62	219.7	49.29
Brahmi	11.41	14.01	6.79	266.4	42.67
Chaibi	12.04	15.12	8.23	254.9	35.71
Jeli	9.76	13.91	7.21	235.1	42.88

reported in Table 5, show that the height ranges from 219.7 cm for cv. Ousleti to 266.4 cm for cv. Brahmi which is tallest tree. Tree size is an important consideration for straddle harvesters which can only harvest trees less than 250 cm tall (Robards and Mailer, 2001; Sweeney, 2003). So cv. Ousleti was the more suited for harvesting. The highest trunk circumference is recorded in Ousleti with 49.29 cm and all cultivars have values between 35 and 50 cm, this parameter is an indicator for the tree vigour (Nieddu *et al.*, 1995). The trees are vigorous from open (cv. Brahmi) to a close growth habit (cultivars Ousleti, Chaibi and Jeli), with dense canopy and few sylleptic shoots.

Shoot elongation reached at the end of vegetative growth period (November-December) an average of 12.58, 11.41, 12.04 and 9.76 cm, for cultivars Ousleti, Brahmi, Chaibi and Jeli, respectively. The number of leaves and internodes per shoot developed during this period give a mean internode length of about 14 mm for all the cultivars.

Leaf description: The shape of the leaves which is determined from the ratio between length and width is elliptic-lanceolate for all the cultivars. The leaf basal angle is acute for all the cultivars while the apical angle is open for Jeli and acute for Ousleti and Chaibi and very acute in Brahmi. The leaves of Jeli are short and wide while for the other cultivars, leaves are long and narrow.

Inflorescence description: A general description of the inflorescence of the four cultivars studied was determined recording their length, number of flowers and perfect flowers (Table 6). The average inflorescence length is medium between 25 and 35 mm for Jeli, Brahmi and Chaibi and short (<25 mm) for Ousleti cultivars. The number of flowers/inflorescence is also medium varying between 18 and 25 flowers (17 for cultivars Jeli and Brahmi cultivars, 19 for cv. Chaibi and 22 for cv. Ousleti).

The percentage of staminate flowers (pistil abortion rate) differed among cultivars; it was very high in cv. Brahmi and low in cv. Jeli. The latest cultivar produced less number of flowers (17 per inflorescence) but less staminate flowers rate (pistil abortion, 28.14%). In the contrary, with the same number of flowers per inflorescence (17), Brahmi flowers exhibit higher percentage of sterility with a highest pistil abortion (39.9%). The number of perfect flowers per inflorescence ranged between 9 (cv. Brahmi) and 12 (cultivars Jeli, Chaibi and Ousleti). A high proportion of sterile flowers is considered as a negative trait in the evaluation of fruit set and olive yield (Lavee *et al.*, 1999). We noted also that the lowest percentage of perfect flowers was generally recorded on the northern side of the tree and in the top of the shoots, while the highest percentage was recorded on the southern side and in the middle of each flowering shoot. The first flowers to open were of better quality than the later-opening ones since they were perfect flowers and gave the highest fruit-set. Physiological and environmental effects such as nutrition, irrigation can be an important determinant of olive flower quality and the differences recorded between cultivars reflect probably genetic differences (Cuevas *et al.*, 1994; Lavee *et al.*, 1996; Lavee, 2004).

Table 6: Average number of flowers, of perfect flowers/inflorescence, pistil abortion and perfect flower rates of four olive cultivars grown in Djebel Ouslet

Cultivars	No. of flowers inflorescence^{-1}	No. of perfect flowers inflorescence^{-1}	Pistil abortion (%)	Perfect flowers (%)
Ousleti	22	12.3	39.7	60.3
Brahmi	17	9.06	46.75	53.25
Jeli	17	12.22	28.14	71.86
Chaibi	19	12.4	34.78	65.2

Fruit description: The symmetry of the fruit is observed on Jeli cultivar but Brahmi, Ousleti and Chaibi fruits are slightly asymmetric. All the cultivars show absence of olive nipple, a central position of maximum transverse diameter of the fruit. The shape of the fruit which is determined from the ratio between the length (L) and the width (W) is elongated except for Ousleti cultivar which is ovoid. Fruit base is truncated for cultivars Jeli and Chaibi and rounded for cultivars Ousleti and Brahmi. The fruit apex is rounded for the cultivars Brahmi, Ousleti and Chaibi and pointed for cv. Jeli. All the cultivars showed absence of olive nipple. Localisation of initial turning is from the base for cultivars Ousleti, Jeli and Chaibi while for cv. Brahmi, the initial turning is uniform under all epidermis. The peduncular cavity is deep in cultivars Ousleti and Brahmi and superficial in cultivars Jeli and Chaibi fruits.

Endocarp description: The endocarp apex of all the cultivars is sharp-point with a short mucro. The cultivars Jeli, Ousleti and Chaibi present a maximum transverse diameter positioned in the central part of the endocarp while in Brahmi endocarp, the maximum transverse diameter is towards apex. The endocarp distribution of fibrovascular sulcus is half uniform for all cultivars. The endocarp is rugose for Brahmi and Chaibi and smooth for Jeli and Ousleti cultivars. Grooving is medium for Brahmi, Ousleti, strong for Jeli and weak for Chaibi. The course of the grooving is longitudinal in Brahmi, Ousleti, Chaibi and not uniform in Jeli endocarp. The depth of grooving is weak in cv. Ousleti and medium in cultivars Brahmi, Jeli and Chaibi.

In the present study, technique based on morphological characters was used to evaluate the level of genetic variation within a mountainous olive population situated in the central-east area of Tunisia which is precisely Djebel Ouslet. Djebel Ouslet varietal structure is somewhat confusing and no systematic studies exist on the olive cultivar performance growing in this area. This work concerned the most representative olive cultivars Jeli, Brahmi, Ousleti, Chaibi and the discrimination of these olive cultivars revealed variability within and between cultivars indicating a wide and diverse genetic background of olive in Djebel Ouslet.

The relative differences within cultivars was based mainly on flowering, fruiting patterns, quality parameters based on IOOC standards (oil yield and fatty acid contents of the fruits) and in fruit and endocarp descriptions. It is clear that morphological characters (using 32 biometric parameters) permit to discriminate between most cultivars but the variation within trees is however less than variation between cultivars. The results obtained in this work showed a high level of precision and different correlations between characters among cultivars was determined. The knowledge of these correlations could also allow the improvement of the method used by reducing the number of parameters (9 most discriminating variables instead of the all set of 32 as recommended by IOOC (1999) as suggested by Pinheiro and da Silva (2005).

The ranges of fatty acid composition for all cultivars studied, fall within the accepted limits for fatty acid composition of Virgin Olive Oil (IOOC, 1997). The results obtained illustrate the link between fruit weight of cultivars and the fatty acid composition. Also, a close link between pollen capacity of four cultivars and their compatibility behaviour was observed. However other important parameters have to be taken in account such as local climatic and edaphic conditions. A high degree of genetic variability was observed among olive cultivars probably due to varying degrees of cultivar adaptability to the pedoclimatic conditions and agronomic practices adopted in the field trials (Patumi *et al.*, 1999, 2002), to fruit size, to commercial use (oil or table uses) and to principal area of cultivation (Sanz-Cortes *et al.*, 2001; Nikoloudakis *et al.*, 2003).

The effectiveness of pomological characters in discriminating the most representative cultivars in Djebel Ouslet seems to be not sufficient but could be exploited for screening synonyms within the olive population and could provide information for cultural purposes and breeding programs. It is important to compare all these physiological and morphological data provided for each cultivar with a DNA fingerprinting results in the future. Correct identification of germplasm resources is of importance to plant material management (Sanz-Cortes *et al.*, 2001). The investigation of Guerin *et al.* (2002) on the genetic identity of Australian olive accessions, has shown that many of the commercially used cultivars known under different names have identical DNA fingerprints so they are synonyms. Intracultivar variability in olives has been detected using the RAPD technique (Fabbri *et al.*, 1995; Mekuria *et al.*, 1999, 2002) and ISSR markers (Terzopoulos *et al.*, 2005). The use of molecular markers can be used in parallel to pomological characters as an alternative tool with optimal results for the olive crop. The main disadvantage of this method is the long analysis process which is subjective and can involves errors.

CONCLUSIONS

The characterization of this olive genetic resource of Djebel Ouslet is very important since both olive productivity and oil quality are traits inherent to a cultivar. The information obtained in this work can serve for varietal improvement and will enable olive producers to make informed cultivar choices from this morphological characterization and the performance of the most representative olive cultivars grown in Djebel Ouslet.

The results obtained can resolve the confusion in olive cultivar identity mainly for local cultivar in Tunisia. Several names exist for some cultivars and the cultivar which is most abundant within Djebel Ouslet grove is Ousleti. This cultivar seems to be mixed up with different names such as El-Horr (from seedlings), El-Guim (when grafted on wild olive trees). Many trees showed signs of rootstock (wild Oleaster) or feral olives (from germinated seeds growing at the base of the trees). Also cv. Chaibi has been identified as synonym of cv. Chetoui, the most widespread olive cultivar in the northern area of Tunisia.

In addition to generate regional employment, olive trees in Djebel Ouslet is an important source of income in these rural areas and provides ecological benefits by contribution to soil retention and reducing erosion.

This diversity within the Djebel Ouslet grove offers an excellent source of genetic material for future research and agriculture. More research and development studies must be achieved on this plant material, since it provides a unique opportunity to study a large range of olive cultivars, a large gene pool of trees representing many of interest cultivars well adapted to this home locality.

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