An-Najah National University
Faculty of Graduate Studies

Morphological and Genetical Characterisation of the main Palestinian olive (O*lea europaea* L.) cultivars

By
Ramiz Jawad Omar

Supervisor
Dr. Hassan Abu Qaoud

This Thesis is Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Plant Production, Faculty of Graduate Studies, An-Najah National University, Nablus, Palestine.

2012

Morphological and Genetical Characterisation of the main Palestinian olive (O*lea europaea* L.) cultivars

By
Ramiz Jawad Omar

This thesis was defended successfully on 14 /2/2012 and approved by:

Defence Committee Members		**Signature**
-Dr. Hassan Abo Qaoud	**(Supervisor)**	**..……**
-Dr. Aziz Barghoothi	**(External Examiner)**	**……..**
-Dr. Hiba Al fares	**(Internal Examiner)**	**……..**

Dedication

This work is dedicated to my father, mother, wife, brothers, sisters and my friends; the completion of this work was not possible without their support and help.

Acknowledgments

I would like to express my deepest respect and most sincere gratitude to my supervisor Dr. Hassan Abu Qaoud for his guidance and encouragement at all stages of my work. In addition I would like to thank my committee members, Dr. Hiba Al Fares and Dr. Aziz Barghoothi.

Another word of special thanks goes for all members of the Department of Plant Production at the Faculty of Agriculture at An-Najah National University.

Last but not least my thanks and gratitude to my family, friends and colleagues in my work for their help and support.

الإقرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان :

Morphological and Genetical Characterisation of the main Palestinian olive (O*lea europaea* L.) cultivars

أقر بأن ما اشتملت عليه هذه الرسالة إنما هي نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها لم يقدم لنيل أية درجة أو لقب علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى .

Declaration

The work provided in this thesis , unless otherwise referenced , is the researcher's own work , and has not been submitted elsewhere for any other degree or qualification.

Student's Name : **اسم الطالب:**

Signature: **التوقيع:**

Date: **التاريخ :**

Table of Contents

List of Figures

List of Tables

List of Abbreviations

Abbreviation	Full Name
AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analysis-of-Variance
Avr.	Average
B.C	Before Christ
cm	Centimeter
ºC	Centigrade
cv	Cultivar
DNA	Deoxyribonucleic Acid
DW	Dry Weight
FRF	Fruit Retintion Force
g	Gram
IOOC	International Olive Oil Council
kg	Kilo Gram
L	length
M	Meter
Meq	Millie Equivalent
MI	Maturation index
mm	Mille Meter
mM	Mille Mole
MOA	Ministry of Agriculture
N	Newton
O.	Olea
PCBS	Palestinian Central Bureau of Statistics
PCR	Polymerase Chain Reaction
RAPD	Random Amplification of Polymorphic DNA
SAS	Statistical Analysis System
SE	Standard error
SPSS	Statistical Package for the Social Sciences
SSR	Simple Sequence Repeat
UV	Ultra Violet
W	Width

Morphological and Genetical Characterisation of the Main Palestinian Olive "*Olea europea L.*" Cultivars

Prepared By

Ramiz Jawad Omar

Supervised by

Dr. Hassan Abu Qaoud

Abstract

A study was conducted to compare morphological, biochemical and genetical characterstics of the main olive cultivars in Palestine. The cultivar studied were; Nabali Baladi , Nabali Mohassan and Souri. Samples were taken from leaves, flowers, fruits and stones for both morphological characters, oil was extracted from the different cultivars for biochemical analysis, for molecular analysis DNA was extracted from leaf tissue and SSR primer analysis was used. Genetic distances between individual trees were calculated using Dice similarity coefficient and the dendrogram based on UPGMA cluster analysis was constructed.

Notable significant differences among the cultivars were observed in all characteristics considered,including ; tree canopy, leaves, inflorescence and fruit characterstics. The acidity, peroxide number and the spectro-photometer absorbencies in ultra-violet were low of the oils of all cultivars were very low. Most cultivars had an oleic content of about 60% or higher except for the cultivar Nabali Mohassan. The sterol composition and content were quite different in the cultivars. The Nabali Baladi cultivar had

a relatively high value of Δ-7stigmastenol. All of the biochemical values (acidity, peroxide number, absorbencies in ultra-violet, fatty acid composition, sterol composition and content) used to evaluate oil quality were within the IOOC trade standards. Microstalite matker was used for fingerprinting and for evaluation of genetic similarity of eight olive sample which collected from Palestine. Seventeen alleles were revealed with five SSR that were selected based on previous literature. The number of allele per locus varied from 2.0 at GAPU-103 and DCA9 to 5.0 at U99-36 and DCA16. The eight olive samples were classified into three major clusters using UPGMA clustering analysis; cultivar Nabali Baladi represent the first group and consisted of four samples. Some morphological and biochemical characteristics of cultivar Nabali Baladi were also distinct from those of the other cultivars; the second cluster consisted of three sample that represent Nabali Mohassan; the third cluster contained only one sample that represent Souri cultivars. The similarity coefficients between the eight olive trees samples varied from 1.0 to 0.31. These SSR loci allowed unequivocal identification of all the cultivars and will be useful for future breeding and olive germplasm management efforts.

Chapter One

Introduction

Introduction

The cultivated Olive (*Olea europaea* L.) is a long-lived evergreen tree native to the Mediterranean basin (Poljuha *et al.*, 2008). It is the most important fruit trees produced commercially in most of the Arab countries. The cultivated olive has developed alongside Mediterranean civilizations and is now commercially produced on more than 9400 million donum in the Mediterranean basin (Paul Vossen 2007).

Palestine is one of the oldest agricultural settlements in history. Evidances revealed by archeological excavations indicated that olives were cultivated befor about 6000 years in palestine. It is not possible to overestimate the importance of olives to the Palestinian economy. Not only are olives the single biggest crop in what remains a largely agricultural economy, but they have deep cultural significance as a symbol of traditional society and ties to the land. It is estimated that olive trees account for nearly 45 percent of cultivated land in Palestine and in good years can contribute as much as 15-19 percent of agriculture output. Given that agriculture accounts for nearly 25 percent of GDP, olives are an important element of the Palestinian economy and estimates suggest that about 100,000 families depend to some extent upon the olive harvest for their livelihoods.(The World Bank 2012). About 90- 95 percent of the Palestinian olive harvest is used to produce olive oil, In the past decade average oil production in good years has been around 20,000-25,000 tons. The quantity of olive oil produced in 2010 reached 23,754 tons (PCBS,

2011). In addition, Palestinian oil is considered to be of high quality among other olive oils in the world. Several factors affect oil quantity and quality , among these are cultivar, cultural practices , harvesting method , processing , handling and storage, and harvesting time. It is well known that oil quality is highly affected by the type of cultivar, it contributes to about 30% of oil quality. Hundreds of olive cultivars are grown in various microclimates and soil types worldwide. Bartolini *et al*. (1993) have ascertained about 1,200 named olive cultivars with over 3,000 synonyms throughout the world. There is much confusion and uncertainty concerning the identity of the olive trees in a region (Ozkaya *et al*. 2008).

In Palestine, there are different olive cultivars known, but the most dominant and most preferred cultivar given by olive growers in Palestinian territories, is the 'Nabali' cultivars, due to it's suitability for picking and oil extraction purposes, and to it's adaptation to the rainfed condition of the region. Other olive cultivars originating from the Mediterranean basin differ morphologically and physiologically. In fact, differences can be found in tree, leaf and fruit shape; oil content and characteristics; productivity; ability to self-fertilizing; susceptibility to certain diseases, etc. In addition, most of the olive trees are non cloned with high variability among the trees within a clone . The wide genetic patrimony and the large number of synonyms and homonyms in olive require precise methods of discrimination for cultivar identification and classification. Different techniques have been used to evaluate olive

diversity. Morphological, agronomical or biochemical characterisation has been adopted for variability evaluation (Leva Annarita 2009).

To date, very few studies have evaluated the morphological, phenological, bio-agronomical and productive characteristics of Palestinian olive varieties. Therefore, the objectives of this study were:

1. To conduct morphological and biochemical description of olive local cultivars in Qalqilia district.
2. To conduct genetic characterization of selected local olive cultivars in Qalqilia district.

Chapter Two

Literature Review

2. Literature Review

2.1. Olive History and Importance

The olive tree originating from the Eastern Mediterranean is one of the oldest cultures, belonging to the family Oleaceae with 30 genera, among which there are certain decorative plants. Most of the olive groves belong to the species *O. europaea*, with 2x = 46 chromosomes. The species *O. europaea* includes many groups and more than 2600 cultivars, many of which may be ecotypes. *Olea europaea* does not seem to be a true species but one group of forms derived from hybridism and mutation. The tropical and subtropical Afro-Asianspecies, such as *O. chrysophilla* and *O. excelsa*, probably participated in the evolution of the culture. Sub-species of olive are distributed in the Mediterranean countries and also in West Africa, Tanzania, the Canary Islands, the Azores, South Africa, etc. Olive trees have been introduced to the USA, Australia, South Africa and China in more recent decades, (Breton *et al.*, 2006). Archeological evidence suggest that olives were being grown in Crete as long ago as 2,500 B.C. From Crete and Syria olives spread to Greece, Rome and other parts of the Mediterranean area." Spain is the world's largest cultivator of olives, producing 970,000 tons of olives annaully. Spain and Italy together account for 50% of the total amount of olive oil produced worldwide. (Therios 2009). (fig. 1).

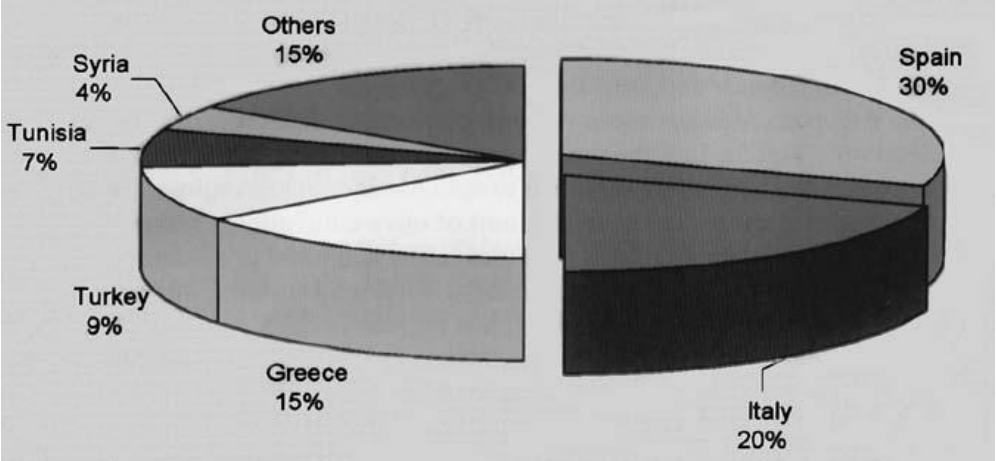


Fig.1. The average annual total world production of olives during the period 1998–2001 (15,090,620 t) (from FAOSTAT, 2003).(from Therios 2009)

2.2. General Morphology of the Olive tree

2.2.1. Leaves

The leaves of olive trees are grey–green and are replaced at 2–3 year intervals during the spring after new growth appears. The olive's feather-shaped leaves grow opposite one another. Their skin is rich in tannins, giving the mature leaf its grey–green appearance. Leaves have stomata on their lower surface only (Fernndez *et al*., 1997). Stomata are nestled in peltate trichomes, restricting water loss and protecting leaves against UV radiation (Karabourniotis *et al*., 1992, 1995). The leaves are covered by a layer of wax and cutin (cuticle). On both surfaces peltate trichomes exist and their concentration is 143/mm^2 on the lower surface but only 18/mm^2 on the upper. Stomates are present (470/mm^2) only on the lower surface (Martin, 1994; Fernndez *et al*.,1997). Leaf age affects stomatal conductance (Gucci *et al*., 1997). Stomata play a significant role in sensing and driving environmental change (Hetherlington and Woodward, 2003).

2.2.2. Inflorescences and flowers

2.2.2.1. Inflorescences in Olives

Inflorescences are born in the axil of each leaf (Fig. 2). Each inflorescence contains 15–30 flowers,. Vegetative buds are induced to become flowering ones after the winter's chilling effects. They then begin to grow, producing inflorescences. The blossoms usually begin to appear in May.

Fig 2: olive infloursence from (Therios 2009)

2.2.2.2. Flowering in Olives

The olive flowers are small, creamy white and hidden within the thick leaves. Each flower consists of a four-segmented calyx, a tubular corolla with four lobes, two stamens and an ovary with two carpels and a short style (Martin, 1994). The flowers are divided between two categories: perfect, having stamen and pistil, and staminate (male) flowers, where the pistil is aborted while the two stamens are functional. In the perfect flower the pistil is large, green in colour and fills the space in the floral tube.

Staminate flowers are very small and do not fill the floral tube; the style is greenish white and small. (Fernndez-Escobar *et al*., 1992; Cuevas *et al*., 1999).

2.2.3. Fruit

The olive fruit is a drupe, spherical or elliptic in shape and consists of the exocarp (skin), which contains stomata, the mesocarp (flesh), which is the edible portion of the fruit, and the endocarp (pit), including the seed. The fruit of the olive tree is purplish black when completely ripe, but a few cultivars are green when ripe and some olives develop the colour of coppery brown. The size of the olive fruit is variable, even on the same tree, and depends on cultivar, fruit load, soil fertility, available water and cultural practices (Therios 2009).

2.3. Moelcular characterization in olives

Several different types of DNA markers are currently available for genetic analysis and new marker types are being developed continuously. Markers differ from each other in many respects: the initial workload and costs for building up the marker system, running costs and ease of use, level of polymorphisms, dominance, number of loci analyzed per assay, reproducibility and distribution on the chromosomes. Detection of polymorphism at the DNA level is usually based either on restriction patterns or differential amplification of DNA. The choice of the best marker system depends on whether it will be used in evolutionary or population studies, genetic mapping or fingerprinting. The ploidy level and reproductive system of the organism studied are also important.

Acomparison of DNA-markers used in barley is shown in table (1).Morphological and biological characters have been widely used for descriptive purposes and are commonly used to distinguish olive cultivars (Barranco & Rallo, 1985; Cantini *et al*., 1999; Barranco *et al.,* 2000). Agronomic characterization also allowed the classification of different olive cultivars (Barranco *et al.*, 2000; Del Rio, 1994). Morphological and RAPD analyses were performed on 8 brown olive populations of Iran using 24 morphological characters. ANOVA test showed significant difference in leaf length and leaf width among different populations and principal components analysis showed that the leaf characteristics (venation, width, trichome, colour in the ventral and dorsal surfaces), number, and distribution of grooves in the endocarp and fruit characteristics (apex, base, and shape) are the most variable characters among the brown olive populations studied. The 38 RAPD primers used produced 541 reproducible bands (loci) out of which 515 bands were polymorphic and 26 bands were common in the populations studied, (Sheidaia *et al.,* 2010).

It is well established in literature that using different molecular markers like RAPD and AFLP explored considerable extent of genetic variation within olive cultivars. For example, in the study of Wiesman *et al*. (1997), genetic differences of about 30% was revealed when comparing eight variants of 'Nabali'. In another study, the comparability of eight olive microsatellite profiles in 17 cultivars generated by four laboratories using different DNA genotyping platforms was tested. In total, 54 alleles

were identified, from a minimum of 3 alleles (DCA15) to a maximum of 12 (DCA9), averaging 6.75 alleles per marker (Doveri *et al.*, 2008). Eighty-four olive accessions in Tunisia, previously evaluated for morphological traits, were analysed with 47 random amplified polymorphic DNA (RAPD) markers. The highest and lowest similarities between genotypes, estimated by simple matching algorithm, were 0.98 and 0.40, respectively. The results showed that most of Tunisian accessions are closely related to olive genotypes originating from the Eastern Mediterranean and some are clustering with genotypes originated from the Western Mediterranean (Zitoun *et al.*, 2008). Amplified fragment length polymorphism (AFLP) analysis was used to evaluate the genetic biodiversity and variability present in some Italian varieties of cultivated olive. A group of 12 genotypes belonging to three varieties was screened using six different AFLP primer combinations. For the varieties analyzed, the data revealed significant genetic diversity in the cultivated olive tree, despite the fact that they come from a limited geographical area (Sensi et al., 2003). DNA fingerprinting (RAPD and ISSR) was performed to access the level of intra-varietal genetic variability within a collection of 120 clones of the Portuguese olive 'Cobrançosa' . The data indicates a wide intra-varietal genetic variability among the clones (Martins-Lopes et al., 2009). Two inter-simple sequence repeat (ISSR) markers (one UBC-818, rich in CA and the other UBC-849, rich in GT) were effeciently used for the differentiation of 31 *Olea europaea* L. cultivars grown in Greece (Terzopoulos et al., 2005). A study was conducted in Turkey to examine

the relationships between accessions considered to represent cv. Derik Halhali and identify the most closely linked one. The results showed that the Derik Halhali accessions collected from Derik–Mardin province differ at various degrees from the standard Derik Halhali cultivar. This classification based on RAPD markers could not be related to known morphological information about the accessions (Ozkaya et al., 2006). Preliminary results of AFLP analysis indicate that olive cultivar Oblica can be regarded as mixture of clonal variants. (Strikic et al., 2010). Morphological and molecular analyses for the characterization of a groupof Italian olive cultivars were studied, the morphological and molecular data led to similar representations of the cultivar relationships. However, only the AFLP and SSR data were able to characterize specific olive varieties and identify erroneous denominations and cases of synonymy.(Rotondi, 2003).

Table 1: Comparison of different DNA-marker systems.

	RFLP	RAPD	SSR	AFLP	ISSR
Principle	Southern blotting of restricted fragments	PCR of rando m pri-mers	PCR of Micro-satellite	Detection of DNA restriction fragments by PCR	PCR of inter simple sequence repeats
Level of polymorphism	Medium	Medium	Very high	Medium	Medium
Codominance of alleles	Codominant	Dom-inant	Codo-minant	Dominant	Dominant
Number of loci analyzed per assay	1-2	3-15	1	40-150	3-12
DNA required per assay	2-10 µg	10-20 ng	20-50 ng	20-500ng	10-20ng
Prior sequence information	Yes	No	Yes	No	No
Developmental cost	High	Low	High	Medium	Low
Running costs per assay	Medium	Low	Medium	Medium	Low
Repeatability	Very high	Fair	Very high	Very high	Medium-high
Ease of use	Labour intensive	Easy	Easy	Difficult initially	Low

SSR markers have been previously used in genetic diversity and relationship studies in olive cultivars (Cipriani *et al.,* 2002; Michele., *et al.*, 2006; Taamalli., *et al.,* 2008; Bracci, *et al.,* 2009; Muzzalupo., *et al.,* 2009; Vietina ., *et al.,* 2011). The codominant nature of SSR marker permitted the discrimination of olive trees samples to their genotypes as indicated in other studies (Belaj *et al., 2003;* Powel *et al.,* 1996). Several DNA marker including RAPD and AFLP used to investigate olive trees

genotype SSR was considered more powerful in many studies. The random amplified polymorphic DNA (RAPD) technique has been applied in several studies to successfully distinguish between olive cultivars (Belaj *et al.,* 2001; Fabbri *et al.,* 1995; Guerin *et al., 2002;* Mekuria *et al.,* 1999). Owen *et al.,* (2005) sampled 65 olive genotypes including most of the important cultivars from Turkey, Greece and the Middle East and selected genotypes from the western Mediterranean area. They obtained a total of 119 polymorphic markers generated from five selective AFLP primer-pair combinations, which resulted in a 41.5% polymorphism ratio. The combined data sets generated by just two primer pairs were adequate to discriminate all 65 genotypes. Sensi *et al.,* (2003) characterized a total 12 olive cultivars originating in Italy using AFLP markers. AFLP analysis of 12 cultivated olive accessions using six pairs of primers provided a total of 274 markers. Grati-Kamoun *et al.,* (2006) characterized 29 olive (*Olea europaea* L.) cultivars including oil and table olive cultivars originating from Tunisia and other Mediterranean countries using AFLP markers. Using nine AFLP primer combinations, they produced a total of 410 AFLP markers, among which 172 revealed polymorphism. The results demonstrated a high degree of polymorphism in the olive germplasm with an average of 39%. Nowadays simple sequence repeat (SSR) have been proven to be very suitable markers for cultivar identification and identity typing in olive as they are transferable, highly polymorphic and co-dominant markers (Carriero *et al.,* 2002*;* Cipriani *et al.,* 2002; Rallo *et al*., 2000).

Chapter Three

Materials and methods

3. Materials and methods

3.1. Plant materials

The study was carried out during the growing season 2010-2011 in kufr qaddoum village in the west of qalqilia distrect in West Bank . Morphological and genotype description of the major cultivated olive cultivars was carried out on three olive cultivars, Nabali Baladi (four trees), Nabali Mohassan (three trees) and Souri (two trees) with a fourty years old trees.The trees were exposed to the traditional agricultural practices incloding plowing, pesticide aplication, pruning. In aditionn, each tree were suplide with two M^3 of water per month.

3.2. Morphological investigation and characterisation

The morphological characteristics were evaluated by using the "methodology for primary characterisation of olive varieties" as proposed by the International Olive Oil Council "IOOC" (Barranco *et al*., 2000). Observations was done on the tree, the fruiting shoot, the leaf, the inflorescence, the fruit and the endocarp, according to the following parameters and foreseen schedule:

3.2.1. Tree

The following parameters were taken into consideration.

3.2.1.1. Height and volume :- for each cultivar were trees in good condition and not pruned, we measured on each stuided tree :-

1.The hieght of the tree (H1) from the ground to the top of canopy.

2. Hieght of trunk (H2) from the ground up to the start of the canopy.

3. Hieght of the lower part of the canopy (H3) from the ground.

4. Diameter of the canopy (D1, D2) at 12:00 a.m., in summertime measurement of the projection of the canopy on the ground.

5. Circumference direct below branching (C1).

6. Trunk circumference (C2) at 30-40 cm from the ground.

3.2.1.2. Vigour: in all areas and when normal cultural practices are applied**,** the following scale was used to measure the tree vigour :

• Weak, the tree growth is modest;

• Medium, the tree displays the average growth expected from an olive tree.

• Strong, the tree displays a vigorous growth and long branches.

3.2.1.3. Growth habit: this is the natural distribution of the scaffold branches and shoots before intervention for shaping the tree for given training system and when vigour exerts little influence. It is divided into three categories:-

• **Dropping**:- characterised by shoots and limbs which are small in diameter and bend downwards from the outset.

• **Spreading**:- characterised by initial orthotropic branching, then the limbs bend down and turn in the direction in which the greatest amount of space and light is available, the canopy becomes a hemispherical shape.

• **Erect**:- a strong apical dominance, the branches tend to grow vertically and have the canopy acquires a pronounced conical shape which becomes cylindrical on reaching maturity.

3.2.1.4. Canopy density: This parameter depends on the interaction among the length of the internodes, the number and vigour of the shoots

and the size of the leaves. this parameter indicates the density of canopy vegetation. It is classified into three categories:-

• **Sparse**:- the fast growing cultivars with long internodes on the shoots, the canopy is observed "spaces" through which light can penetrate are present;

• **Medium:**- the typical density of the species; athick vegetation, but still allowing some light to penetrate the internal parts;

• **Dense**:- the canopy appears as a compact surface and the inner parts are shaded, the shoots with short internodes, abundant branching and heavy foliage.

3.2.2. Fruiting shoot

The following parameter was taken into consideration:-

3.2.2.1. Shoot Length: it was calculated as total shoot length (cm)**,** using 20 shoots per tree for each cultivar, located around the tree at shoulder level**.**

3.2.2.2. Number of Nodes: it was calculated the number of nodes per each calculated shoots.

3.2.2.3. Internode length: it was calculated as total shoot length (cm)/number of nodes, using 20 shoots per tree for each cultivar, located around the tree at shoulder level. It is divided into three categories:

• Short (< 1 cm)

• Medium (1-3 cm)

• Long (> 3 cm)

3.2.3. Leaf

Observations were made on samples of 100 healthy adult leaves/tree for each cultivar, collected from the middle part of one-year-old shoots chosen from among the most representative ones on the south facing side of the tree at shoulder level.

The following characteristics were evaluated and classified according to the options reported for each characteristic.

3.2.3.1. Length:

• Short (< 5 cm)

• Medium (5-7 cm)

• Long (> 7 cm)

3.2.3.2. Width:

• Narrow (< 1 cm)

• Medium (1-1.5 cm)

• Broad (> 1.5 cm)

3.2.3.3. Shape: determined by the **L**ength/**W**idth ratio:

• Elliptic (L/W < 4)

• Elliptic-lanceolate (L/W = 4-6)

• Lanceolate (L/W > 6)

3.2.3.4. Longitudinal curvature of the blade:

• Epinastic

• Flat

• Hyponastic

• Helicoid

3.2.3.5. Apex shape (angle):

• very acute angle (pointed)

• acute angle

• open angle

3.2.3.6. Base shape (angle):

• very acute angle (pointed).

• open angle.

3.2.3.7. Maximum width localization:

• centre

• Centre-Apex

• Centre-Basal**.**

3.2.3.8. Leaf superior face brightness:

• Bright

• Opaque

3.2.3.9. Leaf superior face colour:

• Pale green.

• Dark green.

3.2.3.10. Leaf inferior face colour:

• Green-grey.

• Grey-green.

3.2.4. Peacock eye spot

100 young leave and 100 old leave per cultivar, we dipped the young leaves into NaOH 5% solution during 2-3 minute at 20 – 30^{o}C and we dipped the old leaves into NaOH 5% solution during 2-3 minute at

50 – 60^{o}C, dark spots indicated infections of peacock eye, then counted the leaves that was infected.

3.2.5. Inflorescence

Observations were made on samples of 25 inflorescences/tree at the white stage collected from the middle part of fruiting shoots chosen from among the most representative ones on the south facing side of the tree.

The following characteristics were evaluated and classified according to the options reported for each characteristic.

3.2.5.1. Length:

• Short (< 25 mm)

• Medium (25-35 mm)

• Long (> 35 mm)

3.2.5.2. Peduncle length:

On the same 25 healthy inflorescences wich collected for previous measurement during the white bud stage we measure the peduncle length from the base to the first branch.

3.2.5.3. Maximum width:

On the same previous 25 inflorescences we mesured the maximum width.

3.2.5.4. Structure:

By direct observation:-

• long and spare,

• long and compact,

• short and spare,

• shot and compact.

3.2.5.5. Number of flowers/inflorescence:

• Low (< 18)

• Medium (18-25)

• High (> 25)

3.2.5.6. Time of flowering:

By direct observation when the first flower opening on the tree.

3.2.5.7. Duration of flowering:

On 20 inflorescences / cultivar randomly chosen from the middle part of 1-year old shoots from 4 directions at shoulder height, we was started the observation before the first flower opens and repeated it every 2-3 days, and noted the date that first flower opens, and continue checked 20 inflorescence (randomly chosen) until the last flower will loose the petals.

3.2.5.8. Ovary apportions:

On 50 inflorescences per tree at full bloom randomly chosen, we calculated by direct observation (the number of flower with aborted ovary -male flower- and divided it on the total number of flowers on each inflorescence) *100%, and then we was calculated the percentage of vital ovary (perfect flower).

3.2.6. Fruit

3.2.6.1. Fruit growth:

Observations were made on samples of 50 fruits/tree collected from the middle part of fruiting shoots chosen from among the most representative ones on the south facing side of the tree. Very small or very large olives were discarded from the samples. The samples was taken 2 weeks after

full bloom until pit hardining, evry 15 days, sampiles 50 fruits /tree were collected and stored in plastic bages in a cool place, fresh and dry weight of 50 fruits together were measured.

From pit hardining to november evry 15 days, sample of 10 fruits/tree were collected, fruites should be healthy and not enjured, randomly taken from the external portion of the canopy in 4 direction, measured the fresh and dry wieght of 10 fruits together.

3.2.6.2. Presnce of lenticels:

On the fruits used during the sampling for fruit growth, when the fruits still green.

• Many linticels.

• Few lenticels.

3.2.6.3. Size of lenticels:

• Small linticels.

• Large lenticels.

3.2.6.4. Location of start colour change:

On the fruits used during the sampling for fruit growth, when veraison was started.

3.2.6.5. Fruit Ripening :

When The fruit was described roughly upon completion of colour change which characterises the start of ripening, on 100 fruits / tree for each cultivar taken from the middle part of the most representative fruiting shoots from south facing**.** The following characteristics were evaluated and classified according to the options reported for each characteristic.

3.2.6.5.1. Weight:

• Low (< 2 g)

• Medium (2-4 g)

• High (4-6 g)

• Very high (> 6 g)

3.2.6.5.2. Shape: Determined by the **L**ength/**W**idth ratio:

• Spherical (L/W < 1.25)

• Ovoid (L/W = 1.25-1.45)

• Elongated (L/W > 1.45)

3.2.6.5.3. Symmetry:- determined by the extent to which the two longitudinal halves match:

• Symmetric

• Slightly asymmetric

• Asymmetric

3.2.6.5.4. Apex:

• Pointed

• Rounded

3.2.6.5.5. Nipple:

• Absent

• Tenuous

• Obvious

3.2.6.5.6. Base:

• Truncate

• Rounded

3.2.6.5.7. Stalk cavity:

• Circular shape

• Elliptic shape

3.2.6.5.8. Position of maximum transverse diameter:

• Towards the base

• Central

• Towards the apex

3.2.6.5.9. Colour at full maturity:

• Black

• Violet

• Red

3.2.7. Endocarp (Stone)

Observations were made on samples of 100 endocarps/tree for each cultivar taken from the fruits used for morphological characterization, the following characteristics were evaluated and classified according to the options reported for each characteristic.

3.2.7.1. Weight:

• Low (< 0.3 g)

• Medium (0.3-0.45 g)

• High (0.45-0.7 g)

• Very high (> 0.7 g)

3.2.7.2. Shape: determined by the **L**ength/**W**idth ratio:

• Spherical (L/W < 1.4)

• Ovoid (L/W = 1.4-1.8)

• Elliptic (L/W = 1.8-2.2)

• Elongated (L/W > 2.2)

3.2.7.3. Symmetry, determined by the extent to which the two longitudinal halves match:

• Symmetric

• Slightly asymmetric

• Asymmetric

3.2.7.4. Position of maximum transverse diameter :

• Towards the base

• Central

• Towards the apex

3.2.7.5. Apex :

• Pointed

• Rounded

3.2.7.6. Termination of the apex :

• Without mucro

• With mucro

3.2.7.7. Base:

• Truncate

• Pointed

• Rounded

3.2.7.8. Surface: determined according to the depth and abundance of the fibrovascular bundles:

• Smooth

• Rugose

• Scabrous

3.2.7.9. Number of grooves - determined according to the number of grooves that can be seen from the stalk insertion point:

• Low (< 7)

• Medium (7-10)

• High (> 10

3.2.7.10. Distribution of grooves:

• Regular

• Grouped around the suture

3.2.7.11. Termination of the apex:

• With mucro

• Without mucro

3.3. Phenology :

The phenology was characterised through periodical (every week during flowering, every 2 week during fruit growth) direct observations of the labelled trees. The following phenological phases were reported in the description of the considered cultivars :-

3.3.1. Start of vegetative growth (bud bursting), which corresponds to the time when apical and lateral buds swell and lengthen. New leaves, nodes and internodes are formed at the apex of the new shoots. The new vegetation is easily distinguishable because its green colouration is lighter than that of the previous vegetation.

3.3.2. Full bloom, which corresponds to the time when about 50% of the flowers are opened. Moreover, there is complete separation of petals, lengthening of stamens and stylus, which make the stigma visible, and full opening of the anthers.

3.3.3. Pit hardening, which corresponds to the time when the increase in fruit size, -which has reached about 50% of its final size- slows down and the endocarp progressively lignify (hardening) and we measured it by cutting the fruit with knife.

3.3.4. Fruit turning (veraison), which corresponds to the time when the epicarp turns from green to pale green/pale yellow, due to the reduction of chlorophyll, and pigmentation starts.

3.4. Characteristics of fruit during ripening (ripening indices)

From October to November, every 2 weeks.

3.4.1. Fruit drop was measured for the selected trees by choosen 4 small branches / tree in the 4 directions,we was wrap the branches in a net bag and we was collected the drop fruits every 15 days and count it. During the last observation we counted the number of olives still on the branch.

3.4.2. The fruit detachment force (resistance) was measured by using a hand-held dynamometer on about 50 olives/tree. The fruit detachment force was expressed in Newton (N) and was considered

- low < 4 N
- medium 4 - 6 N
- high> 6 N.

3.4.3. Fresh and dry fruit weight were determined by weighing samples of 100 olives/tree one by one for fresh wieght, then drying them in an oven until constant weight.

3.4.4. Fruit pigmentation was determined, on samples of 50 olives/tree, by using the "Jaen pigmentation index", calculated with the following formula:

Pigmentation index = $\sum_{i=0}^{7} \frac{(i \times ni)}{N}$

ni= number of olives belonging to each class of colour;

N=number of olives in the whole sample.

i=0 - 7 where:-

0 = olive with green epicarp;

1 = olive with yellowish epicarp;

2 = olive with superficial pigment-ation on less than 50% of the epicarp;

3 = olive with superficial pigment-ation on more than 50% of the epicarp;

4 = olive with superficial pigmentation on 100% of the epicarp;

5 = olive with superficial pigment-ation on 100% of the epicarp and pigmentation on less than 50% of the pulp thickness;

6 = olive with superficial pigment-ation on 100% of the epicarp and pigmentation on more than 50% of the pulp thickness;

7 = olive with superficial pigment-ation on 100% of the epicarp and pigmentation on 100% of the pulp thickness;

3.4.5. Pulp/skin firmness (pulp consistency):

Was determined on samples of 50 fruit/tree by using a hand-held penetrometer with a 1.5-mm plunger placed in two positions opposite each

other around the equator of each fruit. The pulp consistency was expressed in grams, with values of,

Low <500 g

Medium 500-550 g

High >550 g.

3.4.6. Pulp (flesh)/pit ratio (fresh and dry wieght):

Was determined on samples of 25 olives/tree,

• **Fresh wieght** by :-

1) wieght the 25 fruits one by one.

2) removed the flesh with cutter and wieght the 25 stones one by one.

Pulp (fresh)/pit = (whole fruit weight – stone weight)/(stone weight).

• **Dry wieght** by :- Weight the stones and the flesh after drying.

The ratio was considered,

Low < 4

Medium 4 – 6

High > 6.

3.5. Oil Characteristics

Samples of oil were extracted from part of the olives collected for evaluating fruit characteristics during ripening (one sample/cultivar). The fruit of olive were crushed with a lab hammer mill, then the mash was malaxed for 30 minutes and centrifuged, the oil was separated, after filtration, the following characteristics were determined on the oil, according to the I.O.C. procedures indicated within parentheses.

3.5.1. Acidity, expressed as % of free oleic acid (EEC Reg. n. 2568/91).

3.5.2. Peroxide number, expressed as meq. of O_2/kg of oil (EEC Reg. n. 2568/91).

3.5.3. Spectrophotometric absorbency in ultra-violet (K232, K270 and ΔK) (EEC Reg. n. 2568/91).

3.5.4. Fatty acid composition, expressed as % (EEC Reg. n. 796/2002).

3.5.5. Sterol composition expressed as % and **content** expressed as mg/kg of oil (EEC Reg. n. 2568/91).

3.5.6. Total polyphenols content of the oil, expressed as mg of gallic acid/kg of oil, (Montedoro G., and Cantarelli C. modified by Solinas et al. methodology).

3.5.7. Organoleptic profile of the oil was determined with a panel test with aradar graph showing the intensity of the main positive attributes (EEC Reg. n. 2568/91 – EC Reg. n. 640/2008).

3.5.8. **Statistical analysis for morphphological data**

Morphological data for the three cultivars were analyzed as one way ANOVA using SAS program (SAS Inst, 1990) followed by mean separation using LSD method at 0.05% P-value level. The data were represented as an average value ± S.E.

3.6. Molecular C**haracterization using simple sequense repeats**

3.6.1. DNA preparation

Approximately 100 mg of fresh leaves of each plant was placed into a 2 ml Safe- Lock microtube. The samples was frozen in liquid nitrogen and grinded in to powder using mortar and pestle, 400 µl of AP-1 buffer

(DNeasy kit, Qiagen) and 4 µl of RNase-A, were added into each tissue-lyser tube and vortex (Biostad, Germany) to remove clumps. The tubes were incubated at 65^{o}C for 10 minutes in water bath for the lyses of cells. The material was mixed by inverting the tubes 2-3 times before, after and during incubation. After incubation at 65 • ^{o}C for 10 minutes, 130 µl of AP-2 buffer was added into the tubes, mixed and incubated on ice for 5 minutes. After incubation on ice the sample was transferred to QIA shredder spin column (lilac) (DNeasy kit, Qiagen) in a collection tube and spun for 2 minutes at 14000 rpm in the centrifuge (Biostad, Germany). 450 µl of the flow-through was transferred in to a clean micro-centrifuge tube and 675 µl of AP-3 buffer was added into the cleared lysate and mixed with tip, flicked and vortex (Gallen Kamp, Spinmix). In the next step 650 µl of the mixture was put into the DNeasy column in a 2 ml collection tube and spun for 1 min at 8000 rpm and flow-through was discarded. The same procedure was repeated with the remaining sample and collection tube was reused to spin again for 2 minutes at 8000 rpm. The collection tube was discarded. The DNeasy column was put into a 2 ml collection tube and 500 µl of AW buffer (DNeasy kit, Qiagen) was added on to the column and spun for 1 min at 8000 rpm. The flow-through was discarded but the tube was kept for reuse, again 500 µl of the AW buffer was added to the DNeasy column and spun for 2 minutes at 14000 rpm to dry the column membrane. At the end the column was removed carefully and collection tube with contents was discarded. The DNeasy column was transferred to a 1.5 ml micro-centrifuge tube and 100 µl of

pre heated AE buffer (DNeasy kit, Qiagen) was added directly on to the column membrane and incubated at room temperature for 5 minutes and then spun for 1 minute at 8000 rpm to collect first elution and same procedure was repeated for the second elution.

3.6.2. DNA quantification

To insure that DNA preparations of the eight samples were of sufficient quality and quantity, DNA quality and concentration were determined using both agarose gel and spectrophotometer. A small aliquot of DNA was run on a 1% agarose gel next to a series of phage λ DNA dilutions ranging from 50 ng to 500 ng. The resulting agarose image allowed visual inspection of DNA integrity. If a substantial smearing appeared below the main band of high molecular weight DNA, the sample DNA quality was considered not suitable for simple sequence repeat (SSR) fingerprinting and the DNA isolation was repeated. Spectrophotometry was also used for quantification and quality checking depending on A260/A280 ratio. An aliquot of 20 µL of each sample was used in a dilution of 1/100 in TE (10 mM Tris-base, 1 mM EDTA, pH 8.0) to measure the DNA concentration (µg/µl) using a spectrophotometer with 260 nm (DU-65 spectrophotometer, Germany) (Vinod, 2004).

3.6.3. Analysis of microsatellites markers

A total of 5 microsatellite markers were used to test the polymorphism in the 8 olive trees. 15 SSR markers out of 17 SSR markers were polymorphic (88.2 %) and used to genotype 8 olive trees. The primers were selected from the literature: DCA9, DCA16 (Sefc *et al.*, 2000;

Bandelj *et al.*, 2004), GAPU103 (Carriero *et al.*, 2002), and UDO99-28, and UDO99-35 (Cipriani *et al.*, 2002). The procedure for SSR amplification was carried out as described by Muzzalupo *et al.* (2006) A list of microsatellite primers along with forward and reverse sequences, used to survey polymorphism is given in Table (2).

3.6.4. Components of polymerase chain reaction mixture

All PCR amplifications were performed in 12.95 µl reaction volume containing 6.5 µl of PCR ReadyMix™ (Abgene, U.K) with 3.0 mM $MgCl_2$, 0.15 µl each of forward primer (2.0 pmol/µl), reverse primer (20 pmol/µl) and optional dye (20 pmol/µl), 5 µl sterilized DNA grade water and 1 µl (5-6 ng/µl) of genomic DNA template per sample. The PCR reactions were setup in 0.2 ml thin wall PCR strip tubes (Lightlabs, USA). The all PCR work was done in PCR work-station (Labcaire, Biocote, USA). The PCR amplification was carried out using the PCR program detailed in Table (3), in GeneAmp PCR System 9700 (Applied Biosystems, Singapora).

3.6.5. PCR Master Mix (2x ReadyMix™)

PCR ReadyMix™ (Abgene, U.K) is a ready-to-use master mix. It is a convenient way of amplifying DNA fragments without the need to thaw individual components, reducing the risk of contamination and pipetting errors. The thermoprime plus DNA polymerase, dNTPs, reaction buffer and $MgCl_2$ are all present in the mix. PCR ReadyMix™ (Abgene, U.K) contained 1.25 unit Thermoprime Plus DNA Polymerase, 75 mM Tris-HCl, 20 mM $(NH_4)_2SO_4$, 3.0 mM $MgCl_2$, 0.01% (V/V) Tween® 20 and

0.2 mM each of dATP, dCTP, dGTP and dTTP respectively. PCR Master mix also contains precipitant and dye to facilitate electrophoresis.

Table (2) : List of SSR tailed primers along with forward and reverse

No.	Marker	Forward Primer	Reverse Primer
1	U99-35	AATTTAATGGTCACAC ACAC	ATTGCGAAATAGATCTA CGA
2	U99-28	CTGCAGCTTCTGCCCAT AC	GCAGCTCATCATTTGGC ACT
3	GAPu-103	TGAATTTAACTTTAAA CCCACACA	GCATCGCTCGATTTTAT CC
4	DCA9	AATCAAAGTCTTCCTTC TCATTTCG	GATCCTTCCAAAAGTAT AACCTCTC
5	DCA16	TTAGGTGGGATTCTGT AGATGGTTG	TTTTAGGTGAGTTCATA GAATTAGC

sequences used in this study.

3.6.6. Description of PCR program used for DNA amplification

Genomic DNA was amplified by using PCR program as given in Table (3)

Table (3) : The PCR program used for the amplification of SSR primers.

	PCR profile for SSR analysis	
Step -1	94^{o}C for 5 minutes	Initial Denaturation
Step -2	94^{o}C for 1 minute	Denaturation
Step -3	55^{o}C for 1 minute	Annealing
Step -4	75^{o}C for 2 minutes	Extension
Step -5	35 times repeated	35 Cycles
Step -6	72^{o}C for 7 minutes	Final extension
Step -7	4^{o}C for ever	Hold
Step -8		End

3.6.7. Preparation of high resolution 2.5% agarose gel

The PCR product of each sample (10µl) was loaded in superfine resolution 2.5% (w/v) agarose gel for the study of polymorphism and scoring of

bands. The 10 kb DNA ladder (Biolab, UK) was also run inside lanes to estimate the size of the amplified fragments. For this purpose 7.5g agarose (Anachem, Lutin, U.K) was gently and thoroughly dissolved in cold 300 ml 1x TBE in a glass flask and was heated initially in microwave oven for 2 minutes at medium to high heating. Then it was swirled, heated at medium to high temperature for 1.5 minutes, swirled and heated again for 30 seconds two to three times swirling in between each heating until solution becomes clear. Then the gel was allowed to cool at 50-60°C and 12.5 ml ethidium bromide (10 µg/µl) was added and gel was poured into the gel tray in fume-hood. The tray was put in the gel tank having 1x TBE buffer and combs were removed from solidified gel. The samples were loaded in the gel for electrophoresis at (100-110) Volt for (1-1.5) hours. After electrophoresis, the gel was photographed on gel documentation system (INTAS, Göttingen, Germany) in the dark room under UV light.

3.6.8. Scoring of gel bands for marker alleles

The DNA bands were scored as ‘1’ for present band and absent band was scored as ‘0’ at each marker.

3.6.9. Statistical analysis of the genomic DNA

Based on SSR profile scoring of each loci as present 1 / absent 0 a similarity matrix among olive trees was calculated using SIMQUAL (Similarity of Qualitative Data), cluster analysis was performed on the estimated similarities using the unweighted pair group method with arithmetic average (UPGMA) and SHAN algorithm, and the resulting

clusters were expressed as a dendrogram using NTSYS-pc (Exeter Software v.2.02k).

Percent polymorphic loci (Ps) were calculated using the following formula:

P_s = Number of polymorphic loci/ total number of loci

The similarity matrix was calculated using the formula of Dice coefficient (Dice, 1945). Dice (GSij) = 2a/(2a+b+c), where a represents the number of shared SSR alleles scored between the genotypes pairs (i and j) considered, b is the number of SSR alleles present in i but absent in j, c is the number of SSR alleles present in j but absent in i.

Chapter Four

Results and Discussions

4.1 Results

4.1.1 General Description of the cultivars

4.1.1.1. Nabali Baladi

Main area of cultivation

Largely diffused in the north and center hilly areas of west bank and partially in gaza strip, and more than 90% of olive variety in qalqilia

Purpose of use	Dual purpose (table and oil)

Morphological Characteristics

Tree

vigour	medium
growth habit	spreading
canopy dinsity	medium

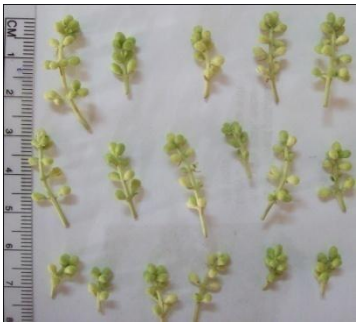
Fruiting shoot

Length of the shoot	16.7 cm
Internodes length	Medium (1.5)

leaves

shape	Elliptic (3.9)
length	Medium (5.58cm)
width	Medium (1.44 cm)
longitudinal curvature of the blade	flat
Apex shape	open
apex angle	open
base shape	open angle (Blunt)
Base angle	Open angle
maximum width localization	center
leaf superior face brightness	bright
leaf superior face color	dark green
leaf inferior face color	green grey

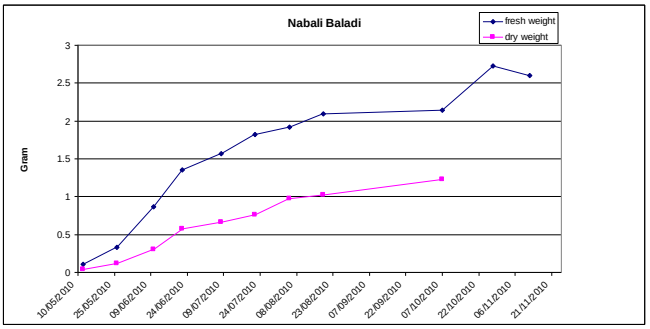
Inflorescences



length	2 cm
peduncle length	0.58 cm
maximum width	0.93 cm
structure	short and compact
number of flowers per inflorescence	12.5 (low)
time of flowering	late march – early April
duration of flowering	30 days
ovary abortion	86% vital ovary (perfect)14% aborted ovary (male)

Fruit

Fruit growth



presence of lenticels	Few
size of lenticels	Small

location of start of color change	Base
shape of fruit	Elongated (L/W=1.51)
Longitudinal symmetry	Asymmetric
Position of max transverse diameter	central
Apex	Pointed
Base	Rounded
Nipple	Absent
stalk cavity	Circular
color at full maturity	Black

Stone

weight	Medium (0.38g)
shape of the stone	Elongated (L/W =2.32)
Longitudinal symmetry	Slightly asymmetric

Position of max transverse	Central
apex	Pointed
base	Pointed
surface	Rugose
number of grooves	Medium (9)
distribution of grooves	Grouped around the suture
termination of apex	With mucro

Fruit ripening

fruit drop	3% until 23/10	
	7/10/2010	**28/10**
fruit retention force	medium	Medium
fruit pigmentation (M.I.)	0.76	1.2
Stone fresh weight (g)	0.48	0.58
fruits fresh weight (g)	2.15g	3.08
pulp/skin firmness	Medium	Low
flesh / pit ratio	3.48	4.4
Fruit dry weight (g)	1.23	
Pulp to pit ratio dry weight	0.099	

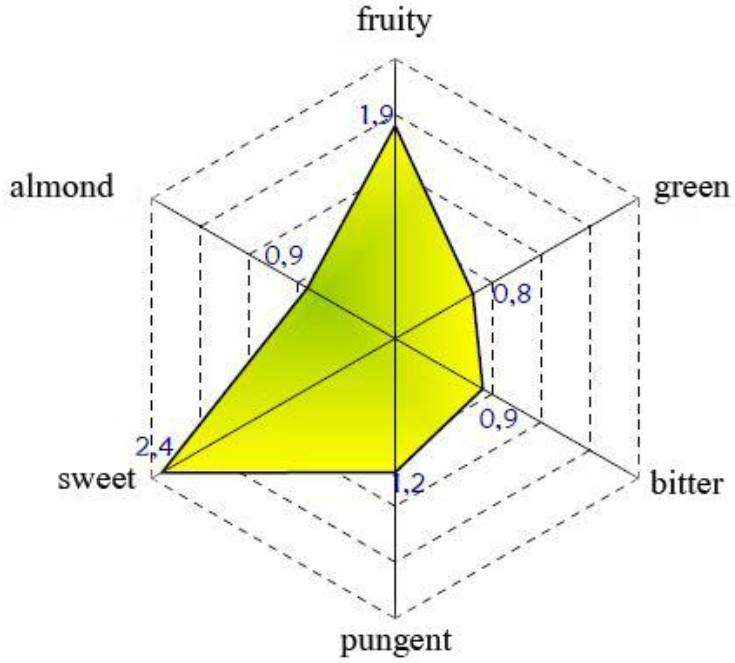
Phenology

Start of vegetative growth	Early February
Full bloom	Late April
Pit hardening	Mid June
Fruit turning	Late October
Tolerance to peacock	medium

Chemical and Physical Characteristics of Oil During Ripening

Chemical analysis	
Free Acidity(%)	0.32
Peroxide (meq o2/kg oil)	6.25
Total polyphenol content (mg/kg oil)	380
Absorption UV	
K232 nm	1.76
K270 nm	0.11
Delta k	- 0.003
Fatty acid composition (%)	
Palmitic	15.5
Palmitoleic	0.90
heptadecanoic	0.12
heptadecenoic	0.15
Stearic	3.56
Oleic	66.20
Linoleic	12.8
Linolenic	0.84
Eicosanoic	0.43
Eicosenoic	0.25
Sterol composition (%)	
Cholesterol	0.4
Brassicasterol	<0.1
24-Metilencolesterol	0.52
Campesterol	2.66
Campestanol	0.3
Stigmasterol	1
Delta-7-Campesterol	0.2
Delta 5,23-Stigmastadienol	<0.1
Clerosterol	1.1
Beta-sitosterol	85.60
Sitostanol	0.37
Delta-5-avenasterol	4.90
Delta-7,9(11)-stigmastadienol	<0.1
Delta-5,24-stigmastadienol	0.5
Delta-7-stigmastenol	1
Delta-7-avenasterolo	1.20
Total Beta-sitosterol	93.2
Erythrodiol + uvaol	2.5
Total sterols (mg/kg oil)	1613.30

Physical analysis
Organoleptic profile of the oil



ACTION:-
Fruity light, basically green, hint of almond. The taste mostly sweet, hints of pungent and bitter.

4.1.1.2. Nabali Mohassan

Main area of cultivation
Largely diffused in the north and center hilly areas of west bank and partially in gaza strip.

Purpose of use	Dual purpose (table and oil)

Morphological Characteristics

Tree

vigour	medium
growth habit	spreading
canopy dinsity	medium

Fruiting shoot

Length of the shoot	16.7 cm
Internodes length	Medium (1.4)

leaves

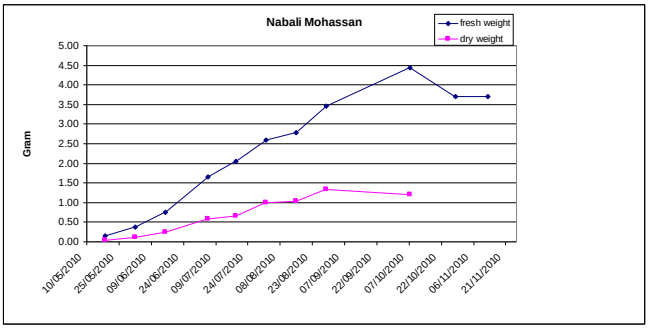
shape	Elliptic (4.6)
length	Medium (5.58cm)
width	Medium (1.44 cm)
longitudinal curvature of the blade	flat
Apex shape	open
apex angle	open
base shape	open angle (Blunt)
Base angle	Open angle
maximum width localization	Center-apex
leaf superior face brightness	bright
leaf superior face color	dark green
leaf inferior face color	green grey

Inflorescences

length	2.8 cm
peduncle length	0.65 cm
maximum width	1.24 cm
structure	Long and spare
number of flowers per inflorescence	17(low)
time of flowering	early April
duration of flowering	31 days
ovary abortion	100% vital ovary (perfect)14% aborted ovary (male)

Fruit

Fruit growth



presence of lenticels	many
size of lenticels	Small

location of start of color change	Apex
shape of fruit	ovoid (L/W=1.4)
Longitudinal symmetry	Slightly Asymmetric
Position of max transverse diameter	central
Apex	Pointed
Base	Rounded
Nipple	Absent
stalk cavity	Circular
color at full maturity	violet

Stone

Weight	Medium (0.44g)
Shape of the stone	Elliptic (L/W =2.16)
Longitudinal symmetry	Asymmetric

Position of max transverse	Central
Apex	Pointed
Base	Rounded
Surface	Rugose
Number of grooves	High (12)
Distribution of grooves	Regular
Termination of apex	With mucro

Fruit ripening

Fruit drop	5% until 23/10	
	7/10/2010	**28/10**
Fruit retention force	medium	Medium
Fruit pigmentation (M.I.)	0.76	1
Stone fresh weight (g)	0.55	0.52
Fruits fresh weight (g)	3.2	2.9
Pulp/skin firmness	High	High
Flesh / pit ratio	4.22	6.5
Fruit dry weight (g)	1.47	
Pulp to pit ratio dry weight	0.11	

Phenology

Start of vegetative growth	Early February
Full bloom	Early May
Pit hardening	Early July
Fruit turning	Early November
Tolerance to peacock	Low

Chemical and Physical Characteristics of Oil During Ripening

Chemical analysis	
Free Acidity(%)	0.17
Peroxide (meq o2/kg oil)	7.6
Total polyphenol content (mg/kg oil)	128
Absorption UV	
K232 nm	1.76
K270 nm	0.1
Delta k	- 0.001
Fatty acid composition (%)	
Palmitic	20.48
Palmitoleic	1.7
heptadecanoic	0.07
heptadecenoic	0.1
Stearic	2.53
Oleic	56.42
Linoleic	17.2
Linolenic	1.02
Eicosanoic	0.4
Eicosenoic	0.26
Sterol composition (%)	
Cholesterol	<0.1
Brassicasterol	<0.1
24-Metilencolesterol	<0.1
Campesterol	3
Campestanol	<0.1
Stigmasterol	1.3
Delta-7-Campesterol	<0.1
Delta 5,23-Stigmastadienol	<0.1
Clerosterol	1.1
Beta-sitosterol	90.1
Sitostanol	0.3
Delta-5-avenasterol	2.4
Delta-7,9(11)-stigmastadienol	<0.1
Delta-5,24-stigmastadienol	0.5
Delta-7-stigmastenol	0.4
Delta-7-avenasterolo	0.5
Total Beta-sitosterol	94.4
Erythrodiol + uvaol	1.7
Total sterols (mg/kg oil)	1583.2

4.1.1.3. Souri

Main area of cultivation
Largely diffused in the north and
Center hilly areas of west bank
And partially in gaza strip.

Purpose of use	For oil purpose

Morphological Characteristics

Tree	
vigour	Medium
growth habit	Erect
canopy dinsity	Medium
Fruiting shoot	
Length of the shoot	14.6 cm
Internodes length	Medium (1.4)

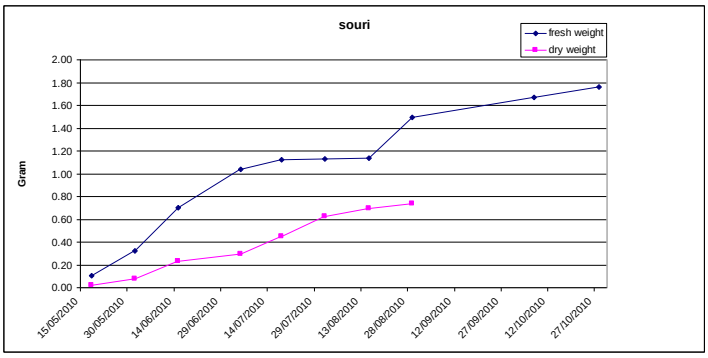
leaves	
shape	Elliptic (3.5)
length	Medium (5.55cm)
width	Broad (1.52 cm)
longitudinal curvature of the blade	flat
Apex shape	open
apex angle	open
base shape	open angle (Blunt)
Base angle	Open angle
maximum width localization	Center-Basal
leaf superior face brightness	Bright
leaf superior face color	Dark green
leaf inferior face color	Green grey

Inflorescences

Length	2.6 cm
Peduncle length	0.64 cm
Maximum width	1.15 cm
Structure	Long and spare
Number of flowers per Inflorescence	17.6(low)
Time of flowering	Early April
Duration of flowering	28 days
Ovary abortion	98% vital ovary (perfect) 2% aborted ovary (male)

Fruit

Fruit growth



Presence of lenticels	Few
Size of lenticels	Small
Location of start of color change	Apex
Shape of fruit	Elongated (L/W=1.67)
Longitudinal symmetry	Asymmetric
Position of max Transverse diameter	Central
Apex	Pointed
Base	Rounded
Nipple	Absent
Stalk cavity	Circular
Color at full maturity	45% Black 55% Violet

Stone

Weight	Medium (0.46g)
Shape of the stone	Elongated (L/W =2.1)
Longitudinal symmetry	Asymmetric

Position of max transverse	Towards the apex
Apex	Pointed
Base	Pointed
Surface	Smooth
Number of grooves	High (13)
Distribution of grooves	Regular
Termination of apex	With mucro

Fruit ripening

Fruit drop	9% until 23/10	
	7/10/2010	**28/10**
Fruit retention force	Medium	Low
Fruit pigmentation (M.I.)	3.3	4
Stone fresh weight (g)	0.46	0.58
Fruits fresh weight (g)	1.67	1.76
Pulp/skin firmness	Medium	Medium
Flesh / pit ratio	2.65	
Fruit dry weight (g)	0.77	
Pulp to pit ratio dry weight	0.05	

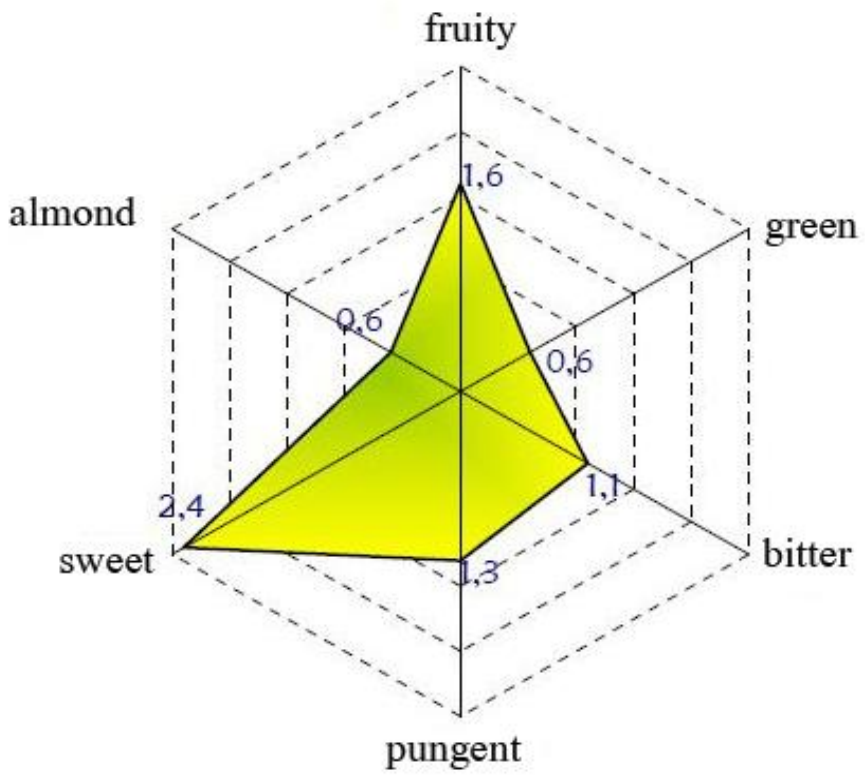
Phenology

Start of vegetative growth	Early February
Full bloom	Early May
Pit hardening	Early July
Fruit turning	Mid October
Tolerance to peacock	Medium

Chemical and Physical Characteristics of Oil During Ripening

Chemical analysis	
Free Acidity(%)	0.28
Peroxide (meq o2/kg oil)	6.1
Total polyphenol content (mg/kg oil)	217
Absorption UV	
K232 nm	1.52
K270 nm	0.087
Delta k	- 0.001
Fatty acid composition (%)	
Palmitic	15.49
Palmitoleic	1.23
heptadecanoic	0.04
heptadecenoic	0.06
Stearic	2.35
Oleic	70.11
Linoleic	9.3
Linolenic	0.97
Eicosanoic	0.26
Eicosenoic	0.19
Sterol composition (%)	
Cholesterol	0.5
Brassicasterol	<0.1
24-Metilencolesterol	0.1
Campesterol	2.6
Campestanol	<0.1
Stigmasterol	1.7
Delta-7-Campesterol	<0.1
Delta 5,23-Stigmastadienol	<0.1
Clerosterol	1.2
Beta-sitosterol	86.1
Sitostanol	0.6
Delta-5-avenasterol	5.4
Delta-7,9(11)-stigmastadienol	<0.1
Delta-5,24-stigmastadienol	0.4
Delta-7-stigmastenol	0.5
Delta-7-avenasterolo	0.9
Total Beta-sitosterol	93.6
Erythrodiol + uvaol	0.8
Total sterols (mg/kg oil)	1673.2

Physical analysis
Organoleptic profile of the oil



ACTION:
Fruity light, mature type, with mild leaves and herbs. The taste mainly sweet, with hints of spicy and bitter. rating 6.5

4.1.2 Morphological and biochemical analysis

Notable significant differences between the cultivars were observed in all characteristics considered (Table 4,5,6,7). Tree canopy was high for the Nabali Baladi cultivar, medium for Souri, and low for Nabali Mohassan but without significant difference among them. Regarding the fruiting shoot, both 'Nabali Baladi' and ' Souri' were the same. For leaf dimensions similar dimensions were observed for the three cultivars. There was difference in the inflorescence characterstics among the three cultivars. The highest inflorescence length was with 'Nabali Mohassan' followed by Souri, the smaller length was with Nabali Baladi. Similar trend was obtained with peduncle dimension, regarding the number of flowers per inflorescence, the smaller number was with Nabali Baladi, percent of perfect flower was high in Nabali Mohassan followed by Nabali Baladi. Larger fruit was obtained in Nabali Mohassan followed by Nabali Baladi, however Souri has the smaller fruit size. Similar trend was obtained with stone dimensions. Fruit drop percent was high in Souri The FRF was high in Nabali Mohassan, the maturation index (MI) was very high in Souri 3.15 compared to the MI of both Nabali Baladi and nabali Mohassan which was 0.522 and 0.72, respectively. The highest pulp/pit ratios values as fresh wt were recorded for Nabali Mohassan followed by Nabali Baladi, followed by Souri. However regarding the pulp/pit ratio as dry wt, bothNabali Baladi and Nabali Mohassan recorded similar value higher than that of Souri cultivar.

	Tree	Canopy					
Cultivar	Height (H1) (m)	Avr. Diameter (m)	Height from the ground 1 (H3.1) (m)	Height from the ground 2 (H3.2) (m)	Height from the ground 3 (H3.3) (m)	Height from the ground 4 (H3.4) (m)	Avr. height from the ground (m)
Nabali Baladi	4.35 ± 0.49	4.77 ± 0.47	0.54 ± 0.07	0.54 ± 0.24	0.32 ± 0.13	0.49 ± 0.22	0.47 ± 0.10
Nabali Mohassan	4.26 ± 0.17	5.17 ± 0.22	0.73 ± 0.10	0.77 ± 0.12	1.05 ± 0.26	0.63 ± 0.10	0.79 ± 0.07
Souri	4.18 ± 0.38	6.34 ± 0.26	0.48 ± 0.38	0.90 ± 0.3	0.97 ± 0.47	1.51 ± 0.05	0.96 ± 0.06

Table 4: Tree canopy characteristics of the different cultivars. Average values of 2-4 trees± SE.

Table 5: Vegetative growth characterstics of the different cultivars. Average value of 2-4 trees ±SE

	Trunk				Fruiting shoot		Leaf		
Cultivar	Height until branching (H2) (m)	Circumference below branching (C1) (m)	Circumference at 40cm from ground (C2) (m)	Length (cm)	Nodes (No.)	Internode length (shoot L / nodes No.)cm	Length (cm)	Width (cm)	Shape (L / W)
Nabali Baladi	1.01 ns ± 0.26	0.62 ± 0.08	0.89 ± 0.05 a	20.04 ±0.15 b	13.21 ±0.20a	1.53 ± 0.02 c	5.58 ±0.16 ns	1.46 ±0.04a	3.85 ±0.10b
Nabali Mohassan	0.91 ± 0.14	0.52 ± 0.02	0.70 ± 0.03 b	20.82 ±0.07a	12.35 ±0.10b	1.69 ± 0.01 b	5.94 ±0.12	1.32 ±0.02b	4.57 ±0.02a
Souri	1.30 ± 0.50	0.55 ± 0.05	0.64 ± 0.10 b	17.87 ±0.42c	9.98 ±0.12c	1.83 ± 0.04 a	5.81 ±0.04	1.52 ±0.0 a	3.88 ±0.02 b

Table 6: Inflorescence characteristics of the different olive cultivars. Average value ±SE

	Inflorescence				Flowers	
Cultivar	Length (cm)	Peduncle length (cm)	Max width (cm)	No. flowers / inflorescence	% of perfect flowers	% of ovary abortion
Nabali Baladi	2.09 ± 0.07 b	0.58 ± 0.02 b	0.93 ± 0.01 c	12.41 ± 0.87 b	86.00 ± 0.58 c	14.00 ± 0.58 a
Nabali Mohassan	2.91 ± 0.09 a	0.73 ± 0.03 a	1.22 ± 0.01 a	17.72 ± 0.90 a	100.00 ± 0.00 a	0.00 ± 0.00 c
Souri	2.65 ± 0.03 a	0.64 ± 0.00 ab	1.14 ± 0.02 b	17.80 ± 0.20 a	97.50 ± 0.50 b	2.50 ± 0.50 b

Table 7 (a): Fruit characteristics of the different cultivars. Average values of 2-4 trees ± SE.

	Fruit			Stone			
Cultivar	Length (cm)	Width (cm)	Shape(L / W)	Length (cm)	Width (cm)	Shape (L / W)	Number of grooves
Nabali Baladi	1.96 ± 0.07 b	1.24 ± 0.08 b	1.61 ± 0.06 ab	1.53 ± 0.01 b	0.60 ± 0.02 b	2.66 ± 0.08 a	8.68 ± 0.12 c
Nabali Mohassan	2.23 ± 0.01 a	1.56 ± 0.02 a	1.46 ± 0.03 b	1.60 ± 0.01 a	0.73 ± 0.01 a	2.22 ± 0.03 b	12.77 ± 0.23 b
Souri	1.60 ± 0.03 c	0.95 ± 0.00 c	1.70 ± 0.03 a	1.51 ± 0.01 b	0.720 ± 00 a	2.10 ± 0.01 b	13.95 ± 0.05 a

Table 7 (b): Fruit characteristics of the different cultivars. Average values of 2-4 ± SE.

	Fruit		
Cultivar	**Fruit drop**	**FRF (N)**	**MI**
Nabali Baladi	0.04 ± 0.01 c	454 ± 23.79 b	0.52 ± 0.02 c
Nabali Mohassan	0.09 ± 0.01b	560.2 ± 13.12 a	0.77 ± 0.01 b
Souri	0.15 ± 0.01a	417.8 ± 13.12 b	3.15 ± 0.15 a

Table 7 (c): Fruit characteristics of the different cultivars. Average valuesof 2-4± SE.

Cultivar	**Avr. Pulp firmness (g)**	**Fruit fresh weight (g)**	**Stone fresh weight (g)**	**Flesh fresh weight (g)**	**Pulp-to-pit ratio (FW)**
Nabali Baladi	526.1 ± 8.73 b	2.47 ± 0.12 b	0.51±0.01 ab	2.01 ± 0.12 b	4.05 ± 0.19 b
Nabali Mohassan	629.5 ± 5.13 a	3.32 ± 0.12 a	0.53±0.02 a	2.94 ± 0.14 a	5.62 ± 0.11 a
Souri	450.4 ± 7.20 c	1.67 ± 0.01 c	0.46±0.01 b	1.22 ± 0.02 c	2.71 ± 0.05 c

Table 7 (d): Fruit characteristics of the different cultivars. Average values of 2-4 trees ± SE.

Cultivar	Fruit dry weight (g)	Stone dry weight (g)	Flesh dry weight (g)	Pulp-to-pit ratio (DW)
Nabali Baladi	1.34 ± 0.05 a	0.38 ± 0.01 a	0.97 ± 0.04 a	2.57 ± 0.07 a
Nabali Mohassan	1.33 ± 0.07 a	0.38 ± 0.01 a	0a.96 ± 0.06 a	2.54 ± 0.08 a
Souri	0.40 ± 0.37 b	0.33 ± 0.02 b	0.47 ± 0.04 b	1.44 ± 0.17 b

The acidity and peroxide number of the oils of all cultivars were very low (Table 8). The spectro-photometer absorbencies in ultra-violet were also low. Most cultivars had an oleic content of about 60% or higher (Table 9). Only the Nabali Mohassan cultivar had a lower value (56.42%) that was associated with relatively high amounts of palmitic and linoleic acids. The sterol composition and content were quite different in the cultivars (Table 10). The Nabali Baladi cultivar had a relatively high value of Δ-7 stigmastenol.

Table 8: Free acidity, peroxide number, spectrophotometer absorbencies in ultra-violet (K 232, K 270, Δk) and total polyphenol of oils of the different olive cultivars. The IOOC trade standard (TS) values for extra virgin olive oils are reported in the last line.

Cultivar	Date	% oil (DW)	Acidity (%)	Peroxide (Meq O2/kg)	K232 (nm)	K270 (nm)	Δ K	T. Polyph. (mg/kg oil)
Nabali Baladi	14/11	55.63	0.32	6.25	1.76	0.11	-0.003	380
Nabali Mohassan	14/11	46.77	0.17	7.6	1.7630	0.1030	-0.001	128
Souri	14/11	40.3	0.28	6.1	1.523	0.087	-0.001	217
IOOC-TS			< 0.8	≤ 20.0	≤ 2.50	≤ 0.22	≤ 0.01	

Table 9: Fatty acid composition of oil of different olive cultivars. The IOOC trade standard (TS) values for extra virgin olive oils are reported in the last line.

Cultivar	Palmitic	Palm-itoleic	Eptade-canoic	Eptade-cenoic	Stearic	Oleic	Linoleic	inolenic	Eico-sanoic	Eicos-Enoic
Nabali Baladi	15.5	0.9	0.12	0.15	3.56	66.2	12.8	0.84	0.43	0.25
Nabali Mohassan	20.48	1.7	0.07	0.1	2.53	56.42	17.02	1.02	0.4	0.26
Souri	15.49	1.23	0.04	0.06	2.35	70.11	9.3	0.97	0.26	0.19
IOOC-TS	7.5-20.0	0.3-3.5			0.5-5.0	55.0-83.0	3.5-21.0	< 1.0		

Table 10 (a): Sterol composition (%) of oil of different olive cultivars. The IOOC trade standard (TS) values for extra virgin olive oils are reported in the last line.

Cultivar	Coles-terol	Brassic-asterol	24-Metilenc-olesterol	Campe-sterol	Camp-estanol	Stigma-sterol	Delta-7-Campe-sterol	Delta 5,23-Stigmas-tadienol	Clero-sterol	Beta-sitosterol
Nabali Baladi	0.4	< 0.1	0.52	2.66	0.3	1	0.2	< 0.1	1.1	85.6
Nabali Mohassan	< 0,1	< 0,1	< 0,1	3	< 0,1	1.3	< 0,1	< 0,1	1.1	90.1
Souri	0.5	< 0,1	0.1	2.6	< 0,1	1.7	< 0,1	< 0,1	1.2	86.1
IOOC-TS	< 0.50	< 0.10		< 4.00		< campe-sterol				

Table 10 (b): Sterol composition (%) of oil of different olive cultivars. The IOOC trade standard (TS) values for extra virgin olive oils are reported in the last line.

Cultivar	Sito-stanol	Delta-5-avena-sterol	Delta-7,9 (11)-stigma-stadienol	Delta-5,24-stigma-stadienol	Delta-7-stigma-stenol	Delta-7-avena-sterolo	Total Beta-sitosterol	Erythridol + uvaiol	Total sterols
Nabali Baladi	0.37	4.9	< 0.1	0.5	1	1.2	93.2	2.5	1613.3
Nabali Mohassan	0.3	2.4	< 0,1	0.5	0.4	0.5	94.4	1.7	1,583.2
Souri	0.6	5.4	< 0,1	0.4	0.5	0.9	93.6	0.8	1,673.2
IOOC-TS					< 0.50				> 1000

4.1.3 Molecular Analysis

A total of 17 alleles over 5 loci were observed. All microsatellites were polymorphic. An average of 3.4 alleles per locus was amplified, ranging from 2.0 at GAPU-103 and DCA9 to 5.0 at U99-35 and DCA16. The smallest allele among the five polymorphic loci was allele 50 bp at DCA16, while the largest allele was 450 bp at U99-35. The level of polymorphism and the associated information content is a crucial criterion for the choice of a particular set of loci. However, marker polymorphism also varies according to the number and origin of the plants analyzed.

4.1.3.1 Genetic relationships between olive cultivars

Olive genotypes were grouped by cluster analysis as shown in the dendrogram (Figure 3) based on SSR data. Three main clusters distinguished individuals at the variety level, in fact, accessions belonging to the same variety clustered together. The first included 1, 2, 3, and 4 that represent Nabali baladi samples, the second consisted from 5, 6 and 7 which showed identity ranging from 0.63 to 1.0 contained all ‘Nabali Mohassan’ samples, the third contained the one sample from Souri cultivar. All cluster can be subdivided in one or three sub-clusters (Table 11) with similarity coefficients for the eight olive trees samples varied from maximum 1.0 to 0.31 minimum.

Table (11) : Similarity index for 8 olive oil trees according to DICE coefficient

	Nabali B	Nabali B	Nabali B	Nabali B	Nabali M	Nabali M	Nabali M	Souri
Nabali B	1.0000000							
Nabali B	0.9090909	1.0000000						
Nabali B	0.9523810	0.9565217	1.0000000					
Nabali B	0.9090909	1.0000000	0.9565217	1.0000000				
Nabali M	0.4210526	0.5714286	0.5000000	0.5714286	1.0000000			
Nabali M	0.4705882	0.5263158	0.4444444	0.5263158	0.8750000	1.0000000		
Nabali M	0.4705882	0.5263158	0.4444444	0.5263158	0.8750000	1.0000000	1.0000000	
Souri	0.3529412	0.3157895	0.3333333	0.3157895	0.6250000	0.7142857	0.7142857	1.0000000

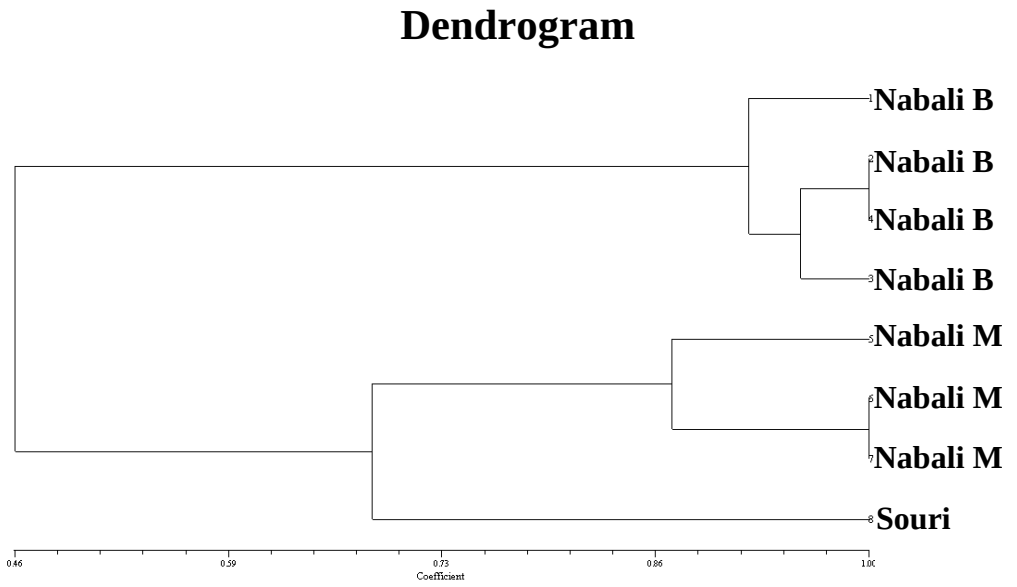


Fig (3) : dendrogram of 8 olive oil trees (B : Baladi, M : Mohassan) based on similarity coefficients using 17 SSR marker produced by five primers

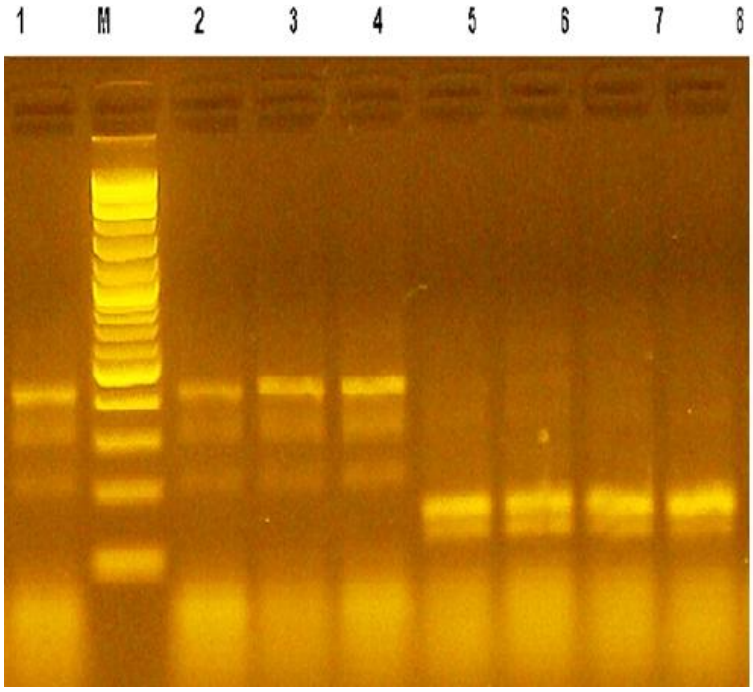


Fig (4a) : SSR pattern obtained among 8 olive oil trees collected from Qalqilya location in Palestine using primer U99-35. M= Molecular weight marker (10 kb DNA ladder).

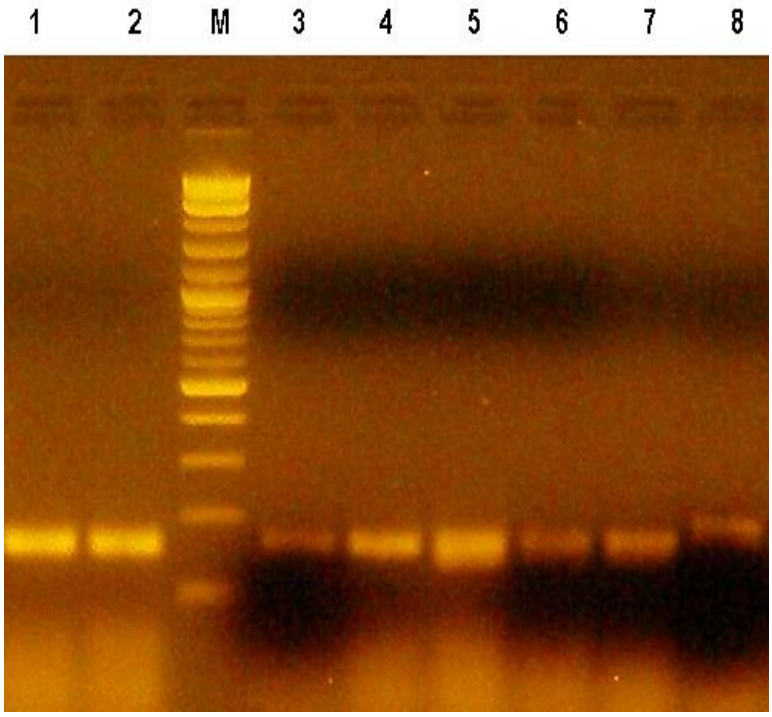


Fig (4b) : SSR pattern obtained among 8 olive oil trees collected from Qalqilya location in Palestine using primer U99-28. M= Molecular weight marker (10 kb DNA ladder).

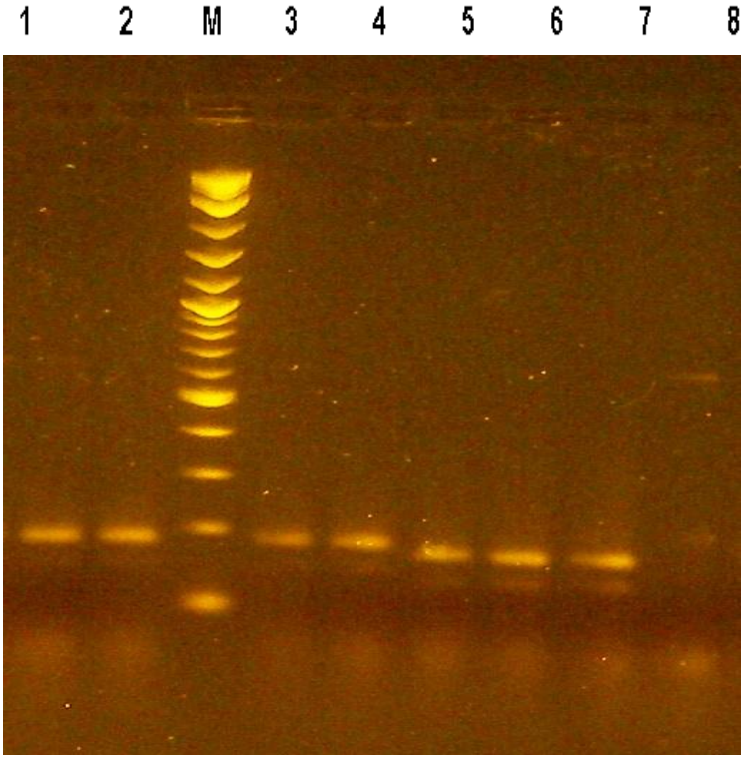


Fig (4c) : SSR pattern obtained among 8 olive oil trees collected from Qalqilya location in Palestine using primer GAPu-103. M= Molecular weight marker (10 kb DNA ladder).

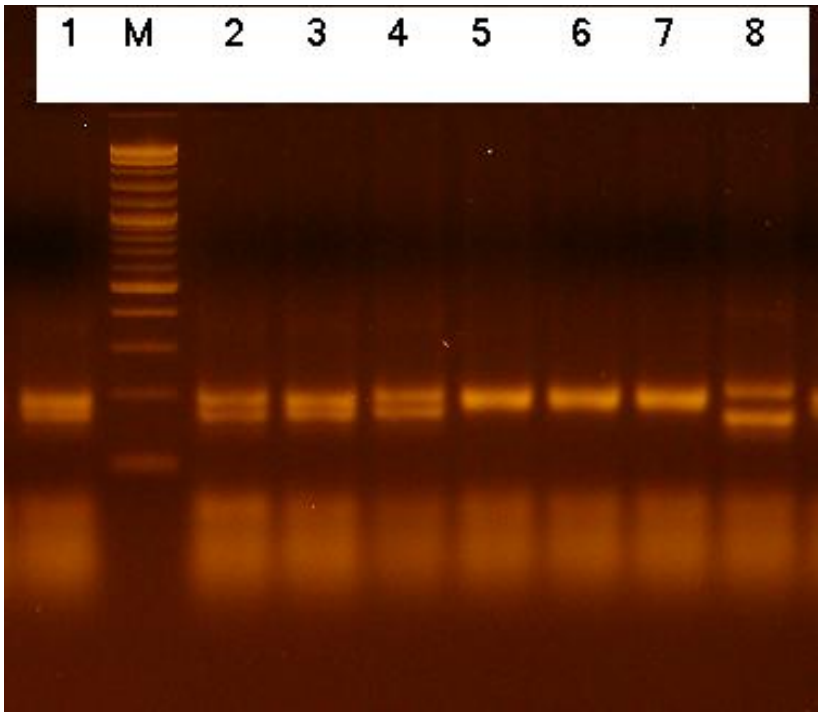
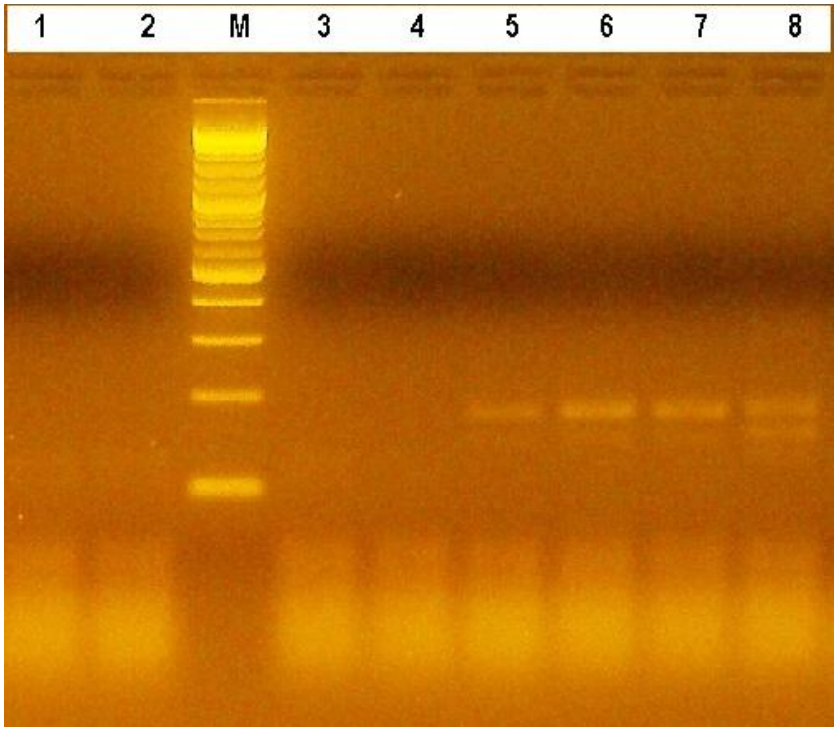


Fig. (4d) : SSR pattern obtained among 8 olive oil trees collected from Qalqilya location in Palestine using primer DCA9. M= Molecular weight marker (10 kb DNA ladder).



Fig, (4e) : SSR pattern obtained among 8 olive oil trees collected from Qalqilya location in Palestine using primer DCA16. M= Molecular weight marker (10 kb DNA ladder).

4.2 Discussion :

The aim of this study was to study three olive cultivars from the semi-costal area in West bank-Palestine, based on morphological, biochemical and molecular characteristics. This study defined for the first time in Palestine the biometric characterstics of local olive tree, leaves flowers, fruits and oil analysis, therefore samples were taken from several trees of each cultivar. Nabali Baladi cultivar is one of the most wide spread local cultivar in Palestine , it is suitable as a table and oil variety. Its name comes from a village (Bier Nabala) near Jerusalem. Nabali Baladi is sensitive to adverse weather conditions during flowering and thereby characterized with an inconstant and alternate bearing. Cultivar Nabali

Mohassan is a nother important local cultivar, It is believed that this cultivars has been originated out side Palestine, it is suitable for both table olive and oil, it bloom earlier than Nabali baladi, therefore this will result in better fruit set. For Souri cultivar is also wide spread in Palestine , it is been originated in Sour in Lebanon, it is a high producing cultivar, used for table and oil production, it is highly influenced by biennial bearing.

4.2.1 Morphological evaluation :

Olive samples collected from the selected site exhibited morphological differences in all characteristics analyzed. The small the standard error within each sample indicated high uniformity of the characteristics measured. Most of the chosen characteristics are suitable for discriminating between varieties.(Polujah 2008). Some characteristics like inflorescence length, flower number, fruit, stone mass, *etc.* can vary due to exogenous factors (environment, cultivation technology,

etc.). In the case of uncertainty in category defining, measuring has been repeated on the larger sample . According to Bartolini et al. (1998) and Barranco et al. (2000), biometric indexes should always be accompanied by a detailed morphological description of the organs (inflorescence, leaf, fruit, and stone) of olive varieties. In fact, biometric values alone were not able to detect differences among some varieties morphologically similar but characterized by different biological and agronomical behavior.

All of the parameter values (acidity, peroxide number, absorbencies in ultra-violet, fatty acid composition, sterol composition and content) used to evaluate oil quality were within the IOOC trade standards. The only

exceptions were in cultivar Nabali Baladi which had Δ-7-stigmastenol value that was higher than 0.5.

4.2.2 Molecular Evaluation :

The five SSR primers (U99-35, U99-28, DCA9, DCA16, and GAPU103) used in this study revealed 15 polymorphic and two monomorphic alleles. This is comparable to the number of alleles among olive cultivars reported by Muzzalupo., et al., 2009 and Cipriani et al. (2002), but somewhat lower than that published by Lopes et al. (2004) and Sarri V. et al. (2006), probably because it included a large number of foreign cultivars. The DNA fingerprints of the eight olive trees was discriminated in to three genotype with similarity coefficient ranged from 100% between sample number 6 and 7 to 32% between samples 8, 4 and 2. The similarity range is consistent with Muzzalupo., et al., (2009) who studied 23 Italian genotypes of Olea europaea using SSR markers . The DNA from the olive cultivars was analyzed using nine pre-selected SSR primers (GAPU59, GAPU71A, GAPU71B, GAPU103A, UDO99-01, UDO99-12, UDO99-28 and UDO99-39) and revealed 29 alleles, which allowed each genotype to be identified.

When compared with the above reported findings, we can say that the polymorphism ratio observed among the three olive cultivars investigated here is close to that of these studies and even higher than in some of these studies. In fact, the three cultivars studied here were found in a very restricted area, and the high polymorphism ratio could be important in using them in future breeding studies.

The dendrogram derived from an UPGMA cluster analysis of the SSR markers is shown in Figure (4). In the dendrogram the five primers allowed the eight olive genotypes to be grouped into three main group and subgroups corresponding to the same cultivar denominations. For example group I consisted of four samples that represent cultivar Nabali Mohassan . In the case of slightly different patterns we considered that such differences were too few to have originated through sexual reproduction, olive being an out-crossing species with a highly heterozygous genome. We could thus suspect the occurrence of mutations at microsatellite loci as it was shown on genotypes of the some individual in Laperrine's olive populations (Baali-Cherif & Besnard, 2005).

This cultivar was also found to be very distinct according to its morphological and biochemical data it has the highest fruit weight value. This cultivar also has high fruit flesh ratio. (Table 7). Therefore, there were some degrees of similarities among molecular, morphological and biochemical data.

Chapter Five

Conclusions and Recommendations

5. Conclusions and Recommendations

The results obtained in this work, aimed at testing the reliability of the morphological parameters for cultivar discrimination and clarifying the local cultivars' identity and their relationships within the local population, the use of SSR markers, has led to very interesting findings. Both, morphological and molecular data, were compared in order to detect the level of reliability for the morphological parameters and to provide information on which parameters should be useful to discriminate olive cultivars.

The high oil content in Nabali Baladi and Souri cultivars shows their high effciency in accumulating oil in the fruit and confrm the high ability of these cultivars in producing oil and is in agreement with the fact that these cultivars together are the main ones for oil production in Palestine. The medium or high fresh fruit weight and pulp/pit ratio for both Nabli Baladi and Nabali Mohassan, as well as the moderate oil content of Nabali Mohassan confirm thier suitability to be used as dual purpose cultivars. It should be noted that all the oils produced by the olive cultivars met the IOOC trade standards applied to extra virgin olive oils. The only exceptions was cultivar Nabali Baldi had excessively high Δ-7-stigmastenol levels. Further evaluation to determine if environment and/or harvesting time affect Δ-7-stigmastenol content are needed. The overall results on oil characteristics are very important considering that in Palestine increasing amounts of oil will be available for export in the next years.

As far as molecular characterization is concerned, no ambiguous cases of synonymy were found. This means that all of the cultivars examined were different from each other. SSR markers can be valuable for distinguishing and identifying olive varieties, since all cultivars are uniquely characterized. The cultivars were able to be distinguished even when they originated from the same area. On the other hand, the marked genetic variability observed among the 8 samples indicated a situation of "cultivar populations", that is, the presence of different clones within the same cultivar. This situation was found in all the cultivars considered. The results of this study have provided important information about Palestinian olive germplasm. until now, only a few studies on very limited sample sets have been carried out. SSR markers are informative descriptors of the genetic variability of Palestinien cultivated varieties of olives studied for the purpose of cultivar identification. These biotechnological tools can provide significant insights for research in crop breeding and germplasm conservation. The high genetic variability of olive trees will hopefully be exploited in breeding programs. The use of microsatellite markers was confirmed to be a powerful tool not only for studying variation between varieties of the *Olea europaea* L. but also for characterizing intra-specific variations among cultivated olive accessions.

Since only one year of observation of olive oil samples was considered for chemical analyses, the reported results are indicative, but a more complete database of chemical characteristics based on several years of observation is needed.

References

- Akash, M. 2003.**Quantitative trait loci mapping for agronomic and fiber quality traits in upland cotton (*Gossypium hirsutum* L.) using molecular markers**. Graduate school of louisiana state university.
- Baali-Cherif, D. and G. Besnard. 2005. **High genetic diversity and clonal growth in relict populations of *Olea europaea* sub sp. laperrinei (Oleaceae) from Hoggae, Algeria**. Annals of Botany, 96: 823-830.
- Bandelj, D., Jakse, J., Javornik, B. 2004. **Assessment of genetic variability of olive varieties by microsatellite and AFLP markers**. Euphytica, 136: 93–102.
- Barranco, D., Cimato, A., Fiorino, P., Rallo, L., Touzani, A., Castañeda, C., Serafín, F. and Trujillo, I. 2000. **World Catalogue of olive varieties. International Olive Oil Council**. Madrid, Spain, 360.
- Bartolini, G., Messeri, C. and Prevost, G. 1993. **Acta Horticulturae**, 356: 116-118.
- Bartolini, G., Prevost, G., Messeri, C., Carignani, G. and Menini, U.G. 1998. **Olive Germplasm. Cultivars and World-Wide Collections**. FAO, Rome.
- Belaj, A., Satovic, Z., Cipriani, G., Baldoni, L., Testolin, R. and Rallo, L. 2003. **Comparative study of the discriminating capacity of RAPD, AFLP and SSR markers and of their effectiveness in establishing genetic relationships in olive**. Theoretical and Applied Genetics, 107: 736–744.

- Belaj, A., Trujillo, I., De la Rosa, R. and Rallo, L. 2001. **Polymorphism and discriminating capacity of randomly amplified polymorphic markers in an olive germplasm bank**. J. Am. Soc. Hort. Sci., 126: 64-71.
- Breton, C., Medail, F., Pinatel, C. and Berville, A. 2006. **From olive tree to Oleaster: origin and domestication of *Olea europaea* L. in the Mediterranean basin**. Cahiers Agriculture, 15(4): 329–336.
- Cantini, C., Cimato, A. and Sani, G. 1999. **Morphological evaluation of olive germplasm present in Tuscany region**. Euphytica, 109: 173–181.
- Carriero, F., Fontanazza, G., Cellini, F. and Giorio, G. 2002. **Identification of simple sequence repeats (SSRs) in olive (*Olea europaea* L.)**. Theoretical and Applied Genetics, 104: 301–307.
- Cipriani, G., Marrazzo, M.T., Marconi, R., Cimato, A., Testolin, R. 2002. **Microsatellite markers isolated in olive (*Olea europaea* L.) are suitable for individual fingerprinting and reveal polymorphism within ancient cultivars**. Theoretical and Applied Genetics,104: 223-228.
- Cuevas, J., Pinney, K. and Polito, V.S. 1999. **Flower differentiation pistil development and pistil abortion in olive *Olea europaea* L. 'Manzanillo'**. Acta Horticulturae, 474: 293–296.
- Del Rio, C. 1994. **Preliminary agronomical characterization of 131 cultivars introduced in the olive germplasm bank of Cordoba in March 1987**. Acta Hortic, 356: 110–115.

- Dice, LR. 1945. **Measures of the amount of ecologic association between species**. Ecology 26: 297-302.
- Doveri S, Sabino Gil F, Diaz A, Reale S, Busconi M, da Camara Machado A, Martin A, Fogher C, Donini P, Lee D (2008). **Standardization of a set of microsatellite markers for use in cultivar identification studies in olive (*Olea europaea* L.)**. Sci. Hortic. (Amsterdam) 116: 367-373.
- Fernandez-Escobar, R., Benlloch, M., Navarro, C. and Martin, G. C. 1992. **The time of floral induction in the olive**. Journal of the American Society of Horticultural Science, 117: 304–307.
- Fabbri, A., Hormaza, J.I. and V.S. Polito. 1995. **Random amplified polymorphic DNA analysis of olive (*Olea europaea* L.) cultivars.** J. Am. Soc. Hortic. Sci., 120: 538-542
- Fernandez, J.E., Moreno, F., Girń, I.F. and Blazquez, O.M. 1997. **Stomatal control of water use in olive tree leaves**. Plant Soil, 190: 179–192.
- Grati-Kamoun, N., Mahmoud, F., Rebaï, A. and Gargouri, A. 2006. **Genetic diversity of Tunisian Olive Tree (*Olea europaea* L.) cultivars assessed by AFLP markers**. Genet. Resour. Crop Evol., 53: 265-275.
- Gucci, R., Massai, R., Casano, S. and Costagli, G. 1997. **The effect of leaf age on CO2 assimilation and stomatal conductance of field-grown olive trees**. Acta Horticulturae, 474: 289–292.

- Guerin, J., Sweeney, S., Collins, G. and Sedgley, M. 2002. **The development of a genetic database to identify olive cultivars**. 1. Amer. Soc. Hort. Sci., 127(6): 977-983.
- Hetherlington, A.M. and F.I. Woodward. 2003. **The role of stomata in sensing and driving environmental change**. Nature, 424: 901–908.
- Karabourniotis, G., Kotsabassidis, D. and Manetas, Y. 1995. **Trichome density and its protective potential against ultraviolet-B radiation damage to leaf development**. Canadian Journal of Botany, 73: 376–383.
- Karabourniotis, G., Papadopoulos, K., Papamarkou, M. and Manetas, Y. 1992. **Ultraviolet-B radiation absorbing capacity of leaf hairs**. Physiologia Plantarum, 86: 414–418.
- Leva, A. 2009. **African Journal of Plant Science**, 3 (3): 037-043.
- Lopes, M.S.; Mendonca, D.; Seft, K.M.; Gil, F.S.; Da Camara Machado, A. 2004.**Genetic evidence of intra-cultivar variability within Iberian olive cultivars**.HortScience, 39: 1562-1565.
- Martin, G.C. 1994. **Mechanical olive harvest use of fruit loosening agents**. Acta Horticulturae, 356: 284–291.
- Mehri, H. and K. R. Mehri. 2007. **The Bioagronomic characteristics of a local olive cultivar gerboui**. American Journal of Plant Physiology, 2 (1): 1-16.
- Mekuria, G., Collins, G. and Sedgley, M. 1999. **Genetic variability between different accessions of some common commercial olive cultivars**. 1. Hort. Sci. Biotech., 74(3): 309-314.

- Michele, LA M., Jennifer, G., Margaret, S., Ettore, B. 2006. **Identification of olive (*Olea europaea* L.) genotypes using SSR and RAPD markers**. Actes Editions, Rabat, 9-14.
- Muzzalupo, I., Lombardo, N., Pellegrino, M., Perri, E. 2006. **Studio della variabilità genetica di ecotipi di olivo dell'Abruzzo e del Molise mediante l'uso di marcatori molecolari SSR**. Alanno, Proceedings. Alanno: PE Italy, 31-35.
- Muzzalupo, I., Stefanizzi, F., Salimonti, A., Falabella, R. and Perri, E. 2009. **Microsatellite markers for identification of a group of Italian olive accessions**. Sci Agric (Piracicaba, Braz), 66: 685- 690.
- Ozkaya, M.T., Cakir, E., Gokbayrak, Z., Ercan, H. and Taskin, N. 2006. **Morphological and molecular characterization of Derik Halhali olive (*Olea europaea* L.) accessions grown in Derik–Mardin province of Turkey**. Scientia Horticulturae, 108: 205–209.
- Ozkaya M.T., Ergulen, E., Ulger, S. and Ozilbey, N. 2008. **Molecular, Morphological and Oil Composition Variability within Olive (*Olea europaea* L.) At Semi-arid Conditios**. Biotechnol. & Biotechnol. Eq., 22 (2): 699-704.
- **Pelestinian Central Bureau of Statistics**, (2006-2007) Agricultural statisitical data. Palestine.
- Poljuha, D., Barbara, S., Karolina B. B., Marina R., Kristina B., Elvino Š., Marin K. and Aldo M. 2008. **Istrian olive varieties characterisation, Food Technol**. Biotechnol, 46 (4): 347–354.

- Rallo, P., Dorado, G. and Martin, A. 2000. **Development of simple sequence repeats (SSRs) in olive tree (*Olea europaea* L.)**. Theoretical and Applied Genetics, 101: 984-989.
- Rotondi, A., Massimiliano, M., Claudia, R. and Luciana, B. 2003. **Morphological and molecular analyses for the characterization of a group of Italian olive cultivars. Euphytica**, 132: 129–137.
- Sarri, V., Baldoni, L., Porceddu, A. and Cultrera, N.G. 2006 . **Microsatellite markers are powerful tools for discriminating among olive cultivars and assigning them to geographically defined populations**. Genome 49: 1606-1615.
- SAS Institute. 1990. **SAS user guide**. Statistics. SAS Institute, Cary, N.C.
- Sefc, K.M., Lopes, M.S., Mendonc¸ D., Rodrigues, D., Santos, M., Laimer, M. and Machado, A. 2000. **Identification of microsatellite loci in olive (*Olea europaea*) and their characterization in Italian and Iberian olive trees**. Molecular Ecology, 9: 1171–1173.
- Sensi, E., Vignani R, Scali, M. and Masi, E. 2003. **DNA fingerprinting and genetic relatedness among cultivated varieties of *Olea europaea* L. estimated by AFLP analysis**. Sci. Hort., 97: 379-388.
- Sheidaia, M., Zahra N., Alireza, D., Farshid, P., Hoda, H. P. and Mehdi H. M. 2010. **Intra-specific morphological and molecular diversity in brown olive (*Olea cuspidata*) of Iran**. Science Asia, 36: 187–193.
- Strikic, F., Dunja B. M., Slavko, P., Zlatko, C., Zlatko, S. and Branka, J. 2010. **Genetic variation within the olive (*Olea europaea* L.) cultivar**

Oblica detected using amplified fragment length polymorphism (AFLP) markers. African Journal of Biotechnology, 9 (20): 2880-2883.

- Taamalli, W., Geuna, F., Bassi, D., Daoud, D. and Zarrouk, M. 2008. **SSR marker based DNA fingerprinting of Tunisian olive (*Olea europaea* L.) varieties**. Journal of agronomy, 7: 176-181.
- Terzopoulos, P.J., Kolano, B., Bebeli, P.J. and Kaltsikes, P.J. 2005**. Identification of (*Olea europaea* L.) cultivars using inter simple sequence repeat markers**. Scientia Hortic., 105: 45-51.
- Therios, I. 2009. **Olives**. Library of Congress Cataloging-in-Publication Data. UK.
- Vinod, K.K. 2004. **Total genomic DNA extraction, purity analysis and quantitation**. Presented in the CAS training program on "Exploiting Hybrid Vigour in Crop Plants Through Breeding and Biotechnological Approaches", Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University. Coimbatore, pp. 92-104.
- Weissman, M.M., Warner, V., Wickramaratne, P., Moreau, D. and Olfson, M. 1997. **Offspring of depressed parents. 10 Years later**. Archives of General Psychiatry, 54: 932-940.
- World Bank, 2012. http://go.worldbank.org/MBK9GU1TD0
- Zitoun B, Bronzini de Caraffa V, Giannettini J, Breton C. 2008. **Genetic diversity in Tunisian olive accessions and their relatedness with other Mediterranean olive genotypes**. Sci. Hortic. 115: 416-419.

جامعة النجاح الوطنية
كلية الدراسات العليا

التوصيف الشكلي والجيني لاصناف الزيتون الرئيسية في فلسطين
(*Olea europea L.*)

إعداد
رامز جواد عمر

إشراف
د. حسان ابو قاعود

قدمت هذه الأطروحة استكمالاً لمتطلبات درجة الماجستير في الانتاج النباتي بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين

2012

التوصيف الشكلي والجيني لاصناف الزيتون الرئيسية في فلسطين

(*Olea europea L.*)

إعداد

رامز جواد عمر

إشراف

د. حسان ابو قاعود

الملخص

أجريت الدراسة لمقارنة الصفات الشكلية، الكيماوية والجينية لأصناف الزيتون الأكثر انتشارا في فلسطين. استخدمت الأصناف التالية : النبالي البلدي، المحسن والسوري. تم جمع عينات من الأوراق، الأزهار، الثمار والبذور من اجل تصنيفها مظهريا، كما تم استخلاص عينات من الزيت من هذه الأصناف لتحليلها كيماويا وحسيا، كما تم تحليلها جينيا، ومن اجل ذلك تم استخلاص الـ (DNA) من انسجة الاوراق وتم تحليلها باستخدام البادئ SSR. ,وتم حساب المسافات الجينية بين كل شجرة باستخدام معامل تشابه النرد والـ dendrogram بالاعتماد على تحليل الكتلة UPGMA .

وقد لوحظت اختلافات كبيره بين الأصناف الثلاثة في معظم المواصفات التي تم دراستها وتضمنت صفات: غطاء الشجرة، الأوراق، الأزهار و الثمار. كما تم قياس درجة الحموضة للزيت والرقم البيروكسايدي وامتصاص الضوء (spectro-photometer) في الأشعة فوق البنفسجية، وجميعها كانت منخفضة في جميع عينات الزيت المفحوصة، معظم الأصناف كانت تحتوي على نسبة حوالي 60% أو أكثر قليلا من حامض الأوليك، باستثناء النبالي المحسن، محتوى وتركيب الستيرولات كان مختلفا بين الأصناف. صنف النبالي البلدي كان يحتوي على نسبة عالية من Δ-7stigmastenol. جميع التحاليل البيوكيماوية (الحموضة، البيروكسايد، الامتصاص للأشعة فوق البنفسجية، الأحماض الدهنية ومحتوى وتركيب الستيرولات) التي استخدمت في تحليل جودة الزيت، هي وفق مواصفات مجلس الزيت والزيتون العالمي (IOOC). في هذه الدراسة تم استخدام تحليل بادئات SSR من اجل الفحص الجيني. لقد تميزت البصمة الوراثية للثمانية أشجار زيتون التي تم دراستها الى انقسامها إلى ثلاثة مجموعات جينية واضحة.