DISSERTATION

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF LIBYAN OLIVE, *OLEA EUROPAEA* L., CULTIVARS (42 LOCAL AND 16 WILD TYPE) IN COMPARISON TO 41 INTRODUCED (WORLD) CULTIVARS

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ABSTRACT

MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF LIBYAN OLIVE, *OLEA EUROPAEA* L., CULTIVARS (42 LOCAL AND 16 WILD TYPE) IN COMPARISON TO 41 INTRODUCED (WORLD) CULTIVARS

Olive (*Olea europaea* L.) consumption and production are important socially and economically in Libya. Olive cultivars that are adapted to local conditions produce olives that have high quality and quantities of oil. Many of the important olive cultivars grown in Libya were evaluated in this research. One goal of this project was to determine the plasticity of morphological traits of olive cultivars that have been grown at diverse locations within Libya. A second goal was to identify a set of traits that are independent of each other and show limited variation (stable traits) regardless of the environmental conditions.

The stable traits were then used in subsequent analyses to correlate genetic and phenotypic characteristics of Libyan olives. Two different groups of olives were compared: the 45 landraces and the 45 cultivars of *Olea europaea* subsp *europaea* var. *sativa.* Morphological data were collected for a total of 39 morphological traits (22 quantitative and 17 qualitative), which were then combined and analyzed to determine phenotypic diversity among different locations.

Differences in many of the morphological traits were observed across the cultivars. These sets of data were used to identify unique and desirable Libyan landraces morphologically. Stable phenotypic traits were used to discriminate between use of fruit (oil or dual-purpose) as well as cultivar origins (local or introduced). This research demonstrates that local Libyan cultivars (landraces) have unique characteristics that differentiate them from imported cultivars.

Ten microsatellite markers were used to differentiate and evaluate the relationships among a total of 91 olive genotypes (39 landraces, 36 introduced cultivars and 16 wild types) collected in Libya. A total of 109 alleles were identified using 10 loci, with the number of alleles per locus ranging from 4 to 20. Three loci (UDO43, DCA16 and GAPU101) had the most alleles with 20, 18 and 16, respectively. The wild types and introduced cultivars had greater numbers of alleles than the local cultivars. Six cases of duplicated genotypes, two cases of synonymy, and thirteen homonyms that were genetically distinct were observed in the Libyan collection.

UPGMA clustering classified the accessions into two main distinct groups. The first group consisted of landraces and the second group included introduced cultivars and wild type accessions. Admixture analysis also distinguished between landraces and wild genotypes. In general, molecular data enables one to separate the Libyan olive accessions based on their orgin but not on their fruit use.

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CHAPTER 1.0 GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Economic impact

Olive trees (*Olea europaea* subsp. *europaea* L.) have been cultivated in the Mediterranean Basin for millennia. It's believed that cultivated varieties of *Olea europaea* supsp. *europaea* var. *sativa* were derived from the wild type *Olea europaea* subsp. *europaea* var. *sylvestris* in the Mediterranean region and then were spread throughout the world (Sesli &Yegenoglu, 2010). The Romans extended the area of olive cultivation from the Greek islands into the Mediterranean Sea countries (Cipriani et al., 2002). Olive cultivars are considered to have great economic significance and may be the most important agricultural oil crop in the Mediterranean region (Terzopoulos et al., 2005). In this region olive orchards cover about 7,000,000 ha (Khadari et al., 2003) and have a worldwide cultivation of about 8,800,000 ha (IOC, 2007 and Haouane et al., 2011).

Approximately 95% of the world olive oil production is concentrated in Southern Europe, North Africa, and the Middle East, and it's considered to be the most extensively cultivated fruit crop in the world (FAO, 2004; FAO, 2012; Hatzopoulos et al., 2002; Jain & Priyadarshan 2009 and http://apps3.fao.org/wiews/olive/intro.jsp). More than 1275 cultivars have been described by Bartolini et al. (1998) in the southern European countries with 538 in Italy, 183 in Spain, 88 in France, 52 in Greece, and 45 in Turkey. Spain, Italy, Greece, Turkey and Tunisia are the largest producers of olive oil in the world.

The number of olive oil consumers has been increasing, especially since recent evidence suggests health and nutritional benefits of virgin olive oil (Poljuha et al., 2008). Virgin olive oil (VOO) is a source of at least 30 antioxidant phenolic compounds and 100 aromatic compounds that contribute to its bitter taste and aroma; also it is the only oil that can be eaten

without refining. Olive oil ranked sixth in level of world cooking oil production. (Navero et al., 2000; Besnard et al., 2007; Kole, 2011 and Aparicio & Harwood 2013).

1.2 Botanical description

The genus *Olea* belongs to the *Oleaceae* family which consists of 30 genera with 600 species of woody plants including the ashes 2n=46 (*Fraxinus)* (Fig.1.0 A), ornamentals such as jasmine 2n = 26 (*Jasminum auriculatum*) (Fig.1.0 B) and agriculturally important plants such olive 2n =46 (*Olea europaea* L.) (Fig.1.1) (Kole, 2011). The genus *Olea* is divided into three subgenera, *Tetrapilus, Paniculatae,* and *Olea*. The subgenus *Olea* has been separated into two sections: *Ligustroides* and *Olea*, The section *Olea* includes just one species of Mediterranean olive tree *Olea europaea* L. that has more than 1,000 sub-species (subsp.) which are cultivated for oil production, table consumption or dual purpose (Rallo et al., 2003).

All of *Olea europaea* subsp. *europaea* var. *sylvestris* (wild types) and *sativa* (cultivated varieties) are diploid and have the same chromosome number: 2n=46. The Euro-Mediterranean olive (*Olea europaea* L. subsp. *europaea*) is found mainly in the Mediterranean Basin. The relationship of the Euro-Med. olive to other subspecies has remained ambiguous. These variations among Mediterranean olive populations probably resulted from genetic variations over years.

The geographic barriers have limited intercrossing resulting in the current five subspecies of *Olea europaea* subsp. *laperrinei*, (it distributed in Saharan massifs in Algeria), *Olea europaea* subsp. *cuspidate* (Egypt to South Africa), *Olea europaea* subsp. *guanchica* (Canary Islands), *Olea europaea* subsp. *maroccana* (Agadir mountains, Morocco), and *Olea europaea* subsp. *cerasiformis* (Madeira Island) (Besnard et al., 2007). Besnard et al. (2008) found tetraploids in supsp. *cerasiformis* as a result of hybridization between subsp. *guanchica* and *europaea*, based on phylogenetic analyses. In addition, hexaploids of *Olea europaea* subsp.

maroccana have been found. This polyploidization is considered an approach to overcome inbreeding depression (Kole, 2011).

Wild olives (*Olea europaea* subsp. *europaea* var. *sylvestris*) are native to the Mediterranean Basin (Kole, 2011). Both wild and cultivated olives grow in similar locations in Spain. However, the wild type oleaster is distinguished from the domesticated types by several characteristics such as long juvenile stage, small fruit size, a higher stone/mesocarp ratio, and relatively low oil content. However, wild type and cultivated olives do have the same botanical descriptions for pollen grains, stones and timber wood frame.

The wild olives are considered important as a genetic resource of genes for improvement of resistance against environmental conditions and diseases for future plant breeding programs (Sesli &Yegenoglu, 2010). The wild type (*Olea europaea* subsp. *europaea* var. *sylvestris*) is used as a rootstock for grafting cultivated *sativa* cultivars. As noted by Belaj et al. (2007), wild types and feral forms can be distingushed from other subspecies. The so called wild forms have originated in natural areas, whereas feral forms are commonly considered either seedlings of cultivated olives or seedlings as the result of hybridization between wild type and domesticated cultivars. Both seed and clonal propagation have played a major role in the evolution and distribution of olive cultivars (Kole, 2011 and Elbaum et al., 2006). Besnard et al., (2007) found greater diversity in wild types than in cultivated varieties.

Wild olive gene pools are sources of genetic diversity for breeding programs. The primary gene pool (GP1) of olive includes both wild and cultivated types of *Olea europaea* subsp. *europaea* var. *sylvestris* and *sativa* that are genetically similar to each other. The secondary gene pool (GP2) is considered to be the related sub-species of *Olea europaea* var. *cerasiformis, maroccana, guanchica, laperrini* and *cuspidate*.

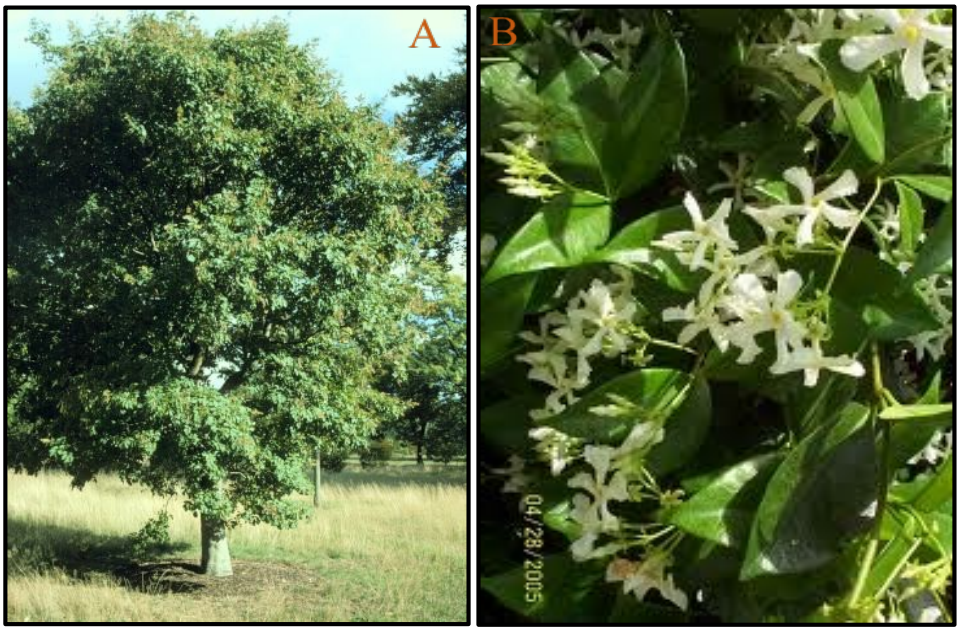


Fig.1.0.The *Oleaceae* family includes important woody species such as ash (*Fraxinus ornus*) A and Ornamental jasmine plants (*Jasminum auriculatum*) B.

Fig.1.1. Agriculturally productive plants such as cultivated olive (*Olea europaea* subsp. *europaea* var. *sativa*) are members of the *Oleaceae* family.

According to Terral et al. (1996), the species include wild relative subspecies of *cuspidata*, *laperrinei*, and *cerasiformis*. A tertiary gene pool (GP3) includes most of the *Ligustroides* species such as *Olea exasperate*, *Olea capensis* subsp. *macrocarpa*, *Olea woodiana*, *Olea paniculata*, and *Olea lancea*, which are considered to be non-related species (Kole, 2011). Also oleaster varieties offers promising sources of genetic diversity for olive breeding.

Olive trees are long-lived perennial evergreens that are likely adapted to a narrow range of environmental conditions such as those that occur in the Mediterranean Basin, Australia, and China (Besnard et al., 2007). Olive tree branches may be upright or pendulous depending on the cultivar. Vigor is highly dependent on tree nutritional status. The olive fruit is a drupe with that is ovoid, spherical and elongated with either pointed or rounded apex end and a truncate or rounded base. Olive trees bear hermaphroditic flowers that are most often self-incompatible and anemophilous; sometimes they may be partially or completely self-fertile.

The degree of outcrossing varies among olive cultivars and environments depending on the genetic background of cultivar and wind direction (Mekuria et al., 2002). Olive pollen distribution is affected by wind and may be transported long distances as demonstrated by gene flow evidence (Ribeiro et al., 2005). Alternative bearing habit is common and is strongly cultivar dependent which impacts annual olive fruit production under normal agronomic conditions. A 5% fruit set in olive results in a full crop. Over the years, olive oil production has varied considerably due to fruiting inconsistencies (Kole, 2011; Navero et al., 2000; Aparicio et al., 2013 and Jain & Priyadarshan 2009).

Olive fruit cannot be consumed fresh because it contains high amounts of the polyphenolic compound oleuropein (5 mg polyphenols per 10g olive oil) and low sugar content 2.6–6%. The oil content varies from 12 to 30% depending on the type of cultivar (Navero et al., 2000; Kole, 2011 and Aparicio & Harwood 2013).

1.3 Domestication and diversity

1.3.1 Phenotypes

Different techniques have been used to characterize olive diversity. Morphological criteria such as leaf, fruit, seed, and growth behavior have been used to evaluate olive diversity as well as to determine the origin of olive trees. An evaluation of phenotypic diversity was used to discriminate olive cultivars with distinct morphological and pomological characters (Ipek et al., 2012). There are many systematic identification procedures that have been developed to help identify genetic diversity in olive trees. These include chemical (fatty acids and oil content) and phenological parameters (dates of first leaves, fruits and flowers) as reported by Lumaret et al., 2004 and Taamalli et al., 2006. Isozyme analysis has also been used to analyze the genetic diversity in cultivated and wild type olives because morphological traits have in general not been able to clearly differentiate between wild olive and feral, or between closely related cultivars (Kole, 2011). The long life cycle and protracted seedling juvenility (15-20 years) of olive (Leon et al., 2005), as well as their routine vegetative propagation, have led to a lack of new genotypes in recent years and has discouraged improvement through plant breeding programs (Cipriani et al., 2002). This lack of breeding programs may affect availability of materials for future generations, particularly if wild diversity and ancient landraces are lost (Sarri et al., 2006 and Ganino et al., 2007).

The relative rates of gene flow by pollen or seed migration determine the exchange of genetic material. This leads to a high level of heterozygosity and genetic diversity among olive cultivars. The mating system of olives has played a major role in the rate of gene flow and exchange of genetic material among wild, feral and cultivated olives, particularly for oleaster types, which were spread by long distance human migration and seed dispersal by birds. As a result, there are more than 2600 olive cultivars described from around the world. However, many of these cultivars are likely synonyms/homonyms of specific genotypes (Cipriani et al.,

2002; Mekuria et al., 2002 and Kole, 2011). Consequently, evaluation and characterization of olive genetic diversity is necessary.

The identification of olive cultivars and their area of origin are very important in order to expand cultivation of those commercial varieties with superior products that are best adapted to specific local environmental conditions (Sarri et al., 2006; Poljuha et al., 2008 and Charafi et al., 2007). The presence of synonymous clones and mislabeling has been reported in olive orchards. Researchers have failed to accurately evaluate these two forms by using morphological studies due to the similarities in phenotypes (Belaj et al., 2007). Various studies have used morphological traits to distinguish specific olive cultivars (Navaro et al., 2000 and Belaj et al., 2002). This information is considered to be of limited value because of the many discrepancies due to environmental influence (phenotypic plasticity) on the specific traits or genetic variations of closely related genotypes.

Olive cultivars are propagated asexually, and sometimes through the process of exchanging plant materials, cultivars have been inadvertently renamed. This has lead to the misidentification of genotypic diversity of intra- and intervarietal cultivated olive trees, which has challenged the establishment of a reliable cultivar database. This has also resulted in numerous names given to a particular clone (denomination) (Baldoni et al., 2009). Consequently, molecular techniques may be more accurate procedures to identify and discriminate genetic variations as well as to improve and support morphological or allozymes analysis. The use of morphological descriptions in combination with molecular marker techniques will help with the identification of olives cultivars (Leon et al., 2005).

1.3.2 Genotypes

DNA-based markers are more reliable for cultivar and subspecies identification than phenotypic traits since they are not influenced by environmental conditions (Sesli andYegenoglu, 2010). Molecular markers have been developed for olives in order to facilitate

accurate cultivar identification (Belaj et al., 2003). This enables clear identification of genetic polymorphism within and among olive cultivars. Previous research clearly indicated that the SSR technique was more appropriate than AFLPs and RAPDs for polymorphic detection which more clearly distingishes among closely related cultivars such as “Frantoio” and “Cellina”. However, they concluded that all three techniques (RAPDs, AFLPs and SSRs) were useful to discriminate all 32 olive genotypes studied (Belaj et al., 2003; Montemurro et al., 2008 and Ganino et al., 2007).

Several different techniques have been used to characterize and evaluate olive diversity such as isozymes (Lumaret et al., 2004 and Kole, 2011), RAPD (Sesli and Yegenoglu, 2010), AFLP (Sanz-Cortés et al., 2003), SSR (Cipriani et al., 2002; Sarri et al., 2006; Sezai et al., 2010) and SNP (Hakim et al., 2009 and Tanyolac, 2013). These techniques along with their markers are currently available and are extremely useful when the morphological traits do not clearly identify the genetic diversity within related genotypes. Olive isozyme analyses determined that western populations of the Mediterranean Basin and Canarian populations of *ssp. guanchica* were genetically similar (Lumaret et al., 2004). A comparison of diversity assessments using SSRs (Baldoni et al., 2009) and allozymes (Lumaret et al., 2004) determined that SSRs illustrated greater allelic diversity than allozymes. These results were similar to reports of using RAPD and ISSR markers (Hess et al., 2000). RAPD markers have been used as an applicable tool for fingerprinting and determination of genetic similarities. Wünsch and Hormaza (2002) pointed out that RAPD marker techniques are still being used for genetic diversity and fingerprinting due to their simplicity of use and low development cost in developing countries when the financial situation is limited.

AFLP has been shown to be reliable, informative, and gives dominant and reproducible markers that can be used to detect genetic similarities in olive (Belaj et al., 2003 and Ercisli et al., (2009). The use of AFLP markers in closely related genotypes have been used to distinguish

the physical location of genes for both qualitative and quantitative traits (Mohler & Schwarz, 2004). The gene pool of cultivated olives in the eastern Mediterranean Basin was revealed by using AFLP techniques, which analyzed 119 polymorphic bands to detect their genetic diversity (Owen et al., 2005). AFLP markers identified synonyms, such as cultivars “Frantoio” and “Correggiolo” (Rotondi et al., 2003).

Simple sequence repeats (SSRs) have proven to be suitable markers for olive fingerprinting and identification. SSR markers are also useful in assigning cultivars to their geographic population of origin (Diaz et al., 2008 and Baldoni et al., 2009). It is the preferred technique for olive discrimination due to the high level of polymorphism, co-dominant inheritance, and ease of detection of a high number of allelic values per locus. It depends on short tandem-repeat sequences (STR) (Rojas et al., 2008; Wu et al., 2004; Kole, 2011).

The discrimination power (PD) of twelve SSR markers has been determined for cultivated varieties in several countries of the Mediterranean Basin. These markers have clearly distinguished more than 100 olive genotypes and could be used to assign them to different genetic populations based on thier origin (Sarri et al., 2006). This could in turn be used to select the most adapted cultivars to each geographical area. In olive trees, microsatellites markers were also used to evaluate chromosome abnormalities (aneuploidy) (Besnard et al., 2008). Ercisli et al., (2011) have recently identified 32 polymorphic alleles that were able to correct and retrieve the original names of 10 olive cultivars grown in Turkey.In olive, all SSRs identified recently have di-nucleotide repeats, mainly AG/CT, because of their high frequency in the genome (Baldoni et al., 2009).

SSR markers can be used to distinguish and identify cultivars as well as indicate genetic relatedness (Mohler et al., 2004 and Mackay et al., 2008). El Saied et al. (2012) indicated that the ISSR technique could be used to provide practical information for breeding and conservation strategies. Genotyping with 13 SSR loci (Erre et al., 2010) determined the

phylogenetic relationships among the oldest wild and cultivated olives (Dı´ez et al., 2011). Differences were found between wild and cultivated olive as well as synonymy cases in local cultivars. Detection of unique alleles was found within wild types as well as local landraces that may be useful for advanced breeding.

Previous research, as noted by Baldoni et al., (2009) with a set of 21 cultivars, tested 37 pre-selected SSR loci to identify those most useful for olive fingerprinting based on reproducibility and quality of scoring. They recommended use of the following 11 SSRs: UDO-043, DCA9, GAPU103A, DCA18, DCA16, GAPU101, DCA3, GAPU71B, DCA5, DCA14, and EMO-90.

1.4 Libyan germplasm

The major agricultural products cultivated in Libya are olives, dates, and almonds (Belaj et al., 2003). Olive oil is one of the most important products in Libyan agriculture, but Libyan agriculture is a small contributor of olive oil and table olive production in the Mediterranean region. Libya is the world's 12th largest olive oil producer with 0.25 % of global oil production in. High quality and quantity of olive oil production in Libya has been noted and is believed to be associated with local landraces that are better adapted to local conditions such as high temperatures and low rainfall in this hot semiarid area (Abdul Sadeg, 2003 and http://www.tripolipost.com). This superior production is very important because both oil quality and olive productivity are traits related to specific varieties. Most of the cultivated areas are located along the coastline (http://www.tripolipost.com). Olive cultivation in Libya is limited due to its dependency on hand labor, especially for harvest, limited water resources and limited arable land. Arable land makes up only 1.7% of Libya. According to 2008 official statistical data and FAOSTAT data, about 205,000 hectare were cultivated with more than 9,000,000 olive trees with an average of 135,000 tonnes of olive fruit in Libya

(FAO,2012;Daham&Ashur, 2008 and http://www.tripolipost.com), representing more than 100 cultivars.

Olives have a high degree of genetic diversity. There are many progeny, which have been developed through recombination of gene flow between cultivated varieties, as well as between cultivated, and either feral or wild types.These methods of cultivation increased the recombination of local landraces with the help of growers' selection. Landraces seem to have superior advantages over introduced cultivars, perhaps as a result of their similarity to wild types. They have useful adaptive traits to local condation that may have been introgressed over the years into local varieties. This has resulted in a high level of genetic diversity through dynamic movment between the wild types and the landraces. Farmers may have taken advantage of these intercrossing and transplanted superior hybrids to new locations. Native or landraces (Fig. 1.2) are thought to hold potential sources of important agronomic variation for Libya. These landraces have superior fruit production, despite limited seasonal rainfall, and high quality and quantity of oil production. Landraces with their local biodiversity may be preferred genotypes for conservation (Daham and Ashur, 2008 and Aparicio & Harwood 2013). A primary analysis based on morphological data may be used to clarify various local olive varieties, but the genetic relationships of varieties, their variability and origins are still limited or unknown in Libya (Abdul Sadeg, 2003).

In Libya, there are two types of olive (*sativa* and *sylvestris*) that are located in the west and east side of Libya, repectively. Wild types (*Olea europaea* subsp. *europaea var. sylvestris*) are native throughout the Green Mountains on the east side of Libya (Fig.1.3). They have very small fruits, leaves, and thorny shoots. These wild genotypes have been used as rootstocks for cultivated olives (var. *sativa*) (Kole, 2011), but now most of the olive nurseries rarely used wild types as a rootstock.

Most of the landraces of Libyan olives originated in western Libya, specifically in Masalata city. The decrease in landraces population sizes is probbally due to expansion of commercial introduced cultivars and the limited resources available for conservation and propagation. Previous studies have genetically characterized some ancient olive cultivars and of these, only a few landraces matched cultivated olive cultivars (Dıez et al., 2011). The genetic characterization of Libyan landraces and wild types may provide unique diversity for breeding and germplasm collections. It is difficult to conserve olive germplasm in Libya due to problems associated with distinguishing specific genotypes (Daham&Ashur, 2008). The presence of genetic intra-varietal variation of closely related olive varieties makes the process of identifying olive cultivars challenging (Sarri et al., 2006). Synonyms, homonyms and mislabeled cultivars are considered one of the most important problems in olive identification. The expansion of cultivation of correctly identified landraces is important with the growing commercial interest in quality products.

In this dissertation I characterize the unique Libyan landraces and wild types of olives using morphological traits and molecular techniques. These approaches were used to determine if there were identifiable domestication patterns between wild types and landraces. SSR markers were used to differentiate and characterize olives from Libya. This information will be useful in the development of a database of olive cultivars in Libya. The purpose of this work is to characterize the landraces and wild types through use of molecular and morphological data as a means to understand and conserve biodiversity of these genotypes as well as the introduced cultivars.

Fig. 1.2. The ancient olive landrace “Rasli” (*Olea europaea* subsp. *europaea* var. *sativa*) located in the Mesalata region.

Fig.1.3 Wild oleaster of olive (*Olea europaea* subsp. *europaea* var. *sylvestris*) as observed in the Green Mountain region In Libya A.

1.5 Objectives

- Determine the plasticity of morphological traits of olive cultivars that grow in Libya in several locations.
- Identify morphological traits that are most stable and independent as a method to identify unique and desirable Libyan cultivars.
- Select the most useful set of SSR loci markers that can be used to assess olive genetic diversity.
- Use molecular markers (SSR) to assess genetic diversity in both Libyan (local cultivars & wild types) and introduced cultivars of olive.
- Identify the correlations between phenotypic and genotypic data in olive.
- Assess the relative relationships among the local, wild and introduced cultivars.
- Identify mislabeled accessions in the Libyan olive collection.
- Perform a systematic assessment of olive varieties as a first step to develop a catalogue of Libyan olive cultivars and germplasm collections.

CHAPTER 2.0 MORPHOLOGICAL CHARACTERIZATION OF LIBYAN OLIVE, *OLEA EUROPAEA* L., CULTIVARS

2.0INTRODUCTION

Olive production has a great economic significance in Libya and throughout the Mediterranean region (Terzopoulos et al., 2005). According to the latest official statistical data, a total of 9,000,000 olive trees are cultivated in Libya (Daham and Ashur, 2008 and http://www.Tripolipost.com). Olive oil is one of the most important products in Libyan agriculture. Olive landraces (Fig. 1.2) are best adapted to specific local environmental conditions. They have superior products and high quality and quantities of oil in comparison to imported cultivars despite limited rainfall. These landraces may contain novel forms of genetic diversity.

The presence of olive landraces have been documented in Libya (Abdul Sadeg, 2003), but very little is known about their morphological diversity. Most olive cultivars that where grown in Libyan are mainly used for oil production of organic virgin oils. Production is based on several accessions of an ancient olive. Olive table production in Libya has no priority due to the negative effect of drought on fruit quality and the time consuming requirements for table olive processing.

Most collected samples from Mesalata city are considered to be landraces, but olive diversity in Gharian city and highland areas located in the Libyan southwest considered to be mixed landraces and cultivated varieties (Fig.2.1). In contrast, most of the cultivated olives in the Tharouna, Zaltin, Tripoli and Libyan coastal cities are cultivars developed in other countries.

The increased interest in intensive cultivation methods has limited the number of cultivars that are planted. For example, Spanish Arbequina olive is preferred by growers because of ease in production under intensive cultivation.

This may lead to loss of local diversity as trees are replanted. Ancient landraces have been vegetatively propagated by olive farmers for millennia. Mislabeling and renaming events may have occurred as plant materials have been exchanged among farmers. It would be valuable to have a reliable and straightforward method to identify olive cultivars that are currently grown in Libya.

Morphological and agronomic characters have been widely used to distinguish olive cultivars (Taamalli et al., 2006; Corrado et al., 2009; Zaher et al., 2011). A set of morphological traits that could be used in cultivar identification would be valuable. It might also be used to evaluate the interaction of genotype and environment on phenotypic morphology. Phenotypic data has been limited in its use to discriminate among cultivars and to estimate relatedness (Corrado et al., 2009).

The main objective of this work was to determine the stability of combined morphological traits. As part of this effort, morphological data were collected from olive cultivars planted in diverse habitats to determine phenotypic diversity as related to different locations. The environmental plasticity/stability of fruit, seed and leaf traits were assessed. Stable traits then used to identify key cultivars and to correlate genetic and phenotypic characteristics.

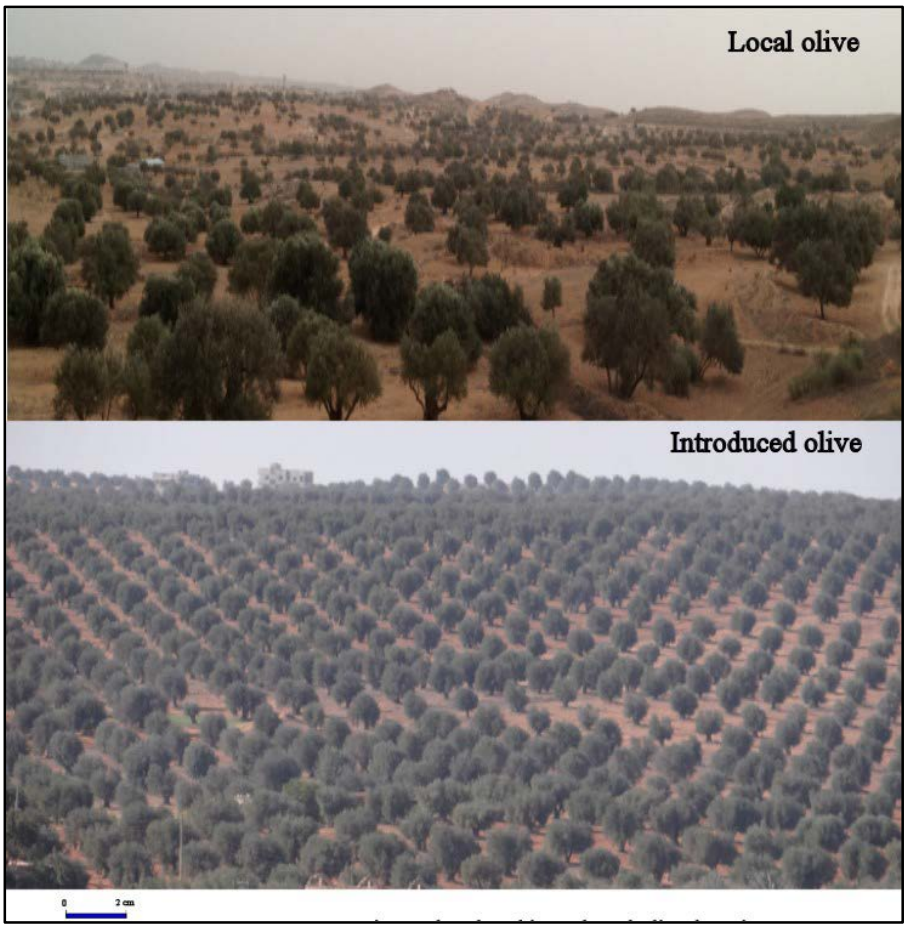


Fig.2.1. Examples of general views of landraces and cultivated olive that located in Mesalata and Tharouna respectively.

2.1 MATERIALS AND METHODS

2.1.1Collection sites

A total of 90 landraces and cultivated varieties of olive (*Olea europaea* subsp. *europaea* var. *sativa*) were collected in two different seasons 2009/2012 from areas near five different Libyan cities: Mesalata, Tripoli, Zaltin, Tharouna and Gharian (Fig. 2.2). These represented 45 landraces and 45 introduced cultivars, primarily imported from Italy in 1953 (Fig 2.1; Table 2.1). These 90 cultivars represent the majority of named olives in Libya. The collection sites represent the primary production regions as well as the diverse environmental conditions in which olives are grown. All of the Libyan landraces were collected near Mesalata from private olive farms while the cultivated varieties were collected from Tharouna and Gharian. These latter areas were government collections. Collections from the Zaltin and Tripoli regions were cultivated varieties from private farmers. Olive accessions were identified based on farmer comments and official government information.

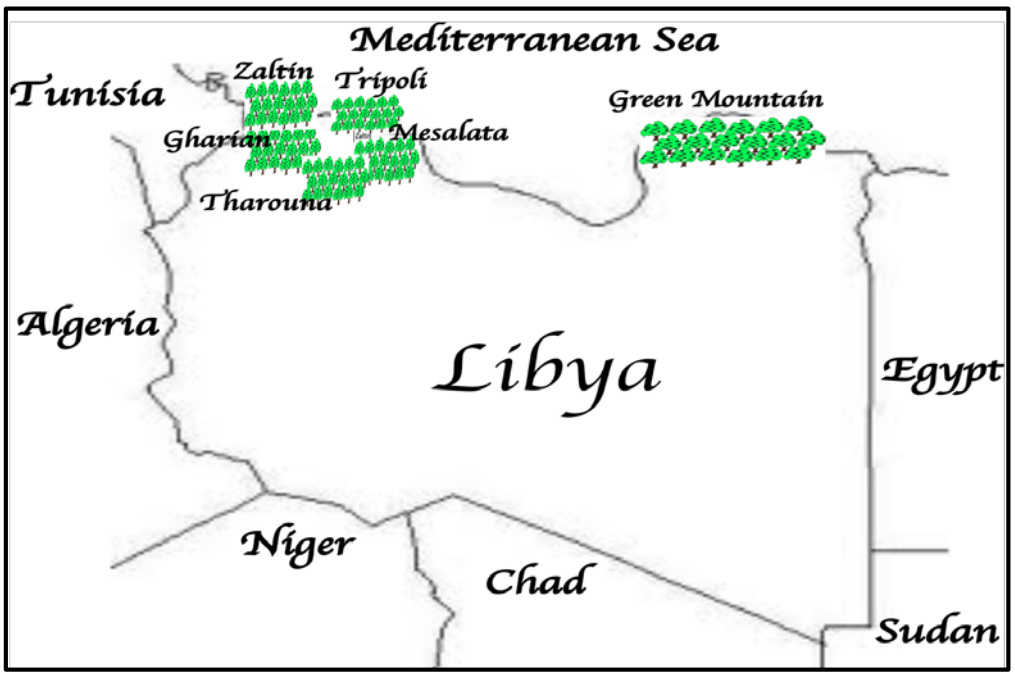


Fig.2.2. Collection sites in the map indicate the locations where olive samples were collected in the West and East side of Libya.

The coastal areas of the collection sites, such as Tripoli, have a Mediterranean climate with warm summers and cold winters. The weather is cooler in the highland areas of Mesalata and Gharian with more rain. The weather in Zaltin and Tharouna is cool but it is a dry climate. Rainfall is limited in all locations and occurs in the winter season from October to March (Table 2.0).

Table 2.0 General climatic conditions, relative precipitation and average temperatures of collection areas as recorded in previous literature (http://www.photius.com/countries/libya/climate/libya).

[z]Locations	Precipitation (mm)	Average Temperatures		Climate
		Summer	Winter	
Mesalata	250 to 500 mm/year	19-30Ċ	2-15Ċ	Warm summers and cooler in winters
Gharian	200 to 450 mm/year	20-32Ċ	3-15Ċ	Warm summers and cooler in winters
Zaltin	<300mm/year	23-37Ċ	5-20Ċ	Dry climate
Tharouna	<300mm/year	21-38 Ċ	5-20Ċ	Dry climate
Tripoli	< 400mm/year	22-35Ċ	9-18Ċ	Mediterranean climate with warm summers and cold winters

z locations (Fig.2.2) (Mesalata, Gharian, Zaltin, Tharouna, and Tripoli).

2.1.2 Plant material and processing samples

One to three trees were representatively sampled for each cultivar. When multiple trees of the same cultivar could not be positively identified, only a single tree was sampled. Fifty fruits and 10 leaves were collected from each tree. All fruit and leaf samples were collected randomly from all sides of the tree. Fruit samples that were used for imaging (Fig.2.4 and Fig.2.5) were collected from immature to mature stages of fruit maturation, while fruit samples that were used to evaluate qualitative or quantitative phenotypic traits were collected form fully mature fruits (black color). All fruit samples were harvested from different developmental stages as fruit started to change color from yellow green to black during October to December, 2012 (Fig.2.3).

Fig.2.3. Stages illustrating the color of harvested fruit samples.

Fig.2.4. Fruit, seed and leaf samples of ‘Zarrasi’ A and ‘Chemlaikussabat’ B that illustrate the descriptive images captured.

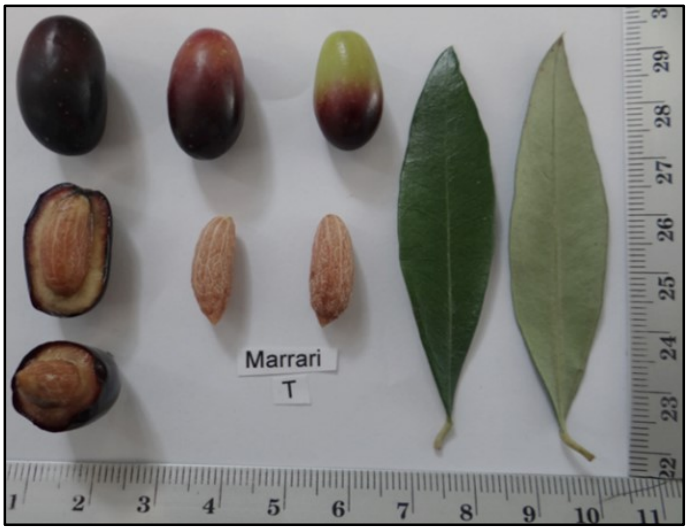


Fig.2.5. Example of fruit, leaf and seed images observed for collected samples of ‘Marrari’.

Information on the cultivar common name, country of origin, and main purpose of use was noted (Table 2.1). The morphological traits were systematically evaluated for thirty-nine (qualitative and quantitative) characters. Fruit and leaf samples from a total of 17 duplicated olive accessions were collected from two different locations (either Tharouna and Mesalata or Tharouna and Gharian). Nine of the replicated accessions (Chemlalikussabat, Gargashi, Marrari, Rasli, Mbuti, Zarrasi, Zaafrani, Hammudi and Jabbugi) were grown in Tharouna and Mesalata while seven others (Maurino, Chemlalisfax, Coratina, Frantoio, Moraiolo, Ouslati and Leccino) were grown in Tharouna and Gharian (Table 2.3) and (Fig.2.1). Data were collected using standardized morphological descriptors according to the International Olive Council (IOC) for trait descriptions and identification of olive varieties (Navero, et al., 2000; Muzzalupo, I. 2012). Scanned images were captured by the TurboScan program (Fig.2.6) and then were analyzed by Image-Pro Plus software to quantify images in order to determine cross sectional area, roundness and Box X/Y for fruit, leaf, and seed samples (Table 2.3).

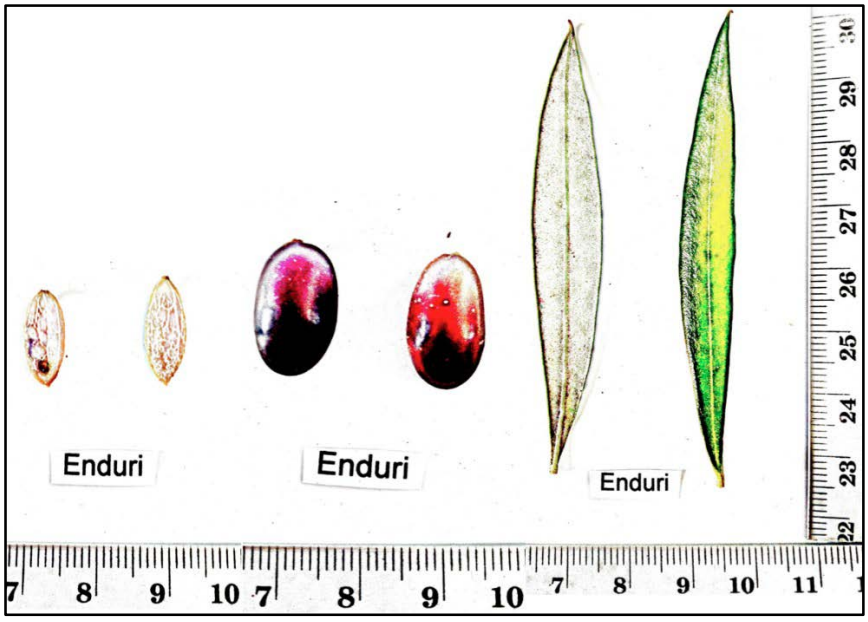


Fig.2.6 An example of scanned images captured by the turboscan program for all accessions.

Table 2.1 The 90 olive accessions used for morphological evaluation with their designated country of origin and fruit use.

Cultivar name	Type of variety	Use of fruit	Country of origin
Arbequina-Tri[Z]	Introduced	Oil	Spain
Ascolanatenera-T	Introduced	Table	Italy
Bella di spagna-T	Introduced	Table	Italy
Caninese-G	Introduced	Oil	Italy
Carmelitana-T	Introduced	Dual-purpose	Italy
Cellina-G	Introduced	Oil	Italy
Chemlalisfax-T	Introduced	Oil	Tunisia
Chemlalisfax-G	Introduced	Oil	Tunisia
Coratina-T	Introduced	Oil	Italy
Coratina-G	Introduced	Oil	Italy
Cucco-T	Introduced	Table	Italy
Enduri-T	Introduced	Oil	Italy
Frantoio-T	Introduced	Oil	Italy
Frantoio-G	Introduced	Oil	Italy
Gragnano-G	Introduced	Oil	Italy
Grossa di sardegna-T	Introduced	Table	Italy
Grossa di spagna-T	Introduced	Table	Italy
Krusi-G	Introduced	Oil	Italy
Leccino-T	Introduced	Oil	Italy
Leccino-G	Introduced	Oil	Italy
Leccinopendulo-T	Introduced	Oil	Italy
Marrari-T	Introduced	Oil	Italy
Maurino-G	Introduced	Oil	Italy
Mbuti-T	Introduced	Dual-purpose	Italy
Mbuti-M	Introduced	Dual-purpose	Unknown
Mignolo-T	Introduced	Oil	Italy
Mignolo-G	Introduced	Oil	Italy
Monopoly-T	Introduced	Oil	Italy
Moraiolo-T	Introduced	Oil	Italy
Moraiolo-G	Introduced	Oil	Italy
Morchiaio-G	Introduced	Oil	Italy
Morellona di grecia-T	Introduced	Oil	Italy

Cultivar name	Type of variety	Use of fruit	Country of origin
Bayyudi-M	Local	Dual-purpose	Libya
Beserri-M	Local	Dual-purpose	Unknown
Chemlali-Za	Local	Oil	Libya
Chemlali-M	Local	Oil	Libya
Chemlalikussabat-T	Local	Dual-purpose	Libya
Chemlalikussabat-M	Local	Dual-purpose	Libya
Farkuti-M	Local	Dual-purpose	Libya
Gaiani-M	Local	Dual-purpose	Unknown
Gargashi-T	Local	Oil	Libya
Gargashi-M	Local	Oil	Libya
Gartomye-M	Local	Oil	Libya
Hammudi-T	Local	Oil	Libya
Hammudi-M	Local	Oil	Libya
Jabbugi-T	Local	Oil	Libya
Jabbugi-M	Local	Oil	Libya
Kalefy-M	Local	Oil	Libya
Karkubi-M	Local	Table	Libya
Keddaui-M	Local	Oil	Libya
Khaddira-M	Local	Oil	Libya
Khaddra-M	Local	Oil	Libya
Marisi-M	Local	Oil	Libya
Maurino-T	Local	Oil	Libya
Marrari-M	Local	Oil	Libya
Mthemr-M	Local	Dual-purpose	Libya
Mukther-M	Local	Oil	Libya
Neb gemel-M	Local	Dual-purpose	Libya
Ninai-M	Local	Oil	Libya
Ouslatikussabat-T	Local	Dual-purpose	Libya
Qalbsarduk-M	Local	Oil	Libya
Rasli-T	Local	Oil	Libya
Rasli-M	Local	Oil	Libya
Rumi-M	Local	Oil	Libya

Nardo-T	Introduced	Oil	Italy		Sahley-M	Local	Oil	Unknown
Nepal-Tri	Introduced	Table	Palestine		Soudia-M	Local	Oil	Libya
Oliardo-G	Introduced	Oil	Italy		Vqos-M	Local	Dual-purpose	Libya
Oliarolasalentina-T	Introduced	Oil	Italy		Yehudi-M	Local	Oil	Libya
Olivastro-G	Introduced	Oil	Italy		Zaafrani-T	Local	Oil	Libya
Ouslati-T	Introduced	Oil	Unknown		Zaafrani-M	Local	Oil	Libya
Ouslati-G	Introduced	Oil	Unknown		Zaglo-M	Local	Oil	Libya
Pendolino-G	Introduced	Oil	Italy		Zalmati-G	Local	Oil	Tunisia
Rosciola-G	Introduced	Oil	Italy		Zalmati-Za	Local	Oil	Tunisia
Santagostino-T	Introduced	Table	Italy		Zarrasi-T	Local	Dual-purpose	Libya
Tombarella-G	Introduced	Oil	Italy		Zarrasi-M	Local	Dual-purpose	Libya
Tunisian-M	Introduced	Oil	Unknown		Znbai-M	Local	Oil	Libya
Anbi-M	Local	Oil	Libya		Wild-G	Wild	Oil	Unknown

z Name of accession attached with their local locations (Fig.2.1) (M= Mesalata, T= Tharouna,G= Gharian, Za= Zaltin, and Tri= Tripoli)

.2.1.3 Phenotypic description

2.1.3.1 Fruit character traits

Quantitative fruit traits included: individual fruit weight, volume, width, length, density and shape. Qualitative fruit traits included the fruit position of maximum transverse diameter, relative fruit shape, base end shape, apex end shape, fruit symmetry, nipple presence, fruit use as well as relative rating of fruit weight (Table A.1).

Fruit were classified as slightly asymmetric, symmetric or asymmetric (Fig.2.7A). The fruit nipple was classified into three observed categories: obvious, tenuous and absent as illustrated in Fig.2.7 B. The relative base end (point of attachment) of olive fruit was classified into one of three categories; pointed, rounded or truncates (Fig. 2.7 C). Olive fruit was rated as to use as table, dual purpose for both, or oil, see Fig2.7 D. The apex of olive fruit was classified into the two categories of rounded or pointed as illustrated in Fig. 2.7 E. The relative ratio of length and width of fruit was used to classify the shape of fruit into three categories: spherical (< 1.25 cm), elongated (> 1.45 cm) and ovoid (1.25- 1.45 cm) (Fig. 2.7 F). The location of maximum transverse diameter was classified into three categories: central, towards apex or towards base (Fig.2.7. G). Relative weight of fruits were placed into 4 categories as follows; low < 2g, medium 2-4g, high 4-6g, very high > 6 g (Table A.1).

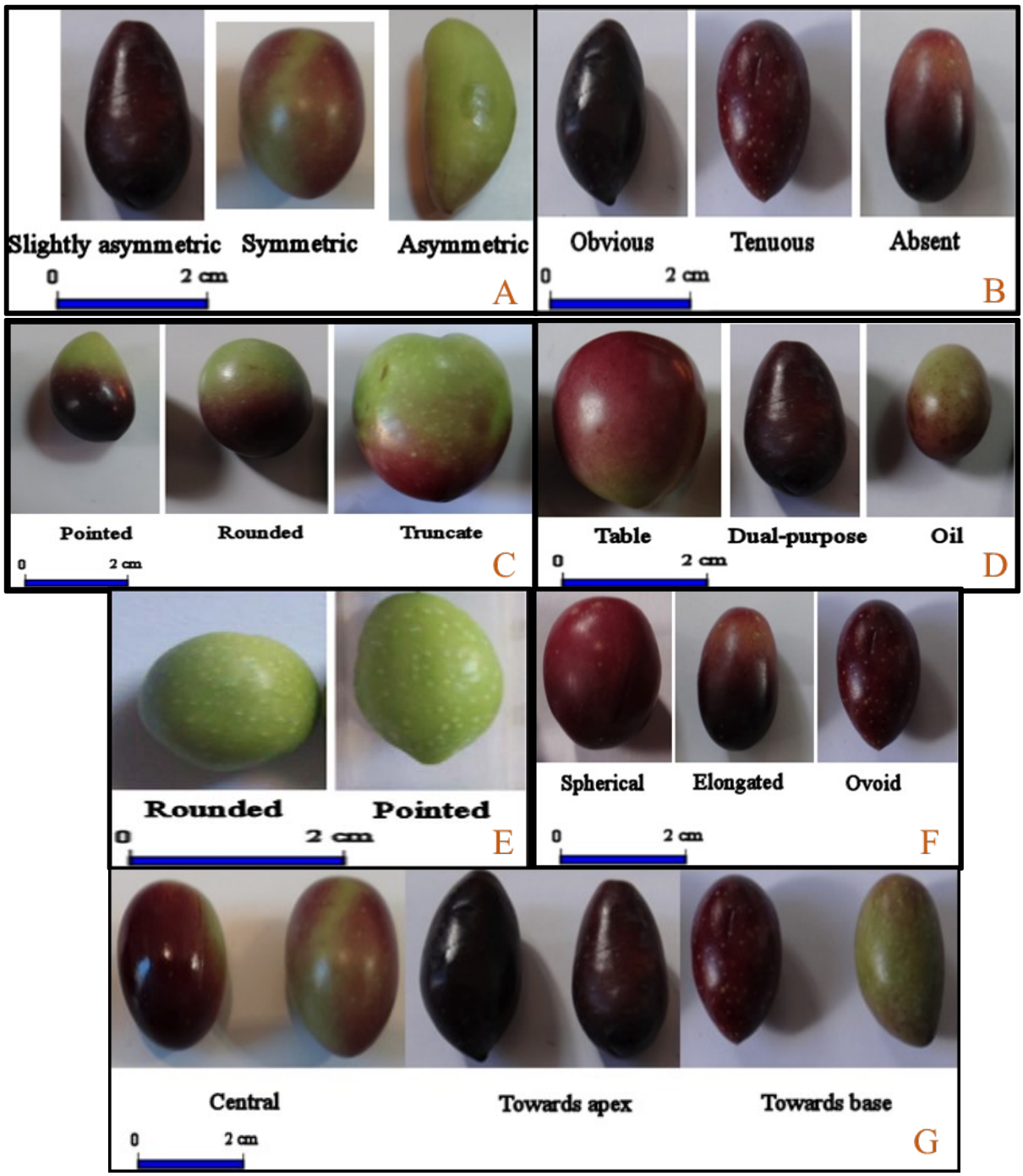


Fig. 2.7 Examples of morphological characteristic used to describe fruit traits, the symmetry of olive fruit A, fruit nipple B, fruit base end C, fruit use D, fruit apex end E, shape of fruit F and location of maximum transverse diameter G.

2.1.3.2 Endocarp character traits

Quantitative seed traits were seed weight, width, length, density and shape. Qualitative seed traits were position of maximum transverse diameter, shape, seed base end, seed apex end, symmetry, surface of seed as well as relative seed weight ranking (Table A.2).

Olive endocarps were classified according to the external surface of each endocarp into one of the three following categories that was dependent on the depth and abundance of the fibrovascular bundles: scabrous, rugose or smooth, (Fig.2.8 A). The apex end of each endocarp was classified into two observed categories: rounded or pointed (Fig. 2.8 B). The location of the maximum transverse diameter was classified into three categories: towards base, towards apex and central, as illustrated in Fig.2.8 C. The base end of each endocarp (point of attachment) was classified into one of three categories dependent on visual observation: pointed, truncated or rounded (Fig. 2.8 D). The symmetry of the endocarp was classified into three categories based on the observation of the match of the two longitudinal halves: symmetric, slightly asymmetric and asymmetric (Fig.2.8 E).The ratio of length to width of endocarps was used to classify the relative shape into the four categories identified as spherical (< 1.40 cm), elongated (> 2.2 cm), ovoid (1.40 -1.80 cm) and elliptic (1.8-2.2cm) (Fig. 2.8 F). The relative weight of stone was classified into three categories: low (< 0.30g), medium (0.30-0.45g), high (>0.45g) (Table A.2).

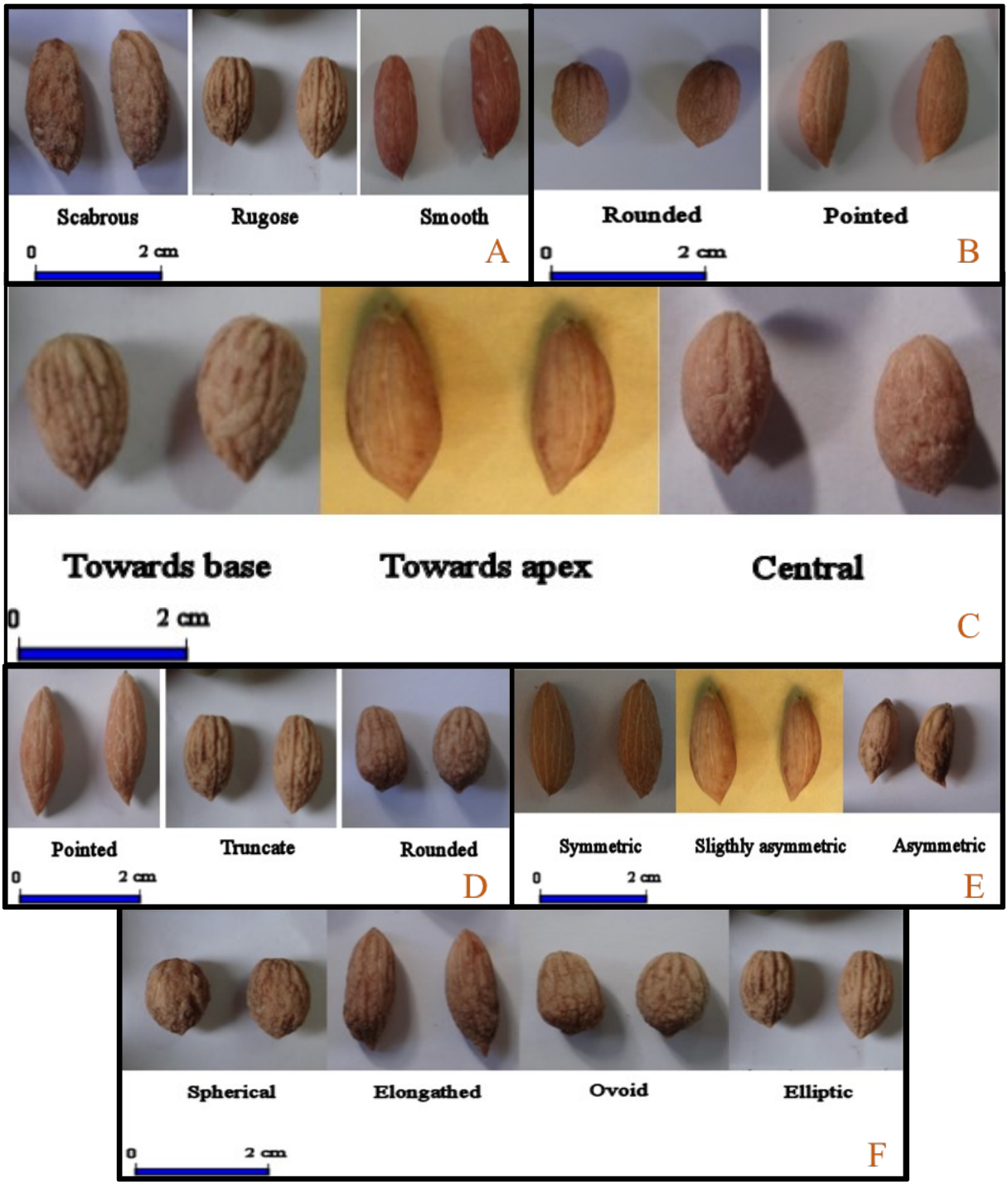


Fig.2.8 Examples of morphological characteristic used to describe endocarp traits, the external surface of endocarp A, apex end B, the location of the maximum transverse diameter C, base end D, symmetry of the endocarp E and shape of endocarp F.

2.1.3.3 Leaf character traits

Quantitative leaf traits were leaf weight, width, length and shape (Table A.7). Qualitative leaf traits were relative shape, length and width (Table A.3).

Leaf ratio of length and width was measured and used to classify blade shape into three categories: elliptic (< 4 cm), elliptic- lanceolate (4-6 cm) and lanceolate (> 6 cm), see Fig.2.9 A. The relative length of leaves were measured and categorized into three categories: Short (< 5cm), medium (5-7 cm) and long (> 7cm), see Fig.2.9 B. The relative width of leaves were also measured for each accession and then placed into one of the following three categories: narrow < 1 cm, broad > 1.5 cm and medium 1-1.5 cm (Fig.2.9 C).

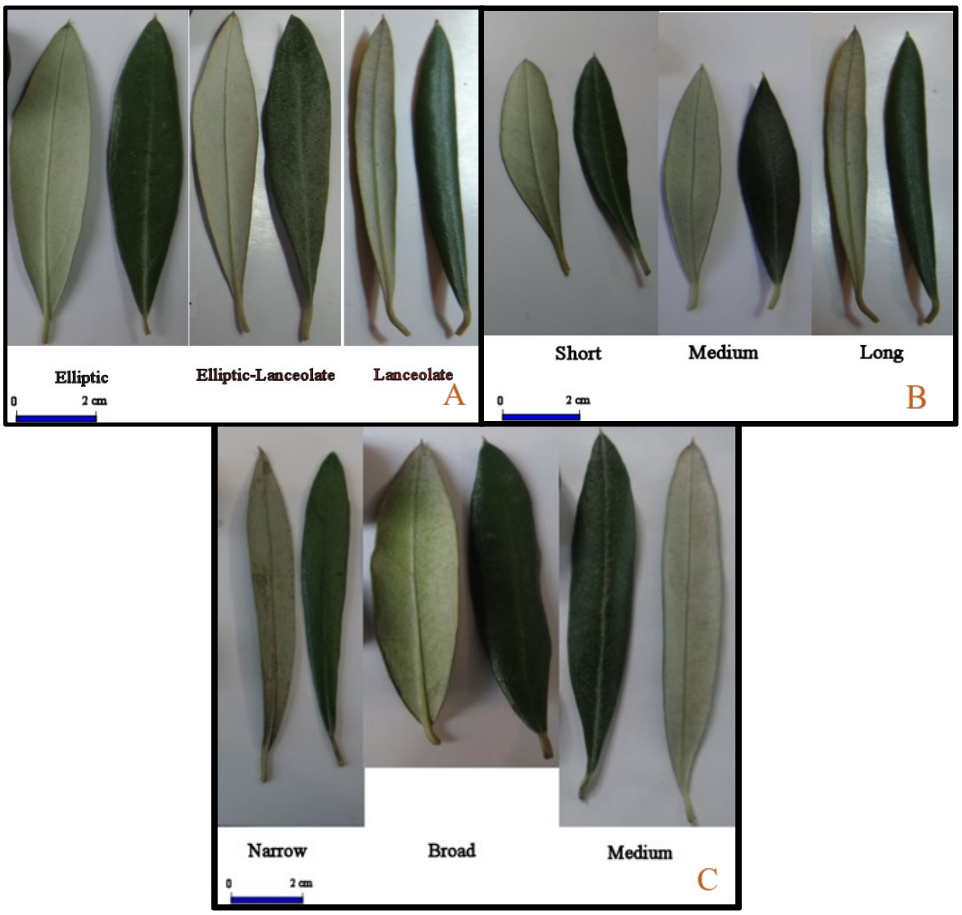


Fig.2.9 Examples of morphological characteristic used to describe leaf traits the shape of blade A, length of blade B and width of blade C.

2.1.4 Phenotypic data analysis

The collected samples from duplicated accessions were measured for a total of 39 morphological traits (22 quantitative and 17 qualitative traits) of fruit, seed and leaf (Table A.4, A.5 and A.6). The t-test was applied for combined morphological traits (Table A.7) in a single analysis using JMP 10 pro software (SAS Institute Inc., Cary, NC) to determine the most independent stable traits across locations. These traits showed a narrower range of phenotypic variation (no difference between locations) for at least 75% of the cultivars. Those were variable (differences observed between the two locations) for more than 25% of the cultivars. The correlations among stable traits were estimated for fruit, seed and leaf traits using multivariate correlation analysis (Table 2.4) (SAS Institute Inc., Cary, NC). Independent stable traits could be used later as useful indicators for phenotypic classification, so we applied this set of stable traits to the larger collection of 90 olive accessions to estimate phenotypic differentiation among all olive accessions. The discriminant analysis was also applied to identify olive accessions in relation to fruit use (oil, table or dual purpose) and origin of cultivars (landraces or cultivated) as covariance variables based on the six most independent and stable traits across locations (Table 2.4) (SAS Institute Inc., Cary, NC).

2.2RESULTS

2.2.1 Plastic traits vs stable traits

Traits that were plastic or stable were identified for duplicated cultivars that were grown at two different locations (Tharouna vs Mesalata or Tharouna vs Gharian). Statistical analysis of combined morphological traits (22 quantitative and 17 qualitative) identified traits as either variable/plastic traits or stable traits in cultivars that were duplicated in 2 locations. Most of the stable traits showed a narrower range of phenotypic variation (no difference between locations for

at least 75% of the cultivars), which seemed to indicate low environment by genetic interaction. These traits were both quantitative: fruit density, fruit shape, seed width, seed length as well as seed roundness and qualitative traits: fruit apex, fruit nipple, seed apex, seed base, relative seed weight and leaf length (Table 2.2). A t-test analysis revealed no significant differentiation ($P<0.0001$***) among all 17 duplicated accessions (9 landraces and 7 cultivated) for the most stable, independent and numeric five traits as shown in (Table 2.3). Seed and leaf samples demonstrated low phenotypic variation (more independent and stable compared to fruit traits) for most of the stable traits that indicated limited genotypic by location interaction. This set of independent stable traits might be useful for olive cultivar identification. This set was applied to the larger dataset of 90 cultivars to differentiate them phenotypically.

A total of 24 out of 39 morphological traits of 9 duplicated landraces grown in Tharouna and Mesalata were significantly different between the two locations. Landraces appeared to be more respbonsive to differing enviroments than major introduced cultivars. This is evident in that most of the morphological traits (23 out of 39) of 7 major duplicated cultivars grown in Tharouna and Gharian (Table 2.2) showed no significant differences and thus were phenotypically more stable across those locations. The cultivated varieties grown in Tharouna and Gharian appeared to have more stable traits (23 out of 39).

Table 2.2 Plastic vs. stable traits observed among duplicated olive cultivars grown in different paired locations (Q= Quantitative traits, S= Scanned traits and D= Descriptive or Qualitative traits).

Plastic traits		Stable traits	
Tharouna vs. Mesalata	Tharouna vs. Gharian	Tharouna vs. Mesalata	Tharouna vs. Gharian
Fruit base D[z]	Fruit base D	Fruit apex D	Fruit apex D
Fruit width Q[x]	Fruit width Q	Fruit density Q	Fruit density Q
Fruit weight Q	Fruit weight Q	Fruit shape Q	Fruit shape Q
Fruit maximum transverse D	Fruit maximum transverse D	Fruit nipple D	Fruit nipple D
Seed maximum transverse D	Seed maximum transverse D	Seed apex D	Seed apex D
Seed shape Q	Seed shape Q	Seed base D	Seed base D
Seed weight Q	Seed weight Q	Seed length Q	Seed length Q
Seed symmetry D	Seed symmetry D	Seed roundness S	Seed roundness S
Leaf area S[w]	Leaf area S	Seed weight D	Seed weight D
Leaf width Q	Leaf width Q	Seed width Q	Seed width Q
Leaf shape Q	Leaf shape Q	Leaf length D	Leaf length D
Leaf weight Q	Leaf weight Q	Fruit length Q	Fruit area S
Fruit area S	Fruit symmetry D	Fruit shape D	Fruit roundness S
Fruit roundness S	Fruit shape D	Fruit symmetry D	Fruit box X/Y S
Fruit weight D	Fruit length Q	Leaf length Q	Fruit weight D
Fruit box X/Y S	Leaf length Q		Seed area S
Seed area S			Seed box X/Y S
Seed box X/YS			Seed shape D
Seed surface D			Seed surface D
Seed shape D			Leaf box X/Y S
Leaf box X/YS			Leaf roundness S
Leaf roundness S			Leaf shape D
Leaf shape D			Leaf width D
24	16	15	23

Table 2.3 Analysis and comparisons means of independent and numeric five stable traits for all duplicated cultivars grown in two different locations using T test.

Accession name[w]	Fruit density Mean	Std Err Mean	Compa-risons means[y]	Fruit shape mean	Std Err Mean	Comar-isons mean[y]	Seed width mean	Std Err Mean	Compa-risons means[y]	Seed length mean	Std Err Mean	Compar-isons means[y]	Seed round mean	Std Err Mean	Compari-sons means[y]
Chemlalikussabat-M[w]	0.98	0.05	A	1.35	0.07	A	0.68	0.02	A	1.44	0.04	A[Z]	1.35	0.03	A
Chemlalikussabat-T[w]	1.08	0.06	A	1.29	0.07	A	0.7	0.02	A	1.26	0.03	B[Z]	1.23	0.01	A
Gargashi-M	0.98	0.06	A	1.57	0.09	A	0.55	0.02	A	1.4	0.04	A	1.45	0.03	A
Gargashi-T	0.98	0.05	A	1.54	0.09	A	0.54	0.01	A	1.26	0.03	A	1.38	0.03	A
Hammudi-M	1.01	0.06	A	1.52	0.08	A	0.7	0.02	A	1.7	0.05	A	1.4	0.05	A
Hammudi-T	0.98	0.06	A	1.47	0.08	A	0.72	0.02	A	1.62	0.04	A	1.4	0.04	A
Jabbugi-M	0.94	0.05	A	1.92	0.11	A	0.6	0.02	A	1.86	0.05	A	1.73	0.01	A
Jabbugi-T	1.04	0.06	A	1.71	0.09	A	0.62	0.02	A	1.8	0.05	A	1.65	0.02	A
Marrari-M	1.05	0.06	A	1.82	0.1	A	0.64	0.02	A	1.7	0.05	A	1.49	0.01	A
Marrari-T	1.1	0.06	A	1.66	0.09	A	0.68	0.02	A	1.78	0.05	A	1.51	0.01	A
Mbuti-M	1.04	0.06	A	1.29	0.07	A	0.76	0.02	A	1.38	0.04	A	1.28	0.01	A
Mbuti-T	1.01	0.05	A	1.43	0.08	A	0.68	0.02	A	1.48	0.04	A	1.35	0.05	A
Rasli-M	1.03	0.06	A	1.51	0.08	A	0.7	0.02	A	1.56	0.04	A	1.36	0.02	A
Rasli-T	1.06	0.06	A	1.52	0.08	A	0.7	0.02	A	1.52	0.04	A	1.31	0.02	A
Zaafrani-M	1.1	0.06	A	1.77	0.1	A	0.64	0.02	A	1.7	0.05	A	1.46	0	A
Zaafrani-T	1.04	0.06	A	1.54	0.08	A	0.8	0.02	B	1.64	0.05	A	1.25	0.03	B
Zarrasi-M	0.99	0.05	A	1.12	0.06	A	0.82	0.02	A	1.32	0.04	A	1.18	0.05	A
Zarrasi-T	1.04	0.06	A	1.16	0.06	A	0.82	0.02	A	1.3	0.04	A	1.16	0.04	A
Chemlalisfax-G	1.06	0.06	A	1.52	0.08	A	0.56	0.02	A	1.28	0.04	A	1.39	0	A
Chemlalisfax-T	0.87	0.05	A	1.7	0.09	A	0.54	0.01	A	1.2	0.03	A	1.36	0.01	B
Coratina-G	1.19	0.07	A	1.4	0.07	A	0.76	0.02	A	1.56	0.04	A	1.33	0.01	A
Coratina-T	1.04	0.06	A	1.33	0.07	A	0.8	0.02	A	1.6	0.04	A	1.3	0.01	A

Table 2.3 Contniued

Frantoio-G	1.08	0.06	A	1.55	0.09	A	0.72	0.02	A	1.54	0.04	A	1.33	0.02	A
Frantoio-T	1.08	0.06	A	1.48	0.08	A	0.8	0.02	A	1.7	0.05	A	1.38	0.03	A
Leccino-G	1.05	0.06	A	1.56	0.08	A	0.76	0.02	A	1.98	0.05	A	1.44	0.06	A
Leccino-T	1.1	0.06	A	1.56	0.09	A	0.78	0.02	A	1.8	0.05	A	1.36	0.02	A
Maurino-G	1.03	0.06	A	1.47	0.08	A	0.68	0.02	A	1.42	0.04	A	1.3	0.05	A
Maurino-T	1.06	0.06	A	1.36	0.08	A	0.72	0.02	A	1.4	0.04	A	1.3	0.03	A
Mignolo-G	0.97	0.05	A	1.3	0.07	A	0.76	0.02	A	1.46	0.04	A	1.23	0.02	A
Mignolo-T	1	0.05	A	1.51	0.09	A	0.64	0.02	B	1.46	0.04	A	1.41	0.01	B
Moraiolo-G	1.07	0.06	A	1.25	0.07	A	0.74	0.02	A	1.22	0.03	A	1.14	0.01	A
Moraiolo-T	1	0.06	A	1.26	0.07	A	0.78	0.02	A	1.28	0.04	A	1.17	0.03	A
Ouslati-G	1.02	0.06	A	1.41	0.08	A	0.78	0.02	A	1.56	0.04	A	1.32	0.09	A
Ouslati-T	1.09	0.06	A	1.27	0.07	A	0.8	0.02	A	1.52	0.04	A	1.25	0.02	A

z varieties with different letters are significantly different.
y comparisons means for all traits shown differences between locations using Tukey-kramer (HSD).
w name of accession attached with their location where grown (Fig.2.1) (M= Mesalata ,T= Tharouna , and Gh= Gharian).

2.2.2 Correlation among stable traits

The correlations among stable traits were estimated by pairwise method for fruit, seed and leaf traits. Seed and leaf traits were more independent and stable across locations as compared to fruit traits. Most of the stable traits were independent of one another. Fruit shape and seed roundness (red colors) were highly correlated while all others had very low correlations (Table 2.4). The correlated trait of fruit shape was omitted. The remaining 6 variables were used in subsequent analysis to identify olive cultivar fruit use and origin as a covariance. This resulted in very high discriminating power for identification of olive cultivars. The stable set of traits was then used in subsequent analyses to correlate genetic and phenotypic characteristics of Libyan olives.

Table 2.4 Multivariate correlations of 7 numeric traits that were observed among duplicated cultivars grown in different locations.

	Fruit length	Seed length	Seed width	Leaf length	Fruit density	Fruit shape	Seed roundness
Fruit length	1	0.5958	0.5123	0.252	0.3498	0.1682	0.0634
Seed length	0.5958	1	0.3445	0.0063	-0.0977	0.2579	0.5089
Seed width	0.5123	0.3445	1	0.0597	-0.0616	-0.603	-0.5792
Leaf length	0.252	0.0063	0.0597	1	0.3603	0.2137	-0.0628
Fruit density	0.3498	-0.0977	-0.0616	0.3603	1	0.4083	-0.0845
Fruit shape	0.1682	0.2579	-0.603	0.2137	0.4083	1	0.736
Seed roundness	0.0634	0.5089	-0.5792	-0.0628	-0.0845	0.736	1

2.2.3 Discriminant analysis based on independent stable traits.

Discriminant analyses were performed using the independent numeric traits (fruit length, seed length, seed width, leaf length, fruit density, and seed roundness) to classify 17 duplicated olive cultivars (P<0.0001***) based on their uses (Fig 2.10 A). These cultivars could be differentiated into the dual use and oil type cultivars Fig 2.10 A. The independent stable traits were then applied to the larger dataset of 90 cultivars. Discriminant analysis also distinguished significant differentiations (P<0.0001***), Fig.2.10 B among those cultivars. These cultivars were differentiated into three groups based on their fruit use (table, oil, dual; Fig.2.10 B).

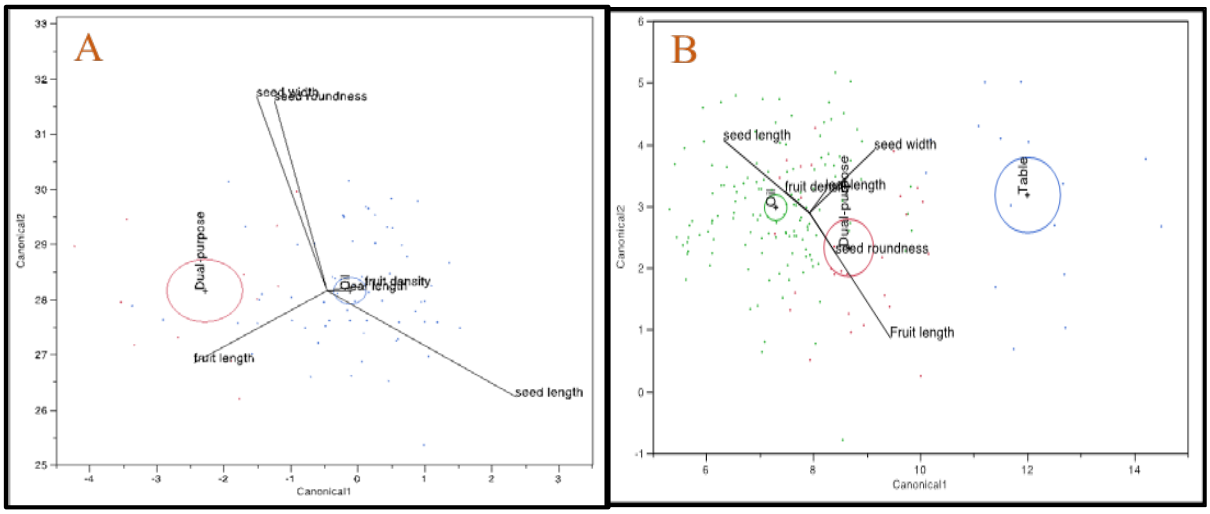


Fig.2.10 Fruit use was clearly identified using discriminant analysis among 17 duplicated genotypes (oil and dual-purpose) (P<0.0001***) A, and among all 90 genotypes using (oil vs. table vs. dual) (P<0.0001***) B.

Landrace and cultivated olive cultivars could also be differentiated using these independent, stable traits (Table 2.4). The origin of cultivars Fig.2.11A and B was not as significant as the fruit use-types in the covariate analyses (17 duplicates; P>0.0164 and 90 cultivars P>0.007).

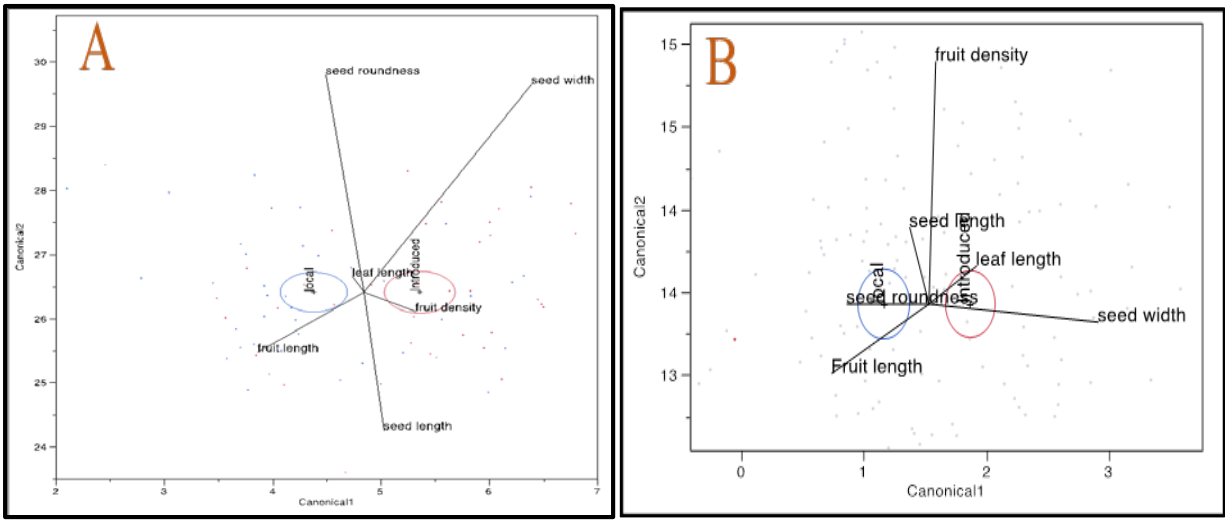


Fig.2.11 Discriminant analysis was applied to differentiate between cultivar origin (local or introduced) among 17duplicated cultivars (P>0.0164) A, and among all 90 cultivars (P>0.007) B.

The discriminant method could be used to distinguish between landraces and cultivated varieties with respect to fruit use (table, oil or dual-purpose) among the 17 duplicated as well as all 90 cultivars across locations using the stable phenotypic traits. Oil use cultivars (Fig.2.12A) and dual-purpose cultivars (Fig.2.12B) of 17 duplicated cultivars showed highly statistically significant discrimination (P< 0.0001***), (P<0.001***) respectively as compared to the fruit use of both traits together (oil vs. dual) (P>0.0164). Oil use cultivars (Fig.2.12C) and dual-purpose (Fig.2.12D) of all 90 cultivars were also found highly significant analysis too (P<0.0002***) and (P<0.0002***) respectively.

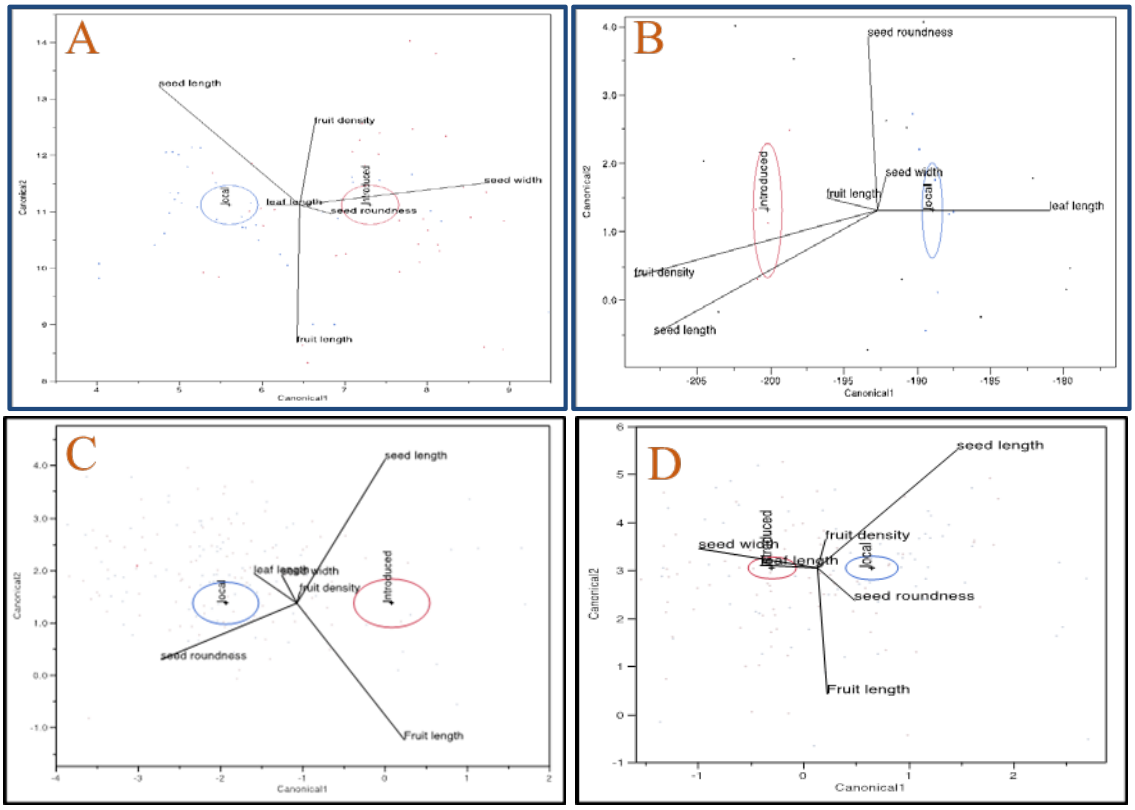


Fig.2.12 Discriminant analysis of oil use cultivars A and dual use cultivars B of 17 duplicated cultivars showed highly statistically significant discrimination (P< 0.0001***), (P<0.001***). Oil use cultivars C and dual-purpose D of all 90 cultivars were also found highly significant analysis (P<0.0002***) and (P<0.0002***) respectively.

There is sufficient variability to discriminate all varieties that originated in different locations based on the stable phenotypic traits. Highly significant differentiations (P<0.0021) and (P<0.0001***) (Fig.2.13 A and B) were observed for both 17 duplicated and all 90 cultivars, respectively. Most of these accessions originally came from different geographic locations, Libya, Italy or Tunisia. All accessions separated into three different geographic locations (Italy, Libya and Tunisia) that have distinctive features for each group based on their morphological characteristics. So, for example, the average fruit weight and volume was (3.27g/fruit and 3.15 ml/fruit), (2.70g/fruit and 2.10 ml/fruit) and (1.43g/fruit and 1.36ml/fruit) for Italy, Libya and Tunisia, respectively.

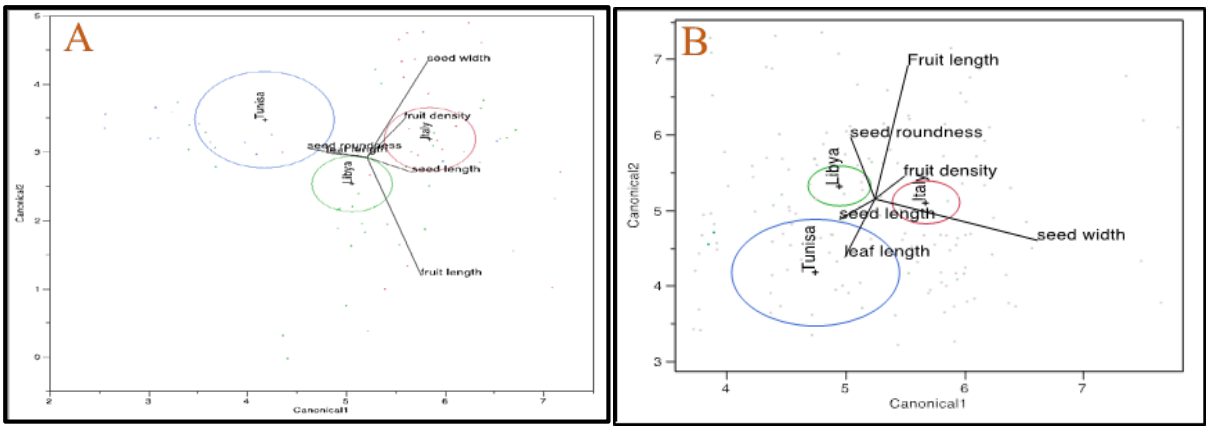
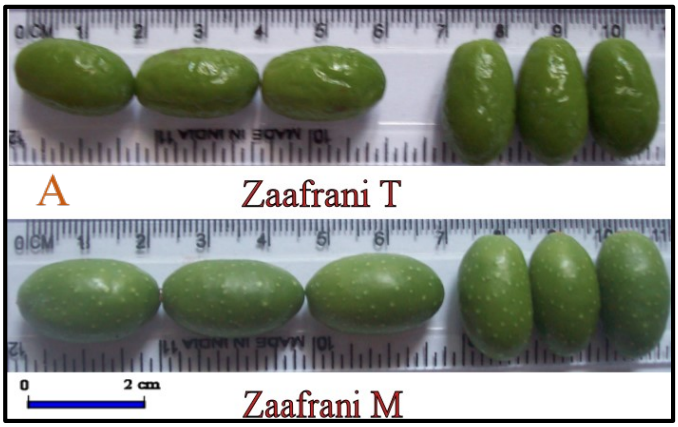


Fig.2.13 Discriminant analysis was used to differentiate genotypes based on their country of origin, highly significant differentiations were observed for both 17 duplicated (P<0.0021) A and all 90 cultivars (P<0.0001***) B.

2.3 DISCUSSION

Identification of phenotypic traits that are both stable and independent will aid in robust cultivar identification. Most of the morphological traits (23 out of 39) of 9 major duplicated landraces that were grown in Tharouna and Gharian (Table 2.2) showed no significant differences and thus were phenotypically more stable across those two locations. This could be due to genetic identity of the genotypes grown in the two locations as well as limited differences in the environment and tree age of the two locations (Rao et al., 2009). Only 15 of 39 morphological traits of 7 major duplicated cultivars that were grown in Tharouna and Mesalata (Table 2.2) were stable across the two locations. This seems likely because most of the Tharouna region were cultivated varieties established years ago by the Italian government whereas accessions in the Mesalata accessions were landraces, so for example the average of fruit weight and volume was (3.09g /fruit and 2.98ml/fruit) and (2.10 g/fruit and 2.04ml/fruit), respectively. These traits were considered to be variable or plastic traits. This plasticity of morphological traits was apparent in the olive accessions since the same cultivars were collected from diverse locations within Libya. Even though the two environments in Tharouna and Mesalata cities were similar, the duplicated accessions that were grown in both cities revealed high phenotypic variations of their morphological traits (24 of 39). This might be related to the fact that these duplicated accessions were in fact not identical and thus mislabeled (Fig.2.14) or were phenotypically different but genetically identical and thus (true duplicates) (Fig.2.15).



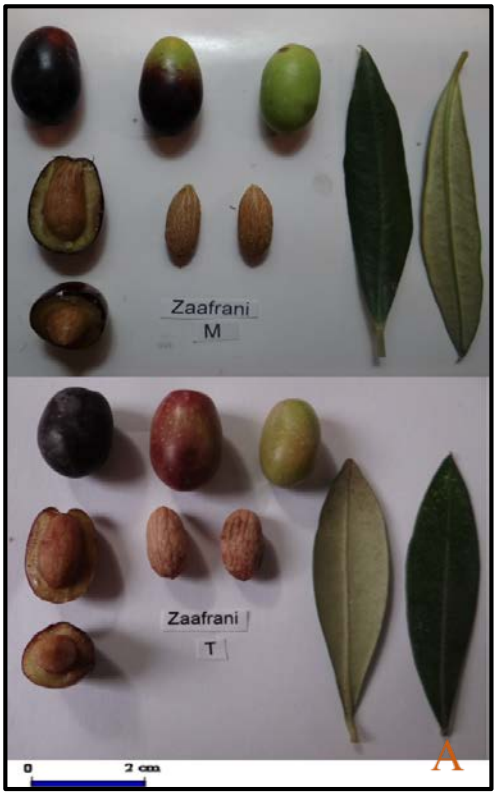


Fig.2.14 Examples of cultivars that appear with the same name ’Zaafrani’, but are morphologically different.

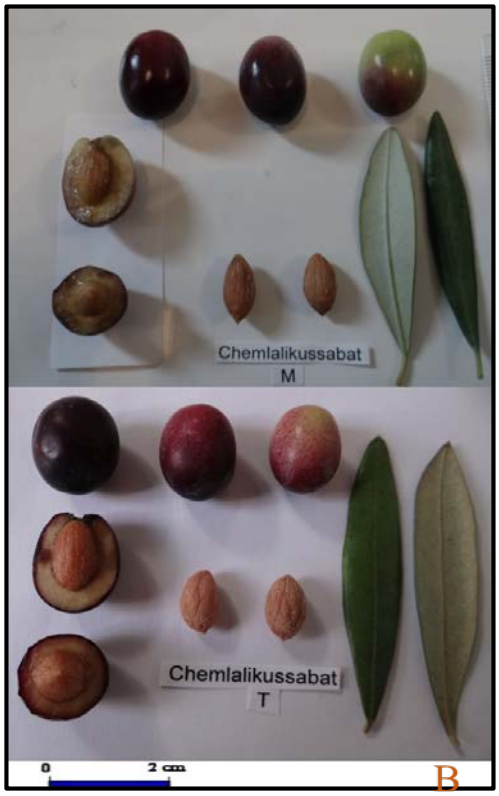


Fig.2.15 Examples of true duplicated cultivars that appear with the same name ‘Chemlalikussabat’ that are morphologically different, but are genetically the same as shown in molecular data elsewhere.

A set of six morphological traits were identified that showed a consistent and narrow range of phenotypic variation across different environments. This set of independent stable traits might be useful in the investigation of phenotypic relationships among olive cultivars within the collection based on their fruit use (oil, table or dual purpose) or origin of cultivars (local or introduced). This study also confirms previous studies on the importance of measuring 26 morphological and pomological traits in Tunisia (Hannachi et al., 2008) and Morpho-physiological traits (quantitative and qualitative) in Italy (Corrado et al., 2009), which successfully classified oleaster and cultivated varieties. Furthermore, the evaluation of agronomic traits may be difficult since it may take as long as 10 years to reach reproductive maturity (Suarez, et al., 2011).

The results of the discriminant analysis of the 17 duplicated as well as all 90 cultivars revealed high phenotypic variation based on their fruit use (oil, dual purpose or table) Fig.2.10 A and 2.10 B. Results of previous studies based on morphological traits classified olive collections into relatedness groups but they were unable to differentiate among similar cultivars (Rao et al., 2009). A combination of morphological traits was assessed for an Italian olive collection revealed phenotypic differentiation among varieties (Corrado et al., 2009). In our work, it was more difficult to differentiate between the landrace and cultivated cultivars than the fruit use types (Fig.2.11A and B). When subsequent analysis of fruit use (oil or dual purpose) was applied one could differentiate between cultivar origins (landraces vs. cultivated). Within each fruit use (dual vs oil), we could discriminate between the landraces and the cultivated varieties among 17 duplicated as well as all 90 cultivars. The significance level of oil use was ($P<0.001$***) (Fig.2.12A) and dual use ($P<0.0001$***) (Fig.2.12B) for the 17 duplicated cultivars, whereas the significance level of oil use was ($P<0.0002$***) (Fig.2.12C) and dual use ($P<0.0002$***) (Fig.2.12D) among all 90 accessions. It also differentiates oil and table types based on their morpho-agronomic traits. Most

of the oil types have a small fruit size and a low flesh-to-stone ratio with high oil content, whereas table types have a larger fruit size and high flesh-to-stone ratio with low oil content. Genetic factor has a greater effect than environmental factors on oil content (Aparicio et al., 2013).

Hannachi et al. (2008) found that there was a genetic basis in olive cultivars related to fruit size and probable fruit use. The discriminant analysis of the morphological stable traits applied to the 17 duplicated cultivars and all 90 cultivars based on their country of origin revealed highly significant differentiations Fig.2.13A and Fig.2.13B, respectively. This indicates that landraces and cultivars differed morphologically. In general, landraces have unique characteristics and certain shapes. Fruit and leaf color or shape as well as stone surface (grooves, basal end and apex end) are key features of landraces. These variations probably are the result of the natural distribution of genetic diversity from which those genotypes arose. Corrado et al. (2009) mentioned that quantitative inherited traits (mono or polygenic) could be used to evaluate genetic diversity, so we can rely on the evaluation of morpho-agronomic traits to evaluate genetic diversity. In Morocco, previous studies based on morphological traits (Zaher et al., 2011) showed similar results in that they differentiated between local and Mediterranean cultivars that have different genetic bases. Also (Belaj et al., 2011 and Durgac et al., 2010) indicated that geographical origin might be an important factor that structures the genetic diversity in olive. This indicates that these cultivars could be phenotypically different, however, we could not confirm whether the existence of phenotypic variation among these genotypes was due to genetic diversity or variation of growing conditions across those locations which favored certain genotypes in one area more than another.

2.4 CONCLUSION

Olive phenotypes are determined by a combination of genetic and environmental factors. It is difficult to use phenotypic traits to differentiate olive cultivars; however, our data did demonstrate that stable, independent traits could be used to differentiate cultivars by use and origin (landrace or cultivar). Oil varieties produce heavier yields of fruit, and have a smaller fruit size, low flesh-to-stone ratio, and high oil content. In comparison, table types have a larger fruit size and high flesh-to-stone ratio with low oil content. Local landraces are adapted to the Libyan environment and provide novel genetic resources that should be conserved.

CHAPTER3.0 CHARACTERIZATION AND IDENTIFICATION OF LIBYAN OLIVE DIVERSITY USING MICROSATELLITE MARKERS.

3.0 INTRODUCTION

Libyan olives (*Olea europaea* subsp. *europaea* var. *sativa* or *sylvestris)* have traditionally been evaluated by leaf, fruit, and seed morphological traits as well as chemical and phenological characteristics. It has been difficult to properly manage and conserve olive germplasm because of the problems associated with clearly distinguishing among cultivars. Further complicating identification of cultivars is the observation that wild populations have likely introgressed with locally adapted cultivars (Mariotti et al., 2010). Dıez (2011) noted the exchange of olive genetic material between North Africa and Europe may be took place during the Arab expansion through Andalusia between the eighth and fourteenth centuries. This offers the archaeological evidence to support the movement of olives with human migration.Olive breeding is a challenge due to the protracted seedling juvenility (15-20 years). Access to outstanding ancient cultivars has further discouraged breeding efforts (Leon et al., 2005; Cipriani et al., 2002; Daham &Ashur, 2008; Sarri et al., 2006 and Taamalli et al., 2006).

There are more than 100 named olive cultivars grown along the coastal region of Libya. Some of these cultivars are likely to be identical due to historical renaming of material. This has lead to the perception that numerous cultivars exist when infact they are actually synonyms or homonyms. Morphological differences associated with specific environmental effects have also lead to mistaken identification of the cultivars. The level of knowledge about cultivar origin, selection and molecular variability is limited because the identification of Libyan olive accessions has previously been based on phenotypic traits.

Recently, morphological descriptions have improved, and are now considered to be complementary tools to molecular markers, aiding in olive cultivar identification.Using both morphological and molecular descriptors have been used to clarify the identy of genotyes within other crops (Corrado et al., 2009). This combination between morphological and molecular traits leads to a more robust results (Leon et al., 2005).

To date, SSR markers have not been used in combination with morphological datat to evaluate and improve the collection of Libyan olive accessions as a genetic resource. In this paper, SSR markers were used to differentiate and classify Libyan olive accessions.

3.1 MATERIALS AND METHODS

3.1.1 Collection sites and plant materials

Accessions were classified into three categories: both 42 local cultivated varieties and 41 introduced cultivars of *Olea europaea* subsp. *europaea* var. *sativa* and 16 wild type of *Olea europaea* subsp. *europaea* var. *sylvestris*. Leaf tissue was collected in 2009 and 2012. Most of the local cultivars (Libyan landraces) were collected from orchards of Masallata city while the introduced cultivars were collected from Tharouna and Gharian government collections as well as from farmers in the Zaltin and Tripoli regions. The wild type accessions were collected from 4 different sites (S1, S2, S3 and S4) in the Green Mountain region (Fig. 3.0) based on a systematic survey of the olive team from Libyan agriculture ministry.

Young leaf samples were collected for each accession from a single tree for DNA extraction. Leaf tissue of each genotype was immediately stored in containers with dry ice to prevent DNA degradation. They were then transferred to the National Medical Research Center in Tripoli, Libya where they were washed with double distilled water and freeze dried. Samples

were then transported to the Horticulture Laboratory at Colorado State University in Fort Collins, CO. USA where they were sto red at –80° C until use.

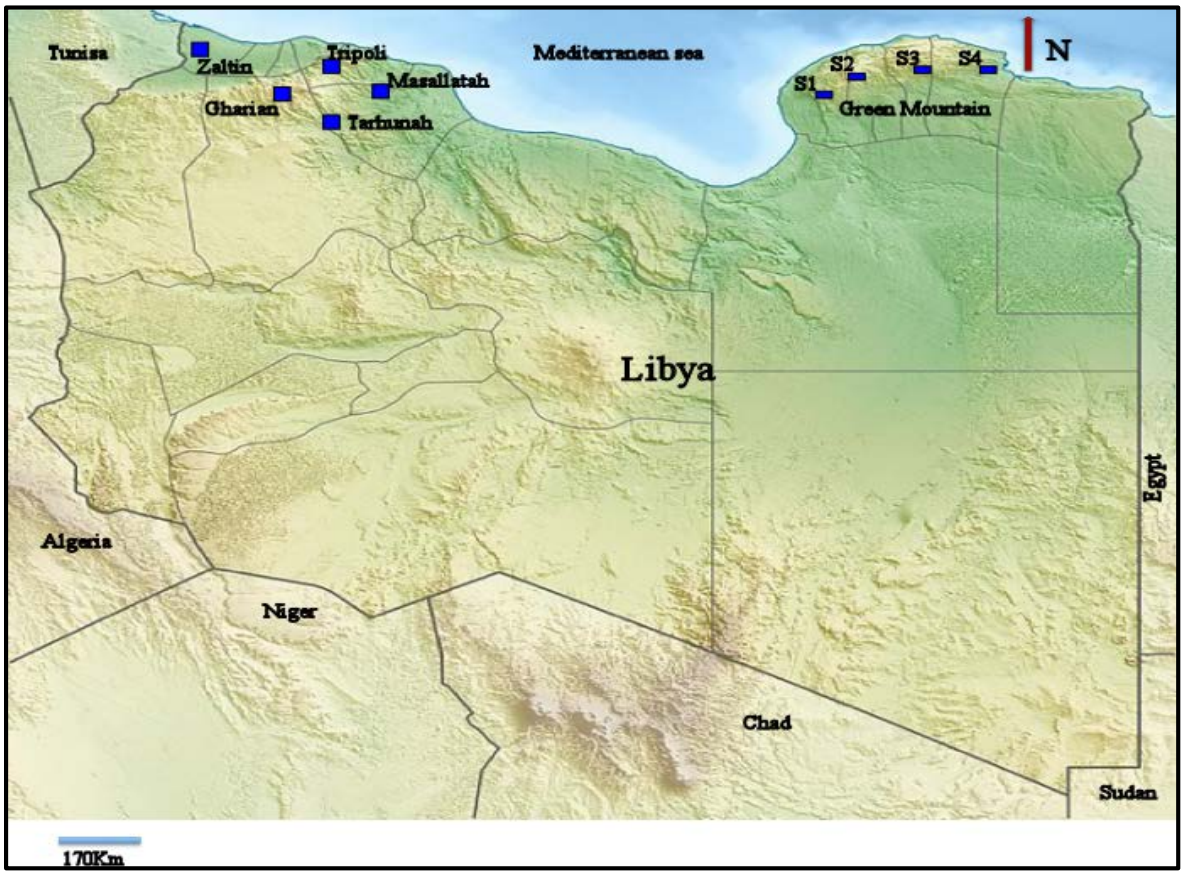


Fig.3.0 Map of Libya that illustrates the collection sites of cultivated and wild olive.

Table 3.1 list of the 99 olive accessions used in the molecular evaluations using SSRs.

Sample number	Cultivar name	Type of variety	Country of origin
1	AnbiM	Local	Libya
2	BeserriM	Local	Unknown
3	ChemlalikussabatT	Local	Libya
4	ChemlalikussabatM	Local	Libya
5	ChemlaliM	Local	Libya
6	ChemlaliZa	Local	Libya
7	FarkutiM	Local	Libya
8	GaianiM	Local	Unknown
9	GargashiM	Local	Libya
10	GargashiT	Local	Libya
11	HammudiM	Local	Libya
12	HammudiT	Local	Libya
13	JabbugiM	Local	Libya
14	JabbugiT	Local	Libya
15	KalefyM	Local	Libya
16	KarkubiM	Local	Libya
17	KeddauiM	Local	Libya
18	KhaddiraM	Local	Libya
19	KhaddraM	Local	Libya
20	MarisMi	Local	Libya
21	MarrariM	Local	Libya
22	MarrariT	Local	Libya
23	MthemrM	Local	Libya

Sample number	Cultivar name	Type of variety	Country of origin
51	FrantoioT	Introduced	Italy
52	FrantoioM	Introduced	Italy
53	GragnanoG	Introduced	Italy
54	GrossadisardegnaT	Introduced	Italy
55	GrossadispagnaT	Introduced	Italy
56	KrusiG	Introduced	Italy
57	LeccinoT	Introduced	Italy
58	LeccinoG	Introduced	Italy
59	LeccinopenduloT	Introduced	Italy
60	MaurinoT	Introduced	Italy
61	MaurinoG	Introduced	Italy
62	MbutiM	Local	Unknown
63	MbutiT	Introduced	Italy
64	MignoloG	Introduced	Italy
65	MignoloT	Introduced	Italy
66	MonopolyT	Introduced	Italy
67	MoraioloG	Introduced	Italy
68	MoraioloT	Introduced	Italy
69	MorellonadigreciaT	Introduced	Italy
70	NardoT	Introduced	Italy
71	NepalTri	Introduced	Palestine
72	OliardoG	Introduced	Italy
73	OliarolasalentinaT	Introduced	Italy

Table 3.1 Continued.

24	MuktherM	Local	Libya
25	NebgemelM	Local	Libya
26	NinaiM	Local	Libya
27	OuslatikussabatT	Local	Libya
28	QalbsardukM	Local	Libya
29	RasliM	Local	Libya
30	RasliT	Local	Libya
31	RumiM	Local	Libya
32	SoudiaM	Local	Libya
33	VqosM	Local	Libya
34	YehudiM	Local	Libya
35	ZaafraniM	Local	Libya
36	ZaafraniT	Local	Libya
37	ZagloM	Local	Libya
38	ZarrasiM	Local	Libya
39	ZarrasiT	Local	Libya
40	ZnbaiM	Local	Libya
41	AscolanateneraT	Introduced	Italy
42	BelladispagnaT	Introduced	Italy
43	CarmelitanaT	Introduced	Italy
44	CellinaG	Introduced	Italy
45	ChemlalisfaxG	Introduced	Tunisa
46	ChemlalisfaxT	Introduced	Tunisa
47	CoratinaG	Introduced	Italy
48	CoratinaT	Introduced	Italy
49	CuccoT	Introduced	Italy
50	EnduriT	Introduced	Italy

74	OlivastroG	Introduced	Italy
75	OuslatiT	Local	Unknown
76	OuslatiG	Local	Unknown
77	PendolinoG	Introduced	Italy
78	RosciolaG	Introduced	Italy
79	SantagostinoT	Introduced	Italy
80	TombarellaG	Introduced	Italy
81	TunisianM	Introduced	Unknown
82	ZalmatiZa	Local	Unknown
83	ZalmatiG	Local	Unknown
84	Ac#08	Wild	Libya
85	Ac#105	Wild	Libya
86	Ac#107	Wild	Libya
87	Ac#13	Wild	Libya
88	Ac#20	Wild	Libya
89	Ac#21	Wild	Libya
90	Ac#32	Wild	Libya
91	Ac#39	Wild	Libya
92	Ac#46	Wild	Libya
93	Ac#48	Wild	Libya
94	Ac#52	Wild	Libya
95	Ac#53	Wild	Libya
96	Ac#56	Wild	Libya
97	Ac#72	Wild	Libya
98	Ac#82	Wild	Libya
99	WildG	Introduced	Unknown

3.1.2 Processing samples

Total genomic DNA was extracted from 100-200 mg of lyophilized of tissue using some minor modifications. Large-scale CTAB extractions were performed according to Mace et al., 2003 with further modification by L. Tembrock (Pers. comm., 2011) (A.11). This protocol was a modification of the CTAB procedure for obtaining purified genomic DNA using using RNase and Proteinase K treatment to eliminate RNA and protein contamination to overcome some limitations such as polyphenols, RNA and binding proteins that inhibit Taq polymerase activity during the amplification cycles.

Concentration and quantity of DNA were determined by using NanoDrop® ND-1000 spectrophotometric methods (NanoDrop Technologies, Inc., Wilmington, DE, USA). A ratio of 1.8 for the 260 / 280 nm reading or greater absorbance was accepted as being of sufficient DNA purity. The final concentrations of DNA samples for further analysis were adjusted to 20ng/ μL for each sample.

Twelve sets of primer pairs were selected (Table 3.2) because of their high resolution in discriminating polymorphism and previous use in the identification of olive genotypes (Ercisli et al., 2011; Ercisli et al., 2012; Sefc et al., 2000; Baldoni et al., 2009; Sarri et al., 2006; Carriero et al., 2002; Cipriani et al., 2002; Belaj et al., 2003; De La Rosa et al., 2002). These were multiplexed using Multiplex Manager 1.2 software (Guichoux et al., 2011) to minimize overlap among the markers and to maximize similarity in the annealing temperature of each primer combination to reduce the variation and total number of PCR reactions. Each cycle of multiplex PCR amplification was performed with combinations of three different primers labeled with specific fluorescent dyes that incorporated during multiplex PCR amplification giving a specific color tag to each PCR product (Table A.8).

Forward primers EMO90-F, DCA3-F, DCA14-F, and GAPU101-F were labeled with blue fluorescent dye (56-FAM) attached to the 5'-end of oligonucleotides from Integrated DNA Technologies (IDT) (IDT, Coralville, IA). The forward primers of DCA18-F, DCA16-F, DCA5-F, and DCA17-F were attached with a green fluorescent dye (VIC) while GAPU103A-F, GAPU71B-F, UDO-043-F, and DCA9-F were attached with red fluorescent dye (PET) (both labeled groups were synthesiszed by Applied Biosystems (AB) (Foster City, CA). The reverse primers for all sets of 12 primer pairs were unlabeled and were obtained from Integrated DNA Technologies. A small tailed oligonucleotide or PIG-tail sequence (GTTTCTT) was added to all the unlabeled reverse primers to promote specific priming,full adenylation and reduce stutter bands (Brownstein et al., 1996) Primer sequences are listed in (Table 3.2 and A.8).

PCR amplifications were carried out in a final volume of 10μL in 2 mL 8-strip PCR tubes with final concentration for those primers at 2μM using a 9600 thermal cycler (Applied Biosystems, Foster City, Calif.). The solution mix for PCR reactions consisted of the following: 2.0 μL of (20 ng/ μL) of genomic DNA; 3 μL of (Type-it microsatellite PCR –Maste mix; QIAGEN, USA); 2.0 μL of (2.0 μM) primer mix; and 3.0 μL of deionized water.

All amplifications of multiplex PCR were performed in a 96-well thermo cycler (Applied Biosystems, Foster City, CA) under the following conditions of touchdown annealing temperature profile (Viljoen et al., 2005): 2 min at 94 °C; 10 cycles of 45 sec at 94 °C, 1 min at 65 °C (annealing temperature was reduced 1 °C after every cycle), and 1 min and 30 sec at 72 °C; 35 cycles of 45 s at 94 °C, 1 min at 55 °C, and 1 min and 30 s at 72 °C; and a final extension step of 5 min at 72 °C. The touchdown procedure was used to reduce non-specific priming during PCR amplification.

Table 3.2 Source lists of SSR loci obtained from previous studies.

SSR loci	Size of allele	Number of Alleles	Heterozygosity	Discrimination Power	Repeat motifs
DCA18	(158-203)	(12-15)	0.91	0.94	(CT) 16 (TG) 4
UDO-043	(165-220)	(5-22)	0.77	0.93	(GT) 12
GAPU101	(192-264)	(3-9)	0.95	-	(AG) 8
DCA9	(160–213)	(13-23)	0.92	0.96	(GA) 7-23
DCA3	(226-255)	(8-13)	0.86	0.95	(GA) 11–19
DCA5	(194-211)	(6-11)	0.68	-	(A) 5 (GA) 5-15
DCA14	(169-191)	(5-11)	0.63	-	(AC) 9-18 (A) 2-9 (TAA) 7-9
DCA17	(101-183)	(0-10)	0.61	-	(GT) 9 (AT) 7AGATA(GA) 38
GAPU103A	(136- 245)	(4-16)	0.64	-	(TC) 15-26
DCA16	(120-222)	(13-37)	0.92	0.96	(GT) 6-16(GA) 9-29
GAPU71B	(118-285)	(2-9)	0.78	-	(AG) 7(AAG) 8
EMO-90	(180-197)	(0 -7)	0.7	-	(AC) 13 (CA) 10

Table 3.2 Continued.

SSR loci	Repeat Sequence	Primer sequence (5’-3’)
		Forward
DCA18	CTCTCTCTCTCTCTCTCTCTCTCTCTCTCT/TGTGTGTG	(F)aagaaagaaaaaggcagaattaagc
UDO-043	GTGTGTGTGTGTGTGTGTGTGTGT	(F)tcggctttacaacccatttc
GAPU101	AGAGAGAGAGAGAGAG	(F)catgaaaggaggggacata
DCA9	GAGAGAGAGAGAGA	(F)aatcaaagtcttccttctcatttcg
DCA3	GAGAGAGAGAGAGAGAGAGAGA	(F) cccaagcggaggtgtatattgttac
DCA5	AAAAA/GAGAGAGAGA	(F)aacaaatcccatacgaactgcc
DCA14	ACACACACACACACACAC/AAAAAAA/TAATAATAATAATAATAATAATAATAA	(F)aatttttaatgcactataatttac
DCA17	GTGTGTGTGTGTGTGTGT/ATATATATATATAT/AGATA/GAGAGAGAGAGAGA…	(F)gatcaaattctaccaaaaatata
GAPU103A	TCTCTCTCTCTCTCTCTCTCTCTCTC	(F)tgaatttaactttaaacccacaca
DCA16	GTGTGTGTGTGT/GAGAGAGAGAGAGAGAGA	(F) ttaggtgggattctgtagatggttg
GAPU71B	AGAGAGAGAGAGAG/AAGAAGAAGAAGAAGAAGAAGAAG	(F)gatcaaaggaagaaggggataaa
EMO-90	ACACACACACACACACACACACACAC/CACACACACACACACACACA	(F)catccggatttcttgctttt

Table 3.2 Continued.

SSR loci	Primer sequence (5'-3') Reverse	Annealing temperature (Tm °C)	References
DCA18	(R)gttttcgtctctctacataagtgac	50	Baldoni et al., 2009;Sefc et al., 2000
UDO-043	(R)tgccaattatggggctaact	55	Baldoni et al., 2009:Cipriani et al., 2002
GAPU101	(R)ggcacttgttgtgcagattg	50	Baldoni et al., 2009; Carrier et al., 2002
DCA9	(R)gatccttccaaaagtataacctctc	55	Baldoni et al., 2009;Sefc et al., 2000
DCA3	(R)tgcttttgtcgtgtttgagatgttg	50	Baldoni et al., 2009;Sefc et al., 2000
DCA5	(R)cgtgttgctgtgaagaaaatcg	50	Baldoni et al., 2009;Sefc et al., 2000
DCA14	(R)ttgaggtctctatatctcccagggg	50	Baldoni et al., 2009;Sefc et al., 2000
DCA17	(R)taaatttttggcacgtagtattgg	51	Sefc et al., 2000
GAPU103A	(R)gcatcgctcgatttttatcc	55	Baldoni et al., 2009; Carrier et al., 2002
DCA16	(R)ttttaggtgagttcatagaattagc	50	Baldoni et al., 2009;Sefc et al., 2000
GAPU71B	(R)acaacaaatccgtacgcttg	55	Baldoni et al., 2009; Carrier et al., 2002
EMO-90	(R)agcgaatgtagctttgcatgt	50	Baldoni et al., 2009;De La Rosa et al., 2002

After successful amplification of the target region of isolated DNA, PCR samples were combined with LIZ 600 internal size standards and Hi-DiTM formamide. Fragment analyses were performed on an Applied Biosystems 3130xL. The fragment data from Genetic Analyzer system was scored using 'GeneMapper' software v.3.7 (Applied Biosystems, Foster City, CA) to size and genotype the alleles.

3.1.3 Analytical methods

3.1.3.1Quality control

Quality control was performed using a set of procedures to ensure integrity, stability and consistency of SSR results. All amplifications of PCR for each sample replicated three times. Negative and positive standard controls were applied.Quality of allele was evaluated, so once the allele sizes were determined (allele calling), the data set was formatted such that it could be converted to the various formats required by the software packages (Convert program Version 1.3.1) (Glaubitz, 2004).Duplicated genotypes that have the same genetic fragment size were excluded to minimize the error estimation of genotyping.The set of loci was filtered to eliminate markers that have a missing data across all genotypes.

3.1.3.2 Population genetic analyses

Descriptive statistics for our data were performed using FSTAT software version 2.9.3.2 (Goudet, 2002) and GDA software version 1.1 (Lewis and Zaykin, 2002).We performed following parameters based on Hardy Weinberg (HW): (observed alleles, observed fragment size, private alleles, probability of identity and power of discrimination) were estimated for each individual locus (Table 3.4) and (probability of identity, power of discrimination, allele richnes, expected heterozygosity, observed heterozygosity and population inbreeding coefficient) for each set of individuals (Table 3.5) (Cipriani et al., 2002; Belaj et al., 2003; D´ıez et al., 2011).

3.1.3.3 Diversity and differentiation

3.1.3.3.1Estimation of population structure and diversity

We used several complementary methods to estimate the dissimilarity or similarity of genetic data based on their populations or type of genotype .The pairwise distance matrix of SSR data was implemented as a (.txt) input file of allelic data in DARwin software v 5.0.158 (Perrier and Jacquemoud-Collet, 2006). The constructed tree from DARwin software applied into the FigTree software v1.4.0 (Rambaut, 2012) to describe the relationship among olive samples using genetic distance that represented as a tree based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) with the support of (1000) bootstrap replicates to assess the uncertainty of the tree structure.

3.1.3.3.2 Estimation of partition by assignment

Structure analysis was used to estimate and partion genetic data and to assign genotypes to specific groups without any prior information. The probability of membership into 1-4 K groups was determined by multiple runs (10 times) using STRUCTURE software Version 2.3.4 by (Pritchard, Falush and Hubisz, 2012; Pritchard et al., 2003). The STRUCTURE HARVESTER program (Earl and Von Holdt, 2012) collect results generated by STRUCTURE program. This method allowe assessment and visualize the likelihood scores of of multiple values of K, to evaluate the most likely level of genetic groups subdivision.

The probability of identity (IP) for each locus and all SSR loci set (accumulated IP) was calculated by means of the CLUster Matching and Permutation Program (CLUMPP) version 1.1.2 (Jakobsson and Rosenberg, 2007). This program was used for aligning multiple replicate runs to assigns the average pair-wise similarity for each individuals on the basis of optimal membership coefficients within clusters.

3.1.3.3.3 Genotype phenotype comparison

Molecular data were combined together with morphological datat of stable phenotypic traits that were blocked by results of structure assignment of molecular data to evaluate the relationship between phenotypic and genotypic data.

3.2 RESULTS

A matrix of 12 SSR primers by 99 indiviuals was used to evaluate the genetic relationships among genotypes of local cultivated, introduced cultivars and wild types. As a result of filtering loci and genotypes that have missing data, allelic data of DCA17 and DCA9 were removed from the dataset due to high failure rate. Eight duplicated accessions, based on their identical genotypes, were also excluded (Table 3.3). Consequently, a total of 10 SSR loci and 91 genotypes (39 local, 36 introduced and 16 wild) remained in the genetic data matrix (Table A.9).

3.2.1 Identification of duplicated genotypes

Ten SSRs loci (Table 3.4) were used to determine if duplicate olive cultivar samples were present in the dataset. Twelve genotypes (6 pairs) had the same names and were genetically identical genotypes, and thus characterized as true duplicates (Table 3.3 and Fig.3.1). Two sets of cultivars had different names but identical genotypes may be due to clonality, and were therefore considered to be (synonyms), genetic similarity among each pair of duplicates or synonyms were based on high frequency of bootstrap values ranging from (59-100%), (Table 3.3 and Fig.3.1). One cultivar from each of these 8 pairs was excluded from further analyses. A review of their morphological data and associated images indicated similarity in phenotypic traits (Fig.3.2).

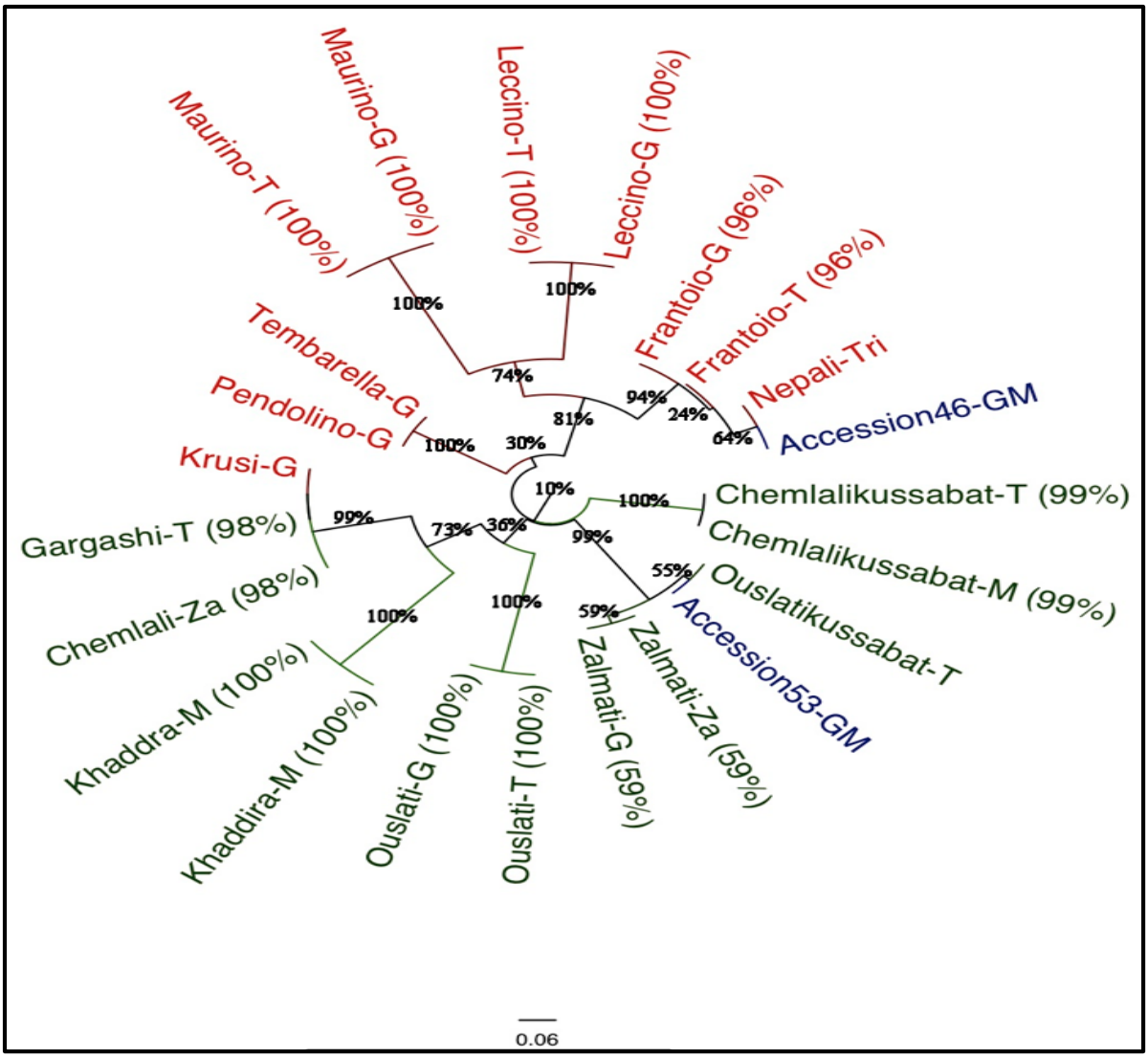


Fig.3.1 Neighbor-joining tree of 23 duplicated olive genotypes; each tip represents a single individual genotype with all pairs of duplicated genotypes similar. The percentage attached to each pair indicate bootstrap values after 1000 replicates

Fig.3.2 Phenotypic traits of the duplicated olive genotypes that illustrates similarity of genotypes.

Fig.3.2B Continued

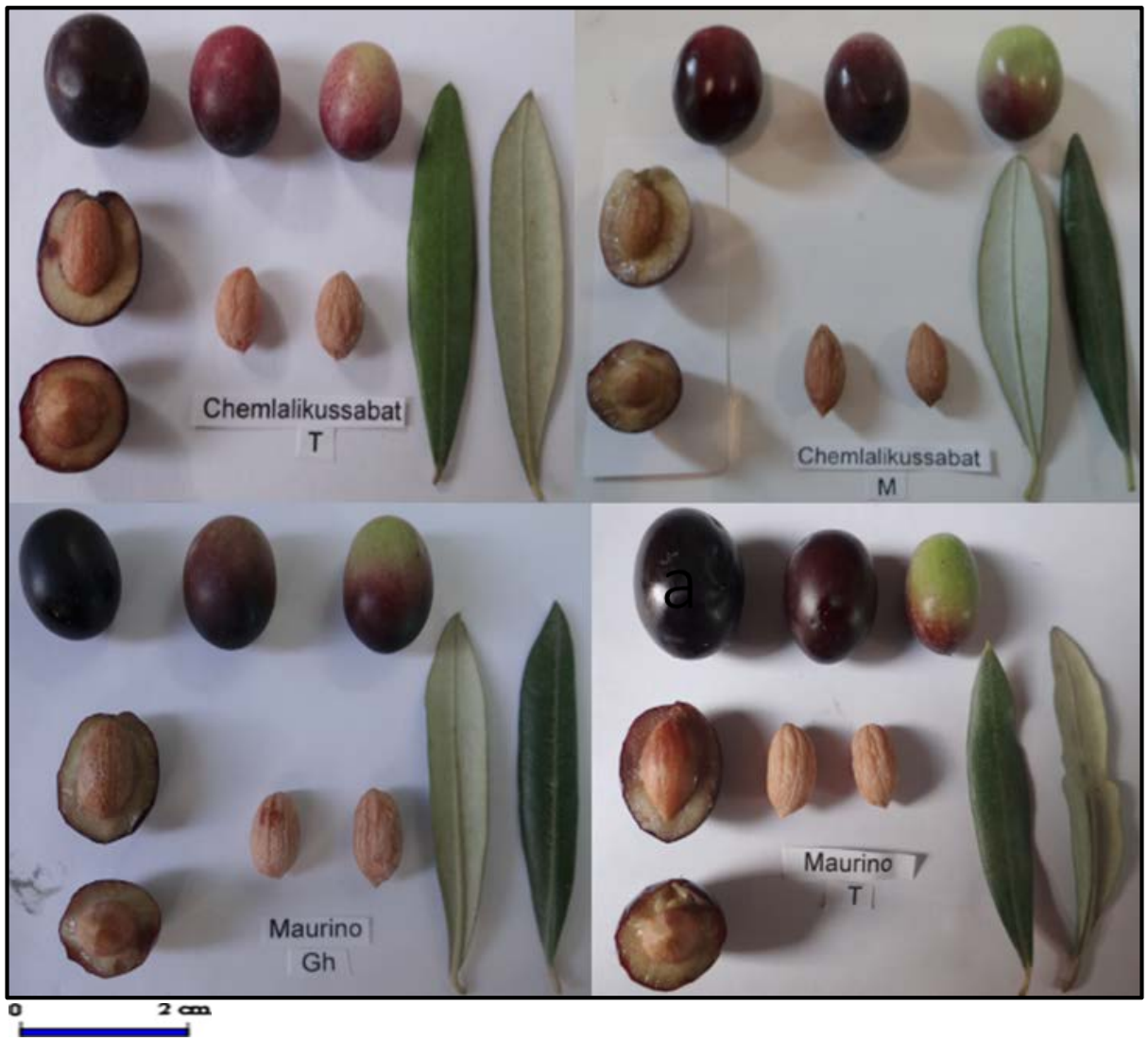


Fig.3.2 Continued

Table 3.3 Eight cultivars with the same fragment size were considered to be genotypes that were duplicated or synonyms.

Variety name	Local location	Relation-ship	Boot -strap values
Chemlkussabat	Tharouna	Duplicated	99
Chemlkussabat	Mesalata	Duplicated	
Khaddira	Mesalata	Synonyms	100
Khaddra	Mesalata	Synonyms	
Ouslati	Tharouna	Duplicated	100
Ouslati	Gharian	Duplicated	
Leccino	Tharouna	Duplicated	100
Leccino	Gharian	Duplicated	

Variety name	Local location	Relation-ship	Boot -strap values
Maurino	Tharouna	Duplicated	100
Maurino	Gharian	Duplicated	
Zalmati	Zaltin	Duplicated	59
Zalmati	Gharian	Duplicated	
Frantoio	Tharouna	Duplicated	96
Frantoio	Gharian	Duplicated	
Chemlali	Zaltin	Synonyms	98
Gargashi	Tharouna	Synonyms	

3.2.2 Descriptive statistics of loci

A total of 109 alleles were identified, and the number of alleles per locus ranged from 4 alleles at the DCA5 locus to 20 alleles at the UDO043 locus, with an average of approximately 11 alleles per locus (Table 3.4). The combined discrimination power for all 10 loci was calculated to be 0.70, indicating that there is a moderate to high discrimination of the markers that were used, so there is a high probability that two individuals have different genotypes for each locus. The average probability of identity for all loci was low, indicating that there is a low (0.30) probability of accessions matching by chance.

Table 3.4 Descriptive statistics of 10 loci based on genetic data from 91 individual olive genotypes collected in Libya.

Locus	Sample size	Observed alleles	Observed fragment size	Private alleles	Probability of identity (PI)	Power of discrimination (PD)
DCA14	90	10	168-188	3	0.22	0.78
DCA16	85	18	121-193	10	0.24	0.76
DCA18	92	10	154-180	3	0.20	0.80
DCA3	84	9	229-252	4	0.49	0.51
DCA5	83	4	194-206	1	0.85	0.15
EMO90	92	5	180-193	0	0.30	0.70
GAPU101	81	16	164-215	6	0.12	0.88
GAPU103A	88	11	134-189	4	0.21	0.79
GAPU71B	92	6	117-140	1	0.23	0.77
UDO043	69	20	154-227	9	0.10	0.90
All	85.6	10.9	161-198	4.1	0.30	0.70

3.2.3 Descriptive statistics of populations

Descriptive analysis of populations using GDA analysis (Table 3.5) revealed a higher inbreeding coefficient in the wild population (0.36) than the two sets of individuals, introduced (0.23) and local (0.24). The private allele frequency in the wild types was higher than the other two populations. However, the discrimination power (PD) of private alleles in local and introduced genotypes was relatively high (0.99 and 0.98) respectively (Table 3.5).

The value of observed heterozygosity (Ho) was less than the value of expected hetreozygosity for all three sets of individuals (Table 3.5) that indicates those population may exhibit a high level of inbreeding within isolated and closely related individuals.

Table 3.5 Descriptive statistics of three sets of individuals (Introduced, local and wild) collected from six locations in Libya[z]

Sets of individuals	Sample size	Number of Private alleles	Probability of identity (PI)	Power of discrimination (PD)	Allele richness	He	Ho	Population inbreeding coefficient
Introduced	36	19	0.02	0.98	5.89	0.71	0.55	0.23
Local	39	4	0.002	0.99	4.88	0.68	0.52	0.24
Wild	16	18	0.13	0.87	5.88	0.64	0.41	0.36
Overall	30.33	13.67	0.05	0.95	5.55	0.68	0.49	0.28

[z] Six locations located as identified in Fig. 3.0.

In general, allelic richness was higher in wild and introduced genotypes (5.89 and 5.88) respectively than in local genotypes (4.88) (Table 3.5). There were more private alleles (observed once) in the introduced genotypes (19 private alleles), than in the wild (18 alleles) and local genotypes (4 alleles) (Table 3.5). Overall, all of the 41 private alleles were considered to be highly polymorphic across locations and could be used to assign individuals into specific population based on their origins (Table 3.5). A total of 42 monomorphic alleles were estimated in all three different populations. These could not be used to assign any genotype to a specific population. Common alleles were most often observed in wild and introduced genotypes.

F-statistics for the three sets of individuals (introduced, local and wild) were estimated by performing a bootstrap analysis across loci to create 95% confidence intervals (Table 3.6). The pairwise F-statistics for the three sets of individuals were significantly different. Genetic diffentation of Fit, Fst and Fis was estimated by bootstrap test over all loci, and it was significant among all loci.

Table 3.6 Genetic differentiations as estimated by Fst with confidence intervals of 95% over all loci and three different loctions.

Source	Fst	Fst confidence interval
Loci	0.025	-0.025-0.077
sets of individuals	0.03	-0.03-0.08

3.2.4 Estimation of diversity and differntation

3.2.4.1 Identification of mislabeled genotypes

Neighbor-joining relationships revealed that the 10 loci failed to distinguish a total of seven cultivars that were similar for 16 alleles, when the molecular data were evaluated, each pair of these cultivars match different genotype. These genotypes were Krusi, Pendolino, Tombarella, Ouslatikussabat, Accession53, Nepal and Aceession46. However, all seven cultivars had missing data for two loci (Table 3.7). A review of their morphological data and associated images (Fig.3.3) indicated large differences in phenotypic traits across all of these cultivars.

Table 3.7 The seven cultivars had missing data that were considered to be similar genotypes.

POP = Introduced	DCA 18	DCA 18	UDO04 3	UDO0 43	GAP U101	GAP U101	DCA 3	DCA 3	DCA 5	DCA 5
KrusiG	168	168	227	227	?	?	240	240	202	202
GargashiT	168	168	?	?	187	193	240	240	202	202
PendolinoG	174	174	204	204	189	203	240	240	202	202
TombarellaG	174	174	?	?	?	?	240	240	202	202
Ac#53	174	174	168	168	189	195	240	240	202	202
OuslatikussabatT	174	174	168	168	189	195	?	?	?	?
Ac#46	?	?	?	?	181	195	240	240	202	202
NepalTri	174	174	177	177	181	195	?	?	?	?

POP = Introduced	DCA 14	DCA 14	GAPU 103A	GAPU 103A	DCA 16	DCA 16	GAP U71B	GAP U71B	EMO 90	EMO 90
KrusiG	182	186	159	159	147	160	124	127	184	184
GargashiT	182	186	159	159	147	160	124	127	184	184
PendolinoG	186	186	150	150	147	147	121	127	183	189
TombarellaG	186	186	150	150	147	147	121	127	183	189
Ac#53	168	186	159	159	?	?	?	?	183	184
OuslatikussabatT	168	186	159	159	147	183	121	140	183	184
Ac#46	178	186	162	174	147	147	121	140	183	189
NepalTri	178	186	162	174	147	147	121	140	183	189

Fig.3.3 Genotypes that were similar based on SSR data available; phenotypic traits illustrating differences and misidentification in those pairs of genotypes.

3.2.4.2 Identification of homonyms genotypes

There were 13 samples that had the same cultivar names, but did not have matching genotypes (Table 3.8; Fig.3.4). This suggests that all of the labeled cultivars were genetically polymorphic for at least (2-15) different alleles. Comparisons of morphology images of each duplicate pair of genotypes showed distinct differences and supported the genetic results of polymorphism (Fig. 3.5). The problems associated with cultivar identification likely in landrace types than in introduced cultivars or wild type olives.

Table 3.8 Thirteen duplicated cultivars with different fragment size that were considered to be mislabeled or homonyms genotypes

Varirty name	Local location	Relation -ship	Number of different alleles
Chemlali	Masallata	Homonyms	10
Chemlali	Zaltin	Homonyms	
Chemlalisfax	Gharian	Homonyms	15
Chemlalisfax	Tharouna	Homonyms	
Coratina	Gharian	Homonyms	9
Coratina	Tharouna	Homonyms	
Gargashi	Masallata	Homonyms	2
Gargashi	Tharouna	Homonyms	
Hammudi	Masallata	Homonyms	3
Hammudi	Tharouna	Homonyms	
Jabbugi	Masallata	Homonyms	9
Jabbugi	Tharouna	Homonyms	
Marrari	Masallata	Homonyms	2
Marrari	Tharouna	Homonyms	

Varirty name	Local location	Relation -ship	Number of different alleles
Mbuti	Masallata	Homonyms	7
Mbuti	Tharouna	Homonyms	
Mignolo	Gharian	Homonyms	14
Mignolo	Tharouna	Homonyms	
Moraiolo	Gharian	Homonyms	10
Moraiolo	Tharouna	Homonyms	
Rasli	Masallata	Homonyms	11
Rasli	Tharouna	Homonyms	
Zaafrani	Masallata	Homonyms	7
Zaafrani	Tharouna	Homonyms	
Zarrasi	Masallata	Homonyms	11
Zarrasi	Tharouna	Homonyms	

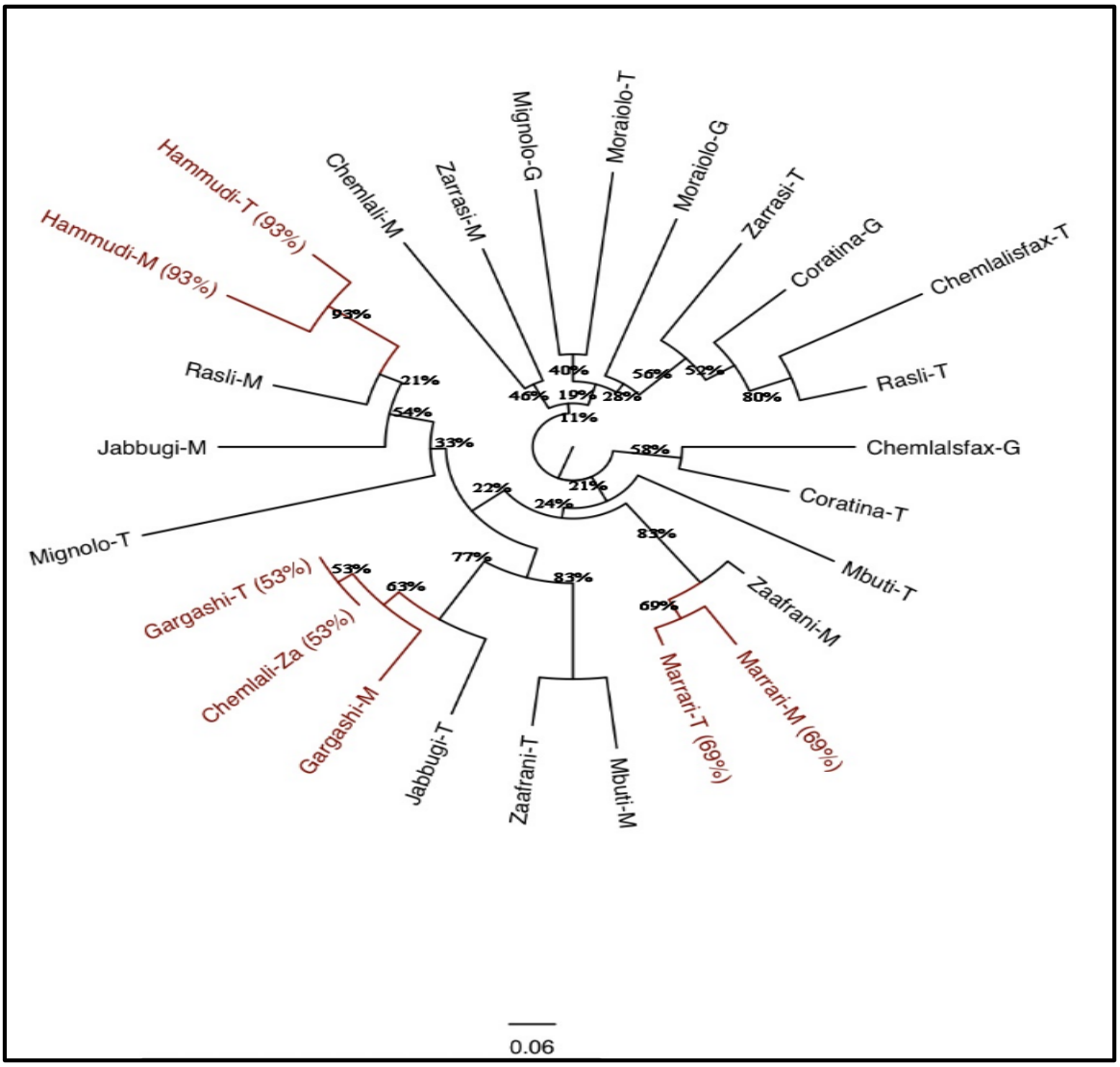


Fig.3.4 Neighbor-joining tree of 13 duplicated pairs of olive genotypes; each tip represents a single individual accession with all pairs of duplicated genotypes different. The percentage attached to each pair indicate bootstrap values after 1000 replicates

Fig.3.5 Accessions identified by the same name but phenotypically were difference (Homonyms accessions).

Fig.3.5 Continued.

Fig.3.5 Continued.

An UPGMA neighbor-joining tree (Fig.3.6) was constructed to study the genetic relationships among the remaining 91 different olive genotypes that were discriminated by the 10 SSR markers .Two primary clusters of individuals were identified (green color = landraces) and (intermixed color, red = introduced cultivars and blue = wild types). Most of the wild types were found within the intermixed wild and introduced genotypes (Fig.3.6).

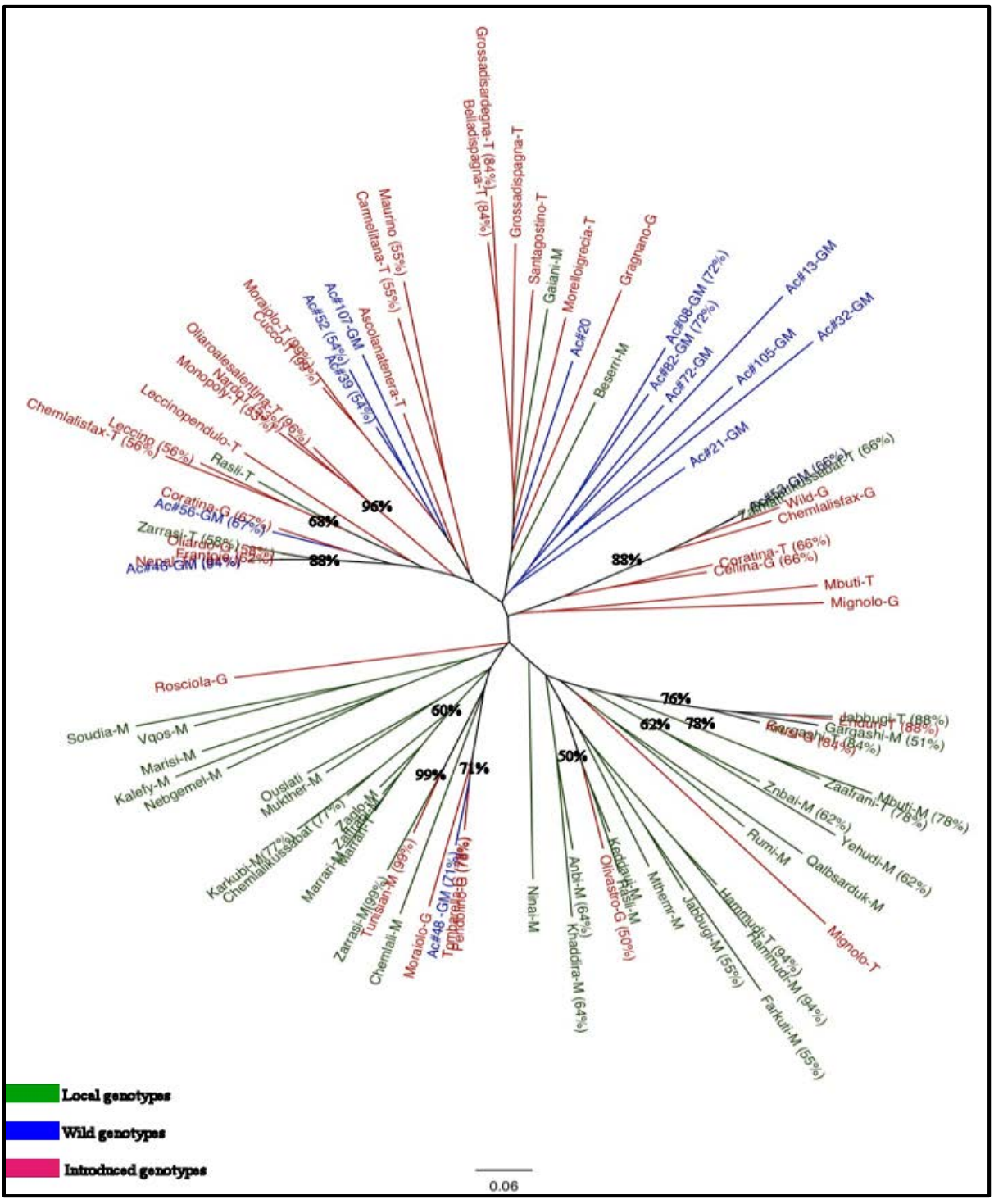


Fig.3.6 Neighbor-joining tree of 91 individuals, each tip represents a single olive genotype and the colors of clades indicate the populations of origin (local, introduced and wild). The percentage attached to the clades indicate bootstrap values after 1000 replicates.

3.2.5 Estimation of partition by assignment

Structure analysis using the admixture model without prior information was used to identify the genetic relationships of Libyan landraces, wild, and introduced cultivars. It was also used to infer the genetic structures of each individuals within each population based on their membership probabilities. The most likely number of clusters inferred by STRUCTURE HARVESTER program was K=3.

The local genotypes clustered together and two distinct sub-groups were identified. The first group consisted of the 20 most popular local genotypes (blue color) that are used mainly to produce olive oil. The second group consisted of 11 hybrid or ambiguous genotypes (blue and red color) between local and introduced cultivars (Table A.10 and Fig.3.7). These accessions are not widely grown and are not preferred for oil production. Those cultivars (20 most popular local genotypes) that were primarily ancient cultivars and grown in the Mesallata region where they are widely grown for their valuable oil characteristics.The first group includes the main two cultivars Rasli and Gargashi that are used mainly for their oil production under extremely dry climates.

There were six genotypes (ZarrasiM, ChemlaliM, MoraioloG, Ac#48, PendolinoG and TombarellaG) that were considered to be local genotypes in neighbor-joining tree cluster (Fig. 3.6) but based on the structure analysis were included in the introduced genotype grouping. This is perhaps best explained by saying that they are really introduced genotypes especially given the derivation of the names of 4 of them is not Arabic but Italian. In the case of ZarrasiM its fruit size is similar to the introduced genotypes that have larger fruit size as compared to the smaller fruit of the local types. The wild and introduced accessions were similarly clustered and intermixed to each other the same as neighbor-joining clusters (Fig. 3.6). They had an intermixed genetic background (red color) as shown in Fig.3.7.

There were 13 genotypes that had a lot of admixture and mixed gentic background of all populations (Table A.10 and Fig.3.7). Some of these genotypes (Beserri-M, Oliarolasalentina-T, Santagostin-T, Mignolo-T, Gragnano-G, Ouslati-T, Nebgemel-M and Kalefy-M) were previously reported with Fig Tree cluster (Fig.3.6) appeared to be distantly related genotypes and were completely different. Finally, the results from population structure analyses clearly distinguished the known ancient local cultivars, introduced cultivars and wild types into specific clusters associated with their origin (local, introduced and wild), but not always due to their use (oil, table and dual purpose) as reported in previous studies (Besnard et al., 2001 and Belaj et al., 2010).

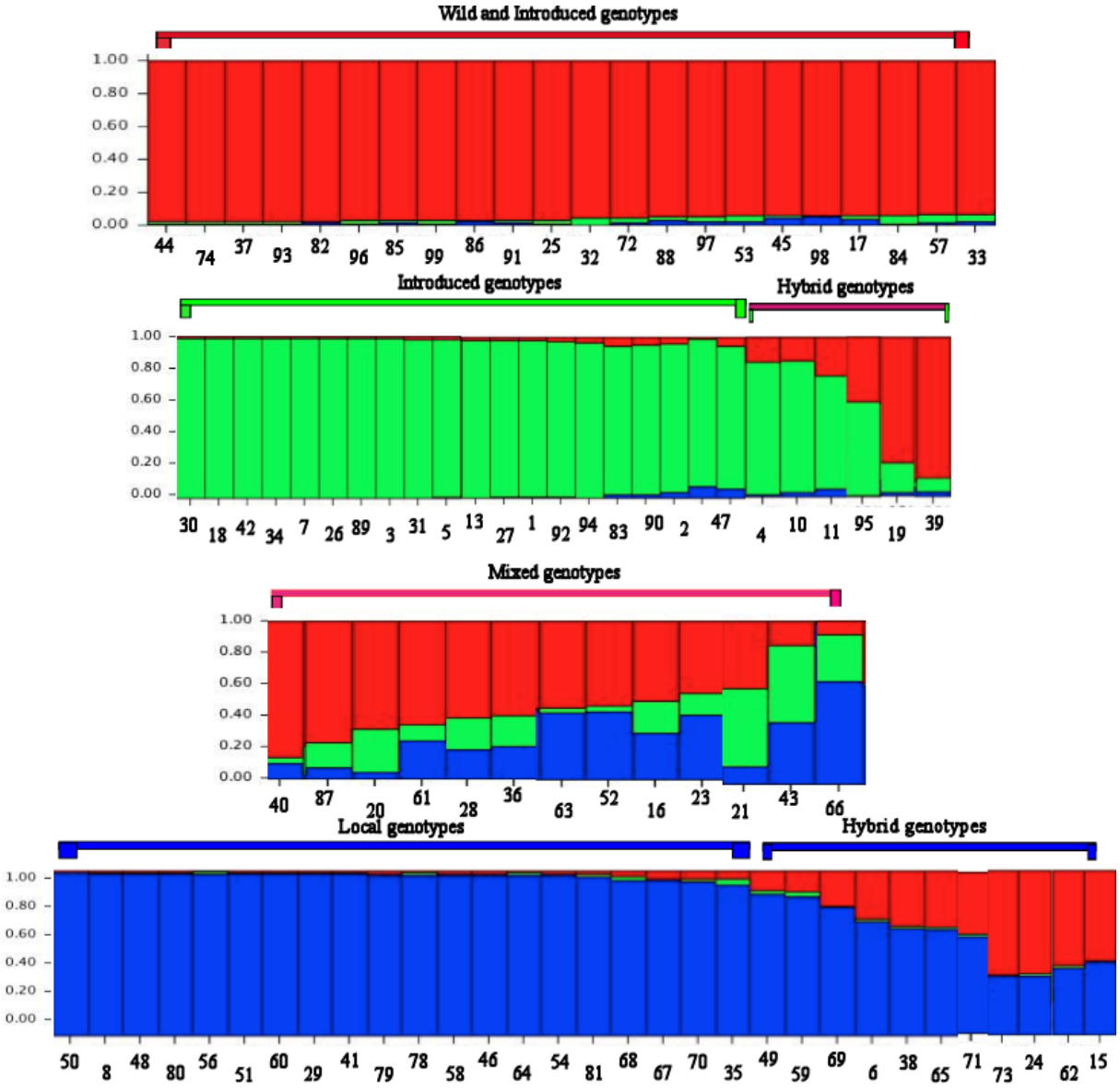


Fig.3.7 Separation of the estimated population structure into specific groups; intermixed group between introduce and wild genotypes (red color), Introduced genotypes (green color) and hybrid genotypes (mixed color) and local genotypes (blue color). Each single vertical strain is represented by an individual genotype (Table A.10).

3.2.6 Correlation between genotypic and phenotypic traits.

We sought to determine if independent stable phenotypic traits data could be used as a covariates or numeric data to predict the genetic classification of olive genotypes to verify if there is a correlation between the phenotypic and genotypic traits as a categories data. Highly significant differentiations (P<0.0001***) (Fig.3.8 A and Fig.3.8 B) of stable phenotypic traits were observed when using the average q values of membership coefficient that were interpreted as a probability of membership in STRUCTURE program to assign each individuals to specific population (1=landraces, 2=mixed and 3=introduced Fig.3.8 A,) or structurama partition assignment (1=mixed, 2=Introduced and 3=landraces, Fig.3.8 B) respectively as a categorical data for all 90 genotypes based on the cultivar origin (introduced or local).

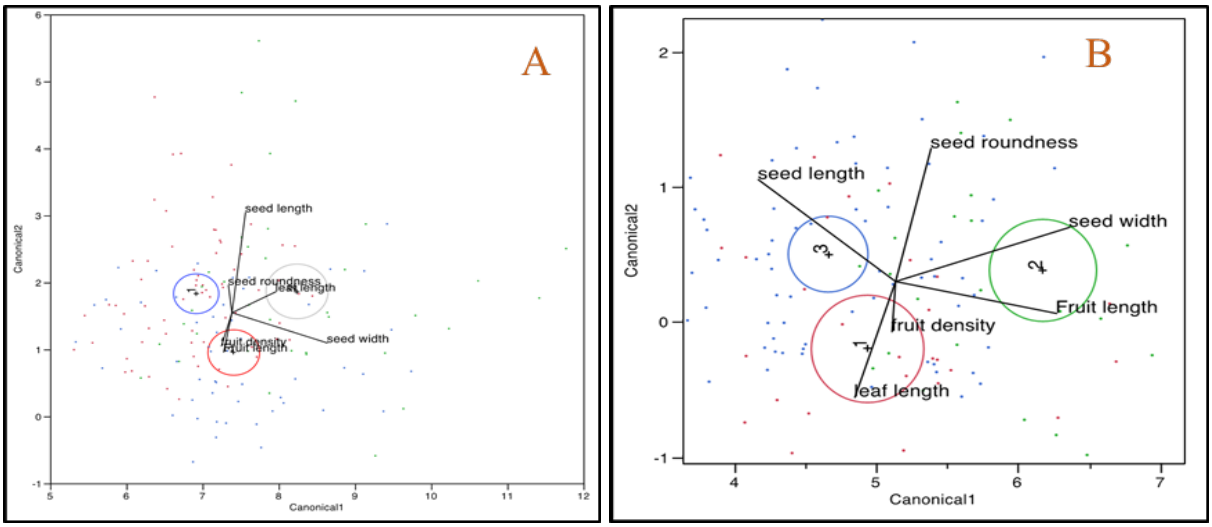


Fig. 3.8 Discriminant analysis was used to differentiate among all 90 genotypes based on membership q values of structure (p<0.0001***) A, and structurama assignment (p<0.0001***) B.

3.3 DISCUSSION

The SSR markers (Table 3.2) used in this study were selected based on previously published reports (Baldoni et al., 2009; Erre et al., 2010; Dı´ez et al., 2011; and Ipek et al., 2012). The identification of duplicated, mislabeled or homonymes genotypes (Table 3.3, 3.7 and 3, 8), respectively found within the Libyan olive collection illustrates one of the most important problems associated with olive production in Libya, which is growers planting genotypes that are not those of greatest yield potential in their specific area. A source of this misidentification may be due to phenotypic variation (Fig 3.2 B) associated with environmental conditions when grown in diverse locations, so the variability of morphological traits in different locations may contribute to the description of the same genotype with different names. In 2009, Rao et al. showed that synonyms and homonyms occur more frequently among landraces than in common cultivars. However, phenotypic data (fruit, seed and leaf) may be important in distinguishing different genotypes when molecular data indicates no differences due to missing or limited data. This is especially true when stable phenotype characteristics indicate that there are differences between genotypes.

Seven cultivars (Table 3.7) were determined to be identical based on the data from 8 loci. However, this data was insufficient to discriminate all seven cultivars due to missing data of two additional loci. The combination of phenotypic traits (Fig.3.3) clearly indicated that these cultivars were different.Descriptive analysis of loci (Table 3.4) identified UDO43 the most informative locus with a total of 20 alleles, lowest probability of identity (0.1) that two individuals share the same genotype at given locus and highest discrimination power (0.90) in which two individuals have different genotypes at that locus. In general, loci that have a high number of alleles were preferred to distinguish between two different individuals

(http://www.mathcs.citadel.edu/trautmand/stuff/dnapapers/little.htm). Whereas the highest probability of identity (0.85) observed was for locus DCA5 that had the lowest power of discrimination (PD) (0.15) with observed number of alleles (1) (Table 3.4).

Overall probability of identity of all loci was generally low (0.30) (Table 3.4), particularly at loci that have high allelic number as noted also in previously published results (Roubos et al., 2010). Overall, the values observed for the expected and observed heterozygosity, Table 3.5 for all three sets of individuals (0.68 and 0.49) respectively were somewhat higher than the number of alleles that were reported by the authors using similar sets of SSR markers (Erre et al., 2010; Belaj et al., 2010; Muzzalupo et al., 2010; Baldoni et al., 2009; Zaher et al., 2011 and Erre et al., 2010). The reason for a high number of alleles observed in our study could be due to use of large number of exotic genotypes or the high discrimination power of selected loci.

Wild types have a higher inbreeding coefficient (0.36) than the two cultivated populations: introduced (0.23) and landrace (0.24). This maybe the result of continued breeding of closely related individuals since the area in which the wild genotypes grow is far away from cultivated genotypes. Also, it has the highest number of private alleles and the highest level of genetic diversity found in this area in spite of the low number of wild types. This may be useful for the preservation of desirable traits of the wild type in the same genetic pool. The result is that the wild type may then be a source of some genes for potential improvement of local cultivars. Genetic diversity studies of the local ancient olive cultivars in Italy (Banilas et al., 2003; Baldoni et al., 2006) have revealed that only a few of these landraces matched current olive cultivars grown today. These studies were comparable to our results, which clearly indicate there are large differences observed in the Libyan collection.

Distinct groups of local landraces differed from introduced and wild genotypes as indicated in both the neighbor-joining tree (Fig.3.6) and the admixture analysis (Fig. 3.7). This was also noted by Zaher et al. (2011) in which distinct clustering of the landraces from the same region has a unique genetic background and did not have matching genotypes form the other two sets of individuals. In contrast, Hannachi et al. (2010) suggested that 'Roumi' could be a progeny of 'Chemlali', but our results from the dendrogram Fig.3.6 and morphological data suggested that they are distantly unrelated genotypes. The major proportion of landraces did not match any other introduced or wild olive genotypes. The local Libyan cultivars likewise may represent early stages of olive cultivation (D´ıez et al., 2011and Belaj et al., 2010) that remain as unexploited genetic diversity and therefore important germplasm resources for breeding that need to be characterized and conserved.

The genetic relationship study among the three sets of individuals (landraces, introduced and wild) that were assumed to be different were not as different as expected. Neighbor-joining tree (Fig.3.6) and the STRUCTURE analysis (Fig. 3.7) demonstrated a strong correlated relationship between wild and introduced genotypes. The wild types were genetically more closely and have common genetic background related to the introduced types than the local genotypes. This was unexpected since one would most commonly assume that the local cultivars were descended from the native wild types. However, accession wild-48 were an exception and they were phenotypically and geneticly more closely related to the landraces than the wild type. This may be due to human errors of the propagation process. Therefore, the idea that Libyan local cultivars may have descended from the wild types is not supported by either the neighbor-joining tree or the STRUCTURE analyses.Our results are compareable with previous studies (Hannachi et al., 2008 and Hannachi et al., 2010) that showed there are close genetic relationships between

oleaster types and cultivated genotypes using SSR data with NJ method. Although, in our data, some of oleaster types were intermixed within cultivated genotypes and others only clustered with wild types.

Most of the wild type accessions were collected from the Eastern side of Libya (Fig.3.1), which is closer to Europe from which introduced genotypes came to Libya in 1954 during the years of colonization by Italy. Wild olive genotypes are currently thought to have a common gene pool in the entire Mediterranean Basin (Kole, 2011). This may be why the wild Libyan accessions being clustered close to the introduced genotypes from Europe with common ancestry and relatedness genetic pool.

Several morphological traits can differentiate between wild and cultivated olive (Hannachi et al., 2008). Our research suggests that phenotypic traits were not as informative as molecular data and were limited in discriminatory power to evaluate the relatedness and the level of genetic similarity of olive genotypes (Corrado et al., 2009, Hannachi et al., 2008). In addition, Rao et al. (2009) reported that biometry values alone were unable to differentiate between similar genotypes that were evaluated by morphological traits.

It seems, there is a strong correlation between the genotype and phenotype data (Fig.3.8 A and Fig.3.8 B) that were based on independent phenotypic stable traits and blocked by structure membership coefficient (1=local, 2=mixed and 3=introduced) or structurama partition assignment (1=mixed, 2=introduced and 3=local) (Fig.3.8 A and Fig.3.8 B) respectively. The results showed that stable phenotypic data could be used the same as genetic data to assign each individual to specific group of cultivars based on their origin (local, introduced or wild). Consequently, phenotypic data accurately estimated all genotypes based on their origin (introduced or local). Molecular and morphological relationships among olive varieties are expected to be similar when

there is a little effect of genetic and environment interaction observed. Recently, both morphological and molecular aspects have been combined together to clarify the identy of genotyes within other crops (Corrado et al., 2009; Hannachi et al., 2010; D´ıez et al., 2011and Belaj et al., 2012).

3.4 CONCLUSION

The study of local ancient cultivars and wild types of the Libyan collection is increasingly important in order to conserve those genotypes as a potential genetic resource; they may have valuable genes that could provide novel and useful phenotypic traits for advanced plant breeding. This study provides useful information in order to establish a general molecular database of Libyan olive cultivars.

There is a high heterozygosity within the Libyan collection studied.The current set of 10 SSR loci amplified the corresponding microsatellite fragments in all the 91 genotypes; also it can be used to genotype the Libyan olive collection and to assign each individual into a genetic relatedness group. In this study, molecular data led to the clear separation of 91 distinct genotypes (39 local, 36 introduced and 16 wild) out of the 99 accessions included in this study, also it revealed the existence of a high level of genetic variability among Libyan collection. It is interesting that changes of the denominations are more frequently within landraces than other cultivated and wild types.

Using additional new candidate loci with the use of a reference sample could lead to a more robust molecular database, which could be used to characterize the Libya olive collection. This may then be used to optimize the management strategy of Libyan olive germplasm.The combination of molecular phenotypic could differentiate olive genotypes.

REFERENCES

Abdul Sadeg, S.M. (2003). Physical and morphological characters of olive cultivars (*Olea europaea* L.) growing in Tarhunah and Masallatah regions and DNA fingerprinting of Zaafrani, Hummudi and Jabbugicvs.Master of Science (M.S.). Master, Thesis.

Aparicio, R., & Harwood, J. (2013). Handbook of Olive Oil. Analysis and Properties. 2nd ed. Springer New York Heidelberg Dordrecht London. DOI 10.1007/978-1-4614-7777-8.

Baldoni, L., Cultrera, N.G., Mariotti, R., Ricciolini, C., Arcioni, S., Vendramin, G.G., Buonamici, A., Porceddu, A., Sarri, V., Ojeda, M.A., Trujillo, I., Rallo, I., Belaj, A., Perri, E., Salimonti, A., Muzzalupo,I., Casagrande,A., Lain,O., Messina,R.,&Testolin,R.(2009). A consensus list of microsatellite markers for olive genotyping. Mol Breeding, 24:213–231.DOI 10.1007/s11032-009-9285-8

Baldoni, L., Tosti, N., Ricciolini, C., Belaj, A., Arcioni, S., Pannell, G., Germana, M. A., Mulas, M., &Porceddu, A. (2006). Genetic structure of wild and cultivated olives in the central Mediterranean Basin. Annals of Botany, 98: 935–942. Doi: 10.1093/aob/mcl178

Banilas, G., Minas, J., Gregoriou, C., Demoliou, C., Kourti, A., Hatzopoulos, P. (2003). Genetic diversity among accessions of an ancient olive variety of Cyprus. Genome 46: 370–376.

Bartolini, G., Prevost, G., Messeri, C., & Carignani, G. (1998). Olive germplasm: cultivars and world-wide collections. *Olive germplasm: cultivars and world-wide collections.*

Belaj, A., del Carmen Dominguez-García, M., Atienza, S. G., Urdíroz, N. M., De la Rosa, R., Satovic, Z., ... & Del Río, C. (2012). Developing a core collection of olive (*Olea europaea* L.) based on molecular markers (DArTs, SSRs, SNPs) and agronomic traits. *Tree Genetics & Genomes*, *8*(2), 365-378.

Belaj, A., Munoz-Diez, C., Baldoni, L., Satovic, Z., &Barranco, D. (2010). Genetic diversity and relationships of wild and cultivated olives at regionallevel in Spain.ScientiaHorticulturae, 124: 323–330.doi: 10.1016/j.scienta.2010.01.010

Belaj, A., Satovic, Z., Cipriani, G., Baldoni, L., Testolin, R., Rallo, L., & Trujillo, I. (2003). Comparative study of the discriminating capacity of RAPD, AFLP and SSR markers and of their effectiveness in establishing genetic relationships in olive. TheorAppl Genet. 107:736–744. DOI 10.1007/s00122-003-1301-5

Belaj, A., Satovic, Z., Rallo, L., & Trujillo, I. (2002). Genetic diversity and relationships in olive (*Olea europaea* L.) germplasm collections as determined by randomly amplified polymorphic DNA. *Theoretical and Applied Genetics*, *105*(4), 638-644.

Belaj, A., Trujillo, I., de la Rosa, R., &Rallo, L. (2001). Polymorphism and discrimination capacity of randomly amplified polymorphic markers in an olive germplasm bank. Am. Soc. Hort. Sci., 126: 64-71.

Besnard, G., Baradat, P., &Berville. A. (2001). Genetic relationships in the olive (*Olea europaea* L.) reflect multifocal selection of cultivars. Theor. Appl. Genet. , 102:251–258.

Besnard, G., Garcia, V.C., Rubio De Casas, R., Treier, U.A., Galland, N., & Vargas, P. (2008). Polyploidy in the olive complex (*Olea europaea*): Evidence from flow cytometryand nuclear microsatellite analyses. Annals of Botany, 101: 25–30. Doi: 10.1093/aob/mcm275

Besnard, G., Henry, P., Wille, L., Cooke, D., &Chapuis, E. (2007). On the origin of the invasive olives (*Olea europaea* L., *Oleaceae)*. Heredity, 99: 608–619.

Besnard, G., Hernandez, P., Khadari, B., Dorado, G., &Savolainen, V. (2011). Genomic profiling of plastid DNA variation in the Mediterranean olive tree. BMC Plant Biology, Doi: 10.1186/1471-2229-11-80

Besnard, G., Khadari, B., Navascués, M., Fernández-Mazuecos, M., El Bakkali, A., Arrigo, N., & Savolainen, V. (2013). The complex history of the olive tree: from Late Quaternary diversification of Mediterranean lineages to primary domestication in the northern Levant. Proceedings of the Royal Society B: Biological Sciences, 280(1756).

Breton, C., Terral, J.F., Pinatel, C., Medail, F., Bonhomme, F., &Berville, A. (2009). The origins of the domestication of the olive tree. C. R. Biologies, 332:1059–1064.doi: 10.1016/j.crvi.2009.08.001

Brownstein, M. J., Carpten, J. D., & Smith, J. R. (1996). Modulation of non-templated nucleotide addition by Taq DNA polymerase: primer modifications that facilitate genotyping. Biotechniques, 20(6), 1004.

Carriero, F., Fontanazza, G., Cellini, F., &Giori, G. (2002). Identification of simple sequence repeats (SSRs) in olive (*Olea europaea* L.Theor Appl Genet, 104:301–307.

Charafi, J., El Meziane, A., Moukhli, A., Boulouha, B., El Modafar, C., &Khadari, B. (2007). Menara gardens: A Moroccan olive germplasm collectionidentified by a SSR locus-based genetic study. Genet Resour Crop Evol.DOI 10.1007/s10722-007-9294-6

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. Theor. Appl. Genet., 104:223–228.

Corrado, G., La Mura, M., Ambrosino, O., Pugliano, G., Varricchio, P., &Rao, R. (2009).Relationships of companion olive cultivars: comparative analysis of molecular and phenotypic data. Genome, 52: 692–700. Doi: 10.1139/G09-044

Daham, M., &Ashur, A. H. (2008). Book of agriculture statistics.League of Arab States.Arab Organization for Agricultural Development. Khartoum. Sudan.

De La Rosa, R., James,C.M.,& Tobutt, K.R. (2002).Isolation and characterization of polymorphic microsatellites in olive (*Olea europaea* L.) and their transferability to other genera in the *Oleaceae* .Molecular Ecology Notes, 2, 265 –267.doi:10.1046/j.1471-8278 .2002.00217.

Diaz, A., Martin, A., De La Rosa, R., & Rallo, P. (2008). Development and characterization of 12 new microsatellites in olive (*Olea europaea* L.). Acta Hort. 791, 87-94.DOI 10.1007/s10722-004-6130-0

Díez, C. M., Trujillo, I., Barrio, E., Belaj, A., Barranco, D., & Rallo, L. (2011). Centennial olive trees as a reservoir of genetic diversity. *Annals of botany*, *108*(5), 797-807.

Doyle, J. J. (1990). Isolation of plant DNA from fresh tissue. *Focus*, *12*, 13-15.

Doyle, J.J., & Doyle, J.L. (1987).A rapid DNA isolation procedure for small quantities of fresh leaf material.Phytochem Bull 19: 11-15.

Durgac, C., Kiyga, Y., &Ulas, M. (2010). Comparative molecular analysis of old olive (*Olea europaea*L.) genotypes from Eastern Mediterranean region of Turkey.African Journal of Biotechnology, 9(4): 428-433. Retrieved from http://www.academicjournals.org/AJB

Earl, D. A. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources, 4(2), 359-361.

El Saied, S. H., Hegazi, A. A., Tawfik, A. A., & Sayed, H. A. (2012). Molecular Characterization of Local and Imported Olive Cultivars Grown in Egypt Using ISSR Technique.

Elbaum, R., Melamed-Bessudo, C., Boaretto, E., Galili, E., Lev-Yadun, S., Levy, A.A., & Weiner, S. (2006). Ancient olive DNA in pits: Preservation, amplification and sequence analysis. Journal of Archaeological Science, 33: 77-88. doi:10.1016/j.jas.2005.06.011

Ercisli, S., Barut, E., &Ipek, A. (2009). Molecular characterization of olive cultivars using amplified fragment length polymorphism markers. Genet. Mol. Res, 8 (2): 414-419.

Ercisli, S., Bencic, D., Ipek, A., Barut, E., & Liber, Z. (2012). Genetic relationships among olive (*Olea europaea* L.) cultivars native to Croatia and Turkey. *Journal of Applied Botany and Food Quality*, *85*(2), 144.

Ercisli, S., Ipek, A., &Barut, E. (2011).SSR marker-based DNA fingerprinting and cultivar identification of olives (*Olea europaea*).Biochem Genet. DOI 10.1007/s10528-011-9430-z.

Erre, P., Chessa, I., Muñoz-Diez, C., Belaj, A., Rallo, L., & Trujillo, I. (2010). Genetic diversity and relationships between wild and cultivated olives (*Olea europaea* L.) in Sardinia as assessed by SSR markers. Genetic Resources and Crop Evolution, 57(1), 41-54.

FAO, J., & FOODS, M. H. I. (2004). Food and Agriculture organization of the United Nations. Rome, URL: http://faostat. FAO. org.

FAO. (2008). Agricultural Statistics of the Food and Agriculture Organization of the United Nations, Rome. Www.FAO.org. Accessed March, 18 2010.

Food and Agriculture Organization of the United Nations. (2012).FAOSTAT.FAO.org

Ganino, T., Beghe, D., Valenti, S., Nisi, R., &Fabbri, A. (2007).RAPD and SSR markers for characterization and identification of ancient cultivars of *Olea europaea* L. in the Emilia region, Northern Italy.Genet Resour Crop Evol , 54:1531–1540. DOI 10.1007/s10722-006-9145-x

Glaubitz, J. C. (2004). Convert: A user-friendly program to reformat diploid genotypic data for commonly used population genetic software packages. Molecular Ecology Notes, 4(2), 309-310.

Goudet, J. "FSTAT, a program to estimate and test gene diversities and fixation indices, ver. 2.9. 3.2." URL: www 2 (2002).

Guichoux, E., Lagache, L., Wagner, S., Chaumeil, P., Léger, P., Lepais, O., & Petit, R. J. (2011). Current trends in microsatellite genotyping. Molecular ecology resources, 11(4), 591-611.

Hakim, I. R., Kammoun, N. G., Makhloufi, E., &Rebai, A. (2009). Discovery and potential of SNP markers in characterization of Tunisian olive germplasm. Diversity, 2: 17-27. Doi: 10.3390/d2010017

Hannachi, H., Breton, C., Msallem, M., Ben El Hadj, S., El Gazzah,M.,&Berville, A.(2010).Genetic relationships between cultivated and wild olive trees (*Olea europaea* L. var. *europaea* and var. sylvestris) based on nuclear and chloroplast SSR markers. Natural Resources, 1: 95-103.doi:10.4236/nr.2010.12010

Hannachi, H., Breton, C., Msallem, M., Ben El Hadj, S., El Gazzah, M., &Berville, A. (2008). Differences between native and introduced olive cultivars as revealed by morphology of drupes, oil composition and SSR polymorphisms: A case study in Tunisia. ScientiaHorticulturae, 116(3), 280–290. doi:10.1016/j.scienta.2008.01.004

Haouane, H., El Bakkali, A., Moukhli, A., Tollon, C., Santoni, S., Oukabli, A., & Khadari, B. (2011). Genetic structure and core collection of the World Olive Germplasm Bank of Marrakech: towards the optimised management and use of Mediterranean olive genetic resources. Genetica, 139(9), 1083-1094.

Hatzopoulos, P., Banilas, G., Giannoulia, K., Gazis, F., Nikoloudakis, N., Milioni, D., Haralampidis, K. (2002). Breeding, molecular markers and molecular biology of the olive tree. Eur. J. Lipid Sci. Technol. 104: 574–586

http://apps3.fao.org/wiews/olive/intro.jsp

http://batzerlab.lsu.edu/genomics/documentation/3130_NanoDrop_tips.pdf support bulletin.

http://en.wikipedia.org/wiki/Tripoli.

http://www.mathcs.citadel.edu/trautmand/stuff/dnapapers/little.htm

http://www.nationsencyclopedia.com/economies/Africa/Libya-griculture.html.http://www.photius.com/countries/libya/climate/libya.

http://www.nfstc.org/pdi/Subject04/pdi_s04_m01_02_f.htm)

http://www.premierbiosoft.com/tech_notes/PCR_Primer_Design.html

http://www.tripolipost.com/articledetail.asp? (Libya Tries to Exploit Olive Oil Sources)

International Olive Council (IOC) (2007) Olive oil production and consumption in the season 2006/2007. Available at: www.internationaloliveoil.org/downloads/

Ipek, A., Barut, E., Gulen, H., & Ipek, M. (2012). Assessment of inter-and intra-cultivar variations in olive using SSR markers. *Scientia Agricola*, *69*(5), 327-335.

Jain, S. M., & Priyadarshan, P. M. (Eds.). (2009).Breeding plantation tree crops: Temperate Species (Vol. 2). Springer.

Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics, 23, 1801–1806.

Khadari, B., Breton, C., Moutier, N., Roger, J.P., Besnard, G.,&Berville, A. (2003).The use of molecular markers forgermplasm management in a French olive collection.TheorAppl Genet, 106:521–529

Kole, C. (2011). Wild crop relatives: Genomic and breeding resources: Temperate fruits.Springer Heidelberg Dordrecht London, New York.

Leon, L., Garrido, V. A., & Downey, G. (2005).Near infrared spectroscopy (NIRS) as a promising selection tool in olive breeding programs.FRUTIC, 05: 12-16

Lewis, P., and D. Zaykin. "Genetic data analysis (GDA): computer program for the analysis of allelic data (Software), version 1.1 (d12)." (2002).

Liphschitz, N., Gophna, R., Hartman, M., &Biger, G. (1991). Thebeginning of olive (*Olea europaea*) cultivation in the old world: A reassessment. J ArchaeolSci 18:441–453

Lumaret, R., Ouazzani, N., Michaud, H., Vivier, G., Deguilloux, M.F.,& Giusto, F.D.(2004) .Allozyme variation of oleaster populations (wild olive tree) (*Olea europaea* L.) in the Mediterranean Basin.Heredity, 92: 343–351

Mace, E.S., Hutokshi K. Buhariwalla, H., & Crouch, J.H. (2003).A high-throughput DNA extraction protocol for tropical molecular breeding programs. Plant Molecular Biology Reporter, 21: 459a–459h.

Mackay, J.F., Wright, C.D., &Bonfiglioli, R.G. (2008).A new approach to varietal identification in plants by microsatellite high resolution melting analysis: Application to the verification of grapevine and olive cultivars. Plant Methods, 4:1-8. Doi: 10.1186/1746-4811

Mariotti, R., Cultrera-Gm, N., Munoz – Diez, C., Baldoni, L., &Rubini, A. (2010).Identification of new polymorphic regions and differentiation of cultivated olives (*Olea europaea* L.) through plastome sequence comparison.BMC Plant Biology, 10:211. Retrieved from http://www.biomedcentral.com/1471-2229/10/211

Mekuria, G.T., Collins, G., &Sedgley, M. (2002).Genetic diversity within an isolated olive (*Olea europaea* L.) population inrelation to feral spread.ScientiaHorticulturae, 94:91–105

Mohler, V., & Schwarz, G. (2004). Genotyping tools in plant breeding: From restriction fragment length polymorphisms to single nucleotide polymorphisms. Biotechnology in Agriculture and Forestry, 55:23-33.

Mohler, V., & Schwarz, G. (2004). Genotyping tools in plant breeding: From restriction fragment length polymorphisms to single nucleotide polymorphisms. Biotechnology in Agriculture and Forestry, 55:23-38

Montemurro, C., Pasqualone, A., Simeone, R., Sabetta, W., & Blanco, A. (2008). AFLP molecular markers to identify virgin olive oils from single Italian cultivars.Eur Food Res Technol, 226:1439–1444.DOI 10.1007/s00217-007-0675-z

Muzzalupo, I., Chiappetta, A., Benincasa, C., & Perri, E. (2010). Intra-cultivar variability of three major olive cultivars grown in different areas of central-southern Italy and studied using microsatellite markers. *Scientia horticulturae*, *126*(3), 324-329.

Navero, D.B., Cimato, A., Fiorino, P., Romero, L.R., Touzani, A., Castaneda, C., Serafini, F., &Navas, I.T. (2000).World catalogue of olive varieties. (1st ed.) .Madrid, Spain: International Olive Oil Council.

Perrier, X., & Jacquemoud-Collet, J. P. (2006). DARwin software.

Poljuha, D., Sladonja, B., Bubola, K.B., Radulovic, M., Brscic, K., Setic, E., Krapac, M., &Milotic, A. (2008).A multidisciplinary approach to the characterisation of autochthonous Istrian olive (*Olea europaea*L.) varieties.Food Technol. Biotechnol, 46 (4): 347–354.

Poljuha, D., Sladonja, B., Setic, E., Milotic, A., Bandelj, D., J. Jakse,J.,&Javornik, B.(2008).DNA fingerprinting of olive varieties in Istria (Croatia) by microsatellite markers.ScientiaHorticulturae, 115:223–230. doi:10.1016/j.scienta.2007.08.018

Pritchard, J. K., Wen, W., & Falush, D. (2003). Documentation for STRUCTURE software: version 2. http://pritch.bsd.uchicago.edu

Rallo, P., Tenzer, I., Gessler, C., Baldoni, L., Dorado, G.,&Martin,A. (2003).Transferability of olive microsatellite loci across the genus *Olea*. TheorAppl Genet, 107:940–946. DOI 10.1007/s00122-003-1332-y

Rambaut, A. (2012). FigTree version 1.4. 0. Computer program distributed by the author, website: http://tree. bio. ed. ac. uk/software/figtree/.

Rao, R., La Mura, M., Corrado, G., Ambrosino, O., Foroni, I., Perri, E., & Pugliano, G. (2009). Molecular diversity and genetic relationships of southern Italian olive cultivars as depicted by AFLP and morphological traits. J. Hortic. Sci. Biotechnol, 84(3), 261-266.

Ribeiro, H., Cunha, M., & Abreu, I. (2005). Airborne pollen of *Olea* in five regions of Portugal. Annals of agricultural and environmental medicine: AAEM, 12(2), 317.

Rojas, G., Méndez, M. A., Muñoz, C., Lemus, G., & Hinrichsen, P. (2008). Identification of a minimal microsatellite marker panel for the fingerprinting of peach and nectarine cultivars. *Electronic Journal of Biotechnology*, *11*(5), 4-5.

Roubos, K., Moustakas, M., & Aravanopoulos, F. A. (2010). Molecular identification of Greek olive (*Olea europaea*) cultivars based on microsatellite loci. Genet. Mol. Res, 9, 1865-1876.

Sanz-Cortes, F., Parfitt, D.E., Romero, C., Struss, D., Llacer, G. &Badenes, M.L. (2003). Interspecific olive diversity assessed with AFLP. Plant Breeding, 122: 173-177.Retrieved from www.blackwell.de/synergy

Sarri, V., Baldoni, L., Porceddu, A., Cultrera, N.G.M., Contento, A., Frediani, M., Belaj, A., Trujillo, I., &Cionini, P.G. (2006). Microsatellite markers are powerful tools for discriminating among olive cultivars and assigning them to geographically defined populations. Genome, 49:1606-1615. Doi: 10.1139/G06-126

Sefc, K.M., Lopes, S., Mendonca, D., Dos Santos, M.R., Machado, M.L.D., & Machado, A.D. (2000). Identification of microsatellite loci in olive (*Olea europaea*) and their characterizationin Italian and Iberian olive trees.Mol. Ecol. 9: 1171–1173.

Sesli, M., &Yegenoglu, E. D. (2010). RAPD assay of wild-type olives in Turkey.Genet. Mol. Res., 9 (2): 966-972.DOI 10.4238/vol9-2gmr783

Sesli, M., &Yegenoglu, E. D. (2010).Genetic relationships among and within wild and cultivated olives based on RAPDs.Genet. Mol. Res., 9 (3): 1550-1556.DOI 10.4238/vol9-3gmr866

Suarez, M.P., Casanova, L., Jimenez, R., Morales-Sillero, R., Ordovas, J., &Rallo, p.(2011).Variability of first flower to ground distance in olive seedlings and its relationship with the length of the juvenile period and the parent genotype.ScientiaHorticulturae 129:747–751

Taamalli, W., Geuna, F., Banfi, R., Bassi, D., Daoud, D., &Zarrouk, M. (2006).Agronomic and molecular analyses for the characterization of accessions in Tunisian olive germplasm collections.Electronic Journal of Biotechnology, 9 (5):467-481.DOI: 10.2225/vol9-issue5-fulltext-12

Tanyolac, M. B. (2013). SNP Discovery in Olive Tree Using Illumina Transcriptome Sequencing. In Plant and Animal Genome XXI Conference. Plant and Animal Genome.

Terral, J.F., Arnold, S. G. (1996).Beginnings of olive cultivation in eastern Spain in relation to Holocene bioclimaticchanges.Quaternary Res 46:176–185

Terzopoulos, P. J., Kolano, B., Bebeli, P.J., Kaltsikes, P. J., &Metzidakis, I. (2005). Identification of olea *europaea* L. cultivars using inter-simple sequence repeat markers.ScientiaHorticulturae, 105, 45-51.doi:10.1016/j.scienta.2005.01.011

Wu, S.B., Collins, G., & Sedgley, M. (2004). A molecular linkage map of olive (*Olea europaea*L.) based on RAPD, microsatellite, and SCAR markers. Genome, 47: 26-35.doi: 10.1139/G03-091

Wünsch, A., &Hormaza, J.I. (2002).Cultivar identification and genetic fingerprinting of temperate fruit tree species using DNA markers. Euphytica 125: 59–67.

Zaher, H., Boulouha, B., Baaziz, M., Sikaoui, L., Gaboun, F., & Udupa, S. M. (2011). Morphological and genetic diversity in olive (*Olea europaea* subsp. *europaea* L.) clones and varieties. Plant Omics Journal, 4(7), 370-376.

APPENDIX

Table A.1. List of fruit descriptive traits were evaluated for 90 olive cultivars.

Cultivar name	Fruit shape	Fruit weight	Fruit symmetry	Position of maximum transverse diameter	Nipple	Apex	Base
Arbequina-Tri[z]	Spherical	Medium	Symmetric	Central	Absent	Roundness	Trancate
Ascolanatenera-T	Spherical	Very high	Slightly asymmetric	Central	Absent	Roundness	Trancate
Bella di spagna-T	Ovoid	Very high	Symmetric	Central	Absent	Roundness	Trancate
Caninese-G	Ovoid	Low	Symmetric	Central	Present	Roundness	Trancate
Carmelitana-T	Elongated	Medium	Asymmetric	Towards apex	Present	Roundness	Trancate
Cellina-G	Ovoid	Low	Symmetric	Central	Absent	Roundness	Roundness
Chemlalisfax-G	Elongated	Low	Symmetric	Towards apex	Absent	Roundness	Roundness
Chemlalisfax-T	Elongated	Low	Slightly asymmetric	Towards apex	Absent	Roundness	Pointed
Coratina-G	Ovoid	Medium	Symmetric	Central	Absent	Roundness	Roundness
Coratina-T	Ovoid	Medium	Symmetric	Central	Absent	Roundness	Trancate
Cucco-T	Elongated	Very high	Asymmetric	Towards apex	Present	Roundness	Trancate
Enduri-T	Elongated	Low	Asymmetric	Towards apex	Absent	Pointed	Trancate
Frantoio-G	Elongated	Low	Symmetric	Towards apex	Absent	Roundness	Trancate
Frantoio-T	Elongated	Medium	Symmetric	Towards apex	Absent	Roundness	Trancate
Gragnano-G	Spherical	Medium	Symmetric	Central	Absent	Roundness	Trancate
Grossa di sardegna-T	Ovoid	Very high	Slightly asymmetric	Towards apex	Absent	Roundness	Trancate
Grossa di spagna-T	Ovoid	Very high	Asymmetric	Towards apex	Absent	Roundness	Trancate
Krusi-G	Elongated	Low	Symmetric	Central	Absent	Roundness	Trancate
Leccino-G	Elongated	Medium	Slightly asymmetric	Central	Absent	Roundness	Trancate
Leccinopendulo-T	Elongated	Low	Asymmetric	Towards apex	Absent	Roundness	Trancate
Leccino-T	Elongated	Medium	Slightly asymmetric	Towards apex	Absent	Roundness	Trancate
Maurino-G	Elongated	Medium	Symmetric	Central	Absent	Roundness	Trancate
Maurino-T	Ovoid	Medium	Slightly asymmetric	Towards apex	Absent	Roundness	Trancate
Mbuti-M	Ovoid	Medium	Asymmetric	Central	Absent	Roundness	Trancate
Mbuti-T	Ovoid	Medium	Asymmetric	Towards apex	Present	Roundness	Trancate
Mignolo-G	Ovoid	Medium	Symmetric	Central	Absent	Roundness	Trancate
Mignolo-T	Elongated	Low	Asymmetric	Towards apex	Present	Pointed	Trancate
Monopoly-T	Ovoid	Low	Slightly asymmetric	Central	Absent	Roundness	Trancate
Moraiolo-G	Spherical	Medium	Symmetric	Towards apex	Absent	Roundness	Trancate
Moraiolo-T	Ovoid	Medium	Symmetric	Central	Absent	Roundness	Trancate
Morchiaio-G	Elongated	Medium	Asymmetric	Towards apex	Present	Roundness	Pointed
Morellona di grecia-T	Ovoid	High	Slightly asymmetric	Central	Absent	Roundness	Trancate

Table A.1. Continued

Nepal-Tri	Spherical	High	Symmetric	Central	Absent	Roundness	Trancate
Oliardo-G	Ovoid	Medium	Symmetric	Central	Absent	Roundness	Trancate
Oliarolasalentina-T	Ovoid	Low	Slightly asymmetric	Central	Absent	Roundness	Trancate
Olivastro-G	Ovoid	Low	Slightly asymmetric	Central	Absent	Roundness	Trancate
Ouslati-G	Ovoid	Medium	Symmetric	Towards apex	Absent	Roundness	Trancate
Ouslati-T	Ovoid	Medium	Symmetric	Central	Absent	Roundness	Trancate
Pendolino-G	Elongated	Medium	Symmetric	Towards apex	Absent	Roundness	Trancate
Rosciola-G	Ovoid	Low	Symmetric	Central	Absent	Roundness	Trancate
Santagostino-T	Spherical	Very high	Symmetric	Central	Absent	Roundness	Trancate
Tombarella-G	Spherical	Low	Symmetric	Towards apex	Absent	Roundness	Trancate
Tunisian-M	Spherical	Low	Symmetric	Central	Absent	Roundness	Trancate
Anbi-M	Elongated	Low	Symmetric	Towards apex	Absent	Roundness	Trancate
Bayyudi-M	Ovoid	Medium	Symmetric	Central	Absent	Roundness	Trancate
Beserri-M	Elongated	Medium	Slightly asymmetric	Central	Absent	Roundness	Trancate
Chemlalikussabat-M	Ovoid	Medium	Symmetric	Central	Absent	Roundness	Trancate
Chemlalikussabat-T	Ovoid	Medium	Symmetric	Central	Absent	Roundness	Trancate
Chemlali-M	Ovoid	Low	Asymmetric	Central	Absent	Roundness	Trancate
Chemlali-Za	Elongated	Low	Asymmetric	Towards apex	Absent	Roundness	Trancate
Farkuti-M	Elongated	Medium	Asymmetric	Towards apex	Present	Roundness	Trancate
Gaiani-M	Elongated	Medium	Asymmetric	Towards apex	Present	Pointed	Trancate
Gargashi-M	Elongated	Low	Slightly asymmetric	Central	Absent	Roundness	Trancate
Gargashi-T	Elongated	Low	Slightly asymmetric	Towards apex	Absent	Roundness	Trancate
Gartomye-M	Elongated	Medium	Slightly asymmetric	Central	Absent	Roundness	Trancate
Hammudi-M	Elongated	Medium	Symmetric	Central	Present	Roundness	Trancate
Hammudi-T	Elongated	Medium	Slightly asymmetric	Towards apex	Present	Roundness	Trancate
Jabbugi-M	Elongated	Low	Asymmetric	Towards apex	Present	Pointed	Roundness
Jabbugi-T	Elongated	Medium	Slightly asymmetric	Central	Present	Roundness	Trancate
Kalefy-M	Elongated	Medium	Asymmetric	Central	Present	Pointed	Trancate
Karkubi-M	Spherical	Medium	Symmetric	Central	Absent	Roundness	Roundness
Keddaui-M	Elongated	Medium	Asymmetric	Towards apex	Absent	Pointed	Trancate
Khaddira-M	Elongated	Low	Asymmetric	Towards apex	Absent	Roundness	Trancate
Khaddra-M	Elongated	Low	Symmetric	Central	Present	Roundness	Trancate
Marisi-M	Elongated	Low	Asymmetric	Towards apex	Present	Roundness	Roundness
Marrari-M	Elongated	Low	Slightly asymmetric	Towards apex	Absent	Roundness	Roundness
Marrari-T	Elongated	Medium	Slightly asymmetric	Towards apex	Absent	Roundness	Trancate

Table A.1. Continued

Mthemr-M	Elongated	Medium	Asymmetric	Towards apex	Present	Pointed	Trancate
Mukther-M	Elongated	Low	Slightly asymmetric	Central	Absent	Roundness	Trancate
Neb gemel-M	Elongated	Medium	Asymmetric	Central	Present	Pointed	Roundness
Ninai-M	Ovoid	Low	Symmetric	Central	Present	Roundness	Trancate
Nardo-T	Elongated	Medium	Symmetric	Towards apex	Absent	Roundness	Trancate
Ouslatikussabat-T	Ovoid	Low	Symmetric	Central	Absent	Roundness	Trancate
Qalbsarduk-M	Elongated	Medium	Slightly asymmetric	Towards apex	Present	Pointed	Trancate
Rasli-M	Elongated	Medium	Slightly asymmetric	Central	Absent	Roundness	Trancate
Rasli-T	Elongated	Low	Slightly asymmetric	Central	Absent	Roundness	Trancate
Rumi-M	Ovoid	Medium	Symmetric	Central	Absent	Roundness	Roundness
Sahley-M	Ovoid	Low	Symmetric	Central	Absent	Roundness	Trancate
Soudia-M	Elongated	Low	Asymmetric	Central	Absent	Roundness	Trancate
Vqos-M	Ovoid	Medium	Slightly asymmetric	Central	Absent	Roundness	Trancate
Yehudi-M	Ovoid	Low	Symmetric	Central	Absent	Roundness	Trancate
Zaafrani-M	Elongated	Low	Symmetric	Towards apex	Absent	Roundness	Trancate
Zaafrani-T	Elongated	Medium	Symmetric	Towards apex	Absent	Roundness	Trancate
Zaglo-M	Elongated	Low	Slightly asymmetric	Central	Absent	Roundness	Trancate
Zalmati-G	Elongated	Low	Symmetric	Central	Absent	Roundness	Trancate
Zalmati-Za	Ovoid	Low	Symmetric	Central	Absent	Roundness	Trancate
Zarrasi-M	Spherical	High	Symmetric	Central	Absent	Roundness	Trancate
Zarrasi-T	Spherical	Medium	Symmetric	Central	Absent	Roundness	Trancate
Znbai-M	Ovoid	Medium	Asymmetric	Central	Present	Roundness	Trancate
Wild-G	Elongated	Medium	Slightly asymmetric	Towards apex	Absent	Roundness	Trancate

z Name of accession attached with their local locations (Fig.2.1) (M= Mesalata h,T= Tharouna ,G= Gharian ,Za= Zaltin and Tri= Tripoli).

Table A.2. List of seed descriptive traits were evaluated for 90 olive cultivars.

Cultivar name	Position of maximum transverse diameter	Seed shape	Weight	Base	Apex	Seed surface	Termination of the apex	Seed symmetry
Anbi-M[z]	Towards apex	Elongated	Medium	Pointed	Roundness	Smooth	With mucro	Asymmetric
Arbequina-Tri	Central	Ovoid	High	Roundness	Roundness	Rugose	With mucro	Sligthly-asymmetric
Ascolanatenera-T	Central	Elliptic	High	Roundness	Roundness	Scabrous	With mucro	Sligthly -asymmetric
Bayyudi-M	Central	Elliptic	Medium	Pointed	Roundness	Smooth	With mucro	Symmetric
Bella di spagna-T	Central	Elliptic	High	Roundness	Roundness	Scabrous	With mucro	Sligthly-asymmetric
Beserri-M	Central	Elongated	High	Roundness	Roundness	Scabrous	With mucro	Asymmetric
Caninese-G	Central	Elliptic	Medium	Pointed	Roundness	Smooth	With mucro	Sligthly-asymmetric
Carmelitana-T	Towards apex	Elongated	High	Pointed	Roundness	Scabrous	With mucro	Asymmetric
Cellina-G	Towards apex	Elongated	Medium	Roundness	Roundness	Smooth	Small mucro	Sligthly-asymmetric
Chemlalikussabat-M	Central	Elliptic	Medium	Pointed	Roundness	Scabrous	With mucro	Asymmetric
Chemlalikussabat-T	Central	Ovoid	Medium	Pointed	Roundness	Scabrous	With mucro	Sligthly-asymmetric
Chemlali-M	Central	Elongated	Low	Pointed	Roundness	Smooth	With mucro	Asymmetric
Chemlalisfax-G	Towards apex	Elongated	Low	Roundness	Roundness	Smooth	Small mucro	Symmetric
Chemlalisfax-T	Towards apex	Elongated	Low	Pointed	Roundness	Smooth	With mucro	Sligthly-asymmetric
Chemlali-Za	Towards apex	Elongated	Low	Pointed	Roundness	Rugose	With mucro	Asymmetric
Coratina-G	Central	Elliptic	High	Roundness	Roundness	Scabrous	Small mucro	Asymmetric
Coratina-T	Central	Elliptic	High	Roundness	Roundness	Scabrous	With mucro	Asymmetric
Cucco-T	Towards apex	Elongated	High	Truncate	Roundness	Scabrous	With mucro	Asymmetric
Enduri-T	Towards apex	Elongated	Low	Pointed	Roundness	\Smooth	With mucro	Asymmetric
Farkuti-M	Towards apex	Elongated	High	Pointed	Pointed	Smooth	With mucro	Asymmetric
Frantoio-G	Towards apex	Elliptic	High	Pointed	Roundness	Rugose	With mucro	Asymmetric
Frantoio-T	Towards apex	Elliptic	High	Roundness	Roundness	Rugose	With mucro	Asymmetric
Gaiani-M	Towards apex	Elongated	High	Pointed	Highly point	Rough	With mucro	Asymmetric
Gargashi-M	Towards apex	Elongated	Low	Pointed	Pointed	Rugose	With mucro	Asymmetric
Gargashi-T	Towards apex	Elongated	Low	Pointed	Pointed	Smooth	With mucro	Sligthly-asymmetric
Gartomye-M	Towards apex	Elongated	High	Truncate	Pointed	Smooth	With mucro	Asymmetric
Gragnano-G	Towards apex	Elliptic	High	Roundness	Roundness	Scabrous	With mucro	Sligthly asymmetric
Grossa di sardegna-T	Central	Elliptic	High	Roundness	Roundness	Scabrous	With mucro	Asymmetric
Morellona di grecia-T	Towards apex	Elliptic	High	Roundness	Pointed	Rugose	With mucro	Asymmetric
Mthemr-M	Towards apex	Elongated	High	Roundness	Pointed	Scabrous	With mucro	Asymmetric
Mukther-M	Towards apex	Elongated	Low	Pointed	Pointed with tip	Smooth	With mucro	Asymmetric
Nardo-T	Towards apex	Elongated	High	Roundness	Pointed	Rugose	With mucro	Asymmetric
Neb gemel-M	Central	Elongated	High	Pointed	Pointed	Scabrous	With mucro	Asymmetric
Nepal-Tri	Towerd base	Ovoid	High	Roundness	Pointed	Scabrous	With mucro	Symmetric
Ninai-M	Central	Elliptic	Medium	Roundness	Pointed	Smooth	With mucro	Symmetric
oliardo-G	Central	Elliptic	High	Roundness	Roundness	Scabrous	With mucro	Asymmetric

Table A.2. Continued

Oliarolasalentina-T	Central	Elliptic	Low	Pointed	Pointed	Smooth	With mucro	Asymmetric
Olivastro-G	Towards apex	Elliptic	Low	Pointed	Pointed	Smooth	With mucro	Asymmetric
Ouslati-G	Towards apex	Elliptic	High	Roundness	Roundness	Scabrous	With mucro	Symmetric
Ouslatikussabat-T	Towards apex	Elliptic	Medium	Roundness	Pointed	Smooth	With mucro	Sligthly asymmetric
Ouslati-T	Towards apex	Elliptic	High	Roundness	Pointed	Scabrous	With mucro	Symmetric
Pendolino-G	Towards apex	Elongated	High	Pointed	Roundness	Scabrous	With mucro	Asymmetric
Qalbsarduk-M	Towards apex	Elongated	High	Roundness	Pointed	Scabrous	With mucro	Asymmetric
Rasli-M	Central	Elongated	High	Roundness	Pointed	Rugose	With mucro	Asymmetric
Rasli-T	Central	Elliptic	High	Roundness	Pointed	Scabrous	With mucro	Asymmetric
Rosciola-G	Towards apex	Elliptic	Medium	Roundness	Roundness	Smooth	With mucro	Symmetric
Rumi-M	Central	Elliptic	High	Pointed	Roundness	Smooth	With mucro	Symmetric
Sahley-M	Towards apex	Elliptic	Medium	Pointed	Roundness	Smooth	With mucro	Symmetric
Santagostino-T	Central	Spherical	High	Roundness	Roundness	Rugose	With mucro	Sligthly asymmetric
Soudia-M	Towards apex	Elliptic	Low	Roundness	Pointed	Smooth	With mucro	Asymmetric
Tombarella-G	Towards apex	Ovoid	Medium	Roundness	Roundness	Scabrous	With mucro	Symmetric
Tunisian-M	Towards apex	Ovoid	Medium	Roundness	Roundness	Rugose	With mucro	Symmetric
Vqos-M	Central	Elliptic	Medium	Pointed	Roundness	Rugose	With mucro	Sligthly asymmetric
Wild-G	Towards apex	Elongated	High	Pointed	Pointed	Scabrous	With mucro	Asymmetric
Yehudi-M	Towards apex	Elliptic	Low	Roundness	Pointed	Smooth	With mucro	Symmetric
Zaafrani-M	Towards apex	Elongated	Medium	Pointed	Pointed	Smooth	With mucro	Sligthly asymmetric
Zaafrani-T	Towards apex	Elliptic	High	Roundness	Roundness	Rugose	With mucro	Sligthly asymmetric
Zaglo-M	Central	Elongated	High	Pointed	Pointed with tip	Smooth	With mucro	Sligthly asymmetric
Zalmati-G	Central	Elongated	Low	Pointed	Pointed	Smooth	With mucro	Symmetric
Zalmati-Za	Towards apex	Elongated	Low	Pointed	Pointed	Rugose	With mucro	Asymmetric
Zarrasi-M	Central	Spherical	High	Roundness	Roundness	Rugose	With mucro	Symmetric
Zarrasi-T	Central	Spherical	High	Roundness	Roundness	Scabrous	With mucro	Symmetric
Znbai-M	Central	Elliptic	High	Roundness	Pointed	Rugose	With mucro	Asymmetric
Grossa di spagna-T	Central	Elliptic	High	Roundness	Pointed	Scabrous	With mucro	Sligthly asymmetric
Hammudi-M	Towards apex	Elongated	High	Roundness	Pointed	Rugose	With mucro	Symmetric
Hammudi-T	Towards apex	Elongated	High	Roundness	Pointed	Scabrous	With mucro	Asymmetric
Jabbugi-M	Towards apex	Elongated	Medium	Pointed	Pointed	Smooth	With mucro	Sligthly asymmetric
Jabbugi-T	Central	Elongated	Medium	Pointed	Pointed	Smooth	With mucro	Asymmetric
Kalefy-M	Towards apex	Elongated	High	Pointed	Pointed	Smooth	With mucro	Asymmetric
Karkubi-M	Central	Ovoid	High	Truncate	Pointed	Rugose	No mucro	Symmetric
Keddaui-M	Central	Elongated	High	Pointed	Highly point	Rugose	No mucro	Asymmetric
Khaddira-M	Towards apex	Elliptic	Medium	Roundness	Pointed	Smooth	With mucro	Asymmetric
Khaddra-M	Towards apex	Elongated	Medium	Roundness	Pointed	Smooth	With mucro	Sligthly asymmetric

Table A.2. Continued

Krusi-G	Towards apex	Elongated	Low	Pointed	Pointed	Smooth	Small mucro	Sligthly asymmetric
Leccino-G	Towards apex	Elongated	High	Roundness	Pointed	Scabrous	With mucro	Sligthly asymmetric
Leccinopendulo-T	Towards apex	Elongated	High	Pointed	Pointed	Scabrous	With mucro	Asymmetric
Leccino-T	Towards apex	Elongated	High	Roundness	Pointed	Scabrous	With mucro	Asymmetric
Marisi-M	Towards apex	Elongated	Medium	Pointed	Pointed	Smooth	With mucro	Asymmetric
Marrari-M	Towards apex	Elongated	Medium	Roundness	Pointed	Scabrous	With mucro	Sligthly asymmetric
Marrari-T	Towards apex	Elongated	High	Roundness	Pointed	Scabrous	With mucro	Asymmetric
Maurino-G	Central	Elliptic	Medium	Roundness	Roundness	Scabrous	With mucro	Sligthly asymmetric
Maurino-T	Towards apex	Elliptic	Medium	Roundness	Roundness	Rugose	With mucro	Asymmetric
Mbuti-M	Central	Elliptic	Medium	Roundness	Roundness	Smooth	With mucro	Symmetric
Mbuti-T	Towards apex	Elliptic	Medium	Pointed	Pointed	Rugose	With mucro	Sligthly asymmetric
Mignolo-G	Towards apex	Elliptic	High	Roundness	Roundness	Rugose	With mucro	Sligthly asymmetric
Mignolo-T	Central	Elongated	Medium	Roundness	Pointed	Smooth	With mucro	Asymmetric
Monopoly-T	Central	Elliptic	Low	Pointed	Pointed	Smooth	With mucro	Sligthly asymmetric
Moraiolo-G	Towards apex	Ovoid	Medium	Roundness	Roundness	Rugose	With mucro	Sligthly asymmetric
Moraiolo-T	Central	Ovoid	High	Roundness	Roundness	Rugose	With mucro	Symmetric
Morchiaio-G	Towards apex	Elongated	High	Pointed	Pointed	Scabrous	With mucro	Asymmetric

z Name of accession attached with their local locations (Fig.2.1) (M= Mesalata h,T= Tharouna ,G= Gharian ,Za= Zaltin and Tri= Tripoli).

Table A.3 List of leaf descriptive traits were evaluated for 90 olive cultivars.

Cultivar name	Length	Width	Shape
Arbequina-Tri[z]	Medium	Medium	Elliptic-Lanceolate
Ascolanatenera-T	Medium	Medium	Elliptic-Lanceolate
Bella di spagna-T	Medium	Medium	Elliptic-Lanceolate
Caninese-G	Medium	Medium	Elliptic-Lanceolate
Carmelitana-T	Medium	Broad	Elliptic
Cellina-G	Medium	Broad	Elliptic
Chemlalisfax-G	Medium	Medium	Elliptic-Lanceolate
Chemlalisfax-T	Medium	Medium	Elliptic-Lanceolate
Coratina-G	Medium	Medium	Elliptic-Lanceolate
Coratina-T	Medium	Medium	Elliptic-Lanceolate
Cucco-T	Long	Broad	Elliptic-Lanceolate
Enduri-T	Long	Broad	Lanceolate
Frantoio-G	Medium	Medium	Elliptic-Lanceolate
Frantoio-T	Long	Broad	Elliptic
Gragnano-G	Medium	Medium	Elliptic-Lanceolate
Grossa di sardegna-T	Long	Medium	Lanceolate
Grossa di spagna-T	Medium	Medium	Elliptic-Lanceolate
Krusi-G	Medium	Medium	Elliptic-Lanceolate
Leccino-G	Medium	Medium	Elliptic-Lanceolate
Leccinopendulo-T	Medium	Medium	Elliptic-Lanceolate
Leccino-T	Medium	Medium	Elliptic
Maurino-G	Medium	Medium	Elliptic-Lanceolate
Maurino-T	Medium	Medium	Elliptic-Lanceolate
Mbuti-M	Medium	Medium	Elliptic-Lanceolate
Mbuti-T	Medium	Broad	Elliptic
Mignolo-G	Medium	Medium	Elliptic-Lanceolate
Mignolo-T	Medium	Medium	Elliptic-Lanceolate
Monopoly-T	Medium	Broad	Elliptic-Lanceolate
Moraiolo-G	Medium	Medium	Elliptic-Lanceolate
Moraiolo-T	Medium	Medium	Elliptic-Lanceolate
Morchiaio-G	Medium	Medium	Elliptic-Lanceolate
Morellona di grecia-T	Medium	Medium	Elliptic-Lanceolate
Nardo-T	Medium	Broad	Elliptic
Nepal-Tri	Long	Medium	Elliptic-Lanceolate
oliardo-G	Medium	Medium	Elliptic-Lanceolate
Oliarolasalentina-T	Long	Medium	Elliptic-Lanceolate

Cultivar name	Length	Width	Shape
Bayyudi-M	Medium	Medium	Elliptic-Lanceolate
Beserri-M	Long	Medium	Lanceolate
Chemlalikussabat-M	Medium	Medium	Elliptic-Lanceolate
Chemlalikussabat-T	Medium	Medium	Elliptic-Lanceolate
Chemlali-M	Medium	Medium	Elliptic-Lanceolate
Chemlali-Za	Long	Medium	Lanceolate
Farkuti-M	Medium	Medium	Elliptic-Lanceolate
Gaiani-M	Medium	Medium	Lanceolate
Gargashi-M	Medium	Medium	Elliptic-Lanceolate
Gargashi-T	Medium	Medium	Elliptic-Lanceolate
Gartomye-M	Long	Medium	Elliptic-Lanceolate
Hammudi-M	Medium	Medium	Elliptic-Lanceolate
Hammudi-T	Long	Medium	Elliptic-Lanceolate
Jabbugi-M	Medium	Medium	Elliptic-Lanceolate
Jabbugi-T	Medium	Medium	Elliptic-Lanceolate
Kalefy-M	Medium	Medium	Elliptic-Lanceolate
Karkubi-M	Long	Medium	Elliptic-Lanceolate
Keddaui-M	Medium	Medium	Elliptic-Lanceolate
Khaddira-M	Long	Medium	Elliptic-Lanceolate
Khaddra-M	Medium	Medium	Elliptic-Lanceolate
Marisi-M	Long	Medium	Elliptic-Lanceolate
Marrari-M	Medium	Medium	Elliptic-Lanceolate
Marrari-T	Long	Broad	Elliptic-Lanceolate
Mthemr-M	Medium	Medium	Elliptic-Lanceolate
Mukther-M	Long	Medium	Lanceolate
Neb gemel-M	Long	Medium	Lanceolate
Ninai-M	Medium	Medium	Elliptic-Lanceolate
Ouslatikussabat-T	Long	Medium	Lanceolate
Qalbsarduk-M	Medium	Medium	Elliptic-Lanceolate
Rasli-M	Medium	Medium	Elliptic-Lanceolate
Rasli-T	Medium	Medium	Elliptic
Rumi-M	Medium	Medium	Elliptic-Lanceolate
Sahley-M	Medium	Narrow	Lanceolate
Soudia-M	Medium	Medium	Elliptic-Lanceolate
Vqos-M	Medium	Medium	Elliptic-Lanceolate
Yehudi-M	Medium	Medium	Elliptic-Lanceolate

Table A.3. Continued.

Olivastro-G	Medium	Medium	Elliptic-Lanceolate
Ouslati-G	Medium	Medium	Elliptic-Lanceolate
Ouslati-T	Long	Medium	Elliptic-Lanceolate
Pendolino-G	Medium	Medium	Elliptic-Lanceolate
Rosciola-G	Medium	Medium	Elliptic-Lanceolate
Santagostino-T	Medium	Medium	Elliptic-Lanceolate
Tombarella-G	Medium	Medium	Elliptic-Lanceolate
Tunisian-M	Medium	Medium	Elliptic-Lanceolate
Anbi-M	Medium	Medium	Elliptic-Lanceolate

Zaafrani-M	Medium	Medium	Elliptic-Lanceolate
Zaafrani-T	Medium	Broad	Elliptic
Zaglo-M	Medium	Medium	Elliptic-Lanceolate
Zalmati-G	Medium	Medium	Elliptic-Lanceolate
Zalmati-Za	Medium	Medium	Elliptic-Lanceolate
Zarrasi-M	Medium	Medium	Elliptic-Lanceolate
Zarrasi-T	Medium	Narrow	Lanceolate
Znbai-M	Short	Medium	Elliptic-Lanceolate
Wild-G	Medium	Medium	Elliptic-Lanceolate

z Name of accession attached with their local locations (Fig.2.1) (M= Mesalata h,T= Tharouna ,G= Gharian ,Za= Zaltin and Tri= Tripoli).

Table A.4 Combinations of fruit, seed and leaf ratio traits of 19 cultivars that grow in two different locations were used to evaluated and identify the rest of olive varieties.

Name of accession	Local Location	Fruit Density	Fruit Shape	Seed Shape	Leaf Shape	Name of accession	Local Location	Fruit Density	Fruit Shape	Seed Shape	Leaf Shape
ChemlaliZa	Za	1.21	1.75	2.34	7.29	MarrariM	M	1.16	2	2.66	5.65
ChemlaliZa	Za	1.1	1.59	2.32	7.27	MarrariM	M	1.05	1.81	2.66	5.67
ChemlaliZa	Za	1	1.44	2.31	7.26	MarrariM	M	0.95	1.64	2.66	5.68
ChemlaliM	M	1.12	1.54	2.47	4.33	MaurinoT	T	1.16	1.5	1.93	5.19
ChemlaliM	M	1.02	1.39	2.44	4.33	MaurinoT	T	1.05	1.36	1.94	5.18
ChemlaliM	M	0.92	1.26	2.42	4.34	MaurinoT	T	0.96	1.23	1.96	5.17
ChemlalikussabatT	T	1.19	1.41	1.79	5.16	MaurinoGh	Gh	1.14	1.6	2.08	5.65
ChemlalikussabatT	T	1.08	1.28	1.8	5.17	MaurinoGh	Gh	1.03	1.46	2.09	5.64
ChemlalikussabatT	T	0.98	1.16	1.81	5.18	MaurinoGh	Gh	0.93	1.34	2.1	5.62
ChemlalikussabatM	M	1.07	1.48	2.11	5.4	MbutiT	T	1.1	1.58	2.17	3.66
ChemlalikussabatM	M	0.97	1.35	2.12	5.42	MbutiT	T	1.01	1.43	2.18	3.67
ChemlalikussabatM	M	0.89	1.23	2.13	5.43	MbutiT	T	0.92	1.29	2.18	3.68
ChemlalisfaxT	T	0.96	1.86	2.24	5.4	MbutiM	M	1.15	1.41	1.82	5.07
ChemlalisfaxT	T	0.87	1.69	2.22	5.42	MbutiM	M	1.04	1.28	1.82	5.08
ChemlalisfaxT	T	0.79	1.53	2.21	5.43	MbutiM	M	0.94	1.16	1.81	5.1
ChemlalisfaxGh	Gh	1.16	1.66	2.3	5.19	MignoloT	T	1.1	1.66	2.28	5.48
ChemlalisfaxGh	Gh	1.05	1.52	2.29	5.18	MignoloT	T	1	1.5	2.28	5.5
ChemlalisfaxGh	Gh	0.96	1.38	2.27	5.17	MignoloT	T	0.91	1.36	2.28	5.52
CoratinaT	T	1.14	1.45	2	4.67	MignoloGh	Gh	1.06	1.42	1.93	4.56
CoratinaT	T	1.03	1.32	2	4.67	MignoloGh	Gh	0.97	1.3	1.92	4.57
CoratinaT	T	0.94	1.2	2	4.66	MignoloGh	Gh	0.88	1.19	1.91	4.59
CoratinaGh	Gh	1.31	1.53	2.07	5.47	MoraioloT	T	1.1	1.38	1.65	4.67
CoratinaGh	Gh	1.19	1.4	2.05	5.45	MoraioloT	T	0.99	1.26	1.64	4.67
CoratinaGh	Gh	1.08	1.28	2.04	5.44	MoraioloT	T	0.9	1.14	1.63	4.66
FrantoioT	T	1.18	1.62	2.13	3.74	MoraioloGh	Gh	1.17	1.37	1.66	4.78
FrantoioT	T	1.08	1.47	2.13	3.75	MoraioloGh	Gh	1.07	1.24	1.65	4.77
FrantoioT	T	0.98	1.34	2.12	3.76	MoraioloGh	Gh	0.97	1.13	1.64	4.76
FrantoioGh	Gh	1.18	1.7	2.13	4.14	OuslatiT	T	1.2	1.39	1.91	5.78
FrantoioGh	Gh	1.07	1.54	2.14	4.14	OuslatiT	T	1.09	1.27	1.9	5.77
FrantoioGh	Gh	0.97	1.4	2.15	4.15	OuslatiT	T	0.99	1.16	1.89	5.76
GargashiT	T	1.07	1.69	2.35	5.01	OuslatiGh	Gh	1.12	1.55	2.01	4.2

Table A.4 continued.

GargashiT	T	0.98	1.54	2.33	5	OuslatiGh	Gh	1.02	1.41	2	4.21
GargashiT	T	0.89	1.4	2.32	4.99	OuslatiGh	Gh	0.92	1.28	1.99	4.23
GargashiM	M	1.08	1.73	2.56	5.37	RasliT	T	1.17	1.66	2.16	3.67
GargashiM	M	0.98	1.57	2.55	5.36	RasliT	T	1.06	1.51	2.17	3.67
GargashiM	M	0.89	1.42	2.53	5.35	RasliT	T	0.96	1.38	2.18	3.66
HammudiT	T	1.08	1.61	2.23	5.07	RasliM	M	1.14	1.65	2.22	4.83
HammudiT	T	0.98	1.46	2.25	5.07	RasliM	M	1.03	1.5	2.23	4.83
HammudiT	T	0.89	1.33	2.27	5.06	RasliM	M	0.93	1.37	2.23	4.84
HammudiM	M	1.11	1.66	2.42	5.4	ZaafraniT	T	1.14	1.69	2.05	3.88
HammudiM	M	1.01	1.51	2.43	5.42	ZaafraniT	T	1.04	1.54	2.05	3.88
HammudiM	M	0.92	1.38	2.44	5.43	ZaafraniT	T	0.94	1.4	2.05	3.88
JabbugiT	T	1.14	1.87	2.9	4.47	ZaafraniM	M	1.21	1.95	2.66	5.82
JabbugiT	T	1.04	1.71	2.9	4.46	ZaafraniM	M	1.1	1.76	2.66	5.83
JabbugiT	T	0.95	1.55	2.91	4.45	ZaafraniM	M	1	1.6	2.66	5.85
JabbugiM	M	1.04	2.12	3.11	4.99	ZalmatiGh	Gh	1.05	1.74	2.52	5.37
JabbugiM	M	0.94	1.91	3.1	5	ZalmatiGh	Gh	0.95	1.58	2.54	5.36
JabbugiM	M	0.85	1.73	3.1	5.01	ZalmatiGh	Gh	0.86	1.43	2.56	5.35
LeccinoT	T	1.21	1.71	2.31	3.67	ZalmatiZa	Za	1.18	1.58	2.31	5.92
LeccinoT	T	1.1	1.55	2.31	3.67	ZalmatiZa	Za	1.07	1.44	2.3	5.91
LeccinoT	T	1	1.41	2.3	3.66	ZalmatiZa	Za	0.97	1.31	2.28	5.9
LeccinoGh	Gh	1.15	1.7	2.63	4.07	ZarrasiT	T	1.14	1.27	1.59	7.74
LeccinoGh	Gh	1.05	1.55	2.61	4.07	ZarrasiT	T	1.03	1.16	1.59	7.75
LeccinoGh	Gh	0.96	1.42	2.59	4.06	ZarrasiT	T	0.94	1.05	1.58	7.76
MarrariT	T	1.21	1.82	2.62	4.24	ZarrasiM	M	1.08	1.23	1.62	5.99
MarrariT	T	1.1	1.66	2.62	4.24	ZarrasiM	M	0.98	1.12	1.61	6
MarrariT	T	1	1.51	2.62	4.23	ZarrasiM	M	0.9	1.02	1.6	6.01

Table A.5 Combinations of fruit, seed and leaf scan traits of 19 cultivars that grow in two different locations were used to evaluated and identify the rest of olive varieties.

Name of accession	Local Location	Fruit area	Fruit roundness	Fruit boxX/Y	Seed area	Seed roundess	Seed box X/Y	Leaf Area	Leaf BoxX/Y	Leaf roundness
Chemlali	Za	1.39	1.22	0.58	0.70	1.36	0.43	3.97	0.11	4.65
Chemlali	Za	1.15	1.13	0.58	0.78	1.40	0.42	3.55	0.14	4.75
Chemlali	M	1.75	1.08	0.68	0.61	1.47	0.38	2.75	0.19	3.31
Chemlali	M	1.90	1.07	0.66	0.59	1.47	0.39	3.23	0.21	2.60
Chemlalikussabat	T	2.15	1.05	0.71	0.96	1.24	0.53	5.64	0.18	2.76
Chemlalikussabat	T	2.17	1.05	0.73	0.90	1.22	0.51	5.07	0.17	2.92
Chemlalikussabat	M	3.39	1.08	0.66	1.10	1.32	0.44	4.73	0.18	2.95
Chemlalikussabat	M	3.29	1.09	0.66	1.14	1.37	0.42	3.74	0.15	3.95
Chemlalisfax	T	1.50	1.14	0.57	0.74	1.36	0.40	3.97	0.13	3.91
Chemlalisfax	T	1.74	1.14	0.56	0.71	1.35	0.43	4.25	0.20	3.34
Chemlalisfax	Gh	1.89	1.13	0.59	0.75	1.39	0.41	3.84	0.17	3.18
Chemlalisfax	Gh	1.63	1.14	0.57	0.74	1.39	0.39	3.46	0.18	3.16
Coratina	T	3.16	1.09	0.69	1.77	1.29	0.45	5.46	0.16	3.19
Coratina	T	3.39	1.05	0.71	1.57	1.30	0.45	5.39	0.15	3.21
Coratina	Gh	2.19	1.14	0.65	1.05	1.32	0.44	3.84	0.13	3.72
Coratina	Gh	2.21	1.20	0.61	1.06	1.33	0.45	3.53	0.15	3.27
Frantoio	T	2.42	1.33	0.58	0.99	1.40	0.40	6.47	0.20	2.64
Frantoio	T	2.88	1.12	0.62	1.04	1.35	0.42	6.28	0.21	2.59
Frantoio	Gh	2.45	1.11	0.60	1.00	1.31	0.45	4.26	0.19	2.70
Frantoio	Gh	2.31	1.11	0.60	0.99	1.35	0.44	4.38	0.22	2.64
Gargashi	T	1.57	1.12	0.62	0.55	1.35	0.42	4.20	0.16	3.19
Gargashi	T	1.48	1.13	0.58	0.51	1.40	0.42	4.23	0.18	2.88
Gargashi	M	2.34	1.15	0.53	0.97	1.42	0.41	4.33	0.15	3.73
Gargashi	M	2.31	1.23	0.49	0.97	1.48	0.36	4.31	0.16	3.25
Hammudi	T	3.31	1.10	0.64	1.01	1.44	0.39	5.70	0.17	3.23
Hammudi	T	3.39	1.08	0.65	1.09	1.36	0.43	5.45	0.18	3.02
Hammudi	M	2.87	1.16	0.55	1.41	1.45	0.42	3.90	0.15	3.53
Hammudi	M	2.97	1.09	0.63	1.33	1.35	0.44	4.02	0.18	3.01
Jabbugi	T	3.03	1.17	0.53	0.95	1.63	0.33	4.93	0.18	3.06
Jabbugi	T	3.16	1.18	0.52	0.92	1.67	0.34	4.65	0.20	2.71
Jabbugi	M	4.20	1.22	0.48	1.22	1.73	0.31	6.27	0.18	2.79
Jabbugi	M	4.04	1.20	0.49	1.33	1.72	0.31	6.42	0.19	2.68
Leccino	T	3.26	1.10	0.61	1.02	1.38	0.40	3.82	0.21	2.64
Leccino	T	3.05	1.09	0.63	1.16	1.34	0.42	4.03	0.23	2.36

Table A.5 Continued

Leccino	Gh	3.61	1.16	0.59	1.60	1.38	0.40	5.70	0.20	2.67
Leccino	Gh	2.92	1.12	0.59	1.44	1.49	0.37	6.12	0.24	2.37
Marrari	T	2.49	1.18	0.54	1.16	1.50	0.35	6.74	0.21	2.72
Marrari	T	2.46	1.14	0.56	1.13	1.51	0.35	6.67	0.21	2.74
Marrari	M	2.65	1.17	0.52	0.95	1.48	0.37	4.25	0.16	3.31
Marrari	M	2.15	1.26	0.46	0.87	1.49	0.36	4.34	0.18	3.14
Maurino	T	2.47	1.10	0.62	1.07	1.27	0.50	4.17	0.18	3.47
Maurino	T	2.81	1.08	0.67	0.95	1.33	0.42	3.72	0.17	3.05
Maurino	Gh	3.52	1.08	0.65	0.87	1.35	0.43	3.59	0.14	3.67
Maurino	Gh	3.34	1.09	0.64	0.77	1.25	0.51	3.55	0.13	3.71
Mbuti	T	4.19	1.15	0.58	0.85	1.39	0.44	6.59	0.22	2.49
Mbuti	T	3.79	1.12	0.61	0.86	1.30	0.46	6.53	0.24	2.25
Mbuti	M	3.82	1.06	0.69	1.05	1.28	0.52	5.52	0.16	3.25
Mbuti	M	4.21	1.07	0.69	0.98	1.27	0.49	5.96	0.17	3.20
Mignolo	T	1.67	1.15	0.57	0.77	1.41	0.41	4.21	0.17	3.32
Mignolo	T	1.83	1.12	0.58	0.67	1.40	0.40	3.55	0.17	3.25
Mignolo	Gh	3.03	1.06	0.72	1.03	1.25	0.51	4.89	0.19	2.94
Mignolo	Gh	2.66	1.06	0.70	0.97	1.21	0.53	4.71	0.21	2.85
Moraiolo	T	3.09	1.06	0.70	0.83	1.20	0.57	5.22	0.17	3.11
Moraiolo	T	3.02	1.05	0.72	0.91	1.14	0.62	5.51	0.16	3.18
Moraiolo	Gh	4.41	1.03	0.80	0.84	1.13	0.59	5.42	0.20	2.90
Moraiolo	Gh	3.54	1.04	0.74	0.81	1.14	0.59	5.35	0.20	2.94
Ouslati	T	3.38	1.05	0.74	1.07	1.23	0.49	5.34	0.14	4.00
Ouslati	T	3.35	1.05	0.72	1.06	1.26	0.47	4.93	0.16	3.29
Ouslati	Gh	3.38	1.09	0.65	1.35	1.41	0.44	4.16	0.17	3.00
Ouslati	Gh	2.97	1.16	0.64	1.20	1.23	0.50	4.38	0.19	2.62
Rasli	T	3.59	1.10	0.60	0.92	1.33	0.45	7.06	0.22	2.32

Table A.5 Continued

Rasli	T	3.57	1.12	0.6	0.9	1.29	0.47	5.75	0.21	2.64
Rasli	M	2.87	1.14	0.57	0.99	1.34	0.45	4.66	0.18	3.22
Rasli	M	3.2	1.12	0.62	0.87	1.37	0.41	4.64	0.18	3.14
Zaafrani	T	3.38	1.09	0.64	1.58	1.22	0.49	6.37	0.22	2.4
Zaafrani	T	3.74	1.12	0.59	1.53	1.27	0.48	6.02	0.23	2.5
Zaafrani	M	2.62	1.18	0.52	1.04	1.46	0.38	4.23	0.15	3.78
Zaafrani	M	2.61	1.17	0.52	1.01	1.46	0.36	4.25	0.15	3.71
Zalmati	Gh	1.77	1.11	0.59	0.79	1.46	0.37	4.37	0.15	3.65
Zalmati	Gh	1.69	1.1	0.62	0.7	1.4	0.42	3.79	0.15	3.61
Zalmati	Za	1.43	1.08	0.66	0.7	1.39	0.42	3.6	0.16	3.33
Zalmati	Za	1.24	1.1	0.64	0.59	1.49	0.41	3.82	0.15	3.58
Zarrasi	T	3.92	1.03	0.77	1.08	1.12	0.64	3.49	0.12	3.82
Zarrasi	T	4.31	1.04	0.76	0.91	1.19	0.56	3.39	0.13	3.88
Zarrasi	M	5.05	3.27	0.82	1.05	1.23	0.52	3.67	0.16	3.45
Zarrasi	M	4.63	3.15	0.83	1.23	1.13	0.62	2.87	0.13	4.11

Table A.6 Combinations of fruit, seed and leaf traits of 19 cultivars that grow in two different locations were used to evaluated and identify the rest of olive varieties.

Variety name	Local Location	Fruit weight g/1 fruit	Fruit length cm/1fruit	Fruit width cm/1fruit	Seed weight(g)/1 seed	seed length(cm)/ 1 seed	Seed width(cm)/1 seed	leaf weight/1 leaf	leaf length(cm)/1 leaf	leaf width(cm)/1 leaf
Chemlali	Me	1.28	1.72	1.12	0.23	1.26	0.51	0.16	5.45	1.26
Chemlali	Me	1.22	1.64	1.18	0.24	1.32	0.54	0.15	5.2	1.2
Chemlali	Me	1.16	1.56	1.24	0.25	1.38	0.57	0.14	4.95	1.14
Chemlali	Za	1.04	1.73	0.99	0.25	1.24	0.53	0.21	8.38	1.15
Chemlali	Za	0.99	1.65	1.04	0.26	1.3	0.56	0.2	8	1.1
Chemlali	Za	0.94	1.57	1.09	0.27	1.36	0.59	0.19	7.62	1.05
Chemkuss	Me	2.65	2.18	1.47	0.38	1.37	0.65	0.21	6.81	1.26
Chemkuss	Me	2.53	2.08	1.54	0.4	1.44	0.68	0.2	6.5	1.2
Chemkuss	Me	2.41	1.98	1.61	0.42	1.51	0.71	0.19	6.19	1.14
Chemkuss	Th	2.15	1.91	1.35	0.36	1.2	0.67	0.21	6.5	1.26
Chemkuss	Th	2.05	1.82	1.42	0.38	1.26	0.7	0.2	6.2	1.2
Chemkuss	Th	1.95	1.73	1.49	0.4	1.32	0.73	0.19	5.9	1.14
Chemsfax	Gh	1.22	1.78	1.07	0.25	1.22	0.53	0.21	5.97	1.15
Chemsfax	Gh	1.16	1.7	1.12	0.26	1.28	0.56	0.2	5.7	1.1
Chemsfax	Gh	1.1	1.62	1.17	0.27	1.34	0.59	0.19	5.43	1.05
Chemsfax	Th	0.64	1.49	0.8	0.21	1.14	0.51	0.16	6.81	1.26
Chemsfax	Th	0.61	1.42	0.84	0.22	1.2	0.54	0.15	6.5	1.2
Chemsfax	Th	0.58	1.35	0.88	0.23	1.26	0.57	0.14	6.19	1.14
Coratina	Gh	2.12	1.98	1.29	0.51	1.49	0.72	0.16	6.29	1.15
Coratina	Gh	2.02	1.89	1.35	0.54	1.56	0.76	0.15	6	1.1
Coratina	Gh	1.92	1.8	1.41	0.57	1.63	0.8	0.14	5.71	1.05
Coratina	Th	3.25	2.24	1.54	0.59	1.52	0.76	0.26	7.33	1.57
Coratina	Th	3.1	2.14	1.62	0.62	1.6	0.8	0.25	7	1.5
Coratina	Th	2.95	2.04	1.7	0.65	1.68	0.84	0.24	6.67	1.43
Frantoio	Gh	1.69	1.94	1.14	0.46	1.47	0.69	0.16	6.08	1.47
Frantoio	Gh	1.61	1.85	1.2	0.48	1.54	0.72	0.15	5.8	1.4
Frantoio	Gh	1.53	1.76	1.26	0.5	1.61	0.75	0.14	5.52	1.33
Frantoio	Th	2.71	2.25	1.39	0.61	1.62	0.76	0.37	7.86	2.1
Frantoio	Th	2.59	2.15	1.46	0.64	1.7	0.8	0.35	7.5	2
Frantoio	Th	2.47	2.05	1.53	0.67	1.78	0.84	0.33	7.14	1.9
Gargashi	Me	1.03	1.71	0.99	0.25	1.33	0.52	0.21	6.18	1.15
Gargashi	Me	0.98	1.63	1.04	0.26	1.4	0.55	0.2	5.9	1.1
Gargashi	Me	0.93	1.55	1.09	0.27	1.47	0.58	0.19	5.62	1.05
Gargashi	Th	0.92	1.61	0.95	0.21	1.2	0.51	0.16	5.76	1.15

Table A.6 Continued

Gargashi	Th	0.88	1.54	1	0.22	1.26	0.54	0.15	5.5	1.1
Gargashi	Th	0.84	1.47	1.05	0.23	1.32	0.57	0.14	5.24	1.05
Hammudi	Me	2.64	2.28	1.37	0.48	1.62	0.67	0.16	6.81	1.26
Hammudi	Me	2.52	2.18	1.44	0.5	1.7	0.7	0.15	6.5	1.2
Hammudi	Me	2.4	2.08	1.51	0.52	1.78	0.73	0.14	6.19	1.14
Hammudi	Th	2.57	2.22	1.38	0.46	1.54	0.69	0.31	7.96	1.57
Hammudi	Th	2.45	2.12	1.45	0.48	1.62	0.72	0.3	7.6	1.5
Hammudi	Th	2.33	2.02	1.52	0.5	1.7	0.75	0.29	7.24	1.43
Jabbugi	Me	1.68	2.33	1.1	0.34	1.77	0.57	0.16	6.29	1.26
Jabbugi	Me	1.6	2.22	1.16	0.36	1.86	0.6	0.15	6	1.2
Jabbugi	Me	1.52	2.11	1.22	0.38	1.95	0.63	0.14	5.71	1.14
Jabbugi	Th	2.4	2.38	1.27	0.38	1.71	0.59	0.16	6.08	1.36
Jabbugi	Th	2.29	2.27	1.33	0.4	1.8	0.62	0.15	5.8	1.3
Jabbugi	Th	2.18	2.16	1.39	0.42	1.89	0.65	0.14	5.52	1.24
Leccino	Gh	3.3	2.5	1.47	0.69	1.89	0.72	0.21	6.39	1.57
Leccino	Gh	3.15	2.39	1.54	0.72	1.98	0.76	0.2	6.1	1.5
Leccino	Gh	3	2.28	1.61	0.75	2.07	0.8	0.19	5.81	1.43
Leccino	Th	2.88	2.36	1.38	0.65	1.71	0.74	0.16	5.76	1.57
Leccino	Th	2.75	2.25	1.45	0.68	1.8	0.78	0.15	5.5	1.5
Leccino	Th	2.62	2.14	1.52	0.71	1.89	0.82	0.14	5.24	1.43
Marrari	Me	1.76	2.2	1.1	0.42	1.62	0.61	0.26	7.12	1.26
Marrari	Me	1.68	2.1	1.16	0.44	1.7	0.64	0.25	6.8	1.2
Marrari	Me	1.6	2	1.22	0.46	1.78	0.67	0.24	6.48	1.14
Marrari	Th	2.3	2.27	1.25	0.48	1.7	0.65	0.21	7.54	1.78
Marrari	Th	2.2	2.17	1.31	0.5	1.78	0.68	0.2	7.2	1.7
Marrari	Th	2.1	2.07	1.37	0.52	1.86	0.71	0.19	6.86	1.62
Maurino	Gh	2.16	2.08	1.3	0.38	1.35	0.65	0.16	6.5	1.15
Maurino	Gh	2.06	1.99	1.36	0.4	1.42	0.68	0.15	6.2	1.1
Maurino	Gh	1.96	1.9	1.42	0.42	1.49	0.71	0.14	5.9	1.05
Maurino	Th	2.43	2.1	1.4	0.42	1.33	0.69	0.16	5.97	1.15
Maurino	Th	2.32	2	1.47	0.44	1.4	0.72	0.15	5.7	1.1
Maurino	Th	2.21	1.9	1.54	0.46	1.47	0.75	0.14	5.43	1.05
Mbuti	Me	2.18	1.91	1.35	0.42	1.31	0.72	0.16	6.39	1.26
Mbuti	Me	2.08	1.82	1.42	0.44	1.38	0.76	0.15	6.1	1.2
Mbuti	Me	1.98	1.73	1.49	0.46	1.45	0.8	0.14	5.81	1.14

Table A.6 Continued

Mbuti	Th	3.16	2.38	1.51	0.38	1.41	0.65	0.26	6.91	1.89
Mbuti	Th	3.02	2.27	1.59	0.4	1.48	0.68	0.25	6.6	1.8
Mbuti	Th	2.88	2.16	1.67	0.42	1.55	0.71	0.24	6.29	1.71
Mignolo	Gh	2.64	2.08	1.46	0.53	1.39	0.72	0.31	6.7	1.47
Mignolo	Gh	2.52	1.99	1.53	0.56	1.46	0.76	0.3	6.4	1.4
Mignolo	Gh	2.4	1.9	1.6	0.59	1.53	0.8	0.29	6.1	1.33
Mignolo	Th	1.57	1.89	1.14	0.34	1.39	0.61	0.21	6.91	1.26
Mignolo	Th	1.5	1.8	1.2	0.36	1.46	0.64	0.2	6.6	1.2
Mignolo	Th	1.43	1.71	1.26	0.38	1.53	0.67	0.19	6.29	1.14
Moraiolo	Gh	2.23	1.88	1.37	0.38	1.16	0.7	0.21	6.5	1.36
Moraiolo	Gh	2.13	1.79	1.44	0.4	1.22	0.74	0.2	6.2	1.3
Moraiolo	Gh	2.03	1.7	1.51	0.42	1.28	0.78	0.19	5.9	1.24
Moraiolo	Th	3.44	2.2	1.59	0.46	1.22	0.74	0.26	7.33	1.57
Moraiolo	Th	3.28	2.1	1.67	0.48	1.28	0.78	0.25	7	1.5
Moraiolo	Th	3.12	2	1.75	0.5	1.34	0.82	0.24	6.67	1.43
Ouslati	Gh	2.77	2.21	1.43	0.57	1.49	0.74	0.21	6.18	1.47
Ouslati	Gh	2.64	2.11	1.5	0.6	1.56	0.78	0.2	5.9	1.4
Ouslati	Gh	2.51	2.01	1.57	0.63	1.63	0.82	0.19	5.62	1.33
Ouslati	Th	2.63	2.02	1.45	0.5	1.45	0.76	0.16	7.86	1.36
Ouslati	Th	2.51	1.93	1.52	0.52	1.52	0.8	0.15	7.5	1.3
Ouslati	Th	2.39	1.84	1.59	0.54	1.59	0.84	0.14	7.14	1.24
Rasli	Me	2.16	2.13	1.29	0.46	1.49	0.67	0.16	6.08	1.26
Rasli	Me	2.06	2.03	1.35	0.48	1.56	0.7	0.15	5.8	1.2
Rasli	Me	1.96	1.93	1.41	0.5	1.63	0.73	0.14	5.52	1.14
Rasli	Th	2	2.04	1.23	0.44	1.45	0.67	0.21	5.76	1.57
Rasli	Th	1.91	1.95	1.29	0.46	1.52	0.7	0.2	5.5	1.5
Rasli	Th	1.82	1.86	1.35	0.48	1.59	0.73	0.19	5.24	1.43
Zaafrani	Me	1.73	2.18	1.12	0.4	1.62	0.61	0.26	7.33	1.26
Zaafrani	Me	1.65	2.08	1.18	0.42	1.7	0.64	0.25	7	1.2
Zaafrani	Me	1.57	1.98	1.24	0.44	1.78	0.67	0.24	6.67	1.14
Zaafrani	Th	2.39	2.18	1.29	0.65	1.56	0.76	0.26	6.91	1.78
Zaafrani	Th	2.28	2.08	1.35	0.68	1.64	0.8	0.25	6.6	1.7
Zaafrani	Th	2.17	1.98	1.41	0.71	1.72	0.84	0.24	6.29	1.62
Zalmati	Gh	0.8	1.57	0.9	0.21	1.26	0.5	0.16	6.18	1.15
Zalmati	Gh	0.76	1.5	0.95	0.22	1.32	0.52	0.15	5.9	1.1

Table A.6 Continued

Zalmati	Gh	0.72	1.43	1	0.23	1.38	0.54	0.14	5.62	1.05
Zalmati	Za	1.12	1.68	1.06	0.23	1.18	0.51	0.21	6.81	1.15
Zalmati	Za	1.07	1.6	1.11	0.24	1.24	0.54	0.2	6.5	1.1
Zalmati	Za	1.02	1.52	1.16	0.25	1.3	0.57	0.19	6.19	1.05
Zarrasi	Me	4.64	2.28	1.86	0.53	1.26	0.78	0.21	6.29	1.05
Zarrasi	Me	4.43	2.18	1.95	0.56	1.32	0.82	0.2	6	1
Zarrasi	Me	4.22	2.08	2.04	0.59	1.38	0.86	0.19	5.71	0.95
Zarrasi	Th	4	2.23	1.75	0.46	1.24	0.78	0.21	6.5	0.84
Zarrasi	Th	3.82	2.13	1.84	0.48	1.3	0.82	0.2	6.2	0.8
Zarrasi	Th	3.64	2.03	1.93	0.5	1.36	0.86	0.19	5.9	0.76

z Name of accession attached with their local locations (Fig.2.1) (M= Mesalata h,T= Tharouna ,G= Gharian ,Za= Zaltin and Tri= Tripoli).

Table A.7 List of quantitative traits of fruit, seed and leaf were measured for 90 olive cultivars that grown in Libya.

Variety name	Fruit weight (g)/1 fruit	Fruit volum/ 1 fruit	Fruit length (cm)/1 fruit	Fruit width (cm)/1 fruit	Fruit shape L/W	Fruit density W/V	Seed Weigh t (g)/1 seeds	Seed wedith (cm)/1 seeds	Seed Length cm/1 seed	Seed shape L/W	Leaf length (cm)/1 leaf	Leaf width (cm)/1 leaf	Leaf weight (g)/1 leaf	Leaf shape L/W
Anbi-M[Z]	1.34	1.2	1.83	1.09	1.68	1.12	0.54	0.78	1.52	0.51	5.5	1	0.05	5.5
Arbequina-Tri	2.31	2.4	1.82	1.49	1.22	0.96	0.46	0.74	1.32	0.56	5.8	1.3	0.2	4.46
Ascolanatenera-T	9.61	9.7	3	2.44	1.23	0.99	1.02	0.96	1.78	0.54	6.8	1.2	0.2	5.67
Bayyudi-M	2.04	2	1.95	1.4	1.39	1.02	0.48	0.82	1.3	0.63	5.8	1.1	0.2	5.27
Bella di spagna-T	6.16	6	2.74	2.09	1.31	1.03	1.04	1.02	1.9	0.54	6.4	1.3	0.15	4.92
Beserri-M	3.29	3.2	2.43	1.65	1.47	1.03	0.34	0.68	1.34	0.51	7.7	1.2	0.25	6.42
Caninese-G	1.73	1.8	1.82	1.31	1.39	0.96	0.5	0.74	1.46	0.51	5.8	1	0.15	5.8
Carmelitana-T	3.83	3.7	2.93	1.55	1.89	1.04	0.88	0.8	2.46	0.33	6.2	2	0.25	3.1
Cellina-G	1.67	1.6	1.81	1.25	1.45	1.04	0.34	0.64	1.3	0.49	6.5	1.8	0.2	3.61
Chemlali-Za	0.99	0.9	1.65	1.04	1.59	1.1	0.26	0.56	1.3	0.43	8	1.1	0.2	7.27
Chemlali-M	1.22	1.2	1.64	1.18	1.39	1.02	0.76	0.84	1.88	0.45	5.2	1.2	0.15	4.33
Chemlalikussabat-T	2.05	1.9	1.82	1.42	1.28	1.08	0.38	0.7	1.26	0.56	6.2	1.2	0.2	5.17
Chemlalikussabat-M	2.53	2.6	2.08	1.54	1.35	0.97	0.24	0.54	1.32	0.41	6.5	1.2	0.2	5.42
Chemlalisfax-T	0.61	0.7	1.42	0.84	1.69	0.87	0.22	0.54	1.2	0.45	6.5	1.2	0.15	5.42
Chemlalisfax-G	1.16	1.1	1.7	1.12	1.52	1.05	0.36	0.64	1.46	0.44	5.7	1.1	0.2	5.18
Coratina-T	3.1	3	2.14	1.62	1.32	1.03	0.62	0.8	1.6	0.5	7	1.5	0.25	4.67
Coratina-G	2.02	1.7	1.89	1.35	1.4	1.19	0.26	0.56	1.28	0.44	6	1.1	0.15	5.45
Cucco-T	6.73	6.7	3.07	2	1.54	1	0.88	0.86	2.02	0.43	8	1.7	0.4	4.71
Enduri-T	0.83	0.8	1.6	0.96	1.67	1.04	0.22	0.56	1.34	0.42	7.5	1.1	0.2	6.82
Farkuti-M	2.25	2	2.28	1.39	1.64	1.13	0.4	0.68	1.44	0.47	6.4	1.4	0.3	4.57
Frantoio-T	2.59	2.4	2.15	1.46	1.47	1.08	0.64	0.8	1.7	0.47	7.5	2	0.35	3.75
Frantoio-G	1.61	1.5	1.85	1.2	1.54	1.07	0.54	0.76	1.56	0.49	5.8	1.4	0.15	4.14
Gaiani-M	2.95	3	2.44	1.52	1.61	0.98	0.48	0.68	1.78	0.38	7	1.1	0.2	6.36
Gargashi-T	0.88	0.9	1.54	1	1.54	0.98	0.22	0.54	1.26	0.43	5.5	1.1	0.15	5
Gargashi-M	0.98	1	1.63	1.04	1.57	0.98	0.48	0.72	1.72	0.42	5.9	1.1	0.2	5.36
Gartomye-M	2.52	2.5	2.25	1.37	1.64	1.01	0.26	0.55	1.4	0.39	7.4	1.4	0.2	5.29
Gragnano-G	3.45	3.2	2.05	1.69	1.21	1.08	0.48	0.72	1.54	0.47	6.9	1.5	0.25	4.6
Grossa di sardegn-T	12.68	12.6	3.38	2.61	1.3	1.01	1.44	1.14	2.22	0.51	8.5	1.3	0.3	6.54
Grossa di spagna-T	7.4	7	2.85	2.2	1.3	1.06	1.22	1.1	2.1	0.52	6.8	1.4	0.2	4.86
Hammudi-T	2.45	2.5	2.12	1.45	1.46	0.98	0.48	0.72	1.62	0.44	7.6	1.5	0.3	5.07
Hammudi-M	2.52	2.5	2.18	1.44	1.51	1.01	0.26	0.54	1.5	0.36	6.5	1.2	0.15	5.42
Jabbugi-T	2.29	2.2	2.27	1.33	1.71	1.04	0.4	0.62	1.8	0.34	5.8	1.3	0.15	4.46
Jabbugi-M	1.6	1.7	2.22	1.16	1.91	0.94	0.5	0.7	1.7	0.41	6	1.2	0.15	5

Table A.7 Continued

Kalefy-M	2.22	2	2.16	1.38	1.57	1.11	0.36	0.6	1.86	0.32	6	1.3	0.2	4.62
Karkubi-M	3.7	3.5	2.15	1.78	1.21	1.06	0.48	0.72	1.66	0.43	7.5	1.5	0.3	5
Keddaui-M	2.84	3	2.45	1.36	1.8	0.95	0.6	0.84	1.38	0.61	6.6	1.2	0.2	5.5
Khaddira-M	1.19	1	1.68	1.07	1.57	1.19	0.52	0.66	1.96	0.34	7.1	1.2	0.2	5.92
Khaddra-M	1.81	1.8	1.96	1.3	1.51	1.01	0.4	0.68	1.44	0.47	6.6	1.1	0.15	6
Krusi-G	1.07	1.1	1.64	1.06	1.55	0.97	0.7	0.84	1.6	0.53	6	1.4	0.15	4.29
Leccino-T	2.75	2.5	2.25	1.45	1.55	1.1	0.68	0.78	1.8	0.43	5.5	1.5	0.15	3.67
Leccino-G	3.15	3	2.39	1.54	1.55	1.05	0.24	0.56	1.34	0.42	6.1	1.5	0.2	4.07
Leccinopendulo-T	1.89	1.7	2.12	1.25	1.7	1.11	0.48	0.7	1.62	0.43	7	1.3	0.15	5.38
Marisi-M	1.65	1.6	1.93	1.28	1.51	1.03	0.3	0.6	1.42	0.42	7.2	1.3	0.2	5.54
Marrari-T	2.2	2	2.17	1.31	1.66	1.1	0.5	0.68	1.78	0.38	7.2	1.7	0.2	4.24
Marrari-M	1.68	1.6	2.1	1.16	1.81	1.05	0.44	0.7	1.58	0.44	6.8	1.2	0.25	5.67
Maurino-T	2.32	2.2	2	1.47	1.36	1.05	0.44	0.72	1.4	0.51	5.7	1.1	0.15	5.18
Maurino-G	2.06	2	1.99	1.36	1.46	1.03	0.72	0.76	1.98	0.38	6.2	1.1	0.15	5.64
Mbuti-T	3.02	3	2.27	1.59	1.43	1.01	0.4	0.68	1.48	0.46	6.6	1.8	0.25	3.67
Mbuti-M	2.08	2	1.82	1.42	1.28	1.04	0.44	0.64	1.7	0.38	6.1	1.2	0.15	5.08
Mignolo-T	1.5	1.5	1.8	1.2	1.5	1	0.36	0.64	1.46	0.44	6.6	1.2	0.2	5.5
Mignolo-G	2.52	2.6	1.99	1.53	1.3	0.97	0.4	0.68	1.42	0.48	6.4	1.4	0.3	4.57
Monopoly-T	1.98	1.8	1.81	1.35	1.34	1.1	0.28	0.62	1.32	0.47	6.9	1.6	0.3	4.31
Moraiolo-T	3.28	3.3	2.1	1.67	1.26	0.99	0.48	0.78	1.28	0.61	7	1.5	0.25	4.67
Moraiolo-G	2.13	2	1.79	1.44	1.24	1.07	0.56	0.76	1.46	0.52	6.2	1.3	0.2	4.77
Morchiaio-G	2.2	2	2.34	1.31	1.79	1.1	0.4	0.74	1.22	0.61	7	1.3	0.2	5.38
Morellona di grecia-T	4.31	4.2	2.34	1.85	1.26	1.03	0.6	0.8	1.5	0.53	6.6	1.4	0.25	4.71
Mthemr-M	3.85	3.7	2.6	1.65	1.58	1.04	0.44	0.76	1.38	0.55	6.2	1.4	0.2	4.43
Mukther-M	0.9	1	1.69	0.98	1.72	0.9	0.48	0.64	1.66	0.39	7.2	1	0.2	7.2
Nardo-T	2.56	2.5	2.16	1.42	1.52	1.02	0.56	0.72	1.68	0.43	6	1.6	0.2	3.75
Neb gemel-M	2.18	2.2	2.63	1.27	2.07	0.99	0.74	0.8	1.9	0.42	7.3	1.1	0.25	6.64
Nepal-Tri	4.47	4.2	2.3	1.87	1.23	1.06	0.9	0.98	1.54	0.64	7.1	1.3	0.25	5.46
Ninai-M	1.87	1.9	1.84	1.35	1.36	0.98	0.52	0.66	2.3	0.29	5.4	1.2	0.2	4.5
Oliardo-G	3.18	2.6	2.11	1.67	1.26	1.22	0.46	0.7	1.74	0.4	5.4	1.2	0.2	4.5
Oliarolasalentina-T	1.91	1.8	1.75	1.36	1.29	1.06	0.26	0.62	1.32	0.47	7.5	1.4	0.3	5.36
Olivastro-G	0.7	0.8	1.38	0.96	1.44	0.88	0.52	0.74	1.52	0.49	5	1.2	0.15	4.17
Ouslati-T	2.51	2.3	1.93	1.52	1.27	1.09	0.52	0.8	1.52	0.53	7.5	1.3	0.15	5.77
Ouslati-G	2.64	2.6	2.11	1.5	1.41	1.02	0.18	0.52	1.12	0.46	5.9	1.4	0.2	4.21
Ouslatikussabat-T	1.82	1.7	1.71	1.37	1.25	1.07	0.3	0.64	1.2	0.53	7.4	1.1	0.15	6.73

Table A.7 Continued

Pendolino-G	2.37	2.1	2.15	1.36	1.58	1.13	0.6	0.78	1.56	0.5	5.8	1.2	0.15	4.83
Qalbsarduk-M	2.98	2.9	2.27	1.5	1.51	1.03	0.3	0.64	1.3	0.49	5.7	1.1	0.15	5.18
Rasli-T	1.91	1.8	1.95	1.29	1.51	1.06	0.46	0.7	1.52	0.46	5.5	1.5	0.2	3.67
Rasli-M	2.06	2	2.03	1.35	1.5	1.03	0.56	0.72	1.8	0.4	5.8	1.2	0.15	4.83
Rosciola-G	1.53	1.5	1.66	1.25	1.33	1.02	0.62	0.68	1.8	0.38	5.6	1.1	0.15	5.09
Rumi-M	2.64	2.5	1.98	1.4	1.41	1.06	0.48	0.7	1.56	0.45	6.5	1.1	0.2	5.91
Sahley-M	1.4	1.2	1.55	1.22	1.27	1.17	0.26	0.6	1.32	0.45	6.1	0.9	0.15	6.78
Santagostino-T	7.34	7.1	2.63	2.2	1.2	1.03	0.8	1	1.5	0.67	6.7	1.2	0.2	5.58
Soudia-M	0.81	0.7	1.52	0.95	1.6	1.16	0.32	0.62	1.48	0.42	6	1.5	0.15	4
Tombarella-G	1.88	1.7	1.7	1.37	1.24	1.11	0.34	0.64	1.3	0.49	5.7	1	0.15	5.7
Tunisian-M	1.71	1.5	1.66	1.34	1.24	1.14	0.3	0.64	1.24	0.52	7	1.25	0.2	5.6
Vqos-M	3.31	3.2	2.15	1.63	1.32	1.03	0.4	0.7	1.22	0.57	6	1.2	0.2	5
Wild-G	2.19	2	2.23	1.32	1.69	1.1	0.4	0.74	1.2	0.62	7	1.3	0.15	5.38
Yehudi-M	0.82	0.9	1.45	1	1.45	0.91	0.38	0.68	1.34	0.51	5.4	1.1	0.1	4.91
Zaafrani-T	2.28	2.2	2.08	1.35	1.54	1.04	0.16	0.2	0.3	0.67	6.6	1.7	0.25	3.88
Zaafrani-M	1.65	1.5	2.08	1.18	1.76	1.1	0.24	0.6	1.12	0.54	7	1.2	0.25	5.83
Zaglo-M	1.65	1.5	2.05	1.16	1.77	1.1	0.42	0.64	1.7	0.38	6.5	1.1	0.1	5.91
Zalmati-G	0.76	0.8	1.5	0.95	1.58	0.95	0.5	0.68	1.76	0.39	5.9	1.1	0.15	5.36
Zalmati-Za	1.07	1	1.6	1.11	1.44	1.07	0.24	0.54	1.24	0.44	6.5	1.1	0.2	5.91
Zarrasi-T	3.82	3.7	2.13	1.84	1.16	1.03	0.68	0.8	1.64	0.49	6.2	0.8	0.2	7.75
Zarrasi-M	4.43	4.5	2.18	1.95	1.12	0.98	0.5	0.66	1.76	0.38	6	1	0.2	6
Znbai-M	2.53	2.5	2.03	1.5	1.35	1.01	0.56	0.82	1.32	0.62	4.8	1.15	0.15	4.17

z Name of accession attached with their local locations (Fig.2.1) (M= Mesalata h,T= Tharouna ,G= Gharian ,Za= Zaltin and Tri= Tripoli).

Table A.8 Final sequences of 12 multiplex primers assigned with their fluorescent dye and PIG-tail

Locus Name	Forward dye label	Company	Primer sequence labeled with fluorescent probe (5' –3 ‘)	Amount of Oligo/nMoles	Primer final concentration µM
EMO-90-F	56-FAM	IDT	5'-/56-FAM/CAT CCG GAT TTC TTG CTT TT-3'	99.80	2
EMO-90-R	PIGtail	IDT	5'-GTT TCT T/AG CGA ATG TAG CTT TGC ATG T-3'	33.80	2
DCA3-F	56-FAM	IDT	5'-/56-FAM/CCC AAG CGG AGG TGT ATA TTG TTA C-3'	114.70	2
DCA3-R	PIGtail	IDT	5'-GTT TCT T/TG CTT TTG TCG TGT TTG AGA TGT TG-3'	31.20	2
DCA14-F	56-FAM	IDT	5'-/56-FAM/AAT TTT TTA ATG CAC TAT AAT TTA C-3'	118.90	2
DCA14-R	PIGtail	IDT	5'-GTT TCT T/TT GAG GTC TCT ATA TCT CCC AGG GG-3'	29.60	2
GAPU101-F	56-FAM	IDT	5'-/56-FAM/CAT GAA AGG AGG GGG ACA TA-3’	96.80	2
GAPU101-R	PIGtail	IDT	5'-GTT TCT T/GG CAC TTG TTG TGC AGA TTG-3'	30.30	2
DCA18-F	VIC	AB	5'-/VIC/AAG AAA GAA AAA GGC AGA ATT AAG C-3'	10.00	2
DCA18-R	PIGtail	IDT	5'-GTT TCT T/GT TTT CGT CTC TCT ACA TAA GTG AC-3'	25.90	2
DCA16-F	VIC	AB	5'-/VIC/TTA GGT GGG ATT CTG TAG ATG GTT G-3'	10.00	2
DCA16-R	PIGtail	IDT	5'-GTT TCT T/TT TTA GGT GAG TTC ATA GAA TTA GC-3'	27.40	2
DCA5-F	VIC	AB	5'-/VIC/AAC AAA TCC CAT ACG AAC TGC C-3'	10.00	2
DCA5-R	PIGtail	IDT	5'-GTT TCT T/CG TGT TGC TGT GAA GAA AAT CG-3'	28.00	2
DCA17-F	VIC	AB	5'-/VIC/GAT CAA ATT CTA CCA AAA ATA TA-3'	10.00	2
DCA17-R	PIGtail	IDT	5'-GTT TCT T/TA AAT TTT TGG CAC GTA GTA TTG G-3'	23.30	2
GAPU103A-F	PET	AB	5'-/PET/TGA ATT TAA CTT TAA ACC CAC ACA-3'	10.00	2
GAPU103A-R	PIGtail	IDT	5'-GTT TCT T/GC ATC GCT CGA TTT TTA TCC-3'	24.50	2
GAPU71B-F	PET	AB	5'-/PET/GAT CAA AGG AAG AAG GGG ATA AA-3’	10.00	2
GAPU71B-R	PIGtail	IDT	5'-GTT TCT T/AC AAC AAA TCC GTA CGC TTG-3'	32.20	2
UDO-043-F	PET	AB	5'-/PET/TCG GCT TTA CAA CCC ATT TC-3'	10.00	2
UDO-043-R	PIGtail	IDT	5'-GTT TCT T/TG CCA ATT ATG GGG CTA ACT-3'	41.00	2
DCA9-F	PET	AB	5'-/PET/AAT CAA AGT CTT CCT TCT CAT TTC G-3'	10.00	2
DCA9-R	PIGtail	IDT	5'-GTT TCT T/GA TCC TTC CAA AAG TAT AAC CTC TC-3'	26.70	2

Table A.9 Matrix of 10 microsatellite markers and 99 olive genotypes obtained from Genemapper program.

ID	POP = Introduced	DCA18	DCA18	UDO43	UDO43	GAPU101	GAPU101	DCA3	DCA3	DCA5	DCA5	DCA14	DCA14	GAPU103A	GAPU103A	DCA16	DCA16	GAPU71B	GAPU71B	EMO90	EMO90
1	AscolanateneT	172	174	172	172	?	?	240	240	202	202	176	186	135	174	152	152	121	140	183	184
2	BelladispagnT	180	180	202	202	197	215	246	250	202	202	176	186	135	135	169	171	119	140	183	183
3	CarmelitanaT	174	180	177	177	181	181	234	250	202	202	178	186	135	162	150	152	121	127	183	183
4	CellinaG	166	166	?	?	189	195	240	240	202	202	186	186	174	176	?	?	121	124	183	184
5	ChemlalisfaxG	174	174	168	168	174	174	240	240	202	202	168	186	159	176	183	184	121	140	183	184
6	ChemlalisfaxT	172	172	212	212	195	197	240	240	194	194	176	178	174	186	147	171	121	140	183	189
7	CoratinaG	172	172	?	?	195	215	240	240	202	202	178	186	135	135	147	147	121	140	183	189
8	CoratinaT	166	166	?	?	?	?	240	240	202	202	168	186	159	159	152	152	121	124	183	184
9	CuccoT	172	174	172	174	174	181	240	240	202	202	169	186	162	162	182	183	121	140	183	183
10	EnduriT	168	168	177	177	187	193	229	240	194	202	182	186	159	159	147	160	124	127	184	184
11	FrantoioG	172	174	?	?	181	195	240	240	202	202	178	186	162	174	147	147	121	140	183	189
12	FrantoioT	172	174	?	?	181	195	240	240	202	202	178	186	162	174	147	147	121	140	183	189
13	GragnanoG	168	168	210	212	187	197	236	246	202	204	176	186	159	159	152	152	119	127	183	184
14	GrossadisareT	176	176	218	218	197	215	?	?	?	?	176	186	135	135	169	171	119	140	183	183
15	GrossadispagT	166	166	174	177	197	215	250	250	202	202	176	176	135	135	152	177	117	121	183	183
16	KrusiG	168	168	227	227	?	?	240	240	202	202	182	186	159	159	147	160	124	127	184	184
17	LeccinoG	172	172	212	216	195	197	250	250	202	202	176	178	174	186	147	171	121	140	183	189
18	LeccinoT	172	172	212	216	195	197	250	250	202	202	176	178	174	186	147	171	121	140	183	189
19	LeccinopendT	174	174	212	216	195	197	250	250	202	202	169	186	174	189	147	169	121	140	183	189
20	MaurinoG	172	172	177	177	181	187	234	250	202	202	169	178	162	186	147	169	140	140	183	183
21	MaurinoT	172	172	177	177	181	187	234	250	202	202	169	178	162	186	147	169	140	140	183	183
22	MbutiM	168	172	216	216	168	172	?	?	?	?	182	186	150	150	152	160	124	127	184	184
23	MbutiT	168	170	168	168	168	168	229	240	194	202	186	186	150	150	147	183	124	127	183	184
24	MignoloG	158	174	189	189	181	189	240	252	194	202	178	186	150	159	121	183	121	127	183	183
25	MignoloT	168	168	177	177	189	215	?	?	?	?	176	182	135	150	181	182	127	127	180	183
26	MonopolyT	154	168	154	168	168	168	240	240	202	202	169	186	159	159	147	147	121	140	189	189
27	MoraioloG	172	174	218	218	174	189	?	?	?	?	169	186	150	150	147	147	121	127	183	189
28	MoraioloT	172	174	172	172	181	197	240	250	202	202	169	186	162	162	182	183	121	140	183	183
29	MorelonadigreciaT	168	176	212	214	168	168	242	250	202	202	176	186	135	150	152	171	121	140	183	184
30	NardoT	154	168	?	?	181	195	240	240	194	202	169	186	159	159	147	147	121	140	189	189
31	NepalTri	174	174	177	177	181	195	?	?	?	?	178	186	162	174	147	147	121	140	183	189
32	oliardoG	172	174	172	174	?	?	240	240	202	202	178	186	162	174	147	147	121	140	183	189
33	OliarolasalentinaT	154	168	177	177	181	187	240	240	202	202	?	?	?	?	147	147	121	140	189	189
34	OlivastroG	172	172	?	?	?	?	240	240	202	202	174	186	150	157	160	160	121	124	183	193
35	OuslatiG	170	170	?	?	189	197	240	240	202	202	174	186	150	150	171	171	124	127	183	184

Table A.9 Continued.

36	OuslatiT	170	170	?	?	189	197	240	240	202	202	174	186	150	150	171	171	124	127	183	184
37	PendolinoG	174	174	204	204	189	203	240	240	202	202	186	186	150	150	147	147	121	127	183	189
38	RosciolaG	168	168	187	214	187	203	240	240	202	202	174	186	174	174	152	152	121	127	180	189
39	SantagostinoT	166	180	174	177	195	197	229	250	202	202	176	186	174	186	171	171	119	124	183	184
40	TombarellaG	174	174	?	?	?	?	240	240	202	202	186	186	150	150	147	147	121	127	183	189
41	TunisianM	168	174	212	214	195	203	240	240	202	202	174	186	150	174	147	147	127	140	184	189
	POP = local																				
42	AnbiM	168	172	212	216	187	197	240	240	202	202	174	182	159	159	147	147	124	127	183	183
43	BeserriM	166	180	?	?	187	193	240	240	202	202	182	186	135	159	?	?	119	127	183	183
44	ChemlalikusM	174	174	187	187	189	195	240	240	202	202	168	186	150	174	147	147	121	127	184	184
45	ChemlalikussT	174	174	187	187	189	195	240	240	202	202	168	186	150	174	147	147	121	127	184	184
46	ChemlaliM	170	174	187	187	189	203	240	240	202	202	186	186	150	150	121	121	124	140	189	189
47	ChemlaliZa	168	168	?	?	?	?	240	240	202	202	182	186	159	159	147	160	124	127	184	184
48	FarkutiM	166	166	166	177	187	193	?	?	?	?	186	186	159	159	152	160	124	124	180	180
49	GaianiM	172	172	174	174	189	197	246	250	202	202	176	186	150	150	152	152	119	121	183	183
50	GargashiM	168	168	177	177	187	193	250	250	202	202	182	186	159	159	147	160	124	127	184	184
51	GargashiT	168	168	?	?	187	193	240	240	202	202	182	186	159	159	147	160	124	127	184	184
52	HammudiM	168	172	177	177	187	189	229	229	194	202	174	182	150	159	160	160	121	124	180	180
53	HammudiT	168	172	?	?	?	?	250	250	202	202	174	182	150	159	160	160	121	124	180	180
54	JabbugiM	172	172	177	177	187	193	240	240	202	202	174	186	159	174	152	160	124	124	180	180
55	JabbugiT	168	168	168	177	187	193	229	240	194	202	182	186	159	174	147	160	124	127	184	184
56	KalefyM	174	174	214	216	174	195	236	240	202	206	174	182	174	174	147	160	124	124	184	184
57	KarkubiM	170	174	187	187	189	195	236	246	202	202	168	186	150	174	147	147	121	127	184	184
58	KeddauiM	168	172	177	177	?	?	240	240	202	202	182	186	150	159	160	160	121	127	183	193
59	KhaddiraM	168	172	216	218	187	193	229	229	202	202	174	182	159	159	147	152	124	127	183	193
60	KhaddraM	168	172	216	218	187	193	229	229	202	202	174	182	159	159	147	152	124	127	183	193
61	MarisMi	172	174	179	179	195	197	236	246	202	202	174	176	?	?	147	147	119	124	184	184
62	MarrariM	166	168	218	218	189	195	250	250	202	202	174	186	150	174	147	152	121	127	184	184
63	MarrariT	166	168	218	218	189	195	240	240	202	202	174	186	150	174	147	152	121	127	184	184
64	MthemrM	166	172	216	218	187	189	240	240	202	202	174	186	150	174	152	152	121	124	180	180
65	MuktherM	166	174	216	218	189	195	240	240	202	202	174	186	150	150	?	?	127	127	183	193
66	NebgemelM	172	174	?	?	172	174	240	240	?	?	174	174	150	174	147	147	124	140	184	184
67	NinaiM	166	168	?	?	187	195	240	240	202	202	188	188	134	134	?	?	124	127	183	184
68	OuslatikussaT	174	174	168	168	189	195	?	?	?	?	168	186	159	159	147	183	121	140	183	184
69	QalbsardukM	166	168	177	177	189	203	250	250	202	202	174	174	150	150	121	184	124	140	184	184
70	RasliM	172	172	177	177	187	189	240	250	202	202	182	186	150	159	160	160	121	124	183	193
71	RasliT	172	172	202	202	195	197	240	240	202	202	176	178	150	159	147	171	121	140	183	189

Table A.9 Continued.

72	RumiM	168	176	177	177	187	203	240	240	202	202	174	174	150	150	147	152	124	127	180	189
73	SoudiaM	166	170	216	218	187	193	229	240	194	204	?	?	159	159	147	147	121	127	180	180
74	VqosM	166	174	216	218	187	195	?	?	?	?	174	186	174	174	147	147	124	127	180	184
75	YehuM	168	168	187	187	?	?	250	250	202	202	168	182	157	157	147	147	124	124	?	?
76	ZaafraM	166	168	?	?	189	195	240	240	202	202	174	186	150	150	147	152	121	127	184	184
77	ZaafraT	166	168	216	216	189	189	250	250	202	202	182	186	150	150	152	160	124	127	184	184
78	ZagloM	166	168	?	?	189	195	240	240	202	202	186	186	150	174	147	147	121	127	184	184
79	ZalmaG	174	174	168	168	189	195	240	240	202	202	168	186	159	159	183	184	121	140	183	184
80	ZalmtZa	174	174	168	168	189	195	240	240	202	202	168	186	159	159	183	184	121	140	183	184
81	ZarrasM	168	174	214	214	195	203	236	240	202	202	177	186	150	150	147	147	127	140	184	189
82	ZarrasiT	172	174	174	174	172	174	240	240	202	202	178	186	162	174	147	147	121	140	183	189
83	ZnbaiM	168	168	?	?	189	195	240	240	202	202	168	174	157	157	?	?	124	127	184	184
	POP = Wild																				
84	Ac#08	176	176	?	?	176	176	240	240	202	202	182	184	159	159	144	144	119	121	183	183
85	Ac#105	164	164	164	164	164	164	240	240	202	202	169	178	135	159	147	160	124	124	183	183
86	Ac#107	172	174	?	?	191	201	240	240	202	202	168	168	?	?	151	151	119	121	183	183
87	Ac#13	168	168	168	168	168	181	?	?	?	?	174	174	135	167	167	167	119	121	183	183
88	Ac#20	176	176	193	221	187	195	240	240	202	202	176	186	135	135	?	?	124	140	183	183
89	Ac#21	168	168	212	216	?	?	240	240	202	202	184	186	159	159	164	164	121	124	183	189
90	Ac#32	164	166	164	166	164	166	245	245	194	194	182	184	159	159	147	147	121	124	183	183
91	Ac#39	172	174	?	?	174	174	240	240	202	202	169	186	?	?	147	169	121	121	183	189
92	Ac#46	?	?	?	?	181	195	240	240	202	202	178	186	162	174	147	147	121	140	183	189
93	Ac#48	174	174	?	?	189	203	240	240	202	202	?	?	134	134	147	147	121	127	183	189
94	Ac#52	172	174	172	172	?	?	240	240	202	202	169	178	159	159	169	169	121	121	183	183
95	Ac#53	174	174	168	168	189	195	240	240	202	202	168	186	159	159	?	?	?	?	183	184
96	Ac#56	172	176	?	?	172	195	240	240	202	202	186	186	135	135	147	147	121	140	183	189
97	Ac#72	172	174	?	?	181	189	240	240	202	202	174	184	135	135	191	193	121	140	183	183
98	Ac#82	170	176	193	218	189	189	240	240	202	202	169	184	?	?	?	?	121	127	183	183
99	WildG	174	174	168	168	189	195	250	250	202	202	186	186	159	159	183	184	121	140	183	184

Table A.10 Final result of admixture model analysis along with their coifficent mempership obtained from STRUCTURE program.

#	Accessions Name	Population Designation	C1	C2	C3	Assignment AMA	Assignmen k3	Type of var	Use of fruit	Structure Type
44	ChemlaliM	2	0.975	0.011	0.014	1	1	Local	Oil	Wild / Introduced
74	Zalmati	2	0.973	0.019	0.008	2	1	Introduced	Oil	Wild / Introduced
37	PendolinoG	1	0.972	0.015	0.012	1	1	Introduced	Oil	Wild / Introduced
93	Accession53	3	0.972	0.01	0.018	1	1	Wild	Oil	Wild / Introduced
82	OuslatikussabatT	2	0.968	0.018	0.014	1	1	Local	Dual-purpose	Wild / Introduced
96	Accession82	3	0.968	0.015	0.017	1	1	Wild	Oil	Wild / Introduced
85	Accession08	3	0.967	0.024	0.009	4	1	Wild	Oil	Wild / Introduced
99	Accession32	3	0.966	0.007	0.027	4	1	Wild	Oil	Wild / Introduced
86	Accession13	3	0.965	0.016	0.018	4	1	Wild	Oil	Wild / Introduced
91	Accession48	3	0.965	0.023	0.012	1	1	Wild	Oil	Wild / Introduced
25	ChemlalisfaxG	1	0.015	0.973	0.012	1	2	Introduced	Oil	Wild / Introduced
32	MignoloG	1	0.952	0.029	0.018	1	1	Introduced	Oil	Wild / Introduced
72	ZarrasiM	2	0.946	0.023	0.031	1	1	Local	Dual-purpose	Wild / Introduced
88	Accession21	3	0.945	0.028	0.027	3	1	Wild	Oil	Wild / Introduced
97	Accession105	3	0.941	0.033	0.026	4	1	Wild	Oil	Wild / Introduced
53	KarkubiM	2	0.939	0.01	0.051	1	1	Local	Table	Wild / Introduced
45	Chemlalikussabat	2	0.938	0.023	0.04	1	1	Local	Dual-purpose	Wild / Introduced
98	Accession107	3	0.936	0.054	0.01	2	1	Wild	Oil	Wild / Introduced
17	MonopolyT	1	0.93	0.054	0.016	1	1	Introduced	Oil	Wild / Introduced
84	WildG	3	0.93	0.043	0.027	1	1	Wild	Oil	Wild / Introduced
57	MarisMi	2	0.911	0.066	0.023	1	1	Local	Oil	Wild / Introduced
33	MoraioloG	1	0.903	0.033	0.063	1	1	Introduced	Oil	Wild / Introduced
30	LeccinoG	1	0.012	0.982	0.006	2	2	Introduced	Oil	Introduced
18	MoraioloT	1	0.012	0.983	0.005	2	2	Introduced	Oil	Introduced
42	ZarrasiT	2	0.011	0.983	0.007	2	2	Local	Dual-purpose	Introduced
34	oliardoG	1	0.012	0.982	0.006	2	2	Introduced	Oil	Introduced
7	CuccoT	1	0.014	0.981	0.005	2	2	Introduced	Table	Introduced
26	CoratinaG	1	0.012	0.981	0.007	2	2	Introduced	Oil	Introduced
89	Accession39	3	0.013	0.981	0.006	2	2	Wild	Oil	Introduced
3	Belladispag	1	0.018	0.977	0.005	2	2	Introduced	Table	Introduced
31	MaurinoG	1	0.011	0.915	0.074	2	2	Introduced	Oil	Introduced
5	ChemlalisfaxT	1	0.956	0.037	0.008	2	2	Introduced	Oil	Introduced
13	LeccinopenduloT	1	0.02	0.973	0.007	2	2	Introduced	Oil	Introduced
27	FrantoioG	1	0.015	0.977	0.009	2	2	Introduced	Oil	Introduced
1	NepalTri	1	0.02	0.968	0.013	2	2	Introduced	Table	Introduced
92	Accession52	3	0.024	0.964	0.012	2	2	Wild	Oil	Introduced
94	Accession56	3	0.032	0.961	0.007	2	2	Wild	Oil	Introduced
83	RasliT	2	0.051	0.926	0.023	2	2	Local	Oil	Introduced
90	Accession46	3	0.05	0.924	0.026	2	2	Wild	Oil	Introduced
2	Ascolanaten	1	0.037	0.923	0.04	2	2	Introduced	Table	Introduced
47	GaianiM	2	0.051	0.888	0.061	2	2	Local	Dual-purpose	Introduced
4	Carmelitana	1	0.156	0.824	0.02	2	2	Introduced	Dual-purpose	Hybrid

Table A.10 Continued.

10	GrossadisardegnaT	1	0.149	0.818	0.033	2	2	Introduced	Table	Hybrid
11	GrossadispagnaT	1	0.24	0.708	0.052	2	2	Introduced	Table	Hybrid
95	Accession72	3	0.405	0.581	0.014	2	2	Wild	Oil	Hybrid
19	MorellonadigreciaT	1	0.79	0.183	0.027	2	1	Introduced	Oil	Hybrid
39	TombarellaG	1	0.881	0.089	0.03	1	1	Introduced	Oil	Hybrid
40	TunisianM	1	0.861	0.032	0.106	1	1	Introduced	Oil	Mixed genotypes
87	Accession20	3	0.765	0.161	0.074	2	1	Wild	Oil	Mixed genotypes
20	NardoT	1	0.68	0.272	0.048	1	1	Introduced	Oil	Mixed genotypes
61	NebgemelM	2	0.654	0.102	0.245	1	1	Local	Dual-purpose	Mixed genotypes
28	GragnanoG	1	0.611	0.203	0.186	3	1	Introduced	Oil	Mixed genotypes
36	OuslatiT	1	0.501	0.204	0.295	1	1	Introduced	Oil	Mixed genotypes
63	QalbsardukM	2	0.549	0.027	0.425	3	1	Local	Oil	Mixed genotypes
52	KalefyM	2	0.531	0.037	0.433	3	1	Local	Oil	Mixed genotypes
16	MignoloT	1	0.455	0.132	0.414	3	1	Introduced	Oil	Mixed genotypes
23	Santagostin	1	0.032	0.707	0.262	2	2	Introduced	Table	Mixed genotypes
21	OliarolasalentinaT	1	0.41	0.476	0.115	1	2	Introduced	Oil	Mixed genotypes
43	BeserriM	2	0.147	0.469	0.385	3	2	Local	Dual-purpose	Mixed genotypes
66	AnbiM	2	0.085	0.285	0.63	3	3	Local	Oil	Mixed genotypes
50	HammudiM	2	0.007	0.005	0.988	3	3	Local	Oil	Local
8	EnduriT	1	0.008	0.005	0.987	3	3	Introduced	Oil	Local
48	GargashiM	2	0.008	0.006	0.986	3	3	Local	Oil	Local
80	JabbugiT	2	0.01	0.005	0.985	3	3	Local	Oil	Local
56	KhaddraM	2	0.008	0.008	0.984	3	3	Local	Oil	Local
51	JabbugiM	2	0.007	0.01	0.983	3	3	Local	Oil	Local
60	MthemrM	2	0.009	0.009	0.982	3	3	Local	Dual-purpose	Local
29	KrusiG	1	0.014	0.006	0.98	3	3	Introduced	Oil	Local
41	ZaafraniT	2	0.012	0.008	0.98	3	3	Local	Oil	Local
79	HammudiT	2	0.01	0.01	0.98	3	3	Local	Oil	Local
78	GargashiT	2	0.016	0.006	0.978	3	3	Local	Oil	Local
58	MarrariM	2	0.013	0.01	0.977	3	3	Local	Oil	Local
46	FarkutiM	2	0.019	0.007	0.974	3	3	Local	Dual-purpose	Local

Table A.10 Continued.

64	RasliM	2	0.012	0.015	0.973	3	3	Local	Oil	Local
54	KeddauiM	2	0.015	0.012	0.972	3	3	Local	Oil	Local
81	MarrariT	2	0.019	0.01	0.971	3	3	Local	Oil	Local
68	VqosM	2	0.031	0.021	0.947	3	3	Local	Dual-purpose	Local
67	SoudiaM	2	0.046	0.009	0.946	3	3	Local	Oil	Local
70	ZaafraniM	2	0.05	0.011	0.939	3	3	Local	Oil	Local
35	OlivastroG	1	0.048	0.034	0.919	3	3	Introduced	Oil	Local
49	MuktherM	2	0.119	0.023	0.858	3	3	Local	Oil	Hybrid
59	MbutiM	2	0.125	0.029	0.846	3	3	Local	Dual-purpose	Hybrid
69	YehudiM	2	0.208	0.009	0.784	3	3	Local	Oil	Hybrid
6	CoratinaT	1	0.287	0.017	0.696	3	3	Introduced	Oil	Hybrid
38	RosciolaG	1	0.33	0.017	0.653	3	3	Introduced	Oil	Hybrid
65	RumiM	2	0.341	0.015	0.644	3	3	Local	Oil	Hybrid
71	ZagloM	2	0.383	0.014	0.603	3	3	Local	Oil	Hybrid
73	ZnbaiM	2	0.631	0.007	0.363	3	1	Local	Oil	Hybrid
24	CellinaG	1	0.623	0.019	0.358	3	1	Introduced	Oil	Hybrid
62	NinaiM	2	0.575	0.013	0.413	3	1	Local	Oil	Hybrid
15	MbutiT	1	0.549	0.008	0.444	1	1	Introduced	Dual-purpose	Hybrid

A.11 Large scale (2x CTAB) protocol for DNA extraction from lyophilized olive leaf tissue.

Reagents and buffers

Homogenization buffer	Per100ml.
2 %(w/v) Cetyl-Trimethyl-Ammonium -Bromide (2x CTAB)	2.0 g
1M Tris-Base	1.6 g
1.4 M NaCl	8.18 g
0.02 M Ethylene-Diamine-Tetraacetic -Acid (EDTA)	0.74 g
2 %(w/v) Polyvinylpyrrolidone-40 (PVP)	2.0 g
1% (w/v) Ascorbic Acid	1.0 g

(Store this solution at room temperature in the dark place pH 8.0)

Lysis buffer	Per100ml.
10 %(w/v) Sodium dodecyl Sulphate (SDS)	10 g
0.25 M Ethylene-Diamine-Tetraacetic -Acid (EDTA)	9.31 g
0.5 M Tris-Base	7.9 g

(Store this solution at room temperature pH 8.

Precipitation buffer (3M Potassium Acetate)	Per100ml.
29.4 % (w/v) Anhydrous Potassium Acetate	29.44 g
0.6 %Glacial Acetic Acid	60 ml

Binding buffer	Per100ml.
2M Guanidine hydrochloride in 95% Ethanol	19.106 g

(Store this solution at room temperature)

Mix and bring volume of above buffers to 100 ml with sterilized water (ddH2O).

Oxidation Inhibitor

1% (w/v) Diethyldithiocarbamic Acid sodium salt (DIECA)

Enzymes

1 mg/ml Proteinase K (store at 4℃)

10mg/ml RNase (store at -20℃)

Elution buffer (TE)

10mM Tris in H2O

Extraction Protocol

1-Weight 100-200 mg lyophilized olive leaf tissue and place 3 grinding beads in 1.5 cm strong Eppendorf tube and grind leaf tissue to powder by using high speed of Electric reciprocating power saw for 30 Seconds.

2- Immediately add 650 µl of pre-heat extraction buffer (300 µl homogenizer buffer 300 µl lysis buffers, 8 µl proteinase K, 8 µl of RNase and 1% DIECA (0.006g)) mix well by invert tubes by hand several times.

3-Incubate in water bath at 65C° for 30-60 minutes mixing occasionally every 20 minutes by using vortex mixer.

4- Add 150-200 µl potassium acetate mix well by invert tubes several times by hand and Incubate them on ice for 10 -20 minutes.

5- Centrifuge tubes at 14,000 RPM for 10 minutes in micro centrifuge.

6- Remove upper aqueous phase and transfer into a new clean tube while avoiding the middle and bottom layers.

7- Add 1.5 volumes of binding buffer (guanidine hydrochloride) into extracted supernatant from previous step and incubate at room temperature for 5 -10 minutes.

8- Transfer 700µl of mixture from previous step into mini spin column, centrifuge tubes at 14,000 RPM for 2 minutes, discard flow-through at the end of each centrifuge cycle, and repeat this step with the remaining sample.

9-Carefully remove the spin column from the collection tube without contact with the flow-through.

10- Place the spin column into a new collection tube; wash the column by passing twice 500 µl of cold 75% ethanol and spin tubes at 14,000 RPM for 2 minutes.

11- Remove any residual ethanol by spin the column for 7 minutes at 14,000 RPM without adding ethanol.

12- Suspend and elute the DNA from the column by adding 100 µl of preheated elution buffer (TE) (65C°) for 5 minutes to elute most of DNA.

13- Store DNA at 4C° for up to 1-3 weeks or at -20c° for a long time.

LIST OF ABBREVIATIONS

Abbreviation	Description
AB	Applied Biosystems
AFLPs	Amplified Fragment Length Polymorphisms
AMOVA	Analysis of Molecular Variance
ANOVA	Analyses of varianc
CLUMPP	CLUster Matching and Permutation Program
CTAB	Cetyl-Trimethyl-Ammonium -Bromide
DIECA	Diethyldithiocarbamic Acid sodium salt
EDTA	Ethylene Diamine Tetraacetic Acid
F	Inbreeding coefficient
GDA	Genetic Data Analysis
GP	Gene pool
GS	Genetic similarity
He	Expected heterozygosity
Ho	Observed heterozygosity
HW	Hardy Weinberg
IDT	Integrated DNA Technologies
IOC	International Olive Council
IP	Probability of identity
Na	Number of alleles
NJT	Neighbor-joining tree
PCR	Polymerase chain reaction
PIC	Polymorphism information content

PVP	Polyvinylpyrrolidone
R	Null allele frequency
RAPD	Random Amplified Polymorphic DNA
SDS	Sodium dodecyl Sulphate
SSR	Simple Sequence Repeats
STR	Short Tandem Repeat
Ta	Annealing temperature
TE	Elution buffer
TE	Tris and EDTA buffer
Tm	Melting temperature
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
VOO	Virgin olive oil