International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:8, No:12, 2014

Isolation and Identification of Diacylglycerol Acyltransferase Type- 2 (GAT2) Genes from Three Egyptian Olive Cultivars

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***Abstract*—**Aim of this work was to study the genetic basis for oil accumulation in olive fruit via tracking DGAT2 (Diacylglycerol acyltransferase type-2) gene in three Egyptian Origen Olive cultivars namely Toffahi, Hamed and Maraki using molecular marker techniques and bioinformatics tools. Results illustrate that, firstly: specific genomic band of Maraki cultivars was identified as DGAT2 (Diacylglycerol acyltransferase type-2) and identical for this gene in *Olea europaea* with 100% of similarity. Secondly, differential genomic band of Maraki cultivars which produced from RAPD fingerprinting technique reflected predicted distinguished sequence which identified as DGAT2 (Diacylglycerol acyltransferase type-2) in *Fragaria vesca* subsp. *Vesca* with 76% of sequential similarity. Third and finally, specific genomic specific band of Hamed cultivars was identified as two fragments, 1- *Olea europaea* cultivar Koroneiki diacylglycerol acyltransferase type 2 mRNA, complete cds with two matches regions with 99% or 2- Predicted: *Fragaria vesca* subsp. *vesca* diacylglycerol O-acyltransferase 2-like (LOC101313050), mRNA with 86 % of similarity.

Keywords*—Olea europaea*, fingerprinting, Diacylglycerol acyltransferase type- 2 (DGAT2**)**.

I. Introduction

OLIVE (*Olea europaea* L.) is one of the oldest agricultural tree crops worldwide and an important source of oil with beneficial properties for human health. Also, a majority and economically important crop for the new reclamation land in Egypt. Genetic patrimony of Mediterranean Basin's olive trees are very rich and are characterized by an abundance of varieties. The cultivated olives (*Olea europaea*, subspecies *europaea*) is a diploid species (2n = 2x = 46) [1]. Interestingly, originally of the olive tree is lost in time, coinciding and mingling with the expansion of the Mediterranean civilizations which for centuries governed the destiny of mankind and left their imprint on Western culture. Many molecular fingerprint techniques could be performed for olive oil, to identify olive cultivars that made up a certain olive oil [2]. Reference [3] traced the cultivar composition of monovarietal olive oils by AFLPs, suggesting that DNA extraction is the most critical step affecting the procedure. Dramatically decrease of DNA quality which extracted from olive oil, with a consequent loss of information a month later from olive oil production [4]. The associating of genetic characteristics and DNA-based molecular markers is very important to select the progeny showing interesting agronomical traits and even specific organoleptic characteristics at the first stages of development which may use as a marker for future olive oil identification. To detect the Triacylglycerols (TAG) biosynthesis is principally accomplished by membrane-bound enzymes that operate in the endoplasmic reticulum through the glycerol-3-phosphate or the so-called Kennedy pathway [5], [6]. The first step in the process involves the acylation of glycerol-3-phosphate (GP) at the sn-1 position to produce lysophosphatidic acid (LPA) by GP acyltransferase (GPAT). LPA is further acylated at the sn-2 position by LPA acyltransferase (LPAT) resulting in the formation of phosphatidic acid (PA). PA is dephosphorylated to produce diacylglycerol (DAG), which is further acylated to produce TAG by diacylglycerol acyltransferees (DGAT), the only enzyme in the pathway that is thought to be exclusively committed to TAG synthesis [7], [8].

A lot of plants accumulate large amounts of triacylglycerols (TAGs) in their seeds as storage reserves for germination and seedling development. Main points in the accumulation of TAGs are the early events of fatty acid biosynthesis and the last and critical events of TAG synthesis [8], [9]. On the other hand there are few fruit crops that deposit most of the oil in the monocarp tissues to attract animals for seed dispersal. Among them olive is of predominant economic importance because its oil is ideal for direct consumption. It is therefore of great importance to elucidate the key-points in the olive oil biosynthesis pathway and storage. This knowledge could speed up the breeding programs aimed at selecting clones with superior fatty acid composition and is also essential for selecting high oil-yielding genotypes more efficiently and rapidly, thus improving decision-making processes. Although, molecular basis of gene regulation underlying olive oil production is far from complete. There is a significant amount of information concerning the regulation of several genes involved in fatty acid synthesis and modification [10]-[12].

The study of DNA is the important factor in the analysis of polymorphisms, either by restriction or amplification profiling has been used to identify different genotypes of *Olea europaea* L. and other agronomic species. It was possible to discriminate between species and between genotypes, [13], [14]. Intensive modes of production favor the use of a few varieties with a stable and regular yield in olive cultivation, over a wide area associated with acceptable organoleptic

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characteristics. Many molecular characterization of Egyptian olive cultivars using RAPD has been done e.g. Mohamed and Yacout study of four Egyptians olive cultivars i.e. Hamed, Wateken, Maraki, Toffahi, and five European cultivars [15].

Reference [16] detected that the gene families have GAT2 (type-2) both of which are ER-localized. DGAT1 genes have been cloned from several plant species, including olive. DGAT2 genes have been cloned from diverse eukaryotes, including the oleaginous fungus *Mortierella ramanniana* and the plant species Arabidopsis [17]. A third member of the DGAT family (type-3), highly unrelated to the previously reported, was identified in peanut that possesses a cytosolic localization [18].

For getting better understanding of genetic bases for oil accumulation in olive oil via tracking DGAT2 (Diacylglycerolacyltransferase type-2) gene in three Olive cultivars namely, Toffahi, Hamed and Maraki, molecular marker techniques and bioinformatics tools were applied in this investigation.

II. Materials and Methods

A. Plant Material

For mining Diacylglycerolacyltransferase type-2 (DGAT2) genes and evaluate its role in fatty acid accumulation in different Olive fruit varieties, molecular and bioinformatical tools were applied.

These varieties are very ancient in origin and come from the Siwa oasis, Egypt. The variety Toffahi is self-compatible and has a low pistil abortion rate. Flowering and harvesting are early. Its productivity is medium. The fruit is freestone and has a very high flesh-to-stone ratio; it is used primarily for green pickling. It is moderately sensitive to damage during transportation and handling. The fruit is large and its oil content is low (5–7%). Hamed is self-compatible and has a low pistil abortion rate. Its productivity is high and constant. The fruit is large and very sensitive to damage during transportation and handling. The flesh-to-stone ratio of the fruit is high. Freestone, it is used for green and black pickling. It is resistant to drought and salinity and is grown on 6% of the Olive crop area. Oil olive content is from 9 to 16%. Maraki used for oil production, fruit is very heavy and has a medium flesh-to-stone ratio. Its oil is very high in oleic acid content and is of medium bitterness and the oil olive content is from 25 to 30%. Accounting for 2% of olive crop area, its distribution is currently limited but it has started to be propagated on a commercial scale and is expected to become Egypt's main oil cultivar.

Differential display molecular technique was employed to detect and compare DGAT2 (Diacylglycerolacyltransferase type-2) gene which catalyse the final step of the triacylglycerol (TAG) biosynthesis Kennedy pathway among three Olive cultivars namely, Toffahi, Hamed and Maraki (with low, moderate and high oil quantity and yield respectively).

B. Nucleic Acid Purification and PCR Reaction Preparation and Amplification

Total RNA Extraction and Construct C-DNA Libraries

Total RNA was extracted from three Olive cultivars using Gene JET™ RNA purification kit (Ferment life sciences. Co) according to manufacturer protocol. Extracted RNAs were turned to DNA through RT-PCR kit (Ready To-Go RT-PCR Beads, Amersham Bioscience). To amplify the central fragment of the degenerate primers FOR 1 (5-CCTTACTGTACTTTCTGGATTATGAAGCC-3) and REV2 (5-CCAGCTACCACCACAGTGACGATATGG-3) was designed for conserved regions of orthologous genes from GeneBank. Amplified products were separated by gel electrophoresis (1.0% Agarose). Resultant RT-PCR products were purified with Microcon spin filters and quantified spectrophotometrically for preparing for sequencing experiment through ABI Prism 7000 instrument based on manufacturer procedure.

Random Amplified Polymiorphic DNA (RAPD) Establishment:

Total genomic DNA was amplified through GeneAmp Polymerase Chain Reaction (PCR) system cycler. PCR for amplified genomic DNA was carried out. The reaction consists of 40 cycles; each cycle consisted of denaturation at 94°C for 30 sec followed by annealing at 30°C for 30 sec and extension at 72°C for 30 sec. There was an initial delay for 15 min at 95°C at the beginning of the first cycle and 10 min delay at 72°C at the end of the last cycle as a post extension step [19]. The product was stored at –20°C or 4°C.

C. Data Analysis

Gel documentation system (Photodoc-it, UVP, England), was applied for data analysis using Totallab analysis software, [20].

D. Nucleotide Sequence Accession Numbers

Nucleotide sequences of the DGAT2 from the RT-PCRs were submitted to Identified through NCBI BLAST program [21] as a single sense-strand contiguous sequence for each cultivar and compared with standard with AD22608.1.

E. Phylogenetic Analysis

Obtaining bands from RAPD fingerprinting technique was represented as 0,1 for absence and presence bands, respectively. Then, phylogenetic tree was constructed through PAST software analysis to detect genetic similarity among cultivars under study [22].

III. Results

Three distinguished genomic specific bands were obtained for DGAT2 gene of three Olive cultivars namely, Toffahi, Hamed and Maraki (with low, moderate and high oil quality and yield respectively). Corresponding to oil yield and quantity level, different band intensity were high, moderate and low intensity for Maraki, Hamed, and Toffahi, respectively. Thus, two characterized band for Maraki, Hamed

were sequenced to detect DGAT2 product. Moreover, differential genomic band which characterize Maraki cultivar for RAPD analysis was sequenced as a potential genomic band which could be play an important role in oil quality and yield metabolic pathways. Only characterized band for Maraki defined as DGAT2 gene after alignment in NCBI BLAST data base (Fig. 1). Sequencing analysis presents a huge help for differential genomic band identification which presence of Maraki cultivar for RAPD analysis and identified it as predicted *Fragaria vesca* subsp. *Vesca*.

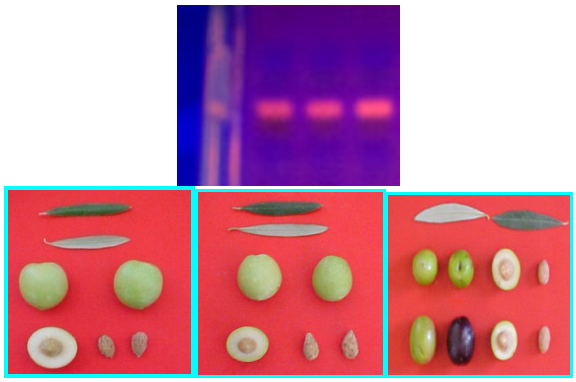


Fig. 1 Specific genomic band for maturity gene for three olive cultivars: 1-Toffahi (with low intensity), 2- Hamed (with moderate intensity), 3- Maraki (with high intensity)

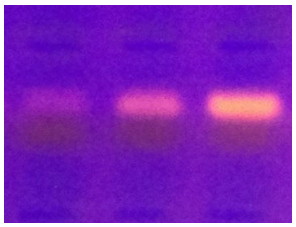
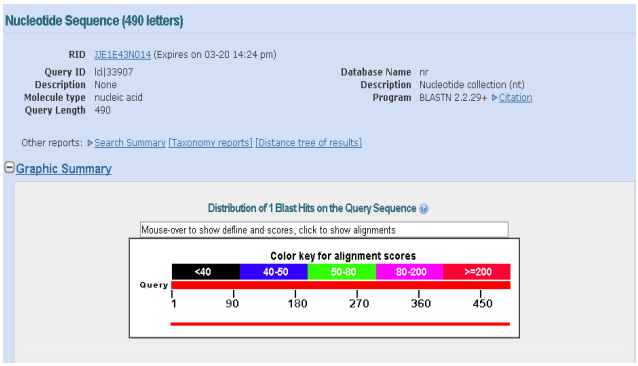


Fig. 2 RNA extracted fragments for all three olive cultivars: 1-Toffahi, 2- Hamed, 3- Maraki

On the other hand, differential genomic band of Maraki cultivars which outputted from RAPD fingerprinting technique reflected predicted distinguished sequence which identified as DGAT2 in *Fragaria vesca* subsp. *Vesca* with 76% of similarity.



Olea europaea cultivar Koroneiki diacylglycerol acyltransferase type 2 mRNA, complete cds

Sequence ID: gb|GU357635.1| **Length:** 1385 **Number of Matches:** 1

Range 1: 101 to 590 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
905 bits(490)	0.0	490/490(100%)	0/490(0%)	Plus/Plus

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Query  1    ATGACGTCGGAGGGGAATGGTGATATGCGGCGGCGGCAGCTGCTGGTGTCTGAGTCGGCG  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  101  ATGACGTCGGAGGGGAATGGTGATATGCGGCGGCGGCAGCTGCTGGTGTCTGAGTCGGCG  160

Query  61   CAACAGCCAACTCCGGCGGAGTTCAAGGGGACTCAAGGATCGATATTCCACACAATACTG  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  161  CAACAGCCAACTCCGGCGGAGTTCAAGGGGACTCAAGGATCGATATTCCACACAATACTG  220

Query  121  GCACTTGTATTGTGGTTGGGATCCGTTCACTTCAACATCATTGTTGTCTTGGCTTCCTTC  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  221  GCACTTGTATTGTGGTTGGGATCCGTTCACTTCAACATCATTGTTGTCTTGGCTTCCTTC  280

Query  181  GTCTTCCTCTCTTTTACTAAGGCTCTCATGGTCATTGGATTGCTTGTGATATTAATGGTA  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  281  GTCTTCCTCTCTTTTACTAAGGCTCTCATGGTCATTGGATTGCTTGTGATATTAATGGTA  340

Query  241  GCTCCAATTGATGATAGGAACAAATGGGGACGAAAATTAGCAAGGTATATATGTAAGCAT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  341  GCTCCAATTGATGATAGGAACAAATGGGGACGAAAATTAGCAAGGTATATATGTAAGCAT  400

Query  301  GCGGTTGGTTATTTTCCAGTGAATTTGTATGTAGAGGACATAAAAGCCTTCGATCCTAAT  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  401  GCGGTTGGTTATTTTCCAGTGAATTTGTATGTAGAGGACATAAAAGCCTTCGATCCTAAT  460

Query  361  GAGTCCTATGTTTTTGGCTACGAGCCACATTCAGTTTGGCCGATTGGAGTTGTTTCTCTG  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  461  GAGTCCTATGTTTTTGGCTACGAGCCACATTCAGTTTGGCCGATTGGAGTTGTTTCTCTG  520

Query  421  GCCGACCTAACAGGTTTCATGCCTCTCCAAAAAATCAAGGTTCTCGCTAGTACTGCTGTG  480
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  521  GCCGACCTAACAGGTTTCATGCCTCTCCAAAAAATCAAGGTTCTCGCTAGTACTGCTGTG  580

Query  481  TTCTACACAC  490
            ||||||||||
Sbjct  581  TTCTACACAC  590
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Fig. 3 Alignment results for Maraki specific band and its identity

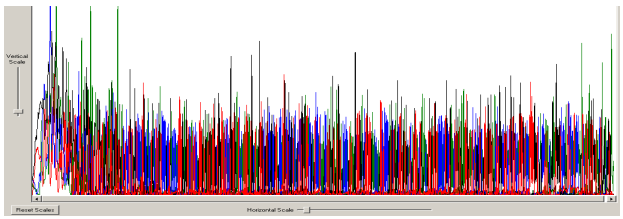

Fig. 4 Chromatogram of DGAT2 gene in *Olea europaea* with 100 % of similarity

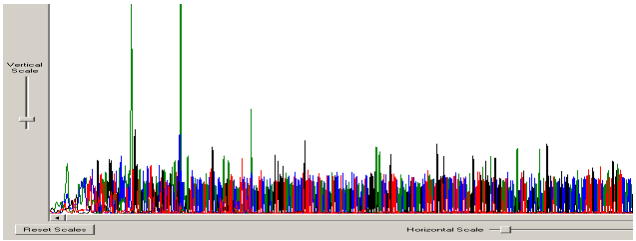

Fig. 5 Chromatogram of DGAT2 gene in *Fragaria vesca* subsp. *Vesca* with 76 % of sequential similarity

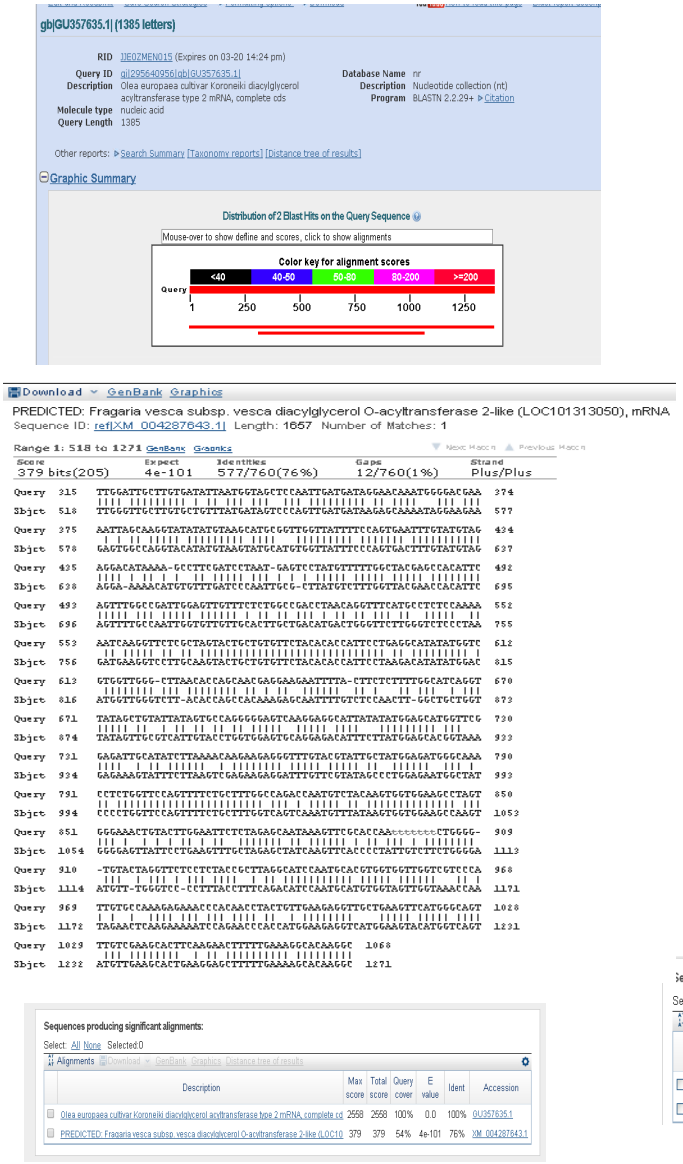

Fig. 6 Alignment results for differential Maraki specific band which produced from RAPD fingerprinting technique and its identity

Moreover, Fig. 7 showed alignment sequence for specific genomic specific band of Hamed cultivars which could be identified as 1- *Olea europaea* cultivar Koroneiki diacylglycerolacyltransferase type 2 mRNA, complete cds with two matches regions with 99% or 2- Predicted: *Fragaria vesca* subsp. *Vesca* diacylglycerol O-acyltransferase 2-like (LOC101313050), mRNA with 86 % of similarity.

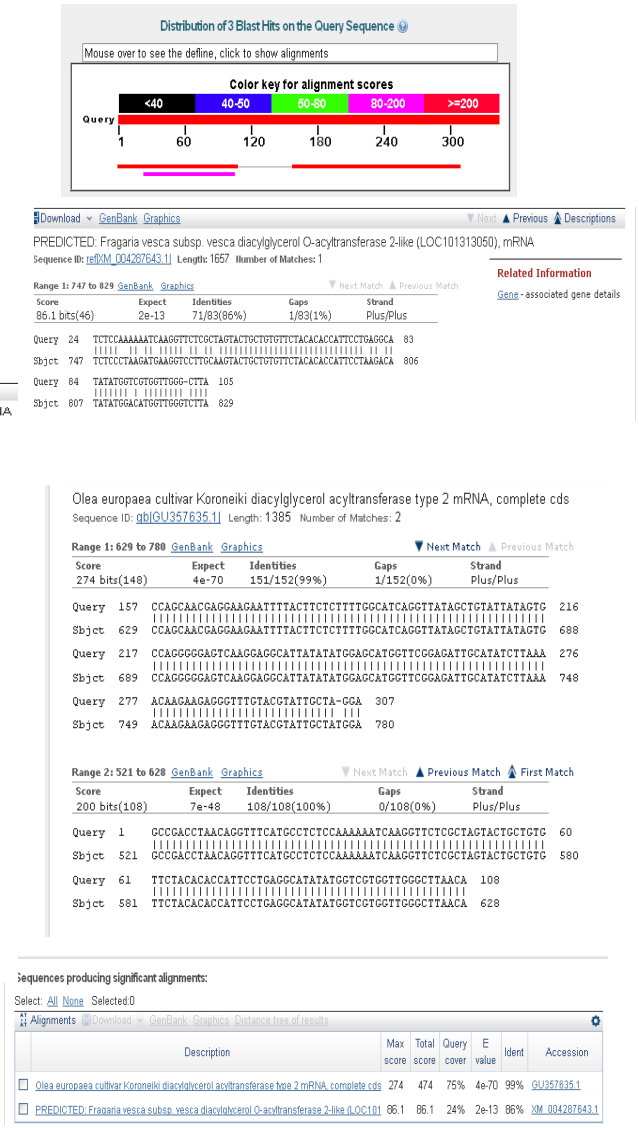

Fig. 7 Alignment sequence for specific genomic specific band of Maraki Hamed cultivar which identified as Koroneiki DGAT2 mRNA in *Olea europaea* cultivar 2) and identical for vesca diacylglycerol O-acyl transferase 2-like in *Fragaria vesca* subsp. with 100 % of similarity

IV. Conclusion

Although many efforts have been made in the last years, genome studies in *Olea europaea* L. are currently behind those of other crops. Several groups have started to work on the olive genome sequencing (i.e., OLEAGEN genomics project, Fundacion Genoma, Spain, [23]. and, thanks to the rapid development of the new sequencing technologies, now the complete sequence of olive genome will be available. The new information's on genome sequence will be very useful to identify genes involved in agronomical traits that could be used to improve the productivity and the nutritional characteristics of this crop. The Maraki Varity under study is cultivate in Siwa Oasis is high oil content and possible studies

of molecular mechanisms of drought and salinity tolerance of thesis Varity, in order to improve the cultivation of this important Varity also in the most arid and semiarid areas of the world. The knowledge of genome nucleotide sequences also could be useful to identify new sequence polymorphisms, which will be very useful in the development of many new cultivar-specific molecular markers (e.g., SNPs) and in the implementation of more efficient protocols for tracking and protect olive oil origin.

The roles of DGAT1 and DGAT2 in oil production, however, do not have to be mutually exclusive. In some plants, DGAT1 may play a more dominant role depending on gene expression patterns and protein accumulation, whereas in plants containing unusual fatty acids, DGAT2 may play a more important role. Nonetheless, the enrichment of enzymes for unusual fatty acid metabolism into distinct regions of the ER would provide an effective mechanism for excluding the unusual fatty acids from membrane lipids, which otherwise could be disruptive to the structure and/or functioning of the membrane lipid bilayer, and for channeling unusual fatty acids into storage oils.

Identification of molecular markers suitable for tracing the genetic identity of olive cultivars from which oil is produced, on the other hand, has a great importance**.** For making decision, which molecular markers will be more useful in obtaining reliable results through the numerous molecular markers existing in the literature, many of them have been practically examined (including RAPDs, AFLPs, SCARs, SSRs, ISSR, SNPs,). A combination of molecular markers (RAPD, ISSR, and SSR) to establish a relationship between small-scale produced monovarietal and commercial *olive oil* samples for certification purposes has been proposed.

REFERENCES

[1] S. Kumar; T. Kahlon and S. Chaudhary. A rapid screening for adulterants in *olive oil* using DNA barcodes, Food Chemi. 2011, 127,1335–1341
[2] C. Consolandi, L. Palmieri, M. Severgnini, E. Maestri, N. Marmiroli, C. Agrimonti, L. Baldoni, P. Donini, G. Bellis and B. Castiglioni. A procedure for olive oil traceability and authenticity: DNA extraction, multiplex PCR and LDR-universal array analysis. Eur. Food Res. Technol. 2008, 227:1429–1438
[3] S. Pafundo, C. Agrimonti and N. Marmiroli. Traceability of plant contribution in olive oil by amplified fragment length polymorphisms. J. Agric. Food Chem. 2005, 53, 6995-7002.S.
[4] Pafundo, M. Busconi, C. Agrimonti, C. Fogher and M. Marmiroli.. Storage-time effects on olive oil DNA assessed by amplified fragments length polymorphisms. Food Chem. 2010, 123:787–793
[5] E. P. Kennedy. Biosynthesis of complex lipids. Federation Proceedings. 1961, 20, 934–940.
[6] J. Browse, and C. Somerville. Glycerolipid synthesis: biochemistry and regulation. Annu. Rev. of Plant Phys. and Plant Molec. Biol. 1991, 42, 467–506.
[7] C. Jako, A. Kumar, Y. D. Wei, J. T. Zou, D. L. Barton, E. M. Giblin, P. S. Covello and D. C, Taylor. Seed-specific over-expression of an Arabidopsis cDNA encoding a diacylglycerolacyltransferase enhances seed oil content and seed weight. Plant Physi. 2001, 126, 861–874.
[8] S. C. Lung, and R. J. Weselake. Diacylglycerolacyltransferase: a key mediator of plant triacylglycerol synthesis. Lipids. 2006, 41, 1073–1088.
[9] B. G. Banials, K. Michael, I. Makariti, A. Kourti and P. Hatzopoulos. The olive DGAT2 gene is developmentally regulated and shares overlapping but distinct expression patterns with DGAT1. J. of Experi. Bot., 2011, 62 (2). 521–532.
[10] P. Hatzopoulos, G. Banilas, K. Giannoulia, F. Gazis, N. Nikoloudakis, D. Milioni and K Haralampidis. Breeding, molecular markers and molecular biology of the olive tree. Euro. J. of Lipid Scie. & Techno. 2002, 104, 574–586.
[11] S. Doveri and L. Baldoni. Olive. In: Kole C, ed. Genome mapping and molecular breeding in plants, fruits and nuts, Vol. IV. Berlin, Heidelberg: Springer-Verlag. 2007, 253–264.
[12] G. Banilas, and P. Hatzopoulos. Developmental perplexity of oil biosynthesis gene expression in olive. In: Berti L, Maury J, eds. Advances in olive resources. Kerala, India: Transworld Research Network, 2009, 1–22.
[13] G. Vergari, M. Patumi and G. Fontanazza. Use of RAPD markers in the characterization of olive germplasm. Olivae, 1996, 60:19-22.
[14] D.S. Hassawi and T. Hdeib. Genetic analysis of olive genotypes (*Olea europaea* L.) using random amplified polymorphic DNA (RAPD). J. Genet. & Breed. 2004, 58: 141-148.
[15] Y. I. Mohamed and M. Yacout. Random Amplified Polymorphic DNA (RAPD) Analysis of Olive *(Olea europaea* L.) cultivars grown in North west coast region of Egypt. Inter. J. of Envir. Engin. 2014,1 (2) 36-40.
[16] K. Giannoulia, K. Haralampids, Z. Poghosyan, D.J. Murphy and P Hatzopoulos. Differential expression of diacylglycerol acyltransferase (DGAT) genes in olive tissues. Biochemi. Soci. Transa. 2000, 28, 695–697.
[17] K. D. Lardizabal, J. T. Mai, N. W. Wagner, A. Wyrick, T. Voelker and D. J. Hawkins. DGAT2 is a new diacylglycerolacyltransferase gene family: purification, cloning, and expression in insect cells of two polypeptides from Mortierellaramanniana with diacylglycerolacyltransferase activity. J. of Biolo. Chemi. 2001, 276, 38862–38869.
[18] S. Saha, B. Enugutti, S. Rajakumari and R. Rajasekharan. Cytosolic triacylglycerol biosynthetic pathway in oilseeds: molecular cloning and expression of peanut cytosolic diacylglyerolacyltransferase. Plant Phys. 2006, 141, 1533–1543.
[19] M. Nelson. Lopera-Barrero, A. Jayme, P. Povh, R. Ribeiro, C. P. Gomes, B. Carolina. Jacometo and L. Tais da Silva. Comparison of DNA extraction protocols of fish fin and larvae samples: modified salt (NaCl) extraction. Cien. Inv. Agr. 2008, 35(1): 77-86.
[20] http://www.totallab.co
[21] http://www.ncbi.nlm.nin.gov/BLAST/
[22] http://www.Past. com
[23] www.chirimoyo. ac.uma.es/olea gen/