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EFFECT OF IRRIGATION TIMES AND TERMINATION ON GENE EXPRESSION OF *OeDGAT1, OeFAD2.1*, AND *OeFAD6* AND OIL QUALITY AND QUANTITY IN OLIVE CULTIVARS

ALI. S. AL-JANABI[1] and THAIRA. KH. AL-RAWI[2*]

[1]Department of Horticulture and Landscape Gardening, University of Kufa, Najaf, Iraq
[2]Department of Horticulture and Landscape Gardening, University of Baghdad, Baghdad, Iraq
*Corresponding author's email: thairaalrawi13@hotmail.com
Email address of coauthor: alialjenaby@yahoo.com

SUMMARY

This study was carried out on four olive (*Olea europaea* L.) cultivars (Khastawi, Nabali, Dikal and Souri) under different irrigation times and terminators to experience the gene expression for *OeDGAT1* - Diacylglycerol acyltransferases (DGATs), *OeFAD2.1* and *OeFAD6* and to determine the oil content (saturated and unsaturated fatty acid) during 2016 at Najaf, Iraq. The orchard of olive trees was grown at Najaf, Iraq and chilled in liquid nitrogen, and stored at -80 ℃. Pure RNA extracted and then cDNA syntheses with specific gene primers. The oil percentage (saturated and unsaturated) was also analyzed. Results showed that genes encoding *OeDGAT1* expression was high in cultivar Souri for all the treatments, while the *OeDGAT1* gene expression was more influenced by water terminator in cultivar Khastawi. The *OeFAD2.1* gene expression was high level in cultivar Souri and appears uninfluenced with different water treatments. However, *OeFAD2.1* gene expression was highly influenced with water terminators in cultivars Khastawi and Dikal. The *OeFAD6* gene expression was significantly high in cultivar Khastawi. Oil percentage (saturated and unsaturated) was significantly high in cultivar Souri, while cultivar Dikal fruits were observed with less content of unsaturated oils.

Key words: Olive, mesocarp fruit (ME), *OeDGAT1, OeFAD*, gene expression

Key findings: For diacylglycerol acyltransferase and oleate unsaturated fatty acid content, the genes *OeDGATI, OeFAD2.1,* and *OeFAD6* expressions were found responsible in olive mesocarp fruits. Cultivar Souri was highly affected by irrigation terminators; however, two other cultivars Dikal and Nibali were less affected. Irrigation treatments with eight and sixteen weeks (after full bloom) were found very important for these genes expressions and oil accumulation.

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INTRODUCTION

Olive (*Olea europaea* L.) is an evergreen species that ranks sixth in the world's production of vegetable oils (Alagna *et al*., 2009). Since ancient times it has been cultivated in the Mediterranean Basin, where ~2600 cultivars have been identified based on morphological traits (Hatzopoulos *et al*., 2002). Virgin olive oil is a natural fruit juice with exceptional organoleptic and nutritional properties. Oleic acid is the major fatty acid in the olive oil (55-83%), whereas linoleic acid accounts for 3.5-21% and α-linolenic acid for <1%. The relative contents of oleic, linoleic, and α-linolenic acids depend mainly on the variety but also on pedoclimatic and culture conditions (Beltran *et al*., 2004). Despite the enzyme activities involved in the biosynthesis and further transformation of these metabolites seem to determine the best cultivar has best quality of virgin olive oil (Williams *et al*., 2008).

In higher plants, fatty acid biosynthesis occurs in the plastid, yielding primarily palmitic and stearic acids by successive addition of two carbon atoms from acetyl-CoA (Harwood, 2005). Still in the plastid, most of the stearic acid is unsaturated by the soluble stearate unsaturated to oleic acid, which is the main product of the fatty plastid acid synthesis. The oleic acid is then incorporated into glycerolipids and can be further unsaturated to linoleic acid by two different Δ12-unsaturated, which differ in their cellular localization, lipid substrates, and electron donor systems (Shanklin and Cahoon, 1998). The microsomal oleate unsaturated (FAD2) is located in the endoplasmic reticulum and uses phospholipids as acyl substrates and NADH, NADH-cytochrome b5 reductase, and cytochrome b5 as electron donors. In contrast, the plastidial oleate unsaturated (FAD6) is located in the chloroplast and uses primarily glycolipids as acyl carriers and NAD(P)H, ferredoxin-NAD(P) reductase, and ferredoxin as electron donors. In olive, two genes encoding two microsomal oleate unsaturated (OepFAD2-1 and OepFAD2-2) have been isolated and characterized (Hernandez *et al*., 2005), whereas only one gene corresponding to the plastidial oleate unsaturated (OeFAD6) (Hernandez *et al*., 2005).

Plant lipids contain polyunsaturated fatty acids, mainly linoleic and α-linolenic acids, which play crucial roles in plant metabolism as storage compounds mainly in the form of triacylglycerols (TAG), as structural components of membrane lipids, and as precursors of signaling molecules involved in plant development and stress response (Ohlrogge and Browse, 1995; Weber, 2002). Linoleic acid, together with oleic acid, is a major fatty acid in vegetable oils and its content greatly affects technological properties such as their oxidative stability (Marqez-Ruiz *et al*., 1990) and nutritional characteristics (Cunnane, 2003).

A number of plants accumulate large amounts of triacylglycerols (TAGs) in their seeds as storage reserves for germination and seedling development. Key points in the accumulation of TAGs are the early events of fatty acid biosynthesis and the last and critical events of TAG synthesis (Bao and Ohlrogge, 1999; Jako, 2001; Lung and Weselake, 2006; Weselake, 2005). There are few fruit crops that deposit most of the oil in the mesocarp 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. Such 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. Nevertheless, the 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 (Banilas and Hatzopoulos, 2009; Doveri and Baldoni, 2007; Hatzopoulos *et al*., 2002), but much less is known about the cellular mechanisms governing the transfer of fatty acids into storage TAGs, not only in olive but generally in plants (Shockey *et al*., 2006). TAG biosynthesis is principally accomplished by membrane-bound enzymes that operate in the endoplasmic reticulum (ER) through the glycerol-3-phosphate or the so called Kennedy pathway (Browse and Somerville, 1991; Kennedy, 1961).

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 acyltransferase (DGAT), the only enzyme in the pathway that is thought to be exclusively committed to TAG synthesis. Inasmuch as DGAT catalyses the final and most critical step for TAG synthesis, it has been suggested that it may constitute a rate-limiting factor in TAG bioassembly in developing seeds (Ichihara *et al*., 1988; Jako *et al.*, 2001; Lung and Weselake, 2006). However, TAGs could also be produced via the transfer of acyl groups from phospholipids to diacylglycerols, an acyl-CoA-independent reaction catalyzed by the enzyme phospholipid: diacylglycerol acyltransferase (PDAT) (Dahlqvist *et al*., 2000; Stahl *et al*., 2004; Zhang, 2009). TAGs are not only produced in seeds or mesocarps. Both TAG accumulation and DGAT activity have been reported in several other organs, such as flowers, developing siliques, germinating seeds, young seedlings, and senescing leaves of *Arabidopsis* (Kaup *et al*., 2002; Zou, 1999), and in stems, flowers, roots, and leaves of tobacco (Zhang *et al*., 2005).

Based on those observations, it has been suggested that TAG may also be implicated in physiological roles other than as a carbon or energy source (Lu and Hills, 2002; Lu *et al*., 2003). Two major unrelated gene families have been shown to encode DGATs, namely *DGATI* (type-I) and *DGAT2* (type-2) both of which are ER-localized. *DGATI* genes have been cloned from several plant species, including olive (Giannoulia *et al*., 2000). *DGAT2* genes have been cloned from diverse eukaryotes, including the oleaginous fungus *Mortierella ramanniana* (Lardizabal *et al*., 2001), human (Cases *et al*., 2001), and the plant species *Arabidopsis* (Lardizabal *et al*., 2001), castor bean (Kroon *et al*., 2006) and

tung tree (Shockey *et al*., 2006). A third member of the DGAT family (type-3), highly unrelated to the previously reported, was identified in peanut that possesses a cytosolic localization (Saha, 2002).

Accumulating data suggest that DGAT activity may have a substantial effect on carbon flow into seed oil of *Brassica napus* (Perry *et al*., 1999; Shah *et al*., 2008), *Arabidopsis thaliana* (Jako *et al*., 2001; Zou *et al*., 1999), and maize (Zheng and Roesler, 2008). Environmental stresses represent limiting factors for plant productivity on the globe. Drought is one of the important abiotic stresses, constraining global crop production and quality seriously and recent global climate change and increasingly erratic weather patterns in the future are likely to enhance this situation more seriously (Secenji *et al*., 2008; Amtmann *et al*., 2009). Abiotic stress factors mainly include temperature, salinity, drought, anaerobic, and mechanical stresses on plants. In most cases, soil water deficits directly result in drought, which is closely linked with natural rainfall (Wan *et al.*, 2004; Materna and Davidson, 2007; Goldgur *et al.,* 2007; Liu *et al*., 2007). Drought is a complex physical-chemical process, in which many biological macromolecules and small molecules are involved ,such as nucleic acids (DNA, RNA, microRNA), proteins, carbohydrates, lipids, hormones, ions, free radicals ,mineral elements (Zhu *et al*., 2005; Karaba *et al*., 2007; Chen and Guo, 2008; Wechwerth, 2008). In an attempt to gain further insight into the role(s) of *DGATI*, *OeFAD2.1* and *OeFAD6* in plant lipid biosynthesis. The expression patterns of *DGATI*, *OeFAD2.1* and *OeFAD6* in several other organs/tissues of the olive tree indicated that genes are differentially regulated to fulfill the needs for TAG and FADs accumulation at certain points of growth and development under different conditions.

Therefore, a research project was planned to study the effects of irrigation times and termination on gene expression and oil quality and quantity in four olive cultivars. The detailed objectives were to: a) determine differences among four olive cultivars for three genes (*OeDGATI*, *OeFAD2.1* and *OeFAD6*) expressions responsible for diacylglycerol acyltransferase and oleate unsaturated in olive mesocarp fruits. b) analyze gene expression using quantitative real time PCR for (*OeDGATI*, *OeFAD2.1* and *OeFAD6*) among four cultivars influenced by irrigation terminators and water levels. c) analyze olive cultivars for oil and compare the oil quantitative traits between cultivars influenced by irrigation terminators and waters levels, and d) analyze the unsaturated fatty acids among cultivars and to compare percent fatty acid between cultivars and their correlation with level expression.

MATERIALS AND METHODS

Plants material and procedure

The mesocarp fruit (ME) of the four cultivars of olive (*Olea europaea* L.) i.e., Khastawi, Nabali, Dikal and Souri was used during 2016 at Najaf, Iraq. The orchard of olive trees was grown and chilled in liquid nitrogen, and stored at -80 ℃.

Irrigation treatments

All olive tree cultivars were irrigated before full bloom and after full bloom divided in to four blocks to compare the four levels of irrigation and their effects on olive cultivars.

I_0 = without irrigation after full bloom for 1 Block of cultivars olive as control

I_1 = irrigation 8 weeks after full bloom (WAF) for 1 Block of cultivars olive.

I_2 = irrigation 8 and16 weeks after full bloom (16 WAF) for 1 Block of cultivars olive.

I_3 = irrigation 8, 16 and 24 weeks after full bloom (WAF) for 1 Block of cultivars olive

All the treatments were replicated three times using whole tree replications in a randomized complete block design (RCBD). All data were recorded on 28 WAF, and subjected to analysis of variance and means were separated by Duncan's Multiple Range Test at the 5% level.

Total RNA extraction and cDNA synthesis

Total RNA for samples were isolated by uses (SV Total RNA Isolation kit/ Promega. USA).The quality of RNA was verified by demonstration of intact ribosomal bands following agarose gel electrophoresis. DNA was removed from RNA samples using the DNase I Mix/ Promega, USA (DNase I, MnCl2, yellow core buffer). First-strand c DNA was synthesized from (16 µl) of total RNA using the (power c DNA Syntheses kit/IntronBio. Inc. USA) with Oligo (dT) 15 primer, following the manufacturer's instructions and quantified using gel electrophoresis.

Quantitative Real Time PCR (qRT-PCR)

Gene expression analyses were performed by qRT-PCR using a Mini Option's System real-time PCR and GO Taq Master Mix SYBR Green kit Q-PCR/ IntronBio. Inc. USA. Primers for gene specific amplification were designed to generate a product of 100-200 bp and to have a Tm (melting temperature) of 60 ℃. PCR reactions were carried out in duplicate in plate. Reaction mix (22.5 µl per well) contained 12.5 µl, Master Mix SYBR Green, 2.5 µl forward and reverse primers,7.5 µl DEPC-D.W and 2.5 µl of c DNA. The thermal cycling conditions consisted of an initial denaturation step of 95 ℃ for 10 min, followed by 40 cycles of 95 ℃ for 30 s, 60 ℃ for 1 min, and 72 ℃ for 30 s. The specificity of the PCR amplification was monitored by melting curve analysis following the final step of the PCR products were also checked for purity by agarose gel electrophoresis. The housekeeping olive Actin gene (*OeActin*) was used to normalize as endogenous reference. The real-time PCR data were analysis by Gene X programs. The gene of the olive tree, used for the design of primers are available in Gen Bank (Gen Bank/EMBL/DDBJ).Primers for QRT-PCR amplification were two parts *FAD2.1* (N. AY733076) forward (GCAATCAAG CCACTAT TAGGTG) and reverse (GACATA AAGACACT CCTTCGC) *FAD6* (N. AY772187), forward (GAACGA GG CTACA TGGAAC) and reverse (GTTGTGAATCTTTGGGT GC) The primers sequences *DGAT1* forward (5-TTGGCTGAATATATTAGCGGAACTTC-3) and reverse (5- CTCATCATA AAAATGTCCA CATCC-3) and *Actin* gene primer were forward (5-

ACCACCTCAGCCGA ACGGGA-3) and reverse (5-TGCTGGGAGCC AAGGC AG TG-3).

Chemical analysis

Chemical analysis were used on fruits like moisture %, oil D.W%, oil F.W and uses Soxhlet method to determination oil percentage (%) in moist and dry of flesh four olive cultivars and addition to saturated and unsaturated fatty acid (%) based on Gas – chromatography.

RESULTS AND DISCUSSION

The results showed the total isolated RNA from flesh mesocarp fruit (ME) of the different olive cultivars studied manner filters and then migrated to agar gel 1%, voltage 100V for 20 minutes noting the success of the method to isolate RNA from this plant part (Figure 1).

The different *DGAT1* gene expression in mesocarp of different olive cultivars were influenced by water terminators during periods of time fruits development (Figure 2a, b, c, d). The I0 and I1 treatments showed that high *DGAT1* was present in cultivar Souri for all treatments reached 4.20, 5.5, 10 and 10.5 fold, respectively. However, the *DGAT1* gene expression in cultivar Khastawi mesocarp was more influenced by water terminator especially I0 and I1 treatments and actively began gradually increase by I2 and I3 treatments, in the other words the level of gene expression for DGAT1 gene in cultivar Khastawi began increase in conjugation with increase water levels was clearly evident in I2 and I3 treatments reached 7 and 9.75 fold respectively. Cultivars Nabali and Dikal observed with less gene expression and less influence by water terminators.

The *FAD2.1* gene expression was high level in cultivar Souri and appear uninfluenced with different treatments for water terminators during the time of fruit development if compared with other cultivars, especially gene expression for I0, I1 and I2 treatments reached 36, 46 and 48, respectively (Figure 3a, b, c, d). The *FAD2.1* gene expression for cultivars Khastawi and Dikal was highly influenced with water terminators and irrigation levels especially in I0 treatment. The *FAD2.1* gene expression gradually began activity and conjugation with increase water level in treatments I1, I2 and I3.

The *FAD6* gene - expression for olive cultivars under study showed the high level gene expression for treatments I0, I1 and I2 in cultivar Khastawi as compare to other cultivars (Figure 4a, b, c, d). On the other hand, cultivar Souri was second after Khastawi in gene expression for I0, I1 and I2 treatments. However, by comparing with other cultivars, the cultivar Souri revealed highest gene expression for I3 treatment. Cultivars Nabali and Dikal exhibited less level of *FAD6* gene expression. Although the *FAD6* gene expression was high for cultivar Souri in I3 treatment but the gene expression was not significant as compare to other cultivars.

The moisture was significantly high in cultivar Dikal fruits for I0, I1, I2 and I3 treatments with values of 50.1, 49.3, 57.9 and 65.7%, respectively (Table 1). However, less moisture was observed in cultivar Souri fruits for treatments I0, I1, I2 and I3 reached 30.6, 31.9, 35.9 and

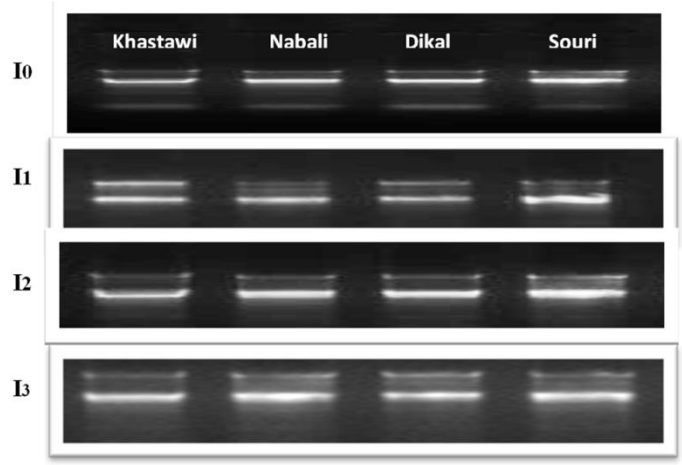

Figure 1. Represents total isolated RNA of the mature mesocarp (MS) of four olive cultivars on agarose gel (1%) and voltage (100 V) for (20 minutes). I0 = without irrigation of cultivars olive as control I1= irrigation 8 weeks after full bloom (WAF). I2= irrigation 8 and16 weeks after full bloom (16WAF). I3 = irrigation 8,16 and 24 weeks after full bloom (WAF).

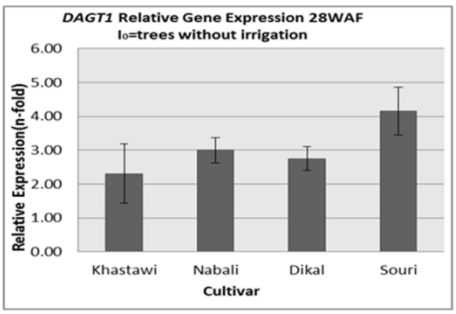

Figure 2a

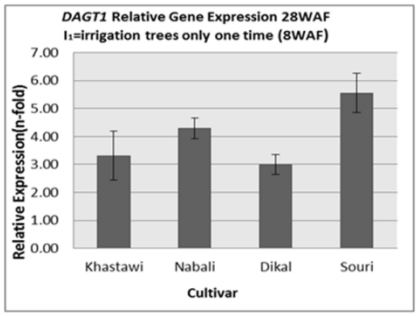

Figure 2b

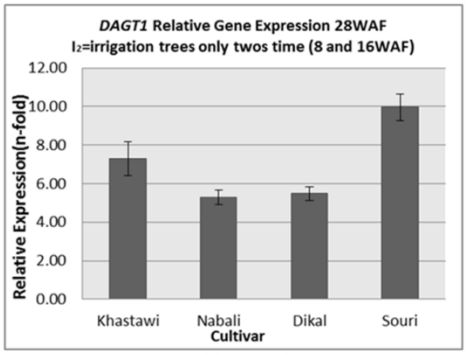

Figure 2c

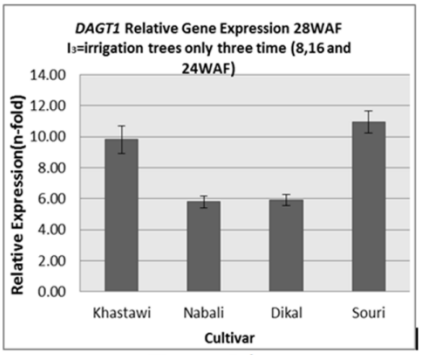

Figure 2d

Figure 2a, b, c, d. Relative gene expression for *DGAT1* gene under different irrigation terminator with different development fruits periods for olive cultivars i.e., Khastawi, Nabali, Dikal and Souri. I0 = without irrigation of cultivars olive as control I1 = irrigation 8 weeks after full bloom (WAF). I2 = irrigation 8 and 16 weeks after full bloom (16 WAF). I3 = irrigation 8,16 and 24 weeks after full bloom (WAF).

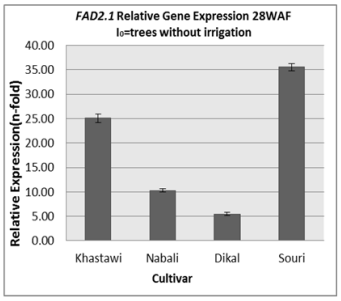

Figure 3a

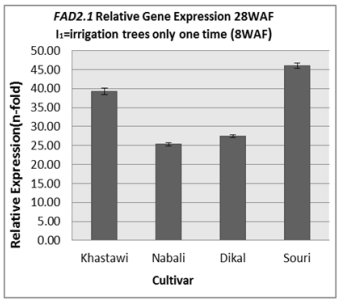

Figure 3b

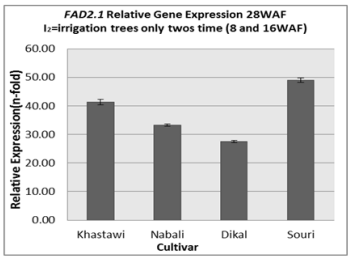

Figure 3c

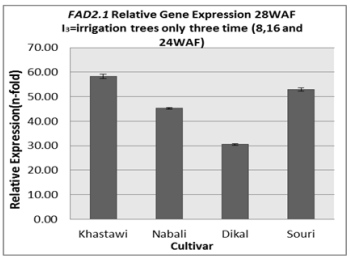

Figure 3d

Figure 3a, b, c, d. Relative gene expression for FAD2.1 gene under different irrigation terminator with different development fruits periods for olive cultivars i.e., Khastawi, Nabali, Dikal and Souri. I0 = without irrigation of cultivars olive as control I1= irrigation 8 weeks after full bloom (WAF). I2 = irrigation 8 and 16 weeks after full bloom (16 WAF). I3 = irrigation 8, 16 and 24 weeks after full bloom (WAF).

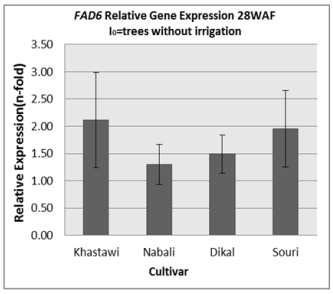

Figure 4a

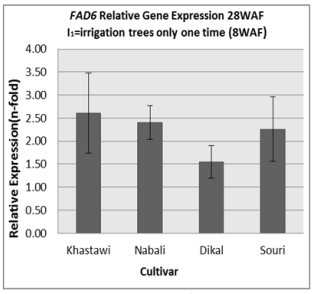

Figure 4b

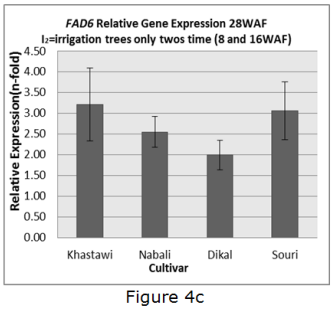

Figure 4c

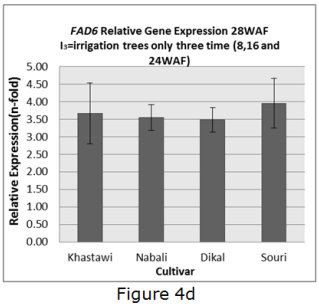

Figure 4d

Figure 4a, b, c, d. Relative gene expression for FAD6 gene under different irrigation terminator with different development fruits periods for olive cultivars i.e., Khastawi, Nabali, Dikal and Souri. I0=Without irrigation of cultivars olive as control I1 = irrigation 8 weeks after full bloom (WAF). I2 = irrigation 8 and16 weeks after full bloom (16 WAF). I3= irrigation 8,16 and 24 weeks after full bloom (WAF).

Table 1. Moisture and oil (dry and fresh) percentage under different irrigation terminator with different development fruits periods.

Traits	Moisture (%)				Oil dry weight (%)				Oil fresh weight (%)			
	I_0	I_1	I_2	I_3	I_0	I_1	I_2	I_3	I_0	I_1	I_2	I_3
Olive Cultivars												
Khastawi	45.3b	50.8a	52.8a	57.4b	14.8c	17.5c	19.3c	20.3c	10.5c	12.3c	12.9c	15.7c
Nabali	39.6bc	38.2b	37.9b	42.5c	30.3b	31.3b	32 b	32.7b	25.5b	26.7b	26.9b	30.9b
Dikal	50.1a	49.3a	57.9a	65.7a	9.3d	10.2d	11.2d	11.5d	4.5d	6.1 d	6.9 d	11.2d
Souri	30.6c	31.9b	35.3b	35.9d	54.4a	60.9a	61.3a	63.8a	40.2a	45.1a	50.6a	53.3a

I_0=Without irrigation of cultivars olive as control I_1= irrigation 8 weeks after full bloom (WAF), I_2= irrigation 8 and16 weeks after full bloom (16 WAF), I_3= irrigation 8,16 and 24 weeks after full bloom (WAF).

for moisture content. The cultivar Nabali was the less affected with different treatments and irrigation levels then the moisture were rose when addition other water levels and the difference value reached 2.9%. The results showed the dry weight for cultivar Souri was high significant with content oil it gradual rose when increase irrigation levels reached 54.4, 60.9, 61.3 and 63.8% and the cultivar Souri fruit was more affected with water terminators with difference value 19.4%. However, the cultivar Dikal fruit was less affected with difference treatments and irrigation levels in fruit, oil content percentage in dry weight reached 9.3, 10.2, 11.2 and 11.5% with difference value 3.2%. The high significant of oil percentage in fresh weight of cultivars appeared in cultivar Souri for all treatments of irrigation treatments reached 12.1%. However, cultivar Dikal fruit was the less affected with different treatments I0, I1, I2 and I3 and provided 4.6, 6.1, 6.9 and 11.2% oil in fresh weight.

The fruit oil analysis for cultivars in this study by gas chromatography showed that fatty acid unsaturated oleic, linoleic and linolenic acid were high percentage in Souri fruit for all water treatments, while cultivar Dikal fruit was less content for fatty acid unsaturated compared with others cultivars (Table 2). The cultivar Souri was more affected with irrigation terminators which gave 1.7% as difference value while less difference value and less affected with irrigation terminators appear in other cultivars Khastawi, Nabali and Dikal reached 1%. Treatments I2 was very important for *OeDGATI, OeFAD2.1,* and *OeFAD6* gene expression and oil accumulations, suggesting that probably the differential response among cultivars back to different genetic materials and gene expressions pattern.

Considering the interacting complexity (at least including water movement, solute transport, information exchange, ion homeostasis regulation, and other related phiso-chemical changes) between plants and their surroundings, it is necessary to generalize the performance of physiological functions for higher

Table 2. Oleic, linoleic, and linolenic acid percentage under different irrigation terminator with different development fruits periods.

Traits	Oleic acid (%) 18:1			Linoleic acid (%) 18:2			Linolenic acid (%) 18:3					
	I_0	I_1	I_2	I_0	I_1	I_2	I_0	I_1	I_2	I_0	I_1	I_2
Olive Cultivars												
Khastawi	60.1a	63.1a	66.0 b	66.3 b	6.12 b	7.33b	8.51c	8.90	0.9 a	1.1a	1.5a	1.9b
Nabali	60.9a	58.1a b	59.0c	60.6 b	10.5 a	10.8 a	11.2 b	12.1	0.5 b	0.9a	1.3a b	1.5b
Dikal	20.4b	22.2 b	27.4 d	30.1c	7.10 b	7.92b	8.80 c	9.20	0.6 b	0.8a	1.1b	1.6b
Souri	60.9a	65.7a	73.4 a	75.5 a	9.77 a	11.80 a	14.11 a	18.80 a	1.01 a	1.2a	1.8a	2.9a

I_0=Without irrigation of cultivars olive as control I_1= irrigation 8 weeks after full bloom (WAF), I_2= irrigation 8 and16 weeks after full bloom (16 WAF), I_3= irrigation 8,16 and 24 weeks after full bloom (WAF).

surroundings, it is necessary to generalize the performance of physiological functions for higher plants under drought stress. Soil water is one of key factors influencing plant production and many reports have proved this clearly (Zhu *et al*., 2005; Karaba *et al.*, 2007; Chen and Guo, 2008; Wechwerth, 2008). Loss of water in soil will lead to great reduction in plant production, which has been reflected from total grain yield of many countries in the world (Asada, 2006; Andrew *et al*., 2006).

Soil water is also the important material for photosynthetic reactions that plants depend on to finish accumulation of photosynthetic products, which are impacted greatly by physiological pathways and environmental factors (such as soil water supply) (Beer and Travazoie, 2004; Bray, 2004; Angela, 2004; Andjelkovic and Thompson, 2007). The influence of water deficits for plant metabolism is very apparent, which is mainly restraining the anabolism by reducing the activity of synthase and strengthening the catabolism by increasing the activity of hydrolytic enzymes. This includes the reduction of protein, chlorophyll, DNA, RNA and plant growth hormone synthesis, which could destroy the normal metabolism and cause growth disorder. So, different soil water supplying will result in quite different physiological pathways, which directly determine the ability for plants to make photosynthetic products.

Water deficits in soil environment also influence solute transport (ion and nutrient uptake of plants) to larger extent, which effects on photosynthetic reactions in plant chloroplasts in many ways (Cushman and Bohnert, 2000; Chaves *et al*., 2003). This is the reason that ion homeostasis and redox state have been brought to attention (Doelle, 2002; De Ronde *et al*., 2004). Recent progress in molecular biology and bioinformatics (especially, DNA microarray technology), genomics, proteomics, metabolomics and transcriptomics) has provided insight into plant gene regulatory network system, which is mainly composed of

inducible-genes (environmental factors and developmental cues), their expression programming and regulatory elements (cis-element and trans-element), corresponding biochemical pathways and diverse signal factors (Sakuma *et al*., 2003; Shinozaki *et al*., 2003; Shinozaki and Dennis, 2003).

The genetic information for drought tolerance is expressed in many prokaryotes and lower eukaryotes, but only in very few higher plants. In higher plants, only seeds can survive for extended periods without water. Exceptional among higher plants is the small group of angiosperm plants termed 'resurrection plants' which can recover from complete dryness within one day of contact with water (Bartels *et al*., 1990). Under the condition of soil water deficits, related stress factors always result in overlapping responses, including anatomical, physiological, biochemical, molecular biological changes, which make plant gene regulatory network system more complicated and difficult to explore. Much information with respect to this topic is from the model plant, *Arabidopsis thaliana*. Much analysis of genomic expression profiling by DNA microarray indicates that the mRNA coding transcriptional element genes in many plants are usually induced to express and accumulated (Munns, 2002; Mark and Antony, 2005).

Most transcriptional element genes involved in plant stress responses have not only completely different expression profiles, but also some overlapping expression profiles, showing the complexity, specificity and crosstalk of plant gene regulatory network system (Bray, 2004; Angela, 2004; Andjelkovic and Thompson, 2007). In other words, one kind of stress may simultaneously activate many transcriptional elements and one transcriptional element may be activated by many types of plant stress responses (Cushman and Bohnert, 2000; Chaves *et al*., 2003; De-Ronde *et al*., 2004). It is mainly in leaf and epicotyl, and its transcription is regulated by water-stress, salt stress and plant hormone at the same time (Yamaguchi *et al.*, 1989).

CONCLUSION

The genes expression of *OeDGATI, OeFAD2.1,* and *OeFAD6* were found responsible for diacylglycerol acyltransferase and oleate unsaturated in olive mesocarp fruits share overlapping but distinct transcription patterns during vegetative growth under different irrigation terminator with different development fruits periods. The cultivar Souri was highly affected by irrigation terminators; however, cultivars Dikal and Nibali were less affected. Irrigation treatments with eight and sixteen weeks (after full bloom) were found very important for these genes expression and oil accumulation.

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