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Cloning of lipase cDNA from *Olea europaea* cv. Gemlik leaves and expression analysis in response to cold stress

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Abstract: The cDNA encoding lipase enzyme was cloned from *Olea europaea* var. *europaea* cv. Gemlik leaves using the RACE method. The full nucleotide sequence was 1546 bp long and encoded a putative protein of 412 amino acid long protein. The amino acid sequence showed identity to Triacylglycerol Lipase 1 sequences from different plants. A phylogenetic tree was constructed showing relations with various plant lipases. Multiple sequence alignment analysis of the putative protein sequence revealed that the enzyme contains conserved GXSXG pentapeptide motif, and the catalytic triad was predicted as Ser-182, His-379, and Asp-350 with bioinformatics tools. Real-time PCR was used to determine relative expression levels of lipase in leaves in response to cold stress.

Key words: Lipase, olive, RACE, stress, real-time PCR

1. Introduction

Lipases (triacylglycerol acylhydrolases, EC3.1.1.3) catalyze the hydrolase triacylglycerols to free fatty acids and glycerol (Sharma et al., 2001). Under certain conditions they can also catalyze esterification, interesterification, and transesterification reactions. Furthermore, characteristics such as stability in the presence of organic solvents, no necessity for cofactors for their action, and high enantioselectivity turn lipases into a group of enzymes with major technological interest (Ke and Klibanov, 1999).

Lipases have applications in food, detergent, oil, organic synthesis, pharmaceutical, cosmetic, and biodiesel industries. Microbial lipases represent the most widely used class of enzymes in biotechnological applications and organic chemistry. Plant lipases have recently begun to attract interest because of their high substrate specificity. One of the most studied plant lipases, *Carica papaya*, has been used for the synthesis of low calorie short and long chain triacylglycerols (Mangos et al., 1999), as well as synthesis of a cocoa butter equivalent from palm oil (Pinyaphong and Phutrakul, 2009). *Brassica napus* lipase is another interesting plant lipase that can catalyze esterification reactions (Hills and Mukherjee, 1990).

Apart from their industrial applications, lipases also began to gain attention for their role in stress responses. Some lipases were reported to be involved in ultraviolet B stress (Lo, 2004), salt and osmotic stress (Ellinger and Kubigsteltig, 2010), desiccation stress (Zhang et al., 2016), pathogen stress (Jakab et al., 2007), disease susceptibility, and abiotic stress tolerance (Hong et al., 2008).

Olive tree (*Olea europaea* L.) is an important oil and food crop for Mediterranean Basin. It is an evergreen species which can endure cold winter conditions. The presence of acylhydrolase activity has been reported in crude olive extracts, exhibiting pH activity around a maximum of pH 8.5 (Olías, 1993). Panzanaro et al. extracted acid lipase activity in olive lipase from the olive fruit mesocarp (Panzanaro et al., 2010). These data suggest that there are different forms of lipases in olive with different characteristics. To date, there is no report on cloning the full length cDNA of any lipase from olive fruits or leaves, or investigation of their relation to other lipases. In this study, we cloned the lipase coding sequence of *Olea europaea* L. var. *europaea* cv. Gemlik (olive) leaves for the first time. We analyzed the phylogenetic relations of this lipase with other lipases. We also analyzed expression levels of lipase in cold stress with real-time PCR.

2. Materials and methods

2.1. Plant materials and RNA isolation

2-year-old *Olea europaea* L. *europaea* cv. Gemlik seedlings were obtained from Ataturk Horticultural Central Research Institute in Yalova.

Total RNA was extracted from olive leaves using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Genomic DNA and chemical

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residues were eliminated by following the Qiagen RNeasy Mini Kit Cleanup Protocol (Qiagen N.V., Venlo, The Netherlands).

2.2. Rapid amplification of cDNA ends (RACE) reactions

EST database was screened with different plant lipase sequences by BLAST analysis for 3' RACE gene specific primer design. One olive EST sequence (GO246365.1) was found to be 77% identical to the lipase sequences. GO246365.1 EST sequence was used for designing gene-specific forward primer (LEF1) 5'-CGTTGAAAGTGGCGCTCTTA-3'.

3' and 5'RACE reactions were performed with a Clontech Smarter RACE Kit (Clontech/Takara Bio, Mountain View CA, USA); 1 µg total RNA was used for first strand cDNA synthesis. 3'RACE PCR was carried out using LEF1 and UPM (included in kit) primers. MyFi™ Polymerase Enzyme (Bioline, Memphis, TN, USA) was used for PCR, and parameters were 3 min of initial denaturation at 95 °C followed by 35 cycles of 95 °C for 40 s, 55 °C for 40 s, 72 °C for 1 min, and final extension at 72 °C for 10 min.

First strand cDNA was synthesized according to the manufacturer's instructions for 5'RACE. The 5' end of the cDNA was amplified using gene-specific reverse primer (LRR1) 5′-TAAAGATATATGGCCATGACACCAG-3′ designed using the 3' RACE sequence. 5'RACE-PCR was performed with UPM and LRR1 primer using MyFi™ DNA Polymerase (Bioline, Memphis, TN, USA). PCR conditions were at 95 °C for 2 min, followed by 95 °C for 30 s, 55 °C for 30 s, 72 °C for 40 s, and final extension at 72 °C for 10 min.

2.3. Cloning in *E. coli* and sequencing

3' RACE and 5' RACE PCR fragments were purified from agarose gels using the Biomatik SpinKlean™ Gel Extraction Kit (Biomatik, Cambridge, ON, Canada). Promega PGEMT Easy Vector (Promega, Madison, WI, USA) was used for T/A cloning of purified products. Plasmids were transformed into the competent *E. coli* DH5α cells prepared according to Inoue ultracompetent cell protocol (Inoue et al., 1990). Positive clones were screened using ampicillin/LB plates that contained IPTG/X-Gal and sent for DNA sequencing.

2.4. Cloning of full length cDNA and 3D modeling

For cloning full length cDNA, a gene-specific forward primer 5′- CGGTTCTTATCGGAAGACGA -3′ (LFF) was designed using sequence knowledge gained from RACE reactions. For reverse primer, oligo-dT primer, which binds to the oligo-A tail of mRNA, was used. First strand cDNA was synthesized according to Bio-rad iScript cDNA Synthesis Kit's protocol (Bio-Rad Laboratories, Inc., Hercules, CA, USA). PCR reaction was performed with MyFi™ DNA Polymerase (Bioline, Memphis, TN, USA). PCR conditions were at 95 °C for 2 min, followed by 95 °C for 30 s, 55 °C for 40 s, and 72 °C for 1 min, with final extension at 72 °C for 10 min.

PCR fragments were purified and ligated with Promega PGEMT Easy Vector (Promega, Madison, WI, USA). Plasmids were transformed into *E. coli* DH5α ultracompetent cells.

2.4. Phylogenetic analysis

3'RACE and 5'RACE sequences were combined with the Emboss Merger online tool and investigated for homology by BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi). A phylogenetic tree was constructed with the MEGA7 program using the neighbor-joining (NJ) method with bootstrap analysis 10,000×. The amino acid sequence retrieved from nucleotide sequence was used for BLAST and selected sequences were aligned with the ClustalW alignment tool.

2.5. Cold stress induced expression analysis by real-time PCR

For expression analysis, 2-year-old olive seedlings were exposed to cold stress under conditions 14 h at 4 °C/10 h at 2 °C cycles. Leaves were collected at 0, 4 h, 6 h, 24 h, 1 week, and 10 days time points. Total RNAs from control and cold-stress–exposed olive leaves were used for cDNA synthesis with SCRIPT cDNA Synthesis Kit (Jena Bioscience GmbH, Jena, Germany). PCR reactions were carried out in replicates for each time point with qPCR Green Master including UNG (Jena Bioscience GmbH, Jena, Germany) using BioRad Connect real-time PCR detection system (Bio-Rad Laboratories, Hercules, CA, USA). Primers are shown in the Table. PCR conditions were as follows: 3 min at 50 °C, 2 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 1 min at 55 °C, and 30 s at 72 °C. All reactions were run in duplicate. A melting curve was done to ensure that only a specific amplification product was obtained. GADPH gene was also amplified as an internal control for normalization of results. The relative amounts of mRNA were calculated using the $2^{-\Delta\Delta C}$ method.

3. Results

3.1. RACE and sequence analysis

The length of the DNA fragment obtained by 3'RACE PCR was 1474 base pairs. BLAST analysis showed it has

Table. Real-time PCR primers. LUF: olive lipase forward primer; LUR: olive lipase reverse primer; OGF: olive GADPH forward primer; OGR: olive GADPH reverse primer.

LUF	5'- TGAACCGCATCCATCATCTA -3'
LUR	5'- CTAAGGCATCATTCCCCGTA -3'
OGF	5'- ACAGCTCCTGGTAAGGGTGA -3'
OGR	5'- GGCTTGCGTCAAGAAGTCTC -3'

73%–79% homology to different plant lipases. This DNA sequence was used for 5' RACE primer LRR1 and a 599bp-long DNA fragment obtained with 5'RACE PCR. ClustalO alignment showed that these two sequences overlapped and were 1640 bp when merged with EMBOSS Merger online tool (Figure 1).

3.2. Cloning of full length cDNA

Full length cDNA (1546 bp) was cloned with LFF and oligo-dT primers. The full length sequence had an open reading frame of 1236 bp which encoded a protein of 412 amino acids (Figure 2). BLAST analysis demonstrate that nucleotide sequence had 90%–99% identity to *Olea europaea* var. *sylvestris* (wild olive) lipase sequences with 99% query coverage.

Lipases have been divided into two types according to conserved sequence motifs GXSXG (Uppenberg et al., 1994) and GDSL (Upton, 1995) on the active site. Olive lipase protein sequence translated from the nucleotide sequence contains GHSXQG pentapeptide surrounding the catalytic serine. Catalytic triad was predicted as Ser-182, His-379, and Asp-350 when the olive lipase protein sequence was aligned with other GXSXG lipase sequences (Figure 3).

3D Modeling of olive lipase was performed using the putative amino acid sequence. Template selection and 3D modelling of olive leaf lipase was carried out using Swiss-Model (http://swissmodel.expasy.org/), which is a fully automated homology model-building server. Since there was no plant lipase model, human gastric lipase (1hlg) was selected as a template with 34%–42% sequence identity. The predicted model was a monomer with α/β fold and catalytic residues were labelled on the model (Figure 4).

3.2. Phylogenetic analysis

A phylogenetic tree was constructed with 25 amino acid sequences retrieved from the NCBI database (Figure 5). *Olea europaea* var. *sylvestris* lipase 1-like isoforms, which were obtained for genomic sequencing, showed 99% and 86% identity respectively to the *Olea europaea* var. *europaea* amino acid sequence. *Olea europaea* var. *sylvestris* is wild-type olive, which is considered an ancestor of cultivated olive trees.

Dorcoceras hygrometricum lipase 1-like is one of the closest relatives to olive lipase. *Sesamum indicum* (sesame) lipase 1 isoforms are other close relatives. Olive lipase has also been shown to be related to *Carica papaya* and *Brassica napus* lipases which are some of the most studied plant lipases as biocatalysts.

3.3. Cold stress induced expression analysis by real-time PCR

Expression profile of lipase in cold stress was analyzed by real-time PCR. Lipase expression was decreased compared to the nonstressed control sample in the first hours of being exposed to cold stress (Figure 6). It shows a slight fluctuation within the first day. Expression level increased

```
   1 ACATGGGGAA ACGTGAAAAC GGCAAAGCAA ATCCACAAAT TGAGGGGCGG ATGAAATTAT
  61 CTGATCATCC TACATTTAAC TGCTGTTACT GAAACCGTTG GAGATCTTCC CGCGGTTCTT

 121 ATCGGAAGAC GATGGAGCTT GCGGCGGCAC AGGCGGCGGT GATGATGTTT CTGTTAAGTA
       LFF                                          LEF1
 181 GTCTCATCCT GTCCGCCGTG AAGGCCGCCG AAGACTTCGA TGGCGCCTCA ATCCTCCTGC
 241 GAAAGTCACA GTCACATGTA GCTGGGCTTT GCGCTCAACT CATCGAACCT GCTGGTTTCC
 301 CTTGCTCAGA ACACAAAACA CAAACAAAAG ATGGATTCAT ACTGGGACTT CAACGCGTGT
 361 CATCTAGCTC TGGAAATCTC AGAAGACAAA GGGGCCCTCC AGTTCTGCTT ATACATGGAC
 421 TTTTCATGGC AGGTGATGCA TGGTTCTTGG ATAACCCAAA TCAATCCTTG GGCTTTATAC
 481 TTGCAAATCG TGATTTTGAT GTCTGGGTTG GTAATGTGCG TGGGACACGC TGGTGTCATG

 541 GCCATATATC TTTATCAGAG AAAGATAAGA AATTCTGGGA TTGGAGTTGG CAGGAATATG

 601 CTCTTTACGA TCTCCAAGAA ATGATTCGTT ATGTATATAC AGTTACAAAC TCTAGAGTGT
 661 TTGTCATTGG ATATTCTCAG GGAACAATCA TTTCTCTGGC TGCCTTTACT GAACCAGATA
 721 CAGTACAAAT GGTTGGAGCA GCTGCGCTTC TTTGTCCTAT AACATATTTG AATCATATGA
 781 CTGCTCGATT GCCTCTTAGA TTAGTTAAAA TGCATCTTAC TCAGGTTTTA CTTGCAGTGG
 841 GCATTCATGA ACTCAATTTC AAAAGTGACT GGGGTACTCG CATCATGGAA ATGATGTGCG
 901 ACAGACATGT AGATTGTGGT GACTTGCTAT CTTCTATTAC AGGGAAGAAT TGTTGTTTCA
 961 ATAGCTCTCG GATTGATTTC TATCTTGAAT ATGAACCGCA TCCATCATCT ACAAAAAATT
1021 TGAATCATCT CTTTCAGATG ATCCGAAAAG GTACTTTTGC GAAGTACGAC TATGGATTTT
1081 GGAAGAACTT GAAGCACTAC GGCCAACTGA AGCCACCAAA GTTCGATCTA AGCCAAATTC
1141 CCAGTTCATT ACCATTATGG ATGGGGTACG GGGGAAATGA TGCCTTAGCA GACGTCACAG
1201 ATTTGCAGCA TACTCTCAAG GAATTACAAT CAAAGCCAGA TTTGCTCTAT CTTGAAAATT
1261 ATGGTCATCT AGATTTCCTT TTAAGCACAA GAGCAAAGGA AGATGTTTAT GACAAAATGC
1321 TTGCATTTTT CGATTCATTG GGAACACACA GCAGTTACTA AATGTTGGAT CGGGGAGGAT
1381 CGGAGAGTCT TGATAGTATT AAGGAAGAAA ATTTTACTCT TGTTCACTCT TGAAGTGAAC
1441 TTGCACATAG GGGTTGTATA CTTGTATAAT TGTGTGTAAA TTGTACGCAT TATGTTTATG
1501 ATGTAAATAT AATTTCATAT TTTCCACGGA TTACTTATTT TCAGCGTCTG TACACTAGCA
1561 TTGTAAATAA CTTTATAATG GTAACACTCT GCCACAAAAA TTTTAATTAA TATCTTAAAG
1621 TTCTAAAAAA AAAAAAAAAA
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Figure 1. Nucleotide and deduced protein sequence of olive leaf lipase. Binding site of forward primers LEF1 and LFF marked with forward arrows and reverse primer LRR1 marked with reverse arrow.

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ATGGAGCTTGCGGCGGCACAGGCGGCGGTGATGATGTTTCTGTTAAGTAGTCTCATCCTG   60
 M  E  L  A  A  A  Q  A  A  V  M  M  F  L  L  S  S  L  I  L
TCCGCCGTGAAGGCCGCCGAAGACTTCGATGGCGCCTCAATCCTCCTGCGAAAGTCACAG  120
 S  A  V  K  A  A  E  D  F  D  G  A  S  I  L  L  R  K  S  Q
TCACCCGTAGCTGGGCTTTGCGCTCAACTCATCGAACCTTCTGGTTTCCCTTGCTCAGAA  180
 S  P  V  A  G  L  C  A  Q  L  I  E  P  S  G  F  P  C  S  E
CACAAAACACAAACAAAAGATGGATTCATACTGGGACTTCAGCGTGTGTCATCTAGCTCT  240
 H  K  T  Q  T  K  D  G  F  I  L  G  L  Q  R  V  S  S  S  S
GGAAATCTCAGAAGACAAAGGGGTCCTCCAGTTCTGCTTATTCATGGACTTTTTATGGCA  300
 G  N  L  R  R  Q  R  G  P  P  V  L  L  I  H  G  L  F  M  A
GGTGATGCATGGTTCTTGGATAACCCAAATCAGTCCTTGGGGTTTATACTTGCAAATCGT  360
 G  D  A  W  F  L  D  N  P  N  Q  S  L  G  F  I  L  A  N  R
GATTTTGATGTCTGGGTTGGTAATGTGCGTGGGACACGCTGGTGTCATGGCCATGTATCT  420
 D  F  D  V  W  V  G  N  V  R  G  T  R  W  C  H  G  H  V  S
TTATCAGAGAAAGATAGGGAATTCTGGGATTGGAGCTGGCAGGATTATGCTCTTTACGAT  480
 L  S  E  K  D  R  E  F  W  D  W  S  W  Q  D  Y  A  L  Y  D
GTGCGAGAAATGATTCGTTATATATACACAGTTACAAACTCGAGAGTGTTTGTCATTGGA  540
 V  R  E  M  I  R  Y  I  Y  T  V  T  N  S  R  V  F  V  I  G
CATTCTCAGGGAACAATAATTTCTCTGGCTGCCTTAACTCAACCAGACATAGTAGAGATG  600
 H  S  Q  G  T  I  I  S  L  A  A  L  T  Q  P  D  I  V  E  M
GTTGGAGCAGCTGCACTTCTTTGTCCTATAACATATTTGAATCATATCACTGCTAGATTA  660
 V  G  A  A  A  L  L  C  P  I  T  Y  L  N  H  I  T  A  R  L
CCTCTTAGATTAGTTAAAATGCACCTTGATCAGGTTTTTCTTGCAATGGGCATTCATGAA  720
 P  L  R  L  V  K  M  H  L  D  Q  V  F  L  A  M  G  I  H  E
CTCAATTTCAAAAGTGACTGGTGTACTCGCATCATGGATATGATGTGCGACGGACATGTA  780
 L  N  F  K  S  D  W  C  T  R  I  M  D  M  M  C  D  G  H  V
GATTGTGGTGACTTGCTAACTTCTGTTATAGGGAAGAATTGTTGCTTCAATAGCTCTCGG  840
 D  C  G  D  L  L  T  S  V  I  G  K  N  C  C  F  N  S  S  R
ATCGATTTCTATCTTGAATACGAACCGCATCCAACATCTACAATGAATTTGAATCATCTC  900
 I  D  F  Y  L  E  Y  E  P  H  P  T  S  T  M  N  L  N  H  L
TTTCAGATGATCCGTGAAGGTACTTTCGCGATGTACAACCATGGAAGGTGGAAAAACATG  960
 F  Q  M  I  R  E  G  T  F  A  M  Y  N  H  G  R  W  K  N  M
TGGCACTATGGCCAACTGAAGCCACCAAAATTTGATCTTAGCCGAATTCCCAGTTCCTTA 1020
 W  H  Y  G  Q  L  K  P  P  K  F  D  L  S  R  I  P  S  S  L
CCATTATGGAGGGGATATGGGGGAAATGACGCGTTAGCAGACATTACAGATTTGCAGCAT 1080
 P  L  W  R  G  Y  G  G  N  D  A  L  A  D  I  T  D  L  Q  H
ACTCTCAAGGAATTACGATCAAAGCCAGATTTGCTCTATCTTGAAAATTATGGTCATCTA 1140
 T  L  K  E  L  R  S  K  P  D  L  L  Y  L  E  N  Y  G  H  L
GATTTCCTTGTGAGCACGAGATCAAAGGAAGATGTTTATGACAAAATGCTTGCATTTTTC 1200
 D  F  L  V  S  T  R  S  K  E  D  V  Y  D  K  M  L  A  F  F
AACTCACTGGAACACTTAGCGGTGGTCACTGATTAACTGCTTGGACGGGTGAACTGGACA 1260
 N  S  L  E  H  L  A  V  V  T  D  *
GCCTCGATAGTGTTAAGGAAGAAAAAAAAAAATTAAAATCTTATTCATTATACTTTCTAAT 1320
ATTGTGTAACAAAAAATGCACATAGGGGTAATGTAGTTGTCTACTTATCATCTAAATTTA 1380
CCGTAGTAGTTAATTTACCATTTAAATGTAATTTCATACTTTTGGTGGACTACTTGTTGC 1440
TCAGTGTCTCTACACGAGTATTGTAAATAACTTTATAATGGTAATGGATTATCACCAAAT 1500
TTCAATAAAGTATTTGAAAGTTTTTGTTTTAAAAAAAAAAAAAAA                1546
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Figure 2. Full length cDNA open reading frame.

after being exposed to cold stress for a week, and reached its highest level during the tenth day of stress. On the tenth day, the expression level was greater than that of the nonstressed sample.

4. Discussion

Lipases are important enzymes for industry. In this study, we have cloned the full length cDNA of lipase gene from *Olea europaea* L. *europaea* cv. Gemlik leaves. The 3'

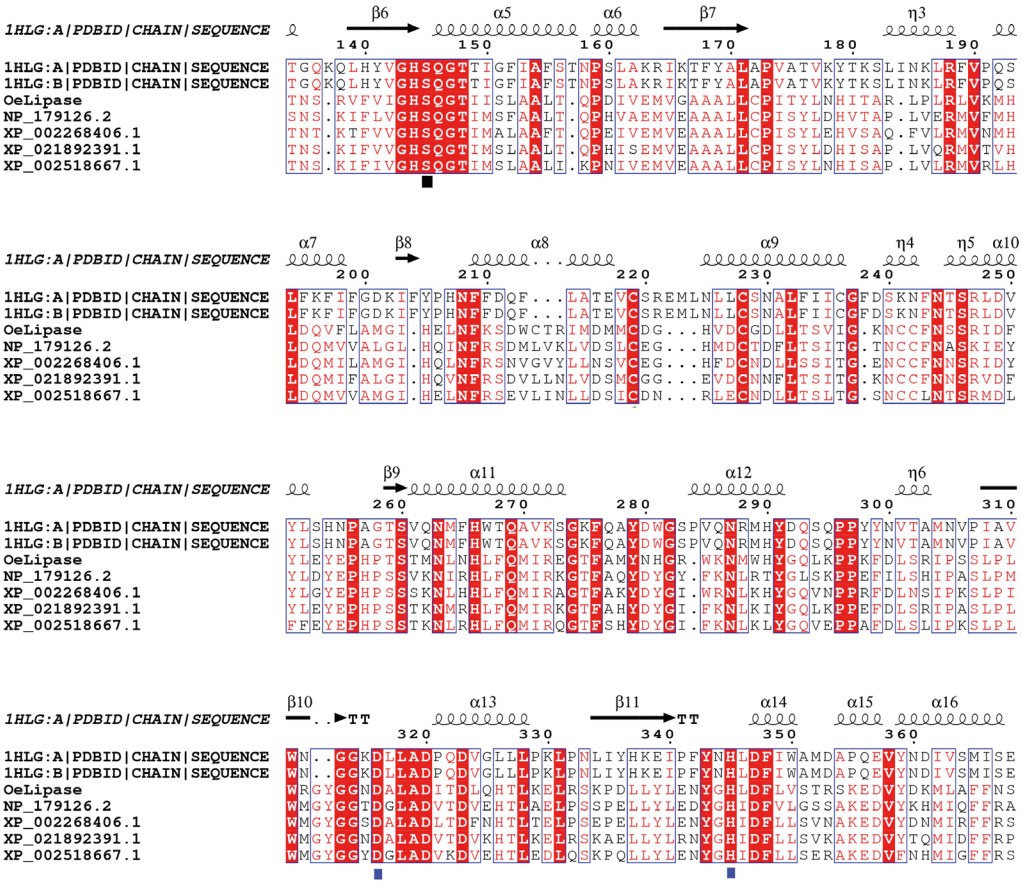

Figure 3. Multiple sequence alignment of lipase. Sequences aligned with ClustalO program and secondary structures were predicted by Espript program using human gastric lipase as a template. Catalytic triad Ser-182 marked with black square; His-379 and Asp-350 marked with blue square. (NP_179126.2 *Arabidopsis thaliana*, XP_002268406.1 *Vitis vinifera,* XP_021892391.1 *Carica papaya*, XP_002518667.1 *Ricinus communis*).

and 5' RACE method was used for retrieving sequence information for olive leaf lipase. Full length cDNA was cloned for the first time with a primer designed using RACE results. Olive lipase nucleotide and amino acid sequences showed 70%–99% homology to different plant lipases according to BLAST similarity analyses. Further bioinformatics tools were used for analyzing the lipase sequence, which revealed olive lipase is a GXSXG type lipase with the predicted Ser-182, His-379, and Asp-350 catalytic triad.

Phylogenetic analysis shows that *Dorcoceras hygrometricum* lipase is one of the closest relatives to olive lipase. *Dorcoceras hygrometricum* is a homiochlorophyllous dicot that can resurrect from desiccation, and its phospholipases play a role in its stress response (Xiao et al., 2015). Lipases can provide substrate for lipoxygenases in jasmonic acid and methyl jasmonate synthesis pathways (Padham et al., 2007). Jasmonic acid and methyl jasmonate participate in signal regulation in stress responses. Another way that lipase is involved in stress response is in

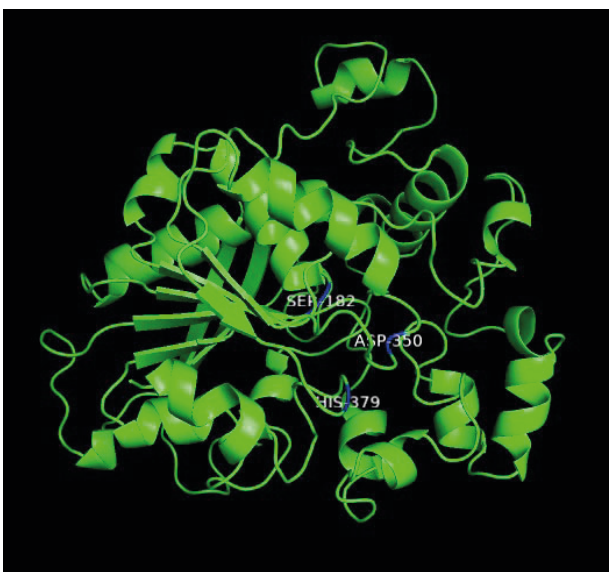

Figure 4. 3D model of olive leaf lipase. Catalytic triad Ser-182, His-379, and Asp-350 labeled.

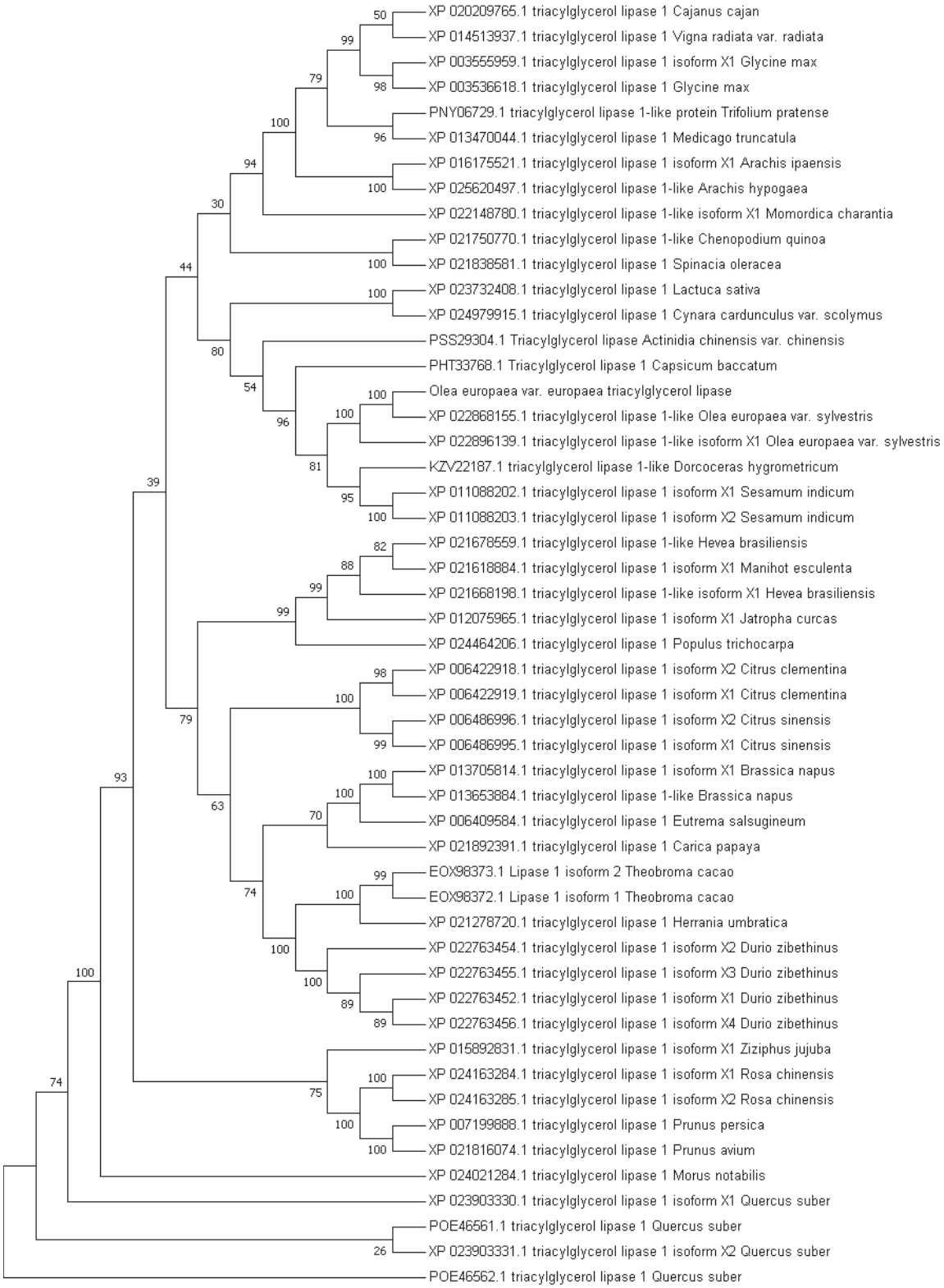

Figure 5. Phylogenetic tree of *Olea europaea* var. *europaea* lipase. Constructed by MEGA7 program using the neighbor-joining (NJ) method with bootstrap analysis 10,000×.

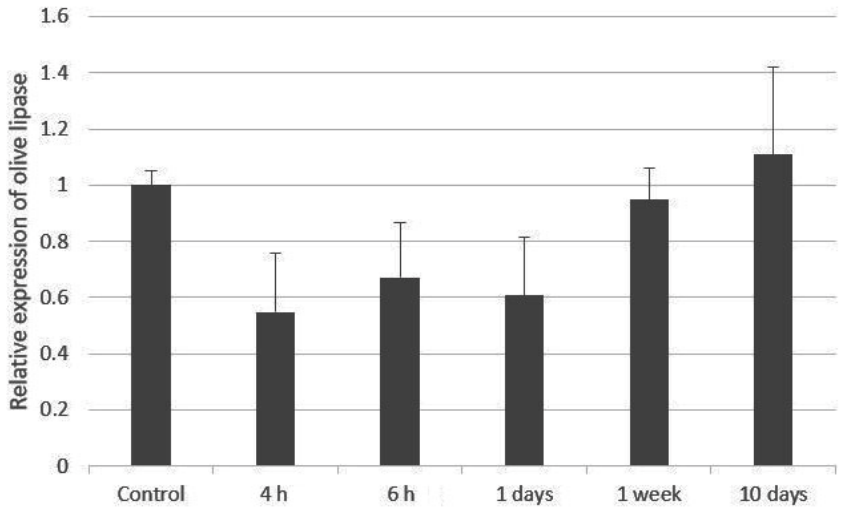

Figure 6. Relative expression of olive leaf lipase in cold stress analyzed by real-time PCR. Cold stress time points are indicated in x-axis. Relative amounts of mRNA which calculated using $2^{-\Delta\Delta C}$ method are indicated in y-axis. The error bars represent the standard deviations of two replicates.

the remodeling of membranes (Lo et al., 2004). Real-time PCR results show that lipase in olive leaves first decreases then increases. Expression level on the tenth day exceeds the nonstressed control's expression level. This profile might be due to its role in membrane adjustments in long term stress exposure. Further experiments should be done to test this hypothesis.

The cDNA we have cloned showed sequence similarity to *Carica papaya* lipase. *Carica papaya* also has more than one form of lipase. One of these lipases presents the optimum pH 8.5, which may be useful for the detergent industry (Rivera et al., 2017). Another lipase form is stable in the pH 5–7 range; it has been suggested that this lipase could be a candidate for a therapeutic tool on patients with pancreatic exocrine insufficiency (Abdelkafi et al., 2009). Another interesting lipase which has similarity to olive lipase is *Brassica napus* lipase. *Brassica napus* lipase can catalyze esterification reactions in the presence of organic solvents. It can catalyze esterification of oleic acid at –22 °C (Hills and Mukherjee, 1990).

Panzanaro et al. (2010), detected acid lipase activity in olive fruit oil body fractions. The optimum pH for this enzyme was pH 5 and optimum temperature was 35 °C. Olías et al. found acylhydrolase activity at optimum pH 8.5 in olive fruit crude extract (Olías, 1993). This difference suggests that more than one form of olive fruit lipase exists (Panzanaro et al., 2010). Since there is no related nucleotide sequence information about the fruit lipases, it's not possible to compare them with our leaf cDNA.

In conclusion, since olive leaf lipase sequence shows similarity to lipases with interesting characteristics, it is possible olive lipase may also be an interesting enzyme for industrial application. Different lipase forms with different characteristics in olive fruit supports this idea. Further analysis of the characterization of olive leaf lipase should be considered.

Acknowledgments

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