



## IDENTIFICATION OF A NEW GDLS LIPASE GENE FROM *Carica papaya* THAT IS REGULATED BY JASMONIC ACID

Ivanna Rivera<sup>a</sup>, Aurora Huerta-Robles<sup>a</sup>, Antonia Gutiérrez-Mora<sup>a</sup>, Carla Sánchez-Hernández<sup>b</sup>, Georgina Sandoval<sup>a</sup>, Abel Gutiérrez-Ortega<sup>a</sup>.  
ivannaiq@gmail.com

<sup>a</sup> Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco (CIATEJ). Av. Normalistas 800, Colinas de la Normal, Guadalajara, Jalisco 44270 México <sup>b</sup> Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Carretera Guadalajara-Nogales Km 15.5, Zapopan, Jalisco 45110, México

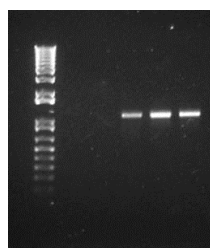
*Key words: Carica papaya genome, lipase, GDSL lipase*

**Introduction.** Lipases are lipolytic enzymes that catalyze the hydrolysis of ester bounds from triglycerides. In addition, under certain conditions they also catalyze a wide range of reactions with potential applications in different fields, like food, pharmaceutical and detergent industry [1]. *Carica papaya* is a remarkable source of plant lipases since its genome presents several lipolytic proteins, which expression, function and applications remain unknown.

In this work, an expression analysis of ten different sequences encoding for lipases was done, for the first time in *Carica papaya* leaves in order to identify new lipases with potential interesting applications.

**Methods.** Ten sequences were previously selected taking in count the hydrophobicity of the sequences and the corresponding PCR primers were designed. Leaves from *Carica* were sprayed with 1 mM Jasmonic Acid solution and the leaf tissue was cut at 0, 1, 4, 8 and 24 h post-treatment. The tissue was immediately frozen with liquid nitrogen and stored at -80°C until RNA extraction. Leaf tissue was ground to a fine powder in liquid nitrogen with mortar and pestle and RNA was extracted. cDNA was generated and PCR amplification was performed.

**Results.** The expression of one gene was identified in *Carica papaya* leaves under JA acid treatment after 24 hours treatment. This gene, named *CpLIP2*, is strongly induced by JA. To investigate *CpLIP2* expression kinetics in *Carica papaya* leaves after JA treatment, samples were taken at 0, 1, 4, 8 and 24 h post-treatment and its relative transcript levels were detected by end-point RT-PCR. *CpLIP2* transcripts accumulated from 4 h to 24 h post-treatment; the highest expression levels were observed at 8 h post-treatment and there was a slight decrease after 24 h (fig 1).



a b c d e f

**Fig1.** RT-PCR analysis of CpLip2 expression profile in *Carica papaya* leaves under JA treatment. a) MWM b) t= 0, c) t= 1h, d) t= 4h, e) t= 8h, f) t= 24h

CpLIP2 is a member of the GDLS lipase family with homology with different plant GDSL lipases such a CpEst from *Carica papaya* latex [2].

Concerning the characteristics of this gene, *CpLIP2* gene has an open reading frame of 1125 nucleotides encoding a 375 amino acid protein with a predicted molecular mass of 40.83 kDa and a theoretical isoelectric point (pI) of 8.2. Localization of the critical residues for CpLIP2 enzyme activity was done by comparing it with a previously reported lipase denominated CpEst. The residues from the catalytic triad (Ser47, Asp345 and His348) and the oxyanion hole residues (Ser47, Gly77 or Gly82, Asn179) are highly conserved between both enzymes. Also, a 32 amino acid signal peptide was predicted using the Expasy Signal P V4.0 program and it is estimated that the N-terminal sequence of the mature protein starts at amino acid G33.

**Conclusions.** Analysis of the expression of different lipase sequences found in *Carica papaya* genome was performed for the first time in *Carica papaya* leaves. Ten sequences from *Carica papaya* encoding putative lipases were selected and analyzed for their expression after JA treatment of papaya leaves. Only one sequence, named *CpLIP2*, was expressed and its transcription was detected at 4, 8 and 24 h after JA induction. Since *CpLIP2* gene was induced by JA, it might be involved in biotic or abiotic stress response and its physiological role must be determined.

**Acknowledgements.** The authors thank CONACYT (Mexico) and projects ECOS-ANUIES M08-A03 and CB-2008-01-104429 for financial support.

### References.

1. Casas-Godoy L, Duquesne S, Bordes F, Sandoval G, Marty A (2012) Lipases: an overview, Sandoval G (ed) Methods in molecular biology, Lipases and phospholipases, 1: 547, pp 3-30, Springer. ISBN: 1940-6029
2. Abdelkafi S, Ogata H, Barouh N, Fouquet B, Lebrun R, Pina M, Scheirlinckx F, Villeneuve P, Carriere F (2009) Identification and biochemical characterization of a GDSL-motif carboxylester hydrolase from *Carica papaya* latex. BBA-Molecular Cell Biol L 1791:1048-1056