Increasing oil accumulation in olive mesocarp: Cloning and characterization of phospholipid: diacylglycerol acyltransferase (PDAT) genes from olive fruit

García-Conde, Úrsula[1], Sicardo, M. Dolores[1], Martínez-Rivas, José M.[1]

1. Department of Biochemistry and Molecular Biology of Plant Products. Instituto de la Grasa (CSIC), Campus Universidad Pablo de Olavide, Ctra. Utrera km 1, 41013 Seville, Spain.

INTRODUCTION

The majority of plants accumulate large amounts of triacylglycerols (TAGs) in their seeds as storage reserves. However, there are few fruit crops that deposit most of the oil in the mesocarp tissues. Among them, olive is of predominant economic importance because the beneficial effects on human health and the exceptional organoleptic properties of its oil (Banilas et al., 2011).

It is therefore of great importance to elucidate the key-points in the olive oil biosynthesis pathway and storage. TAG biosynthesis is principally accomplished by membrane-bound enzymes that operate in the endoplasmic reticulum through the Kennedy pathway. However, TAGs could also be produced via the transfer of acyl groups from phospholipids to diacylglycerols, an acyl-CoA-independent reaction catalysed by the enzyme phospholipid:diacylglycerol acyltransferase (PDAT) (Banila et al., 2011).

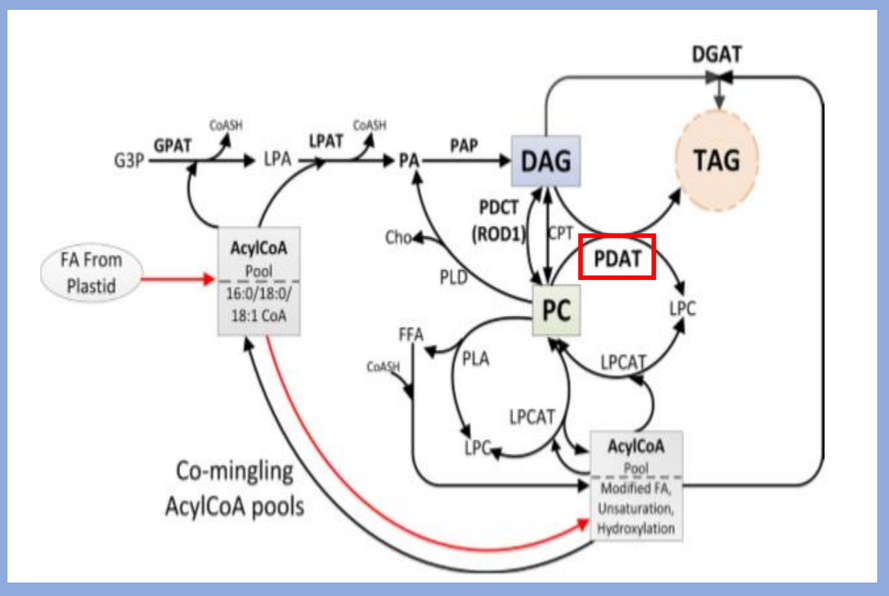

Figure 1. TAG biosynthesis in plants. Picture taken from Chapman and Ohlrogge, 2012.

OBJECTIVES

To identify, characterize and study the regulation of genes and enzymes involved in the biosynthesis of triacylglycerols, in order to increase oil accumulation in olive mesocarp. In particular, to investigate the contribution of olive phospholipid:diacylglycerol acyltransferase (PDAT) genes (Dahlqvist et al., 2000). To achieve this objective, we will address the following points:

- Isolation and sequence analysis of *PDAT* genes from olive.
- Transcriptional regulation of *PDAT* genes in olive fruit.
- Functional expression of olive *PDAT* genes in a mutant of *Saccharomyces cerevisiae* (Sandager et al., 2002).

EXPERIMENTAL

1. Identification of an olive sequence with a high degree of similarity with the other phospholipid:diacylglycerol acyltransferase (PDAT) genes of olive previously identified, OepPDAT1-1 and OepPDAT1-2. The oleaster genome was used as the database (Unver et al., 2017).

2. Design of specific oligonucleotides and a fragment, designated *OepPDAT2*, has been isolated from olive by nested PCR.

3. Analysis of olive *PDAT* genes expression levels by qPCR (quantitative real-time PCR) in Picual and Arbequina cultivars using different olive tissues, and in mesocarp under different abiotic stresses such as draught, low and high temperature, darkness and wounding.

4. Functional expression using pYES2 vector in a quadruple mutant of *S. cerevisiae* that cannot synthesize triacylglycerols to confirm gene identity.

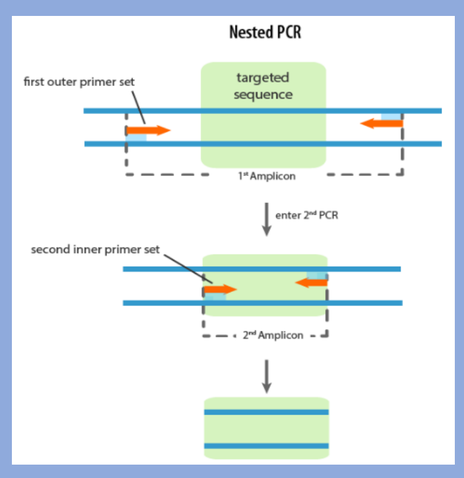

Figure 2. Nested PCR.

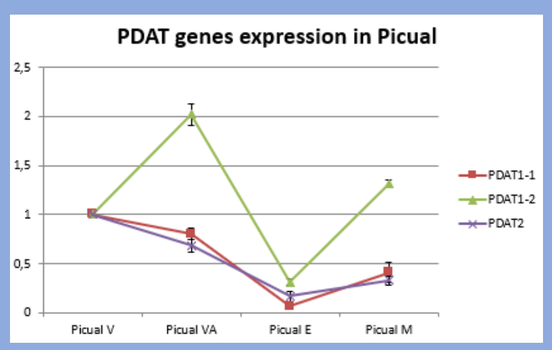

Figure 3. PDAT genes expresión levels in Picual.

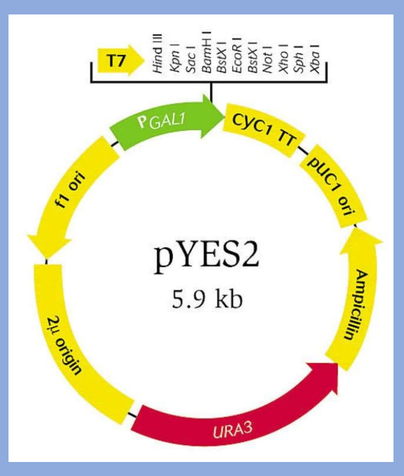

Figure 4. Map of pYES2 vector containing an inducible GAL1 promoter.

Figure 5. Different stages of maturation picual variety.

CONCLUSIONS

Regarding the results obtained, the sequence analysis suggests that the *OepPDAT2* gene encodes a PDAT enzyme. In addition, gene expression analysis in olive seed and mesocarp tissues indicates that *OePDAT2* participates in triacylglycerol synthesis during development and ripening of olive fruit, and it is transcriptionally regulated by different environmental factors.

REFERENCES

1. Banilas et al., (2011) The olive DGAT2 gene is developmentally regulated and shares overlapping but distinct expression patterns with DGAT1. J. Exp. Bot., 62 (2) 521–532.
2. Chapman, K. and Ohlrogge, J.B. (2012) Compartmentation of Triacylglycerol Accumulation in Plants. J. Biol. Chem., 287 (4) 2288–229.
3. Dahlqvist et al. (2000) Phospholipid:diacylglycerol acyltransferase: An enzyme that catalyzes the acyl-CoA-independent formation of triacylglycerol in yeast and plants. Proc Natl Acad Sci USA, 97 (12) 6487-6492.
4. Sandager et al. (2002) Storage Lipid Synthesis Is Non-essential in Yeast. J. Biol. Chem., 277, 6478-6482.
5. Unver et al. (2017) Genome of wild olive and the evolution of oil biosynthesis. Proc Natl Acad Sci USA, 114 (44) 9413-9422.

Francisca Reyes Ramírez