IDENTIFICATION AND EXPRESSION DYNAMICS OF KEY GENES INVOLVED IN THE BIOSYNTHESIS OF MAJOR VIRGIN OLIVE OIL PHENOLICS DURING OLIVE DEVELOPMENT

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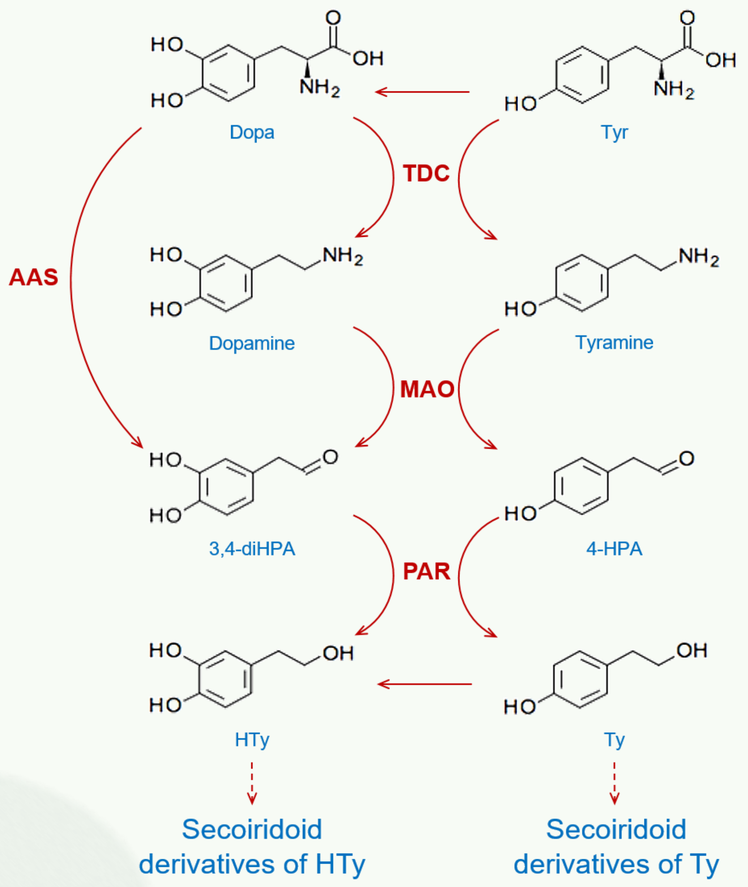
INTRODUCTION

Virgin olive oil (VOO) is one of the oldest known vegetable oils and underpins many of the recognized health benefits of the Mediterranean diet. These benefits are primarily attributed to its phenolic fraction, widely used as a quality indicator. In olives, the majority of phenolics are glucosylated secoiridoid derivatives that include tyrosol (Ty) and hydroxytyrosol (HTy) residues. During the VOO extraction process, endogenous β-glucosidases remove glucose from the molecule, increasing the solubility of the compounds in oil and converting Ty- and HTy-based molecules into the predominant phenolics in VOO. Correlation data suggest that the levels of Ty and HTy derivatives in olive fruit largely determine their contents in the resulting oil. Despite growing interest in Hty and Ty derivatives as functional components of VOO, the biosynthesis of these compounds in olive is still not fully understood.



OBJECTIVE

This work focused on the identification of key genes involved in the synthesis of HTy and Ty and their relationship with the accumulation of Hty- and Ty-derived phenolics throughout the ontogeny of the olive fruit.

MATERIALS & METHODS

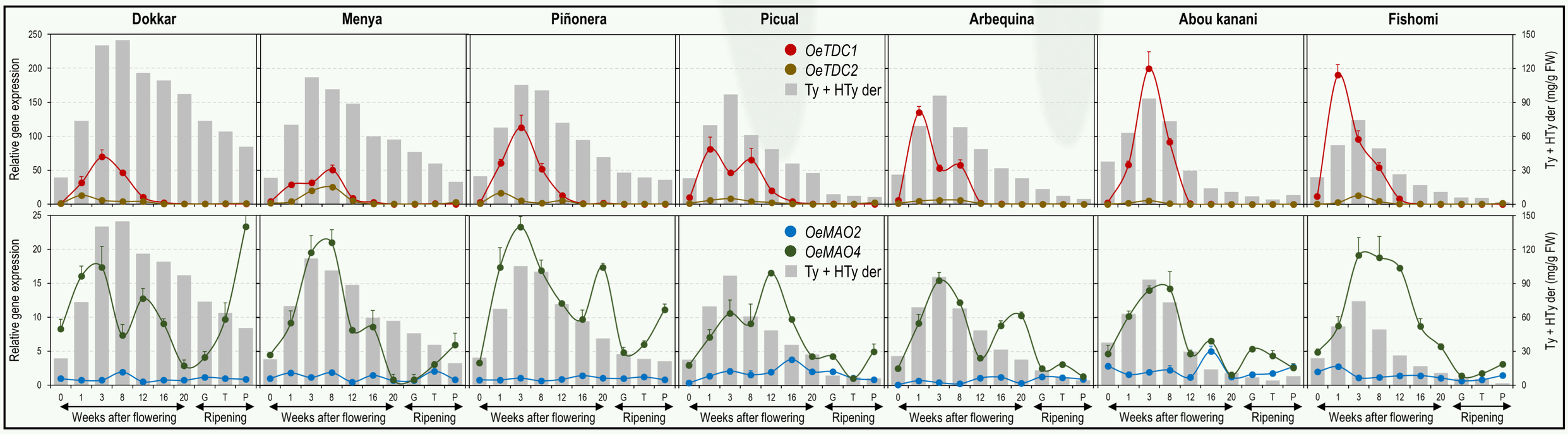
Different genes identified in a previous transcriptomic study as potentially related to the Hty/Ty synthesis pathway (see scheme) were synthesized, heterologously expressed and their products functionally validated. Their role in the biosynthesis of phenolics was studied by coupling metabolic, transcriptomic and functional validation data using seven olive cultivars with divergent phenolic profiles along their ontogeny (0-20 weeks after flowering; Green, Turning and Purple fruits).

RESULTS

TDC/AAS - We have found that some genes of the olive tyrosine decarboxylase family, such as *OeAAS*, *OeTDC1* and *OeTDC2*, encode proteins that exclusively accept aromatic amino acids with phenolic side chains such as tyrosine and 3,4-dihydroxyphenylalanine (Dopa). *OeAAS* has very low expression levels compared to those of the *TDC* gene type (data not shown). Their encoded proteins displayed higher affinity and catalytic efficiency for Dopa than for tyrosine. The expression analyses indicate that both *OeTDC1* and *OeTDC2* genes are temporally regulated in a cultivar-dependent manner (see graphs below). Gene expression peaked in the first weeks after fruit set when HTy and Ty derivatives accumulate maximally.

MAO - Two monoamine oxidase genes have been identified in the olive genome: *OeMAO2* and *OeMAO4*. Functional validation of the *OeMAO4* product shows that it only accepts amines with phenolic side chains, converting tyrosine to tyramine and Dopa to dopamine, while the OeMAO2 product has not yet been functionally validated. The results show that the expression level of *OeMAO4* is much higher than that of *OeMAO2* and is regulated both by cultivar and time (see graphs below), reaching one of its maximum expression peaks in the first weeks of fruit development, coinciding with the expression of TDC-type genes.

PAR - Previous studies showed that the products of the phenylacetaldehyde reductase genes, *OePAR1.1* and *OePAR1.2*, also accepted only aromatic substrates with phenolic side chains. *OePAR1.2* showed much higher expression levels than *OePAR1.1*, and this high level of expression appears to be constitutive throughout olive fruit ontogeny (data not shown).



* The relative gene expression levels were scaled using as calibrator the expression level of the MAO2 gen from Dokkar at 0 weeks after flowering.

CONCLUSIONS

The results indicate that the synthesis of the main phenolic compounds in olives occurs in the first eight weeks after fruit set, with *OeTDC1* and *OeMAO4* apparently being the key genes for the biosynthesis of Ty and HTy in olives and, finally, in their oil. Furthermore, functional validation studies show that the enzymes encoded by these genes are more efficient using diphenolic than monophenolic substrates to ultimately produce HTy and Ty, respectively, mirroring the typically higher abundance of HTy- than Ty-derivatives in the olive fruit and VOO.

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