Poster

Increasing the vitamin E content of virgin olive oil: cloning and molecular characterization of genes involved in the biosynthesis of phytyl-diphosphate in olive fruit

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ABSTRACT

Virgin olive oil (VOO) is characterized by its distinctive organoleptic characteristics and exceptional nutritional properties due to its well-balanced fatty acid composition, as well as the presence of minor components such as natural antioxidants. Among them, alpha-, beta-, and gamma-tocopherols have been detected, with alpha-tocopherol representing more than 95% of the total tocopherol content, which ranges between 50 and 300 mg/kg. VOO with improved functional quality, such as the increase in vitamin E (alpha-tocopherol) content, is considered an important target for generating new cultivars in olive breeding programs. With this purpose, the identification of molecular markers associated with high vitamin E content is needed. Therefore, the general objective of this study is to improve the VOO nutritional quality by increasing the alpha-tocopherol content of olive fruit. To achieve this, we focus on the isolation and characterization of genes involved in alpha-tocopherol biosynthesis in olive fruit.

Tocopherols are generated in photosynthetic organisms from the condensation of phytyl-diphosphate (PDP) and homogentisic acid, followed by cyclization and methylation reactions. The precursor PDP can be synthesized from phytol by the successive action of two kinase enzymes, phytol kinase (PK) and phytyl-phosphate kinase (PPK)(1).

In the present work, the identification of olive genes with a high degree of similarity with Arabidopsis PK(2) and PPK(3) genes was carried out using the olive transcriptome and genome. From the identified sequences, a pair of specific oligonucleotides for each sequence were designed and the full-length cDNA clones encoding for olive PK and PPK were obtained using a PCR approach. Alignment of the sequences and phylogenetic analysis indicated that they code for PK and PPK enzymes. In addition, expression analysis by qRT-PCR was performed using different olive tissues, particularly in mesocarp and seeds from Picual and Arbequina at distinct developmental stages, as well as in mesocarp from cultivars with low and high tocopherol content. Furthermore, PK and PPK transcript levels were determined in olive mesocarp from fruits subjected to different abiotic stresses, including water deficit, low and high temperature, darkness, and wounding. Overall, this study constitutes a significant advance in elucidating the factors that regulate the tocopherol biosynthesis in olive fruit to obtain VOO with enhanced alfa-tocopherol content.

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