Tocopherol biosynthesis dynamics in almond kernel development

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INTRODUCTION

***Prunus dulcis* (Miller) D.A. Webb**, known as almond, is one of the most important tree nut crops in terms of commercial production in Mediterranean climate areas [1]. This crop is widely cultivated for its edible seed, named kernel, which has a high nutritional value as it contains high concentration of lipids, mainly unsaturated fatty acids, and tocopherols.

Tocopherol (Vitamin E) is an antioxidant who prevents the peroxidation of unsaturated fatty acids, increasing storage-life of almond [2]. As an antioxidant, it is highly effective preventing cardiovascular diseases by inhibiting platelet aggregation [3]. There are 4 homologues of tocopherol (α-, β-, γ-, δ-tocopherol), being the α-tocopherol the most abundant [1]. Genes involved in its biosynthesis have been characterized in other species, but few research have been made in almond.

Objective: The aim of this work was to study tocopherol accumulation during almond kernel maturation, and to characterize a candidate *tocopherol cyclase* gene (*PdVTE1*), putatively involved in tocopherol biosynthesis.

METHODS

Tocopherol analysis

Extract preparation → HPLC analysis → Statistic analysis

Sequence analysis

In silico analysis → Primer design

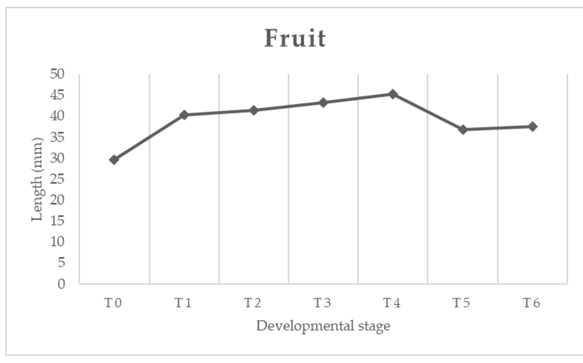
RNA extraction → cDNA synthesis → Sanger sequencing → Alignment and Phylogeny

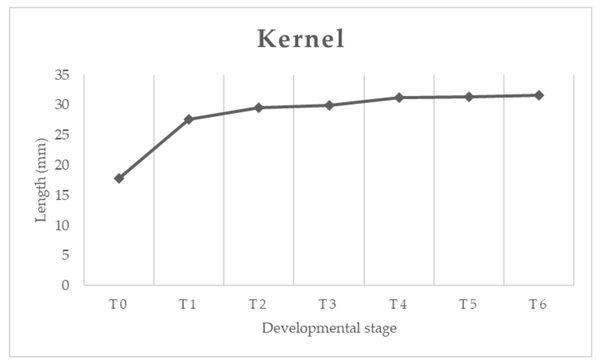
PLANT MATERIAL

Almonds at different developmental stages were collected, from March to September, from four-year-old 'Soleta' trees. (A) - Fruit with shell; (B) - Almond kernel; (C) - Kernel cut lengthwise. Scale bars = 1 cm.

RESULTS & DISCUSSION

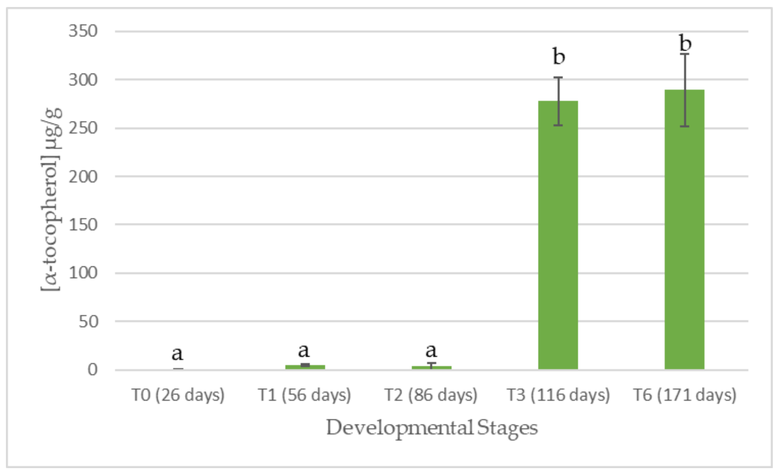
Morphological analysis





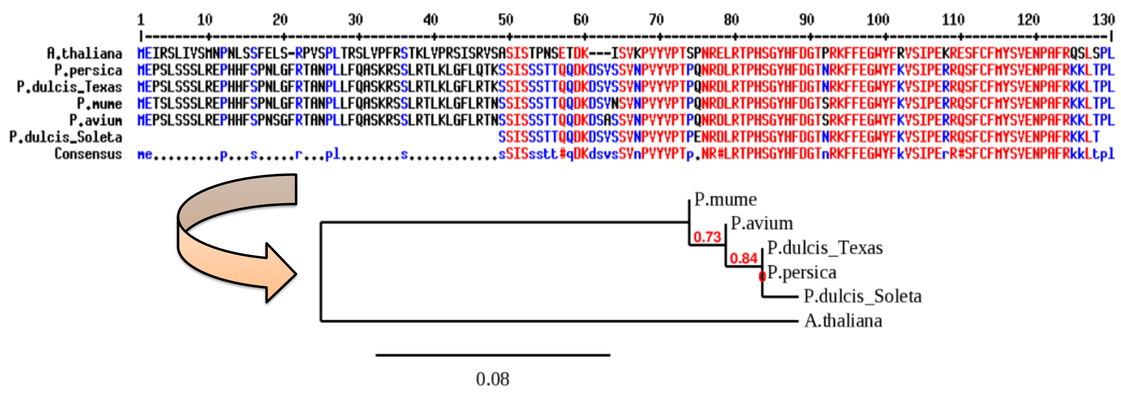
The biggest growth occurs between March (T0) and April (T1), growing slightly over the remaining months. The decrease between T4 and T5 is due to the fact that measurements were done in fruits without mesocarp.

α-Tocopherol content



In the early stages, the amount of tocopherol present was quite residual (2.91 µg/g). In stage T3, the highest content was reached (281 µg/g). In the mature stage T6, the concentration remained constant, which indicates that the production of tocopherol was greatly reduced from T3 to T6.

Sequence analysis



- ✓ The sequences present high similarity among Prunus species and slightly vary compared to *A. thaliana;*
- ✓ A higher proximity between *Prunus persica* and *Prunus dulcis* sequences were confirmed;
- ✓ The variations in VTE1 sequences between species, may not influence protein activity, keeping its cyclase activity.

CONCLUSIONS AND ONGOING WORK

- ✓ The period of time where α-tocopherol has the biggest accumulation is between T2 and T3;
- ✓ The sequence of *PdVTE1* transcript was identified in Soleta and a high similarity with other Prunus sequences was confirmed;
- ✓ For better understanding tocopherol biosynthesis pathway, the differential expression level of candidate PtVTE1 by RT-qPCR is ongoing.

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