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IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN THE FLESH OF BLOOD AND BLOND CULTIVARS OF ORANGE AND CLONING OF A cDNA SEQUENCE HOMOLOGUE TO THE R2R3 DOMAIN OF THE MYB TRANSCRIPTION FACTOR FAMILY

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Deep red colour in blood oranges is due to the presence of anthocyanins, secondary metabolites which synthesis level varies notably depending on the different genotype's response to environmental factors.

Genes encoding for CHS, ANS and UFGT were assayed in experiments of Real-time RT-PCR, in order to test their synthesis level in the flesh of blood and blond oranges (*Citrus sinensis*) during seven different fruit maturation stages and in 11 different genotypes at the end of winter season. Results show that the target genes mRNA transcripts are positively related to the synthesis level of the pigment.

A subtractive cDNA library of sweet orange was constructed through Suppression Subtractive Hybridization (SSH) - PCR Select and T/A cloning and used to identify differentially expressed genes in the flesh of two different cultivars: Moro 58-8D-1, a blood cultivar (used as tester) and Cadenera, a blond cultivar (used as driver). The library consisted of 1248 clones, 309 of them resulted to be up-regulated in Moro, while 41 were up-regulated in Cadenera. All the clones were blotted in nylon filter for reverse-Northern analysis and then hybridized with specific probes, in forward (Moro probe) and in reverse (Cadenera probe). Fragment size was between 300 bp and 1000 bp. All differential clones were sequenced and screened for homology in GenBank non-redundant databases. Some of the up-regulated genes are involved in the anthocyanins pathway (biosynthetic (41%) and regulatory mechanism (0.7%)), some others are related to energetic metabolism (9.6%), acidity (3%) and signal trasduction mechanism (10.4%). About 8.4% of the clones were annotated as unknown proteins. The redundancy of some clones could be an indication of the high level of gene expression. We isolated 25 times a glutathione S-transferase (that is involved in the transport of anthocyanin from the cytoplasm to the vacuole), two clones of putative S receptor kinase [OSJNBa0079M09.4 (21 times) and OSJNBb0018A10.11 (4 times)], a cytochrome b5 (13 times), an alcohol acyltransferase (10 times), a 10-hydroxygeraniol oxidoreductase (6 times). We suppose that some of these genes could have an important, but still undefined function in *Citrus*. We validated the result of the different expression of these clones through semi-quantitative RT-PCR, and are going to test samples harvested in different periods in Real-time RT-PCR.. The results demonstrate the importance of Suppression Subtractive Hybridization in identifying genes responsible for blood pigmentation in orange fruit. The isolated sequences will be used to study the influence of different environmental conditions on ripening process.

Anthocyanin pigmentation pattern in different plant species is controlled in part by members of the *myb* and *myc*-like regulatory gene family. We report the cloning of the full length cDNA of one sequence

(*Cs1*) from total cDNA of Moro orange showing structural similarities with proteins of the Myb transcription factors family. We are tempting to characterise *Cs1* and to verify his implication in the regulation of anthocyanin's biosynthetic pathway.