

VALIDATION OF DIFFERENTIALLY EXPRESSED GENES IN THE FLESH OF BLOOD AND BLOND CULTIVARS OF SWEET ORANGE [*C. SINENSIS* (L.) OSBECK]

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SSH-PCR select, anthocyanins, PCR Real time

A subtractive cDNA library of sweet orange was constructed through Suppression Subtractive Hybridization - PCR Select on Moro (blood cultivars, tester) and Cadenera (blond cultivars, driver). After reverse Northern screening, a total of 230 clones were found to be up-regulated in blood orange, while 30 were up-regulated in common one. Some genes are involved in the anthocyanins pathway, biosynthetic (44%) and regulatory (1%) mechanism; some others are related to flavour's biosynthesis, signal transduction mechanisms, defense and primary metabolism. Almost the 9% of clones encoded proteins with insufficient similarity to proteins of known function, and we classified them as being of unknown and unnamed function (most of them are incomplete and not full length). Almost the 36% of ESTs produced from the subtracted library were redundant and this situation could be an indication of the high level of gene expression. EST identities were determined by sequence comparison to the nonredundant GenBank database using BLASTN and BLASTX and sequence homology informations were used to assign putative functions. We validated the result of the different expression of some clones (GST, cytochrome b5, PAL, alcohol acyl transferase, 10-hydroxigeraniol oxidoreductase, valencene synthase, bHLH, MADs box, putative Ser receptor kinase and pectinesterase) firstly through semiquantitative RT-PCR. Then we used Real-time PCR to confirm the differential expression pattern of selected candidate genes (GST, putative Ser receptor kinase and pectinesterase). We chose four samples harvested in different periods to investigate the behaviour of gene expression level during maturity period. Real-time PCR showed that GST transcript levels in 'Moro' orange increased constantly during the entire period of maturation, while in common orange no detectable levels of transcripts could be detected at the first time point of sampling, according also semiquantitative RT-PCR results. We isolated many clones of different kinases (10%) and especially for the clone of a putative Ser receptor kinase (isolated 25 times), we can suppose that maybe it could be involved in regulation mechanisms. Semiquantitative RT-PCR and Real time results revealed that this clone is slightly up-regulated in blood oranges during the late phases of sampling (even if a lower level of transcripts are detected also in common oranges samples). According Real time data of pectinesterase, it is clear that in all ripening sampling of 'Cadenera' and in the first two sampling of 'Moro' there's no traces of gene expression of pectinesterase, but in 'Moro' become evident in the third sampling and increase in ripening sampling. We also concentrated our attention on lengthen and verifying the sequence of alcohol acyl transferase, 10-hydroxigeraniol oxidoreductase and a clone of an unknown sequence,

respectively, using 5'-3' RACE PCR technique, cloning and sequencing. Our object is to obtain the complete ORF sequence and to verify the difference with the corresponding sequences deposited in GenBank. According the 'unknown' clones, the possibility to lengthen and verifying them could allow to obtain many informations about their sequences and their functions. Recently it was constructed a microarray, in which there were spotted all sequences isolated with SSH and cDNA-AFLP procedures and other regulatory genes (MYC and MYB): our aim is using differential sampling period of Moro and Cadenera oranges to will allow a better understanding of their role during ripening. First results will be presented.