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Poster Abstract - E.20

IDENTIFICATION OF GENES SHOWING MODULATED EXPRESSION DURING FRUIT DEVELOPMENT IN *MALUS X DOMESTICA* BORKH

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In 2003 an European project named HiDRAS (High-Quality Disease Resistant Apples for a Sustainable Agriculture) aimed at the identification of the genetic factors controlling apple fruit quality has started. HiDRAS involves 11 European groups and the present study is part of the project.

Fruit development and ripening are fundamental processes determining fruit quality, therefore the knowledge of the genes involved would allow the production of apples that better comply with consumer's expectations. The chosen experimental approach to identify the genes involved in fruit development is cDNA microarray. Such technique allows analyzing the expression of a large number of genes simultaneously. Two subtractive cDNA libraries have been constructed. The first library was generated by selective subtraction of leaf cDNA from fruit cDNA (a mixture of cDNA from three developmental stages) to enrich for fruit specific genes. The second library was produced from fruit cDNA, using Clontech PCR-Select cDNA Subtraction kit to normalize the sample therefore reducing sequence redundancy and enriching to some extent for less expressed genes. Ninety-six clones per library were sequenced as a quality check. 80% and 97% of the sequences from fruit versus leaf and fruit versus fruit libraries appeared to be unique sequences, respectively. About 1600 clones, derived from the two fruit cDNA libraries have been printed in duplicate on microarray slides. Microarray hybridizations were performed adopting an experimental design that involved the comparison of a control RNA sample (isolated from fruits of the cultivar Prima, picked in May 2003) with different RNA samples extracted at later developmental stages (RNA isolated from Prima fruits picked in June, July, August or September, 2003). The adopted strategy allows performing indirect comparisons among all intermediate stages. RNA samples were labelled using the indirect method to avoid the different incorporation of fluorophore modified nucleotides. The collected data were normalized and filtered using specific software. The analysis of the expression profiles suggests that about 10% of the genes present on the slides show a modulated expression during fruit development. As expected, the number of genes differentially expressed increases from the June to September stage. Those genes have been sequenced to identify their putative function. Molecular markers are going to be generated from the most interesting ones, their map position on the apple reference map (Fiesta x Discovery) will be determined and eventually it will be possible to correlate the allelic state of the markers with QTLs controlling fruit quality traits.