Progress in molecular characterization of members of the apple lipoxygenase (LOX) gene family involved in volatile metabolism

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Lipoxygenases (LOXs) are nonheme iron-containing enzymes that catalyze the dioxygenation of fatty acids and are ubiquitous among eucaryotes. LOX catalyzes the addition of molecular oxygen to polyunsaturated fatty acids to produce respective hydroperoxides and have many different putative physiological functions in higher plants. Plant LOXs have been proposed to form biologically active compounds both during normal developmental stages such as germination or growth as well as during responses to environmental stress such as wounding or pathogen attack. LOXs also play a decisive role in the production of volatiles that influence the flavour and aroma of fruits and vegetables.

By a bioinformatic LOX gene mining about 40 different apple sequences were identified as putative members of the LOX family. After a second round of screening for putative functional genes, a phylogenetic tree of the LOX gene family in *Malus*, including totally 23 genes, was calculated. Two sub-trees were found which differentiate the LOX sequences according to their positional specificity of linoleic acid oxygenation (9-LOX or 13-LOX). The positional information of these sequences published in the frame of the '*Malus* x *domestica* Whole Genome Assembly and Annotation' project was used to create a map of the LOX genes with their positions on the apple chromosomes. Some LOX genes are located on apple chromosomes that show strong collinearity, as for instance the chromosomes 2 and 7 or 4 and 12, respectively. It is suggested by several researchers that the apple genome was duplicated during a genome-wide duplication event. Therefore, some of the LOX genes located on these chromosomes might be of the same evolutionary origin.

Cloning of full-length LOX genes was performed by a PCR-based strategy. Based on the sequences of 16 LOX genes, gene-specific PCR primers have been developed and used for semi-quantitative RT-PCR to determine LOX gene expression patterns in apple tissues during fruit ripening and pathogen attack. A variety of SNPs was detected by direct sequencing of PCR fragments amplified with the gene-specific primers in apple cultivars. Presently, SNPs of some candidate genes are analyzed by the TASSEL software package for their association with quantitatively and qualitatively assessed apple volatiles putatively involved in fruit aroma.