

FUNCTIONAL INVESTIGATION OF TWO APPLE LIPID ASSOCIATED GENES IN ENHANCING COLD STRESS RESISTANCE IN TOMATO

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In the commercial production, immediately after harvest, fruits are usually stored at low temperatures to prolong their shelf life and marketability. However, low temperatures may lead to different post harvest related disorders as a result of cold injuries. In apple, one of the most important is known as superficial scald, a physiological disorder causing brown or black patches on the fruit skin. It is suggested that facing chilling conditions, changes in fatty acids composition are recognized to be a crucial adaptation trait in response to temperature stress, because of their role in enhancing membrane integrity, stabilization and fluidity.

In this study, we develop a biparental mapping population crossing the sensitive variety to superficial scald "Granny Smith", with the resistant cultivar "Pinova", to get insight into this occurrence. Apples were held at 4°C for six months after harvest and kept at 24°C for seven days to accelerate the occurrence of the symptoms. The seedlings of the population were genotyped with an Illumina 20K SNP array, enabling a high dense marker coverage of the 17 linkage groups, and phenotyping allowed a more comprehensive metabolite profiling, especially related to lipids, phenols and volatile organic compounds. The co-variant analysis between the markers and the lipids identified a set of QTLs associated, in particular, to two unsaturated fatty acids as major metabolites mainly implicated in cold

stress adaptation: linolenic and vaccenic acid.

A subsequent System Genetics approach, implementing a genetical genomic analysis carried out by profiling the genome-wide transcriptome on the individuals of the bi-parental population through RNA-Sequencing, allowed the identification of a list of differentially expressed genes (DEGs) involved in fatty acid metabolism. Two DEGs related to a member of the Cytochrome p450 family and an Esterase/Lipase were identified and co-locating within the interval of the QTL associated to Linolenic and Vaccenic acid. The role of these two genes was finally functionally validated by the means of heterologous expression in the cultivar Moneymaker of *Solanum lycopersicum*, the model species for fleshy fruit.

To explore the magnitude and the functionality of these components in fostering cold stress resistance, we develop both a constitutive expression and fruit specific expression lines, together with orthologous gene silencing in tomato by CRISPR-Cas9 techniques.

The combination of different OMICS techniques, as well as the functional characterization of two fatty acids related genes will elucidate their role in promoting cold stress resistance in apple.