3213 - Genomics, Molecular Genetics and Biotechnology

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TRANSCRIPTIONAL AND METABOLIC PROFILING OF TWO APPLE TREE CULTIVARS CONTRASTING IN CHILLING REQUIREMENT

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Objective - Considering the limited information about bud dormancy control, this work aimed to investigate the differential gene expression and metabolic profile between Gala and its derived bud sport Castel Gala, which are apple tree cultivars displaying respectively medium and low chilling requirement. In a previous work, a suppressive subtractive hybridization (SSH) assay yielded 28 candidate genes associated to dormancy cycling. Methodology - We performed RT-gPCR in order to validate differential expression on buds from 2007, 2008 and 2009. Samples from 2007 and 2008 were harvested with approximately the same phenological patterns of the ones used for SSH libraries construction. In 2009, sampling was performed on six dates at a commercial orchard with both Castel Gala and Royal Gala trees. Total RNA was isolated by LiCl precipitation, and cDNA synthesis was carried out using the GeneAmp kit (Applied Biosystems). RT-gPCR was performed on a StepOne Plus thermal cycler (Applied Biosystems) with SYBR Green chemistry (Ambion). In parallel, the first two of the six dates from 2009 sampling were screened for a range of metabolites, mainly carbohydrates and organic acids. Frozen tissues were ground in liquid nitrogen and extracted in methanol:chloroform:water (12:5:1) with 20 mg/ml adonitol as internal standard. Derivatized samples were analyzed in a GC-MS system (Agilent). Results - Of the 28 candidate genes identified by SSH, 18 showed differential expression at the predicted dates in 2007 and 2008 samples. The expression of the 18 genes was tested in buds from 2009 sampling, and 12 of them were differentially regulated between cultivars as expected. The final 12 genes were annotated as ARP6, CONSTANS-LIKE, Dormancy-associated MADS box, GAST-like, two histones H2A variant H2A.Z, NAC transcription factor, galactinol synthase, ESKIMO and three dehydrins. In the GC-MS analysis, raffinose, galactinol, pinitol and quinic acid showed the overall most contrasting results, mainly between dormant and non dormant samples of Royal Gala. Conclusions - The results displayed major differences in gene expression between cultivars through the winter, along with metabolic contrasts associated to cell water metabolism. The 12 genes selected are strong candidates to play key roles on dormancy process in apple trees, and further studies are underway to better understand their functions. Financial support: CAPES, CNPq, FINEP, ABPM.