

Identification of dormancy-associated MADS-box genes in apple

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Keywords: *Malus x domestica*, apple, MADS-box, dormancy, RT-qPCR.

Apple (*Malus x domestica*) genetic breeding encompasses the understanding of specific regulatory mechanisms that involve environmental factors, such as cold dependent dormancy progression. MADS-box genes code for a family of transcription factors with conserved DNA-binding domain with a key role in floral meristem determination and in transition from vegetative to reproductive growth. Dormancy-associated MADS-box (*DAM*) genes were identified in peach as candidates for the regulation of growth cessation and terminal bud formation. In this work, we aimed to identify possible *DAM* orthologous genes in apple, exploiting bioinformatic tools and the first available public version of apple genome, besides to investigate their expression patterns during an annual cycle. Deduced amino acid sequences of six peach *DAM* genes were used as baits for BLAST searches of the genomes of apple, *Arabidopsis* and poplar and the best hits, their scores and e-values were registered. Six potential orthologs (*MdDAM1* to *6*) were found in the apple genome and a phylogenetic analysis was performed to compare these sequences to the *DAM* genes of *Prunus persica*, *Arabidopsis thaliana*, *Populus trichocarpa*, *Ipomea batatas* and *Petunia x hybrida*. Domains analysis was carried out using MEME suite program. *MdDAM1* to *3* genes represent possible candidates for regulators of dormancy process in apple because they cluster to the same clade and present similar domains to the peach *DAM* genes. *MdDAM4* and *5* genes are possible orthologous to *Arabidopsis SVP* gene, one of the responsible for suppression of the flowering gene regulator *FT*. *MdDAM6* gene seems to have an incomplete MIKCC domain, although it has been included in *DAM* clade. Specific primers were designed for each *MdDAM* from the available nucleotide sequences of the apple genome. Three primer pairs (*MdDAM 2, 4* and *6*) led to amplification products. However, only *MdDAM6* has shown estimated primer efficiency sufficient to allow quantitative expression analyses. *MdDAM6* gene expression was evaluated by RT-qPCR in buds of Fuji Standard cultivar, sampled throughout the year 2009, including vegetative and dormant plant stades. A progressive increase in *MdDAM6* expression was observed from summer to winter. At the end of cold season, *MdDAM6* expression levels declined and, in mid-spring, it reached basal levels similar to those found in the summer. This profile was coincident to that of genes whose function has been associated with establishment and / or maintenance of endodormant bud state.

Financial support: CNPq, CAPES, EMBRAPA, FINEP.