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Type-B response regulators of the cytokinin-signaling pathway link hormonal stimulus and molecular responses to overcome dormancy in apple

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Type-B response regulators (TBRRs) are essential transcription factors acting in the final steps of the plant cytokinin-signaling pathway. Cytokinin (CK) is an important phytohormone that stimulates cell division and is involved in plant dormancy overcome. Our previous studies revealed the presence of TBRR binding sites in the promoter region of *MdoDAM1 and MdoFLC genes,* important regulators of dormancy establishment/maintenance and flowering inhibition, respectively. The aim of this study is to evaluate if apple TBRRs could modulate the expression of *MdoDAM1* and *MdoFLC* in order to promote dormancy overcome. The transcript levels of the *TBRRs MdoFLC and MdoDAM1* genes were evaluated by RT-qPCR in 'Royal Gala' apple buds exposed to controlled chilling (3°C) and growth condition (25°C). Results indicated that two *TBRRs* have higher responses during chilling accumulation, contrasting with *MdoDAM1* expression. When growth conditions were applied, transcripts of other *TBRRs* accumulated at the same point when *MdoFLC* expression dropped. In the Castel Gala cultivar, the expressions of *TBRR* genes were in agreement with higher quantities of endogenous CK. The exogenous application of CK in ecodormant 'Royal Gala' buds increased *TBRR* expression at the same time that decreased *MdoDAM1* and *MdoFLC* transcript levels when compared to untreated samples. The ability of TBRRs to bind to *MdoDAM1* promoter was evaluated by a transactivation assay using *Arabidopsis thaliana* protoplasts. Results showed that TBRRs were able to bind to *MdoDAM1* promoter, possibly acting as repressors. Altogether, these findings suggest an important connection between hormonal stimulus and molecular response mediated by TBBRs to overcome apple dormancy.

Keywords: *Apple, Type-B Response Regulators, Cytokinin, Dormancy*