Reference gene selection for gene expression studies in apple

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Apple (*Malus x domestica* Borkh.) is the most important deciduous tree fruit crop grown around the world, and also in the Southern of Brazil. Researches on apple genetic breeding include disease resistance mechanisms, grafting, chilling requirement, fruit ripening and production of nutraceutical compounds. Comparisons among profiles of gene expression of different tissues, conditions or cultivars are valuable scientific tools to better understand the genetic basis of important agronomic and forestry traits. The quantitative reverse transcriptase PCR (RT-qPCR) offers a robust resource to quantify, accurately, changes in gene expression, being specific and sensitive to low abundance mRNAs. However, its accuracy is dependent on the evaluation of stable reference genes for normalization of data, which must be validated previously to the analysis of samples. The choice of inappropriate reference genes may result in deviation on gene expression profiles, statements of statistical significance undue or incorrect conclusions and characterizations. The objective by this work was to select the best genes to be used as references for gene expression studies by RT-qPCR in apple trees. Vegetative and reproductive tissues of Gala cultivar were evaluated during their seasonal cycle of growth and dormancy. Were investigated the expression of 23 genes, traditional housekeeping or suggested as constitutive genes by microarray data: *ACT2, ACT11, ACTfam* (gene family), *ARC5, C3HC4, CDC48, CKL, DLD, EF1α, EF1β, GAPDH, KEA1, MDH, PCS, PP2-A1, PP2A-A3, SAND, THFS, TMp1, TUBα5, TUBβ6*, *UBC10* and *WD40*. RT-qPCR assays were performed in *StepOnePlus™ Real-Time PCR System* (Applied Biosystems). All tested combinations of primers allowed specific amplification and suitable efficiency curves for gene expression studies by RT-qPCR, except primer pairs for *CDC48* an *PP2A-A3*. The stability of the 21 genes was determined by two different statistical descriptors, *geNorm* and *NormFinder*. The expression of *EF1β*, *MDH*, *SAND*, *THFS* and *WD40* were the most stable through all samples: dormant and open buds, flowers, young and expanded leaves, fruitset, skin and pulp or unripe and mature apple fruits. Specific combinations of two or three reference genes were shown to be sufficient to normalize each apple sample set analyzed. Furthermore, *PAL* expression was used to validate the selected normalizers.

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