Title Validation of reference genes for RT-qPCR studies of gene expression in banana fruit under different experimental conditions

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Abstract

Reverse transcription quantitative real-time PCR (RT-qPCR) is a sensitive technique for quantifying gene expression, but its success depends on the stability of the reference gene(s) used for data normalization. Only a few studies on validation of reference genes have been conducted in fruit trees and none in banana yet. In the present work, 20 candidate reference genes were selected, and their expression stability in 144 banana samples were evaluated and analyzed using two algorithms, geNorm and NormFinder. The samples consisted of eight sample sets collected under different experimental conditions, including various tissues, developmental stages, postharvest ripening, stresses (chilling, high temperature, and pathogen), and hormone treatments. Our results showed that different suitable reference gene(s) or combination of reference genes for normalization should be selected depending on the experimental conditions. The *RPS2* and *UBQ2* genes were validated as the most suitable reference genes across all tested samples. More importantly, our data further showed that the widely used reference genes, *ACT* and *GAPDH,* were not the most suitable reference genes in many banana sample sets. In addition, the expression of *MaEBF1*, a gene of interest that plays an important role in regulating fruit ripening, under different experimental conditions was used to further confirm the validated reference genes. Taken together, our results provide guidelines for reference gene(s) selection under different experimental conditions and a foundation for more accurate and widespread use of RT-qPCR in banana.