Identification of reference genes for quantitative real-time PCR in different developmental stages and under refrigeration conditions in soursop fruits (*Annona muricata* L.)

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Abstract

The quantitative real-time polymerase chain reaction (qRT-PCR) is a technique to quantify the gene expression. However, data normalization needs suitable references genes that show a constant expression under different conditions. Up to date, no reference genes have been evaluated in soursop fruits (*Annona muricata* L.). The objective of this work was to analyze the transcript stability of some genes in different developmental stages and under refrigeration conditions in soursop fruits. We created a database from a public transcriptome data of soursop leaf, identify homologous gene in soursop and designed primers to amplify the reference genes: *Ubiquitin carrier-like protein* (*UBCc*), *Ubiquitin-conjugating enzyme E2 2* (*UBCg*), *Elongation factor 1α* (*EF1α*), *b-tubulin* (*TUB*) and *18 S ribosomal RNA* (*18S*). Total RNA was extracted from soursop mesocarp at 0, 3 and 6 days from fruits stored at 25±1 °C and 0, 3, 6 and 9 days from fruits stored at 15±1 °C. cDNA was synthesized through SuperScript III kit following the manufacturer instructions. The gene expression was analyzed by qRT-PCR using the Rotor-Gene Q equip, the geNorm and RefFinder web-tool were used to classify the most stable transcript for each condition. The results indicated that the *UBCc* was the most stable transcript during the fruit development at 25±1 °C. On the other hand, *EF1α* showed the highest stability in soursop fruits stored at 15±1 °C. Global analysis of both conditions demonstrated that *UBCc* and *EF1α* were the most stable transcripts and can be used as reference genes for the normalization of the data in soursop during fruit development and under refrigeration.