



Notes

Reference genes for RT-qPCR expression studies in wild and cultivated *Arachis* species

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Plant transcriptome analysis under specific experimental conditions helps the understanding of cellular processes related to development, stress response, and crop yield. The validation of these studies has been accomplished by RT-qPCR which requires normalization of mRNA levels among samples. This can be achieved by comparing the expression ratio between a gene of interest and a reference gene which is constitutively expressed. Nowadays there is a lack of appropriate reference genes for wild and cultivated *Arachis*. In the present work, a simplified RT-qPCR protocol based on SYBR reagent was used for the identification of genes with minimal expression variation in *Arachis*. Ten reference genes were analyzed in four *Arachis* species (*A. magna*, *A. duranensis*, *A. stenosperma*, and *A. hypogaea*) subjected to biotic (root-knot nematode and leaf spot fungus) and abiotic (drought) stresses, in two distinct plant organs (roots and leaves). By the use of three algorithms (GeNorm, NormFinder and BestKeeper), five genes (ACT1, UBI1, GAPDH, 60S and UBI2), emerged as top reference genes in eight samples. The former three were the most stable across all species, organs and treatments studied. To ratify the expression stability of candidate reference genes, the expression profile of an *A. magna* gene induced by water deficit was analyzed using reference genes (60S and UBI2) selected in this study. The use of the appropriate reference genes characterized here should improve the accuracy and reliability of gene expression analysis in peanut and other legumes and contribute for the better understanding of gene expression.

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