Validation of Reference Genes for Gene expression Studies by RT-qPCR in leaf-cutting ant *Atta sexdens rubropilosa.*

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INTRODUCTION. Quantitative real time PCR (qPCR) is a sensitive, accurate and reproducible tool in gene expression analysis. To bypass the several problems in standardization of the sample, reliable reference genes should be used. **OBJECTIVE:** Select suitable genes to be used as a reliable reference genes in RT-qPCR analysis of gene expression from leaf-cutting ant *Atta sexdens rubropilosa*. **MATERIAL AND METHODS:** The samples from *A. sexdens rubropilosa* were picked from a laboratory nest kept at Centro de Estudos de Insetos Sociais (UNESP de Rio Claro/SP). The gene profile expression was evaluated in two groups, developmental stages (larva, pupa and worker) and tissues (head, thorax, abdomen and worker without abdomen). To this, total RNA was extracted, quantified and its integrity was analysed. The samples were treated with DNase and used in a reaction to obtain cDNA. Primers from five different candidate reference genes (RPL18, EF1BETA, EF1ALPHA, GAPDH and ACT) were synthetized and qPCR was performed with diluted cDNA. The best primer concentration and efficiency reaction were evaluated for each gene. The raw values of C_q obtained were used with five algorithm (geNorm, BestKeeper, NormFinder, comparative ΔC_t method and RefFinder) to analyse gene expression stability and rank them in each group. **RESULTS AND DISCUSSION**: The expression stability of candidate reference genes were almost the same when ranked by each algorithm. Furthermore, the optimal number of reference genes was determined for both groups by geNorm, displaying that two reference genes are sufficient to normalize expression level experiments. RefFinder integrated the results of four previous analyses and ranked RPL18 and EF1BETA as the suitable reference genes to be used for different developmental stages and tissues. **CONCLUSIONS:** The validation of reference genes for *A. sexdens rubrupilosa* showed that two genes, RPL18 and EF1BETA, are reliable to be used as reference genes in expression level studies.

Keyword: Reference gene,expression level, qPCR, *Atta sexdens rubropilosa*.
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