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Reference Genes Validation in *Phenacoccus solenopsis* under Biotic and Abiotic Stress Conditions

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ABSTRACT: *Phenacoccus solenopsis* (Mealybug) is a devastating phloem sap feeder. It is now recognized as an aggressive invasive pest of various economically important crops. Due to economic significance of this species has led to great insight in basic research involving gene-expression studies. Real-time PCR (RT-qPCR) expression analysis is a powerful analytical technique, but for normalization of data requires the use of stable reference genes. However, suitable reference genes are still not known in the case of *P. Solenopsis*. This study proposed to test the expression stability of 6 commonly used candidate reference genes including *β-TUB*, *GAPDH, RPL32*, *α-TUB, ACT and SDH* subjected to various regimes of experimental treatments. In the validated samples, expression stability of the reference genes was analyzed by employing three different statistical software's geNorm, NormFinder, and Refinder. Moreover, pairwise analysis showed that two reference genes were not sufficient to normalize the gene expression data under different condition. By contrast, ACT and GAPDH were the least stably expressed genes tested. The suitability of particular combination of reference genes was empirically validated by performing normalizations of expression data for the MIP's and *Zn_MP* genes under development and host feeding assay's. This study provides a list of reliable reference genes for transcript normalization in *P. Solenopsis* in different abiotic and biotic stress treatments, which will facilitate genetic studies in other closely related species.

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