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Evaluation and Validation of housekeeping genes in two contrast species of thyme plant to drought stress using real-time PCR

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Abstract

To decrease errors and increase accuracy and reliability of quantitative real-time PCR (qRT-PCR) results, the use of a reference gene is inevitable. Despite the industrial importance of genus *Thymus*, not any validated reference gene has not been reported for *T. kotschyanus* and *T. vulgaris* which could limit such investigations. In this study, the expression stability of seven housekeeping genes including *Actin*, *Cyclophilin-18*, *elongation factor-1A*, *glyceraldehyde-3-phosphate dehydrogenase*, *18S ribosomal RNA*, *Cullin*, and *Polypyrimidine tract-binding protein* were evaluated in *T. kotschyanus* and *T. vulgaris* which grown at four levels of drought stress using geNorm, NormFinder, and BestKeeper algorithms. *Histone deacetylase-6* (*HDA-6*) gene was also used for validation of evaluated reference genes. In *T. vulgaris*, all of the algorithms similarly ranked elongation *factor-1A* and *glyceraldehyde-3-phosphate dehydrogenase* as the two most stably expressed genes. In *T. kotschyanus*, only NormFinder and BestKeeper had a similar ranking and identified *Actin* and *glyceraldehyde-3-phosphate dehydrogenase* as the two most stably expressed genes, but geNorm algorithm ranked elongation *factor-1A* and *glyceraldehyde-3-phosphate dehydrogenase* as the best two reference genes. On the other hand, all algorithms ranked *18S rRNA* and *Cyclophilin-18* as the least stable genes in *T. kotschyanus* and *T. vulgaris*, respectively. Validation results indicated that there was a significant change (0.53 to 3.19 fold change) in relative expression of *HDA-6* normalized by the best stable gene compare to the least ranked gene. Our study presented the first systematic validation of reference gene(s) selection in *T. kotschyanus* and *T. vulgaris* and provided useful information to obtain more accurate qRT-PCR results in these species.

Key words: *Thymus kotschyanus*, *Thymus vulgaris*, gene expression analysis, reference genes, abiotic stress.