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Evaluation of reference genes for RT-qPCR studies in the leaves of rice seedlings under salt stress

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ABSTRACT. To obtain accurate and reliable results for the expression of genes of interest using quantitative real-time polymerase chain reaction (RT-qPCR) techniques, it is necessary to normalize the data by comparing them to constitutive genes that exhibit uniform expression levels under experimental conditions. In this study, the stability of expression was evaluated for the following ten candidate reference genes in rice leaves (*Oryza sativa* L.) from the BRS Bojuru and BRS Ligeirinho genotypes that were subjected to salt stress (150 mM): actin 11 (*ACT11*), beta-tubulin (β-TUB), eukaryote elongation factor 1-α (*Eef-1*), eukaryotic initiation factor 4-α (*eIF-4-α*), E2 ubiquitin-conjugating enzyme (*UBC-E2*), ubiquitin 5 (*UBQ5*), ubiquitin 10 (*UBQ10*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), *TIP41-like*, and cyclophilin. The stability of expression for the aforementioned genes was then compared to that of three *LTP* genes using *UBQ10*, *Eef-1*, and *eIF-4-α* as references. After analyzing the

expression levels using analysis of variance tests, the results indicated that *UBQ10* was the most stable in all treatments (M = 0.404 and SV = 0.327). Furthermore, the *eIF-4-α*, *TIP41-like*, and cyclophilin genes exhibited the highest total coefficient of variation (CV = 269, 169.2, 179.2, respectively), which signifies that they exhibited the least stable expression. The expression levels of each candidate gene (*LTP7*, *LTP10*, and *LTP13*) were in contrast to the reference genes. Therefore, we concluded that *UBQ10* is the best reference gene for RT-qPCR reactions under the experimental conditions. The expression analysis of *LTP7*, *LTP10*, and *LTP13* confirmed the importance of validating reference genes to achieve accurate RT-qPCR results.

Key words: *Oryza sativa* L.; Salinity; Ubiquitin; Real-time quantitative PCR