Reference Genes For Transcript Quantification In Gracilaria Tenuistipitata Under Drought Stress

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INTRODUCTION. *Gracilaria tenuistipitata* is a red macroalgae often found in intertidal environments, where it is frequently under drought stress due to tidal water scarcity. **OBJECTIVE:** The aim was to study the molecular mechanisms involved in *G. tenuistipitata* dehydration resistance**. MATERIAL AND METHODS:** It was performed a dehydration assay, tested different RNA extraction protocols, and selected suitable reference genes for RT-qPCR. **DISCUSSION AND RESULTS**: After testing the 4 different methods, we found that an adapted TRIzol method resulted in the highest quantities of pure RNA from less biomass. This method yielded an average of 783.6 $ng \cdot mg^{-1}$ of clean RNA from 25 mg of fresh tissue in only 1.5 hours. Additionally, DNAse I treatment and silica spin column purification were performed prior to RT-qPCR. Of the 9 putative reference genes tested for expression stability under control and drought-like conditions, a combination of 4 genes: *β-GALACTOSIDASE* B (*GLB*), *TRANSCRIPTION INITIATION FACTOR IIB* (*GTF2B*), *EUKARYOTIC ELONGATION FACTOR 2* (*EEF2*), and *β-TUBULIN* (*TUB*) were selected. This allowed us to measure the transcription levels of *THIOREDOXIN* (*TRX*), *OXYGEN-EVOLVING ENHANCER 1* (*OEE)*, and *NITRATE REDUCTASE 2* (*NIA2*). *TRX* was up-regulated after 1 h dehydration, and maintained higher transcriptional levels even 4 h later. In contrast, *OEE1* transcriptional levels were slowly down-regulated, and *NIA2* transcriptional levels were down-regulated 1 h after dehydration, but returned to regular levels 4 h after. **CONCLUSION:** This suggests that 40% dehydration of *G. tenuistipitata* induced transcriptional responses without changing the relative growth rate or affecting thalli mortality.

Keywords: Rhodophyta; Dehydration; geNorm;RT-qPCR

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