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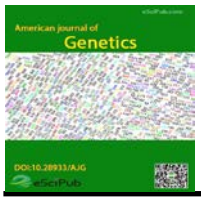

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Selection of Reference Genes in Vitis Vinifera Inoculated With Xanthomonas Campestris

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ABSTRACT

Introduction: The selection of reference genes (RGs) is considered as one of the most critical steps for RT-qPCR since these genes must show stable expression under varying experimental conditions, cell types, and developmental stages, among other factors. Despite the fact that there are publications for this topic in the grapevine, the majority of them are based on cultivars infected by fungi or subject to abiotic stress. To our knowledge, there is no study investigating the selection of RGs in vine affected by X. campestris. Objective: To select for suitable RGs for gene expression normalization of RNA-Seq expression data from two V. vinifera cultivars with contrasting phenotypes for resistance to this pathogen. Methodology: A search in the literature for publications that selected explicitly for RGs in grapevine was performed, further including those articles using conditions of biotic (preferentially) or abiotic stress. After an initial prospection, the retrieved primers sequences were evaluated by an in silico PCR assay using the Primer-BLAST software at NCBI aiming to align the sequences in the V. vinifera genome and its specific amplification, thus confirming, therefore, gene annotation. Finally, the RGs were tested by both conventional PCR and RT-qPCR and using a negative control (NTC) reaction each tested primer pair. Results and Discussion: From 14 RGs selected, nine (CYP, VATP16, EF1α, TRU5, TCPB, TIF-GTP, GAPDH, ACT, and 60SRP) met the criteria all the criteria described above and its sequences were sent for primer synthesis. The detection by both conventional PCR and RT-qPCR confirmed the amplification of all tested RGs. However, under the RT-qPCR, it was possible to not unspecific amplification on NTC of two RGs (CYP and VATP16), therefore confirming the resolution of the assay. Conclusion: A selection of putative candidate RGs was carried out by showing satisfactory results on initial tests on both convencional PCR and RT-qPCR. However, it remains necessary to assure the stability of these genes by using specific software (Genorm, NormFinder, and BestKeeper).

Keywords:
Gene Expression; Grapevine; Grapevine Bacterial Canker; PCR; RT-qPCR

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