Identifying and validating housekeeping hybrid Prunus spp. Genes for root gene-expression studies

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Prunus rootstock belonging to subgenera Amygdalus (peach), Prunus (plum) and Cerasus (cherry) are either from the same species as the scion or another one. The number of inter-species (including inter-subgenera) hybrids has increased as a result of broadening the genetic basis for stress (biotic and abiotic) resistance/tolerance. Identifying genes associated with important traits and responses requires expression analysis. Relative quantification is the simplest and most popular alternative, which requires reference genes (housekeeping) to normalize RT-qPCR data. However, there is a scarcity of validated housekeeping genes for hybrid Prunus rootstock species. This research aims to increase the number of housekeeping genes suitable for Prunus rootstock expression analysis. Twenty-one candidate housekeeping genes were pre-selected from previous RNAseq data that compared the response of root transcriptomes of two rootstocks subgenera to hypoxia treatment, 'Mariana 2624' (P. cerasifera Ehrh.× P. munsoniana W. Wight & Hedrick), and 'Mazzard F12/1' (P. avium L.). Representing groups of low, intermediate or high levels of expression, the genes were assayed by RT-qPCR at 72 hours of hypoxia treatment and analyzed with NormFinder software. A sub-set of seven housekeeping genes that presented the highest level of stability were selected, two with low levels of expression (Unknown 3, Unknown 7) and five with medium levels (GTB 1, TUA 3, ATPase P, PRT 6, RP II). The stability of these genes was evaluated under different stress conditions, cold and heat with the hybrid 'Mariana 2624' and N nutrition with the hybrids 'Colt' (P. avium × P. pseudocerasus Lindl.) and 'Garnem' [P. dulcis Mill.× (P. persica L.× P. davidiana Carr.)]. The algorithms of geNorm and BestKeeper software also were used to analyze

the performance of these genes as housekeepers. Stability rankings varied according to treatments, genotypes and the software for evaluation, but the gene GBT 1 often had the highest ranking. However, most of the genes are suitable depending on the stressor and/or genotype to be evaluated. No optimal number of reference genes could be determined with geNorm software when all conditions and genotypes were considered. These results strongly suggest that relative RT-qPCR should be analyzed separately with their respective best housekeeper according to the treatment and/ or genotypes in Prunus spp. rootstocks.