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Review

Plant reference genes for development and stress response studies

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Many reference genes are used by different laboratories for gene expression analyses to indicate the relative amount of input RNA/DNA in the experiment. These reference genes are supposed to show least variation among the treatments and with the control sets in a given experiment. However, expression of reference genes varies significantly from one set of experiment to the other. Thus, selection of reference genes depends on the experimental conditions. Sometimes the average expression of two or three reference genes is taken as standard. This review consolidated the details of about 120 genes attempted for normalization during comparative expression analysis in 16 different plants. Plant species included in this review are *Arabidopsis thaliana*, cotton (*Gossypium hirsutum*), tobacco (*Nicotiana benthamiana* and *N. tabacum*), soybean (*Glycine max*), rice (*Oryza sativa*), blueberry (*Vaccinium corymbosum*), tomato (*Solanum lycopersicum*), wheat (*Triticum aestivum*), potato (*Solanum tuberosum*), sugar cane (*Saccharum* sp.), carrot (*Daucus carota*), coffee (*Coffea arabica*), cucumber (*Cucumis sativus*), kiwi (*Actinidia deliciosa*) and grape (*Vitis vinifera*). The list includes model and cultivated crop plants from both monocot and dicot classes. We have categorized plant-wise the reference genes that have been used for expression analyses in any or all of the four different conditions such as biotic stress, abiotic stress, developmental stages and various organs and tissues, reported till date. This review serves as a guide during the reference gene hunt for gene expression analysis studies.

Keywords. Biotic and abiotic stress; normalization; plant; real-time PCR; reference genes; stable expression

1. Introduction

Comparative gene expression analysis requires one or more reference genes to reflect the amount of RNA or cDNA transcribed under a particular condition or treatment in an experiment. While analysing two samples of different experimental conditions, or tissues, expression of the reference gene is taken as internal standard. Formerly, relative quantification of transcription was done by Northern blot analysis or by reverse transcription polymerase chain reaction (RT-PCR). Northern blot often used the amount RNA loaded as a reference. The problem associated with RNA was that the visible band was of rRNA and not mRNA (Shah *et al.* 2009). So several laboratories re-probed the Northern blots with cDNA of reference genes like housekeeping genes (HKG) (Dean *et al.* 2002). Such reference genes were amplified for comparison of RT-PCR results as well. The images of Northern blots and RT-PCR gels were captured using digital cameras and the information was processed by software (example, NIH Image program), which normalized the band intensity of gene of interest with that of reference gene and calculated the fold change (Dean *et al.* 2002). The limitations of this method were that the calculation was not precise, was less sensitive, and could not detect extremely low expression. Soon, Northern hybridization and RT-PCR evolved into microarray and real-time PCR, respectively. While the former could analyse thousands of genes at a time, only one gene could be analysed in the later method. One requirement which was inevitable all throughout this evolution was that of an appropriate reference gene to normalise the RNA levels.

Microarray and real-time PCR techniques are highly sensitive and can calculate the precise fold change during a comparative expression analysis. This precision is highly dependent on the expression of the reference gene. It is most important that the reference gene exhibited stable expression during various treatments considered for comparison. There are reports of about 120 genes, analysed for their potential to be used as reference genes in plants subjected to various treatments. In this review we have consolidated details of these genes, the nature of stress/treatment and, the stability of these genes as well.

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