Reference genes for quantitative real-time polymerase chain reaction studies in soybean plants under hypoxic conditions

T.J. Nakayama[1,2], F.A. Rodrigues[1], N. Neumaier[1], F.C. Marcelino-Guimarães[1], J.R.B. Farias[1], M.C.N. de Oliveira[1], A. Borém[3], A.C.B. de Oliveira[4], B.M. Emygdio[4] and A.L. Nepomuceno[1-5]

[1]Laboratório de Biotecnologia Vegetal, Embrapa Soja, Londrina, PR, Brasil
[2]Departamento de Biologia Geral, Universidade Estadual de Londrina, Londrina, PR, Brasil
[3]Departamento de Fitotecnia, Universidade Federal de Viçosa, Viçosa, MG, Brasil
[4]Embrapa Clima Temperado, Pelotas, RS, Brasil
[5]Plant Gene Expression Center, Embrapa Labex USA Plant Biotechnology, Albany, CA, USA

Corresponding authors: A.L. Nepomuceno
E-mail: alexandre.nepomuceno@embrapa.br

ABSTRACT. Quantitative real-time polymerase chain reaction (RT-qPCR) is a powerful tool used to measure gene expression. However, because of its high sensitivity, the method is strongly influenced by the quality and concentration of the template cDNA and by the amplification efficiency. Relative quantification is an effective strategy for correcting random and systematic errors by using the expression level of reference gene(s) to normalize the expression level of the genes of interest. To identify soybean reference genes for use in studies of flooding stress, we compared 5 candidate reference genes (CRGs) with the NormFinder and GeNorm programs to select the best internal control. The expression

stability of the CRGs was evaluated in root tissues from soybean plants subjected to hypoxic conditions. Elongation factor 1-beta and actin-11 were identified as the most appropriate genes for RT-qPCR normalization by both the NormFinder and GeNorm analyses. The expression profiles of the genes for alcohol dehydrogenase 1, sucrose synthase 4, and ascorbate peroxidase 2 were analyzed by comparing different normalizing combinations (including no normalization) of the selected reference genes. Here, we have identified potential genes for use as references for RT-qPCR normalization in experiments with soybean roots growing in O_2-depleted environments, such as flooding-stressed plants.

Key words: Endogenous genes; Internal control genes; Flooding; Housekeeping genes; Gene expression