# Quantification of mRNAs and Housekeeping Gene Selection for Quantitative Real-Time RT-PCR Normalization in European Beech (*Fagus sylvatica* L.) during Abiotic and Biotic Stress

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Z. Naturforsch. **63c**, 574–582 (2008); received March 12/April 30, 2008

Analyses of different plant stressors are often based on gene expression studies. Quantitative real-time RT-PCR (qRT-PCR) is the most sensitive method for the detection of low abundance transcripts. However, a critical point to note is the selection of housekeeping genes as an internal control. Many so-called 'housekeeping genes' are often affected by different stress factors and may not be suitable for use as an internal reference. We tested six housekeeping genes of European beech by qRT-PCR using the Sybr Green PCR kit. Specific primers were designed for 18S rRNA, actin, glyceraldehyde-3-phosphate dehydrogenase (GAPDH1, GAPDH2), $\alpha$-tubulin, and ubiquitin-like protein. Beech saplings were treated with increased concentrations of either ozone or $CO_2$. In parallel, the expression of these genes was analyzed upon pathogen infection with *Phytophthora citricola*. To test the applicability of these genes as internal controls under realistic outdoor conditions, sun and shade leaves of 60-year-old trees were used for comparison. The regulation of all genes was tested using a linear mixed-effect model of the R-system. Results from independent experiments showed that the only gene not affected by any treatment was actin. The expression of the other housekeeping genes varied more or less with the degree of stress applied. These results highlight the importance of undergoing an individual selection of internal control genes for different experimental conditions.

*Key words:* Abiotic/Biotic Stress, European Beech (*Fagus sylvatica*), Housekeeping Genes, Quantitative Real-Time RT-PCR