Seasonal quantification of Grapevine fanleaf virus by one-step RT real-time PCR

U. Čepin, I. Gutiérrez-Aguirre, M. Pompe-Novak, K. Gruden, M. Ravnikar

Department of Biotechnology and Systems Biology, National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia
Contact: urska.cepin@nib.si

Objective
Design and validate an RT quantitative real-time PCR (RT-qPCR) assay, to efficiently quantify GFLV virus in grapevine tissues through the whole season.

Results & Conclusions
Validation of MGB-probe-based one-step RT real-time PCR (RT-qPCR) assay, developed for relative quantification of GFLV during the season showed:
• a dynamic range of up to 10 orders of magnitude (Figure 3),
• LOQ down to ≈ 100 genome copies (Table 1),
• $R^2$ was higher than 0.995 and the amplification efficiency (E) between 90.6% and 104%.

For relative quantification of GFLV in phloem during the growing season, the expression stability of two reference genes (COX and 18S) was validated. M value of 0.488 (by GeNorm software) indicated high seasonal correlation and stability of their expression, which makes them suitable candidates as normalisers. Their geometric mean was used for normalisation (Vandesompele et al., 2002).

Table 1: The performance of RT-qPCR assay evaluated after analysing three independent runs using 10-fold serially diluted RNA. $\Delta$Cq: Difference in quantification cycles between 10-fold dilutions, CV: Coefficient of variation of calculated GFLV concentration.

For relative quantification of GFLV in phloem during the growing season, the expression stability of two reference genes (COX and 18S) was validated. M value of 0.488 (by GeNorm software) indicated high seasonal correlation and stability of their expression, which makes them suitable candidates as normalisers. Their geometric mean was used for normalisation (Vandesompele et al., 2002).

The relative expression ratio ($r$) was calculated based on the $E=10^{1/\text{slope}}$, where the slope means $\Delta$Cq between 10- and 100-fold dilutions, of both GFLV and the reference genes and expressed as GFLV concentration in each sample compared to the GFLV concentration in a defined calibrator sample (Pfaffl, 2001).

The $r$ values were proportional to the GFLV genome concentration and showed the lowering of viral RNA amount during the season in the majority of the tested grapevines, except in one plant Ref DU 3/13 (Figure 4) which also showed different symptoms.

For relative quantification of GFLV in phloem during the growing season, the expression stability of two reference genes (COX and 18S) was validated. M value of 0.488 (by GeNorm software) indicated high seasonal correlation and stability of their expression, which makes them suitable candidates as normalisers. Their geometric mean was used for normalisation (Vandesompele et al., 2002).

The relative expression ratio ($r$) was calculated based on the $E=10^{1/\text{slope}}$, where the slope means $\Delta$Cq between 10- and 100-fold dilutions, of both GFLV and the reference genes and expressed as GFLV concentration in each sample compared to the GFLV concentration in a defined calibrator sample (Pfaffl, 2001).

The $r$ values were proportional to the GFLV genome concentration and showed the lowering of viral RNA amount during the season in the majority of the tested grapevines, except in one plant Ref DU 3/13 (Figure 4) which also showed different symptoms.

References