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

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Figure 1: *Grapevine fanleaf virus (GFLV) is the causal agent of the fanleaf degeneration disease (see the symptoms), which confronts grape growers worldwide.*

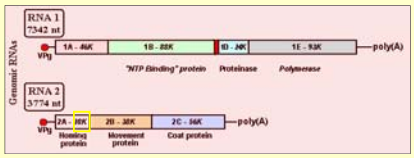

Figure 2: *The genome of GFLV virus is composed of two single-stranded positive-sense RNA molecules. Yellow square represents location of qPCR amplicon.*

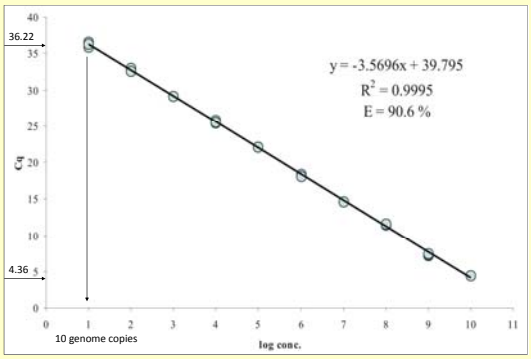

Figure 3: *Performance of the assay using 10-fold dilutions of plasmid DNA (of known concentration carrying GFLV RNA2 molecule).*

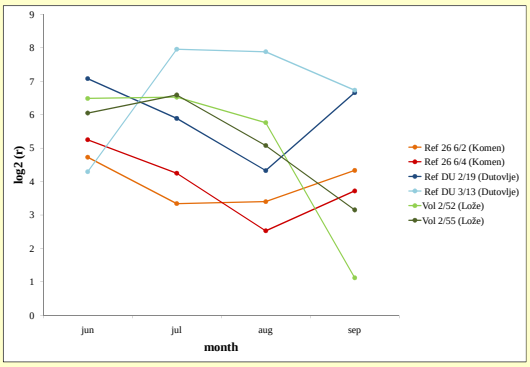

Figure 4: *GFLV was relatively quantified in six grapevine samples from Slovenia at 4 time points during the season.* ***r:*** *relative expression ratio*

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%.

		Type of analysis						
		Intra-assay					Inter-assay	
Dilution RNA (isolate F13)	Theoret. conc.	Signal ratio (positive/total No. of reactions)	Average C_q	ΔC_q	Average calculated conc.	CV (%)	Signal ratio (positive/total No. of runs)	Average calculated conc.	CV (%)
non diluted	10^7	3 of 3	16.67	NA	10 102 440	4.2	3 of 3	9 158 103	14.7
10^{-1}	10^6	3 of 3	20.13	3.46	924 320	12.5	3 of 3	941 688	2.8
10^{-2}	10^5	3 of 3	23.48	3.35	91 013	4.1	3 of 3	111 000	31.8
10^{-3}	10^4	3 of 3	26.23	2.75	13 527	6.3	3 of 3	13 225	5.3
10^{-4}	10^3	3 of 3	30.03	3.80	980	10.7	3 of 3	993	3.4
10^{-5}	LOQ 100	3 of 3	33.48	3.45	91	22.8	3 of 3	86	10.4
10^{-6}	LOD 10	2 of 3	37.73	4.25	5	16.5	3 of 3	9	39.4
10^{-7}	1	0 of 3	/	/	/	/	0 of 3	/	/
0 (water control)	0	0 of 3	/	/	/	/	0 of 3	/	/

Table 1: *The performance of RT-qPCR assay evaluated after analysing three independent runs using 10-fold serially diluted RNA.* ***Cq:*** *Quantification cycle,* ***ΔCq:*** *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 the 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/slope), where the slope means ΔCq 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

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- Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F., (2002) Genome Biol. 3 (7), research0034.1–research0034.11