

Statistical modeling of long-term grapevine response to ‘*Candidatus Phytoplasma solani*’ infection in the field

Ana Rotter, Petra Nikolić, Neža Turnšek, Polona Kogovšek, Andrej Blejec, Kristina Gruden, Marina Dermastia*

National Institute of Biology, Department of Systems Biology and Biotechnology, Večna pot 111, SI-1000 Ljubljana, Slovenia

*Corresponding author

Marina Dermastia, National Institute of Biology, Department of Systems Biology and Biotechnology, Večna pot 111, SI-1000 Ljubljana, Slovenia

E-mail: marina.dermastia@nib.si

Table S2.

Table S2.

A. Genes analysed in this study together with their annotated metabolic pathways and protein functions. Expression of genes was followed through several subsequent years, with the exception of year 2006, when no samples were analysed. The genes used for validation of model predictions were analysed additionally in 2010. qPCR amplicon details are given as forward (FP) and reverse (RP) primer and probe sequences and their concentrations used in the final reactions. Efficiency of amplification was calculated for each amplicon from the standard curve from the season 2007. Probes were modified with 5' – FAM reporter and a) 3' - TAMRA or b) 3' - MGB quencher. Amplicons were designed and produced by *MWG Biotech AG or ** Applied Biosystems. *** these gene were as reference genes.

Metabolic pathway	Protein function	Gene name	Sequence (5'-3')	Conc. (nM)	Amplicon length	Target sequence (location of the amplicon) and ID number from microarrays	Efficiency of amplification (2007)	Followed in years
Photosynthesis	Apocytochrome f precursor	Vv ACYT*	FP cga gga ctt cca aac aca tc	900	900	TC57415 (2060-2156 bp)	93,34	2004-2009
			RP act ggg tca ttc ata gca tta c	900	97 bp	Vv_10003724		
NAD regeneration	Alcohol dehydrogenase	Vv ADH1*	FP aag gtg atc ttg ggt gac ttt ca	900	900	AF194173 (1220-1291 bp)	102,95	2004-2009
			RP caa cca gac aga tgc tct ctt tca	900	72 bp	Vv_10000593		
Carbohydrate metabolism	Large subunit of ADP-glucose pyrophosphorylase	Vv AGPL*	FP gag tgt agt ttg gaa gac gat g	300	300	TC64860 (499-649 bp)	95,35	2004-2010
			RP aca gga cag ata cca ctt gc	300	111 bp	Vv_10001368		
	Callose synthase	Vv CASY**	FP cctgcaagcctgtgttgag	900	900	TC78751 (1355-1416 bp)	86,71	2004-2009
			RP cccacgagcaagtgttgcga	900	900	Vv_10001615		
			Probe ^b accgatgcccaaac	250	62 bp			
			FP cca acc aag ggc atc tat g	900	900	TC52500 (721-796 bp)		
	Vacuolar acid invertase	Vv INV2*	FP ttg agg cag tga tgc tgg	900	900	Vv_10004311	93,32	2004-2009
			Probe ^a agc tca gct cta tgt ctt caa caa tgc tac	100	76 bp			
	Sucrose synthase	Vv SUSY*	FP tgt taa ggc tcc tgg att tca att a	900	900	TC38393 (2741-2817 bp)	88,25	2004-2009
			RP agc caa atc ttg gca agc a	900	71 bp	Vv_10000177		
	Cytokinin oxidase involved in cytokinin degradation	Vv CKO**	FP tgg gac gac ggc aca tc	900	900	TC75658 (1222-1281bp)	105,02	2004-2009
			RP cag ggc cac aag gta aaa cac	900	900	Vv_10009579		
	Ethylene receptor	Vv ETR*	Probe ^b cct ctg ggc taa cca c	250	60 bp		103,89	2004-2009
			FP ttc gga agt tca gga gcc	300	300	TC52433 (606-705 bp)		
(Hormone) regulation	Histidine-containing phosphotransfer protein involved in cytokinin signal transduction	Vv HP*	RP cca ttc att cct atc tcc aag c	300	100 bp	Vv_10001788	102,81	2004-2010
			FP agc aac aaa ttt tgg cag c	300	300	TC62606 (562-649 bp)		
	Lipoxygenase	Vv LOX*	RP gcc att tgt cct ctt gct c	300	88 bp	Vv_10001604	99,84	2004-2009
			FP gcc atg agg aca aga aag atg	900	900	TC59834 (1227-1342 bp)		
	S-adenosyl-L-methionine:salicylic acid carboxyl methyltransferase	Vv SAMT*	RP gtt gac ggc agc atg gtc	900	116 bp	Vv_10003710	90,3	2004-2009
			Probe ^a acg gtg aat tct gcc cat cag atg ctc ata	100	128 bp			
	Transcription factor WRKY54	Vv WRKY*	FP tcc cat att aga aag gaa gag g	300	300	TC66580 (944-1031 bp)	93,06	2004-2009
			RP tcc gtc tac acc gca gtc	300	88 bp	Vv_10004898		
	b-1,3-glucanase (PR-2 protein)	Vv GLC1**	FP tgc cat gtt tga gga gaa	900	900	TC77213 (963-1037 bp)	95,29	2004-2009
			RP ggt tgt ttg tta ggg agg aag agc	900	900	Vv_10000389		
	b-1,3-glucanase (PR-2 protein)	Vv GLC2**	Probe ^b atg ctt ctc caa ttc tg	250	75 bp		90,07	2004-2010
			FP cca tca tca gct tcc tgg tca aaa	900	900	TC76238 (618-703 bp)		
PR proteins	b-1,3-glucanase (PR-2 protein)	Vv GLC3**	RP gtc ccg ggt gtt acc aat gta	900	900	Vv_10002068	92,75	2004-2009
			Probe ^b ccc cac tgc ttg tta ac	250	86 bp			
	Osmotin (PR-5 protein)	Vv OLP*	FP cgc ttc tgg cga ata tat acc ctt a	900	900	TC92012 (624-694 bp)	93,21	2004-2010
			RP agc gta ggg aag aga tat gtc ctt	900	71 bp	Vv_10010418		
	2-Oxoglutarate and Fe(II)-dependent oxygenase superfamily protein 6 (unknown function)	Vv DMR6 *	Probe ^b act cag gca acc cc	250	120 bp		94,88	2004-2010
			FP tgc cca gtc taa act act agg	300	300	TC63891 (759-878 bp)		
	Ascorbate peroxidase	Vv APX**	RP cgt aga aaa gtt gtt gca tga g	300	117 bp	Vv_10000872	91,1	2007-2010
			FP gca ggc tct atg gtt ttt tcc	300	300	TC53331 (347-463 bp)		
	Glutathione peroxidase	Vv GPX**	RP gct tca tct tct cct cca cc	900	900	Vv_10002511	90,99	2007-2010
			FP tgc aag att tca aga atg cct aca tca	900	900	TC82462 (595-687 bp)		
	Glutathione S-transferase	Vv GST1**	RP ctg ttg aaa cgt tgt tgt tca gga at	900	900	Vv_10002134	89,42	2007-2009
			Probe ^b ttg cac cag aat tca c	250	76 bp			
Redox metabolism	Glutathione S-transferase	Vv GST3**	FP cgt gta aat ggc cct gat gct	900	900	TC75607 (473-540 bp)	87,14	2007-2009
			RP cca aga aat cca ctt ttg tgt gct t	900	79 bp	Vv_10000459		
	Cytochrome oxidase	COX	Probe ^b cca gtc tac aaa ttc c	250	68 bp		98,79	2004-2010
			FP aga caa gat gat tgc caa gtt cca	900	900	TC84318 (651-729 bp)		
Endogenous genes***	Eukaryotic 18S rRNA	18S rRNA	RP tga ggc ggt tca ttt aga tgc t	900	900	Vv_10004267	88,72	2004-2010
			Probe ^b tgc tga tgc caa act t	250	76 bp			

Table S2.
B. Details on RT-qPCR reactions and normalization.

Sample/Template	Check list	Comment
Source	y	grapevine leaf midrib
Method of preservation	y	liquid N2
Storage time (if appropriate)	y	1 to 2 years
Handling	y	frozen
Extraction method	y	Rneasy Plant Mini kit (Qiagen) following manufacturers' recommendations
RNA: DNA-free	y	DNA degradation using DNaseI (Invitrogen) following manufacturers' recommendations
Concentration	y	Nanodrop (Nanodrop Technologies)
RNA: integrity	y	Bioanalyser (Agilent)
Inhibition-free	y	testing 10 times and 100 times dilluted samples
Assay optimisation/validation		
Accession number	y	Table above
Amplicon details	y	Table above
Primer sequence	y	Table above
<i>Probe sequence*</i>	y	Table above
<i>In silico</i>	n	
empirical	y	Table above
Priming conditions	y	random hexamere
PCR efficiency	y	
Linear dynamic range	n	
Limits of detection	n	
Intra-assay variation	n	
RT		
Protocols	y	30 µl of denaturated RNA (80 oC, 5 min) was added

		to 20 µl of reaction mix
Reagents	y	High Capacity cDNA Reverse Transcription Kit (ABI) following manufacturers' recommendations
qPCR		
Protocols	y	2 µl of diluted cDNA was tested in two parallel reactions in 10 µl of final reaction volume. 384-well reaction plates were used and analysed on Lightcycler LC480 (Roche) using real time data collection. Standard cycling conditions were used with added melting curve stage for SybrGreen reactions
Reagents	y	Universal Master Mix (TaqMan or SybrGreen, ABI)
Duplicate RT	n	
NTC	y	In none of the NTCs amplification was detected.
NAC	n	
Positive control	n	
Data analysis		
Specialist software	n	Lightcycler 480 software (Roche) and standard curve method was used for quantification of relative gene expression
Statistical justification	y	biological replications

Transparent, validated normalisation	y	Relative transcript copy number was scaled to the geometrical mean of COX or COX and 18S rRNA gene expression Baebler et al 2011.
--------------------------------------	---	---

SUPPLEMENTAL REFERENCE

Baebler Š, Stare K, Kovač M, Blejec A, Prezelj N, Stare T, Kogovšek P, Pompe-Novak M, Rosahl S, Ravnikar M, et al. 2011. Dynamics of Responses in Compatible Potato - Potato virus Y Interaction Are Modulated by Salicylic Acid (G Bonaventure, Ed.). PLoS ONE 6, e29009