International Journal of Horticultural Science 2015, 21 (1–2): 21–30.
Agroinform Publishing House, Budapest, Printed in Hungary
ISSN 1585-0404

Primers designed for the detection of grapevine pathogens spreading with propagating material by quantitative real-time PCR

Czotter, N.[1], Manduláné Farkas, E.[2], Lózsa, R.[3], Ember, I.[3], Szűcsné Varga, G.[2], Várallyay, É.[1] & Szegedi, E.[2#]

[1]National Agricultural Research and Innovation Centre, Agricultural Biotechnology Institute, 2100 Gödöllő, Szent-Györgyi Albert út 4., Hungary;
[2]National Agricultural Research and Innovation Centre, Research Institute for Viticulture and Enology, Experimental Station of Kecskemét, 6000 Kecskemét-Katonatelep, Katona Zsigmond út 5., Hungary;
[3]Corvinus University of Budapest, Faculty of Horticulture, Department of Viticulture, 1118 Budapest, Villányi út 29-43., Hungary
[#]author for correspondence: szegedi.erno@naik.hu

***Summary*:** Several grapevine pathogens are disseminated by propagating material as systemic, but latent infections. Their detection and identification have a basic importance in the production and handling of propagating stocks. Thus several sensitive and reliable diagnostic protocols mostly based on molecular techniques have been developed. Of these methods quantitative real-time PCR (q-PCR) has recently got an emerging importance. Here we collected primer data for the detection and identification of grapevine pathogens which are important in the production of propagating stocks by q-PCR. Additional novel techniques that use DNA amplification, hybridization and sequencing are also briefly reviewed.

Keywords: bacteria, fungi, phytoplasmas, polymerase chain reaction, primer sequences, viroids, viruses, *Vitis vinifera*

Introduction

The use of healthy planting stock is a basic prerequisite to prevent spreading of several grapevine diseases including viroids, viruses, phytoplasmas, bacteria and fungi which systemically and frequently infect their host plant in latent form. To obtain pathogen-free plants several protocols have been developed which include various diagnostic methods that include biological, -serological and molecular assays to detect and identify, and curative treatments to eliminate the various pathogens (*Bisztray* et al. 2012, *Szegedi* et al. 2012). Due to its high sensitivity, cost efficiency and easy application the polymerase chain reaction (PCR) has become the most widely used diagnostic protocol during the last 25 years in plant pathology (*Louws* et al. 1999). To increase the sensitivity, reliability and specificity of conventional PCR methods several novel technologies have been introduced into the plant quarantine studies during the recent years. In addition to increased sensitivity, most of these methods allow also the simultaneous detection of multiple pathogens.

Novel molecular diagnostic techniques

Loop-mediated isothermal amplification: In spite of the high convenience, sensitivity and cost-efficiency of conventional PCR several novel molecular techniques have been developed and introduced to increase the sensitivity, reliability and/or applicability of diagnostic and identification protocols. The loop-mediated isothermal amplification (LAMP) originally developed by *Notomi* and coworkers (2000) uses a set of inner and outer primers and *Bst* polymerase that amplifies DNA at 65 °C. The elongation is followed by a colourimetric reaction using, e. g., hydroxynapthol blue (Goto et al. 2009). Thus the use of thermal cycler, gel electrophoretic separation and ethidium-bromide staining, and even DNA extraction is not necessary for scoring the results. Among grapevine pathogens this protocol has already been used for the detection of *Xylella fastidiosa* (*Harper* et al. 2010) and phytoplasmas (*Kogovšek* et al. 2015, *Tomlinson* et al. 2010). Further improvement includes addition of reverse transcriptase into the reaction which allows detection of RNA targets, RNA viruses and viroids. Among grapevine viruses an RT-LAMP based method was developed for GLRaV-3 and proved to be

as sensitive as nested PCR (*Walsh and Pietersen,* 2013). Besides its high specificity and sensitivity a great advantage of LAMP is that it can be easily used in mobile laboratories, for example in the field and state border quarantine stations.

Micro and macroarray techniques: The microarray and macroarray technologies involve the hybridization of PCR amplified and labelled samples to immobilized oligonucleotide probes specific for the various pathogens. Engel and coworkers (2010) printed 70-mer oligonucleotides of 570 probes specific for 44 grapevine viruses onto the surface of a microscope slide. These probes included both family-, and species-specific sequences. Thus each virus was covered by several specific sequences. Viral sequences were enriched by random primed PCR prior to hybridization. Using this step the detection becomes more sensitive than labelling total plant RNA. Since microarrays allow the detection of ten-thousands of specific sequences in a single hybridization step, this technique allows the detection of mixed infections or even the complete virus population present in the tested plant. Thompson and co-workers (2014) used the macroarray technology to detect and identify grapevine viruses. They used similarly 60-70-mer oligonucleotide virus-specific probes. Approximately 1600 probes specific for 38 viruses and plant internal controls were immobilized onto a nylon membrane and used for the subsequent hybridization experiment with PCR enriched and labelled plant cDNA. Although macroarrays allow the application of approximately only one tenth of probes than that of microarrays, it is still far sufficient to detect viruses infecting grapevines and the method does not require expensive equipments and the membrane blots can be reused up to 20-30 times. Results of the micro-, and macroarray detections were consistent with ELISA and/or reverse transcription PCR assays. The great advantages of array technologies over the conventional and real-time PCR that they it cover much longer nucleotide sequences (several 70-mer oligonucleotides for a given virus) thus the reaction is highly specific for the given pathogen. In simple nucleic-acid amplification based detections the reactions are determined by short (usually 21-25 nucleotide long) primers thus a few mutations may cause false negative results. A further hybridization-based technique applied a „polyprobe" established from four tandemly cloned viroid sequences. This probe was then labelled with digoxygenin (DIG) and hybridized to grapevine RNA extract blotted onto nylon membrane. This method allowed the simultaneous detection of four viroids (*Zhang* et al. 2012). To detect fungal diseases Martos and coworkers (2011) dot-blotted the PCR products onto a nylon membrane followed by hybridization with a *Phaeomoniella*-specific probe. The low density-array uses microwell plates (with 384 sample wells) precoated with a set of virus specific primers and TaqMan probes thus allowing the simultaneous detection of several viruses from a large number of samples by quantitative real-time PCR (*Osman* et al. 2008).

Barcodes: A recent technique called barcoding combines PCR amplification and sequencing. A basic prerequisite of a barcode is that it should contain highly conserved regions for a given taxonomic group but sequences bordered by these regions should be variable enough to discriminate species or strains. In the first step of barcoding a relatively short DNA fragment is amplified using primers designed for the conserved borders. Subsequently, this fragment is sequenced that allows the precise identification of the given organism. DNA barcodes for phytoplasmas based on *tuf* gene sequences (*Makarova* et al. 2012) and for fungi based on the ITS region (*Schoch* et al. 2012) have already been published, and barcode database for plant pathogens has also been established (*Bonants* et al. 2010, www.qbol.org). This database contains data for some grapevine pathogenic bacteria such as *Xylella fastidiosa* subsp. *fastidiosa* and *Xanthomonas viticola* as well.

Deep (Next generation) sequencing: Fast evolution in sequencing techniques established the possibility to get metagenomic information of the investigated plants. Next generation sequencing serves as a base of finding all of the expressed RNA (including the pathogens also) in the host and get a virome of the plant. Different platforms were used for the description of new grapevine viruses: Roche 454 for Grapevine Syrah-1 virus (*Al Rwahnih* et al. 2009), while Illumina for Grapevine virus F (*Al Rwahnih* et al. 2012, *Al Rwahnih* et al. 2013). This later platform was used also to create the viromes of a vineyard (*Coetzee* et al. 2010). RNA interference, the RNA based defense reaction of the plant, opens a new possibility in virus diagnosis using deep sequencing of small RNA libraries of infected plants (*Donaire* et al. 2009, *Kreuze* et al. 2009). During virus infection small interfering RNAs having sequence similarity to the infecting viruses are formed and sequencing them make it possible to identify viroids (*Navarro* et al. 2009) or viruses (*Pantaleo* et al. 2010) even if they are alien on the plant or never described (*Giampetruzzi* et al. 2012, *Wu* et al. 2012, *Zhang* et al. 2011). Deep sequencing offers a unique opportunity to reveal any viruses or viroids present in the sample, either expected or not. It needs a major investment at the beginning, running costs are relatively high, but barcoding the libraries for pooled sequencing, improvement of data analysing pipelines (*Ho and Tzanetakis,* 2014) and serious drop in the sequencing costs can make this method suitable for large scale sample testing in the near future.

Changes in the expression profile of grapevine host genes: Pathogen infections may cause specific changes in the expression profiles of infected grapevine plants (*Albertazzi* et al. 2009, *Choi* et al. 2010b, *Espinosa* et al. 2007, *Fung* et al. 2008, *Hren* et al. 2009, *Santi* et al. 2013, *Zhao* et al. 2011). Therefore analysis of host mRNAs may provide valuable information for pathogen diagnosis. Choi and coworkers (2010a) found four grapevine genes which were specifically up-regulated following *Xylella fastidiosa* infections, thus monitoring these genes indicates latent infections prior to symptom development. In symptomless *Eutypa lata*-infected grapevine plants 10 genes were found which were differentially up-, or down- regulated (*Camps* et al. 2014).

GC-MS analysis of volatile compounds in healthy and galled (*Agrobacterium vitis*-infected) grapevine cuttings provided further indirect evidence for the pathogen-induced changes in plant gene expression. Diseased plants accumulated styrene, a phenylalanine derivative that was not present in healthy samples (*Blasioli* et al. 2010). In phytoplasma infected grapevine leaves genes involved in plant defence mechanisms such as callose synthase, sugar transporters and cell wall invertase showed higher expression (*Santi* et al. 2013).

The quantitative real-time PCR: Soon after the introduction of conventional PCR a more developed technique, the quantitative real time-PCR (q-PCR) has also been introduced for the detection of plant pathogens (*Boonham* et al. 2014, *Christense*n et al. 2013, *Hren* et al. 2010, *Schena* et al. 2004). The q-PCR is more sensitive than the conventional PCR method and the amplification and detection is combined in one step, thus subsequent gel-electrophoretic separation of the amplified product is not necessary. The amplification is continuously detected by various fluorescent reporters

Table 1. Viroid specific primers

Viroid*	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Probe	Reference
CEVd	CEVdF CEVdR CEVdTAQ	GCGTCCAGCGGAGAAACA CAGCGACGATCGGATGTG FAM-TCGTCTCCTTCCTTTCGCTGCTGG-BHQ1	60 60 70	68	TaqMan	Papayiannis 2014
HSVd	HSVdF HSVdR HSVdTAQ	GCCTTCGAAACACCATCGA CACCGGTCGCGTCTCATC HEX-CGTCCCTTCTTCTTTACCTTCTCCTGGCTC-BHQ2	60 60 69	71	TaqMan	Papayiannis 2014
HSVd	HSVd FP HSVd RP HSVd probe	CCGCGGATCCTCTCTTGA CCGGGGCTCCTTTCTCAG FAM-CTGGGGAATTCTCGAGTTGCCGCA-BHQ1	55	106	TaqMan	*Sun* et al. 2014
HSVd	HSVd-H-long HSVd-C-long HSVd-Probe	CCGGGGCAACTCTTCTCAGAATCCAGCGAG GAAGAAGGGACGATCGATGGTGTTTCGAAG AGAGAGGGCCGCGGTGCTCT-BHQ1	60	65	TaqMan	*Luigi & Faggioli* 2013
GYSVd1	GY FP GY RP GY probe	CTTGTGGTTCCTGTGGTTTCAC CCTCTGCCCCTATCTTCTTCTTT FAM-AGAGAGGGCCGCGGTGCTCT-BHQ1	55	69	TaqMan	*Sun* et al. 2014

*CEVd = Citrus exocortis viroid, HSVd = Hop stunt viroid, GYSVd-1, = Grapevine yellow speckle viroid-1

Table 2. Virus specific primers

Virus*	Primer	Primer sequence (5'-3')**	At/Tm (°C)	Fragment length (nt)	Target gene	Probe	Reference
ArMV[a]	ArMV FP ArMV RP **ArMV probe**	GCACTGTAGCCCTTGGAGATAATCC CCCTCCAAATCCCACATTAACTTA **CTCACATGATAGCTTGTCATGGACTCC**	55	100	RNA2-polyprotein	TaqMan	*Sun* et al. 2014
ArMV	ArMV i1 ArMV i2 **ArMV p S3[b]** **ArMV p ES[b]**	AATTATATGCTGAGTTTGAG AAAATTATACACCTTATGAGTA **ACCAGTGCCTACAAGAGTGTGTCC** **ACCAGTGCTTATAAGAGTGTTTCC**	60	203	RNA2-polyprotein CP	TaqMan	*Bertolini* et al. 2003 *Bertolini* et al. 2010
ArMV	ArMV III D ArMV III R **ArMV III P**	TAGCCCTTGTACTTATGGCA TATTTAAACAGTTGATTCCA **TTGTTAGTGAATGGAACGGGGTCA**	60	84	RNA2-polyprotein P2V	TaqMan	*López-Fabuel* et al. 2013
GFkV	GFkVPrep220F GFkVPrep261R **GFkVPrep230P**	ACGTGAAGACCAACGTCGAAT CGGTGATGCGCATGCA **CCAATTTGGCCCTCTC**	52	56	Replicase	TaqMan	*Pacifico* et al. 2011
GFKV[c]	Fleck 239 f Fleck 328 r **Fleck 261 p1** **Fleck 261 p2** **Fleck 261 p3**	CAACATCGAATGCCAATTTGG GCCAGGCTGTAGTCGGTGTTGT **CCTCTCACGTGCATGCGCATC** **CCTCTCACGTGCATGCGGATC** **CCTCTGACGTGCATGCGCATC**	60	89	Replicase	TaqMan	*Osman* et al. 2008
GFKV	GFkV OB F GFkV OB R **GFkV OB S**	CGAGAACTCTCTTTTCACCTC CCGGCGTGGATGTAGAG **ACCCTCGCCCTCATGCA**	60	146	Replicase	TaqMan	*Bertolini* et al. 2010
GFLV	GFLV F GFLV R **GFLV probe1[d]** **GFLV probe2[d]**	AGCTGCGGCACTYTTTGC TCATCACTRGTGTCATACCACTTCCT TGCTCAARCATACCACTTG ATGCTTAARCATACCACTTG	60	128	RNA2-polyprotein 2AHP	Taqman	*Čepin* et al. 2010
GFLV	GFLVPpoly 617F GFLVPpoly 660R GFLVPpoly 640P	CTCGTCCACTGCTGGAATTAGA ATCCTGCCTCCAGAGCTTTTT **TTTTTGAAACGTGGATTTG**	52	64	RNA1-polyprotein RdRP	TaqMan	*Pacifico* et al. 2011

Continuation of ***Table 2***

Virus*	Primer	Primer sequence (5'-3')**	At/Tm (°C)	Fragment length (nt)	Target gene	Probe	Reference
GFLV	GFLV-769f GFLV-868r **GFLV-799p**	GGGACCACTATGGATGGAATGA TTCGGTGATATGGAGAGCGAAT **AAGTATCCCGGGGTGTATGTGGAAGAGGA**	60	99	RNA2-polyprotein CP	Taqman	*Osman & Rowhani* 2006
GFLV	GFLV-769f GFLV-868r **GFLV-**	GGGACCACTATGGATGGAATGA TTCGGTGATATGGAGAGCGAAT **AGTGGAACGGGACCAC**	60	99	RNA2-polyprotein CP	Taqman	*López-Fabuel* et al. 2013
GFLV	GFLV CP2-671f1[e] GFLV CP2-671f2[e] **GFLV CP2-822 r** **GFLV CP2-761p**	GTTGTGTGTTAGGTATGGGAGGTACTATTA TGTGTTTTGGGTATGGGAGGTACTATTA **TTCCACATACACCCCGGGATA** **AGTGGAACGGGACCAC**	60	149	RNA2-polyprotein CP	TaqMan	*Osman* et al. 2008.
GLRaV-1	GLRaV1Ppoly167F GLRaV1Ppoly206R **GLRaV1Ppoly190P**	CTGACCCTATCGCTGCTACTGA TCCCATCAACCCAGGTATCAA **CTCGCCAGCTTGTTT**	52	60	RNA dependent RNA polymerase	TaqMan	*Pacifico* et al. 2011
GLRaV-1	HSP70-149 F HSP70-293 R **HSP70-225 P**	ACCTGGTTGAACGAGATCGCTT GTAAACGGGTGTTCTTCAATTCTCT **ACGAGATATCTGTGGACGGA**	60	144	HSP70-like protein	Taqman	*Osman* et al. 2007
GLRaV 2	198 F 290 R **233 P**	CATTATATTCTTCATGCCTCTCAGGAT GATGACAACTTCTGTCCGCTATAGC **TTGCTACTGATCGACTGTGCAGCTCACA**	60	92	HSP70-like protein	TaqMan	*Osman* et al., 2007
GLRaV-2	P19qtF4 P24qtR	CTAACAATTTCTTCTTTGGATCGCAT AGAATGTCTTCAGCTTCATAAGGAG	61	154	p19 and p24 kDa protein	SYBR Green	*Beuve* et al. 2007
GLRaV2-RG	Redglobe-227 f Redglobe-319 r **Redglobe-250 p**	GCGACTCCAGCAACTTTAGTGA CCGTATCATTCAGACCAGTACCTATTT **TACTTTGATCGCCTGAAGCCACACTATGC**	60	92	HSP70-like protein	TaqMan	*Osman* et al. 2008
GRLaV-3	GLRaV3Ppoly36F GLRaV3Ppoly84R **GLRaV3Ppoly54P**	GGCGGAGGTGACGGAAA CCCTTTTGTCCAACCAATCT **CCATTTGTCCAGCAACGCGACGT**	52	67	RNA dependent RNA polymerase	TaqMan	*Pacifico* et al. 2011
GLRaV-3	GLRaV3-56f GLRaV3-285r **GLRaV3-181p**	AAGTGCTCTAGTTAAGGTCAGGAGTGA GTATTGGACTACCTTTCGGGAAAAT **CAGGTAATAGCGGACTGAGACTGGTGGACA**	60	229	HSP70-like protein	Taqman	*Osman & Rowhani* 2006
GLRaV-3	LR3 _6995F LR3 _7138R LR3 _14586F LR3 _14725R LR3 _18345F LR_18488R	GGGRACGGARAAGTGTTACC TCCAAYTGGGTCATRCACAA ATGAAYGARAARGTYATGGC CTAAACGCYTGYTGYCTAG CCTCACGGTTTAATACTCTG ATTGTCGATAAGTTAGCCTC	53 50 54	143 139 143	ORF1a Coat Protein 3'UTR	SYBR Green	*Bester* et al. 2014
GLRaV-4	LR4 hsp-85 f LR4hsp-178 r **LR4hsp-120 p**	ATATACATACCAACCGTTGTGGGTATAA CCCTATAAACTAGCACATCCTTCTCTAGT **TGGAACATATACCATTGGGCTTGGTGCT**	60	93	HSP70- like protein	TaqMan	*Osman* et al. 2007
GLRaV-5	GLRaV-5 26 f GLRaV-5 188 r **GLRaV-5 131p**	AACACTCTGCTTTTCTGCTGGC CTTTTTATGTCCCGATAAACGAGTACA **CAATAGGTTTGGGGGCTTTGCTAGAAAAGG**	60	161	HSP70	TaqMan	*Osman* et al. 2007
GLRaV-9	LR9-114 f LR9-196 r **LR9-136 p**	CGGCATAAGAAAAGATGGCAC TCTTTATGTCACGGTAGACCAACAC **TATACCATAGGATTGGGAGCTCTGCTGGAGA**	60	82	HSP70	TaqMan	*Osman* et al. 2007
GVA	GVAPrep121F GVAPrep169R **GVAPrep143P**	AATGTCATGGACTGGGTCGAA GATATGTCGATGAGATTCAC **ATGACAGCGCGAACACCCTCAGTG**	52	73	Replicase (RdRp)	TaqMan	*Pacifico* et al. 2011
GVA[f]	GVA-77 f1 GVA-77 f2 GVA-192 r1 GVA-192 r2 **GVA-104 p**	CGACCGAAATATGTACCTGAATACTC CGACCGAA**C**TATGTACCTGAATACTC TTTGCTAGCTTTAGGACCTACTATATCTACCT CTTGCTAGCCTTAGGTCCTACTATATCTACCT **CTTCGGGTACATCGCCTTGGTCGG**	60	111	Coat protein	TaqMan	*Osman & Rowhani* 2008
GVA[g]	GVAmu-77f1 GVAmu-77f2 GVAmu-192r1[h] GVAmu-192r2[h] **GVAmu-104p**	CGACCGAAATATGTACCTGAATACTC CGACCGAACTATGTACCTGAATACTC AGGTAGATATAGTAGGTCCTAAAGCTAGCAA GGTAGATATAGTAGTAGGACCTAAGGCTAGCAA **TCGGGTACATCGCCTTG**	58	110	Coat protein	TaqMan	*Osman* et al. 2013
GVB[f]	GVB-92 f1 GVB-92 f2 GVB-95 f3 GVB-202 r1 GVB-202 r2 **GVB-119 p1** **GVB-119 p2**	CTAGGAGTGCGGCTAAACGAA GGAGTGCGGCCAAACGA CAAGGAGTGCGGCTAAACGAA CCTTAACCTCGTCCTGTGATATGGT CCTTCACCTCATC**Y**TGGGATCGTGT **CTCGTTATGGTCGCTGTTACTGTTGTGGTAG** **ACCGTTACGGCCGTTGTTACTGTTGTGGTAG**	60	110	RNA binding protein	TaqMan	*Osman & Rowhani* 2008

Continuation of ***Table 2***

Virus*	Primer	Primer sequence (5'-3')**	At/Tm (°C)	Fragment length (nt)	Target gene	Probe	Reference
GVB[g]	GVBmu-91f1[j] GVBmu-91f2[j] GVBmu-91f3[j] GVBmu-202r1[h] GVBmu-202r2[h] **GVBmu-119p1** **GVBmu-119p2**	CTAGGAGTGCGGCTAAACGAA GGAGTGCGGCCAAACG CAAGGAGTGCGGCTAAACG CCATATCACAGGACGAGGTTAAGG ACGATCCCAYGATGAGGTGAA **TGGTCGCTGTTACTGTT** **ACCGTTACGGCCGTTG**	58	108	RNA binding protein	TaqMan	*Osman* et al. 2013
GVD	GVD-554 f GVD-631 r **GVD-580 p**	AGGTGTATTCAACGCCCAGTCG CCTGCGAGAAATGATGGGTCA **TTGGAGAGCAGGCAGTTGAGATATAAATGGA**	60	77	Coat Protein RNA binding protein	TaqMan	*Osman & Rowhani* 2008
GVD[g]	GVDmu-554f GVDmu-661r[h] **GVDmu-581p**	AGGTGTATTCAACGCCCAGTC GTAGTTATGCTAAGAAGCGTAGGGC **TGGAGAGCAGGCAGTT**	58	107	Coat Protein, RNA binding protein	TaqMan	*Osman* et al. 2013
GRSPaV[f]	RSPaV-52 f1 RSPaV-52 f2 RSPaV-52 f3 RSPaV-130 r1 RSPaV-130 r2 **RSPaV-75 p1** **RSPaV-75 p2** **RSPaV-75 p3**	AGACGGGAATACCACCAGCTAA AGACGGGAATTCCACCCGCTAA AGACGGGGATACCACCAGCTAA AGGAAGAAGTCAAAGGCTGCAA AAGAAAAAATCAAAGGCTGCAA **TGGGCCAAGAAAGGATTTAATGAGAATGAA AAG** **TGGGCCAAGAAAGGGTTTAATGAGAATGAA AAA** **TGGGCCAAGAAGGGATTTAATGAAAATGAG AAA**	60	78	Coat protein	TaqMan	*Osman & Rowhani* 2008
TBRV	TBRV-70F[i] TBRV-70R[i] **TBRV-70P**	GCTCGTAACAGTTGCGGAGATAT TGTCCACACTGTCATGGGA **TGCATAGGCTCACTCCTTGGGA**	62	72	RNA2-Polyprotein	TaqMan	*Harper* et al. 2011
ToRSV	ToRSV-UTRf ToRSV-UTRr **ToRSV-UTRp**	GAATGGTTCCCAGCCACT AGTCTCAACTTAACATACCAC **AGGATCGCTACTCCTCCGTCAAC**	60	182	RNA2-3' UTR		*Lebas & Ward* 2012
ToRSV	ToRSV-1590 f ToRSV-1661 r **ToRSV-1610 p**	GCCACCCGAGAACGTTAGC GCCTGCTGAGTCTGCTGTAGAG **CCACGGGCCCCGGTAGTCAATATG**	60	71	RNA2-Polyprotein-CP	TaqMan	*Osman* et al. 2008

*ArMV: Arabis mosaic virus, GFLV: Grapevine fanleaf virus, GFkV: Grapevine fleck virus, GLRaV-1, -2, -3, -4, -5, and -9: Grapevine leafroll-associated virus-1, -2, -3, -4, -5, and -9, respectively, GRSPaV: Grapevine rupestris stem pitting-associated virus, GVA: Grapevine virus A, GVB: Grapevine virus B, GVD: Grapevine virus D, TBRV: Tomato black ring virus, ToRSV: Tomato ringspot virus

** For TaqMan oligonucleotide probes (show in bold) we only refer for the sequence and not show either the fluorophore or the quencher.

[a]ARMV in this case was tested in lily leaf not in grapevine

[b]two different probes mixed together were used for succesful detection of highly variable isolates

[c]In order to increase sensitivity different probes were used to detect all or at least the majority of diverse isolates

[d]Two different probes were used separately

[e]Two different forward primers mixed together were used for succesful detection of variable isolates

[f]The mixture of the multiple primers were used to increase the efficiency of the detection

[g]Oligos were used in different combinations

[h]Be aware that these published reverse oligos are in sense orientation, for PCR reaction reverse complementer of them must be used

[i]Be aware that these published forward and reverse oligos are mixed up

[j]The forward oligos are the same as in the previous paper

bound to the primers (Molecular bacon and Scorpion probes) or to internal complementer oligonucleotide sequences (TaqMan® probes) or bound specifically only to the amplified double stranded DNA (SYBR® Green). Until annealing or insertion of primer or probe sequences to the target DNA the light emission is blocked by specific quenchers. The quenchers are removed during amplification from the reporters allowing the fluorescent light emission. Due to its high sensitivity and specificity the application of q-PCR has been widely used in plant quarantine laboratories despite of its relatively high costs.

At present the various versions (TaqMan®, SYBR Green®) of q-PCR protocols combined with traditional PCR are most extensively applied in pathogen detection and identification. In a previous survey we have collected a primer „databank" for the detection of the most important grapevine pathogens by conventional PCR methods (*Manduláné Farkas* et al. 2014). As a subsequent part of this work here we summarize primer data available for q-PCR detection of grapevine pathogens spreading with propagating material (Tables 1–5).

Table 3. Phytoplasma specific primers

Phytoplasma	Primer	Primer sequence (5'-3')	At/Tm (°C)	Fragment length (nt)	Target gene	Probe	Reference
Universal	Forward Reverse Probe	CGTACGCAAGTATGAAACTTAAAGGA TCTTCGAATTAAACAACATGATCCA FAM-TGACGGGACTCCGCACAAGCG-TAMRA	60	n. g.*	16SrRNA	TaqMan	*Christensen* et al. 2004
Universal	UniRNAfw UniRNArev Probe	AAATATAGTGGAGGTTATCAGGGATACAG AACCTAACATCTCACGACACGAACT FAM-ACGACAACCATGCACCA-NFQ**	60	73	16SrRNA	TaqMan	*Hren* et al. 2007
Bois noir Stolbur 16SrXII-A Tuf-type a	FStol4-tA RStol4-tA Probe	GATCCACCCTTCGCTTTATTT CTTGGAATAACTGAAGCGACA FAM-GTCAAACACCACCTTTTATCATTCCT-BHQ-1	60	222	Stol4 fragment	TaqMan	*Daire* et al. 1997, *Fahrentrapp* et al. 2013
Bois noir Stolbur 16SrXII-A Tuf-type b	FStol4-tB RStol4-tB Probe	GATCCACCCTTCGCTTTATTT CTTGGAATAACTGAAGCGACA HEX-ATCAAAAACAACAACCTTTTATCATTC-BHQ-1	60	222	Stol4	TaqMan	*Fahrentrapp* et al. 2013
Bois noir Stolbur 16SrXII-A	mapBN-F mapBN-R mapBN-VIC	ATTTGATGAAACACGCTGGATTAA TCCCTGGAACAATAAAAGTYGCA VIC-AAACCCACAAAATGC	60	72	*map*	TaqMan	*Pelletier* et al. 2009
Bois noir Stolbur 16SrXII-A	190F 660R 210F 280R BN-P	GAGATAAGAAGGCATCTTCTTA AACAGTTTTTATAGCATCACAA CTTCTTATTTTTAAAAGACCTAGCAATAGG GTCTTGGTAGGCCATTACCC FAM-TTAGGGAAGAGCTTGCGTCA-BHQ-1	53 58	470 70	16S rRNA	TaqMan	*Margaria* et al. 2009***
Bois noir Stolbur 16SrXII-A	F R P	GGTTAAGTCCCGCAACGAG CCCACCTTCCTCCAATTTATCA FAM-AACCCTTGTTAATTGCCATCATTAAG-TAMRA	60	98	16S rRNA	TaqMan	*Angelini* et al. 2007
Bois noir Stolbur 16SrXII-A	F R P	AAGCAGGTTTAGCGATGGTTGT TGGTACCGTTGCTTCATCATTT FAM-TTAATACCACCTTCAGGAAA-NFQ**	60	71	Stol11 genomic fragments	TaqMan	*Hren* et al. 2007
BN 16SrXII-A-VK_I	qBN-AD-F qBN-AD-R qBN-VKI	CCTTTCTTAATGCCAGTCGAA TGTCCTCTTTCAACTCTACCAGTAA FAM-TCCTCTACCGGTGATAG	57	80	*tuf*	TaqMan	*Berger* et al. 2009
BN 16SrXII-A-VK-II	qBN-AD-F qBN-AD-R qBN-VKII	CCTTTCTTAATGCCAGTCGAA TGTCCTCTTTCAACTCTACCAGTAA VIC-TGTTCCTCTACCAGTGAT	57	80	*tuf*	TaqMan	*Berger* et al. 2009
Flavescence dorée 16SrV-C, D and E	mapFD-F mapFD-R mapFD-FAM	TCAAGGCTTCGGBGGTTATA TTGTTTTAGAAGGTAATCCGTGAACTAC FAM-TTGTATTTCAGTGAATGAAG	60	71	*map*	TaqMan	*Pelletier* et al. 2009
FD	395F 480R FD-P	GCCGCGTGAACGATGAA GAATAACGTCAAGATAGTTTTTCCACT FAM-TTTCGGTATGTAAAGTTCT-TAMRA	58	85	16SrRNA	TaqMan	*Margaria* et al. 2009
FD	F R P	AAGTCGAACGGAGACCCTTC TAGCAACCGTTTCCGATTGT FAM-AAAAGGTCTTAGTGGCGAACGGGT-TAMRA	60	103	16SrRNA	TaqMan	*Angelini* et al. 2007
FD	F R P	TTATGCCTTATGTTACTGCTTCTATTGTTA TCTCCTTGTTCTTGCCATTCTTT FAM-ACCTTTTGACTCAATTGA-NFQ	60	85	*secY*	TaqMan	*Hren* et al. 2007
FD 16SrV-D, C	F1024 F1112 probe	GTGAGATGTTAGGTTAAGTCCTAAAACGA GGACTTTAGCGAGACTGCCAA FAM-AACCCCTGTCGCTAGTTGCCAGC-TAMRA	60	89	16SrRNA	TaqMan	*Bianco* et al. 2004
Aster Yellows 16SrI	Forward Reverse Probe	TTGGGTTAAGTCCCGCAAC CCCACCTTCCTCCAATTTATCA FAM-CCAGCACGTAATGGTGGGGACTT -TAMRA	60	102	16SrRNA	TaqMan	*Angelini* et al. 2007

*n. g.: not given, **: non fluorescent MGB quencher, ***: In BN detection, due to the low sensitivity of the 210F/280R primers, prior to qPCR an RT-PCR has to be performed using 190F/660R primers.

Table 4. Bacterium specific primers

Species	Primer	Primer sequence (5'-3')	At/Tm (°C)	Fragment length (nt)*	Target gene*	Probe	Reference
Xylophilus ampelinus	Xamp 14F Xamp 104R Xamp14F /104MGB	CCCGATGATAAATACCGAAAACTC TGTCTTCTGGTTGTTTTGGTTTTAAT FAM-AGCGCCTGACGCAT-MGB	60	91	Xamp 1.27A	TaqMan	*Dreo* et al. 2007
Xylella fastidiosa	XF-F XF-R XF-P	CACGGCTGGTAACGGAAGA GGGTTGCGTGGTGAAATCAAG TCGCATCCCGTGGCTCAGTCC	62	70	*rimM*	TaqMan	*Harper* et al. 2010
Xylella fastidiosa	XfF1 XfR1 XfP1	AAAAATCGCCAACATAAACCCA CCAGGCGTCCTCACAAGTTAC FAM-ACCTATGCCAACATCAACCCTGAATGCA-TAMRA	62	n. g.	ITS	TaqMan	*Schaad* et al. 2002
Xylella fastidiosa	XfF2 XfR2 XfP2	CTCGCCACCCATGGTATTACTAC CTGGCGGCAGGCCTAAC 6FAM-ATGTGCTGCCGTCCGACTTGCATG-TAMRA	62	n. g.	16S	TaqMan	*Schaad* et al. 2002
Xylella fastidiosa	HL5 f HL6 r probe	AAGGCAATAAACGCGCACTA GGTTTTGCTGACTGGCAACA FAM-TGGCAGGCAGCAACGATACGGCT-BHQ1	60	221	n. g.	TaqMan	*Francis* et al. 2006
Agrobacterium vitis	VIRD59F26 VIRD59R122	ATTGGAATATCTGTCCCG GGCGAGATCGCGGATATT	52	96	*virD2*	SYBR Green	*Bini* et al. 2008
Agrobacterium vitis	VIRD62F23 VIRD62R135	AACCATTCAGCAGGTTAT TGGTAATATGATCAGGCG	52	102	*virD2*	SYBR Green	*Bini* et al. 2008
Agrobacterium vitis	virD2.For1 virD2.Rev1	TTGGAATATCTGTCCCGGAAG CTTGTACCAGCAGGGAAGCTTA	50	n.g.	*virD2*	SYBR Green	*Johnson* et al. 2013

*n. g. = not given

Table 5. Fungus specific primers

Fungus	Primer	Primer sequence (5'-3')	At/Tm (°C)	Fragment lenght (nt)	Target gene	Probe	Reference
Phaeomoniella chlamydospora	pchITS417F pchITS495R pchITS439P	TGGCGGCGTCAAGAATGA CAATAGGGCCAGGCCAAG AGGTGCAGCGAGCAATCAAGCATACA	60	78	ITS2 region	TaqMan	*Martin* et al. 2012
Phaeomoniella chlamydospora	Pmo1f Pmo2r	GTTACATGTGACGTCTGAACG CAGTGTATGCTTGATTGCTCG	56	320	ITS region	SYBR Green	*Overton* et al. 2004
Phaeoacremonium aleophilum (Pa), *P. parasiticum* (Pp), *P. mortoniae* (Pm) and *P. viticola* (Pv)	F1btfw F2btfw R1bt Probe-Pa Probe-Pp Probe-Pm Probe-Pv	CCCTGARTTACCCCACCATC VAGCTTCGACRWCCTCGACG GCTACTTACRCAYTGRCCGGTCTG FAM-CAGAATCTACCCCAGATCATCGA CCAGC- 6-TAMRA TET-CGACTCTGACCCCAAAAGCATCGAC-6-TAMRA VIC-CGTGAGCGTCACCTCTAAGTCATTGA CC-6-TAMRA VIC-CAAAATCAATTCAAATCATTGAACAGCT TGCTA-6-TAMRA	55	approx. 130-170	*B*-tubulin gene	TaqMan	*Aroca* et al. 2008
Phaeoacremonium aleophilum	palFI1 palRI1 palI1probe	CCTCGACGAGCCCAGAATC GTAAAAACCCCACCGGTTAGC CCCAGATCATCGACC	60	63	*B*-tubulin gene	TaqMan	*Martin* et al. 2012
Phaeoacremonium spp.	Pac1f Pac2r	ACCCTTTGTGAACATACCTG TACTGCGCTCGGAGTCCTG	56	410-428	ITS region	SYBR Green	*Overton* et al. 2004
Rosellinia necatrix	R10fw R7rev	R10 CCCCTG TTG CTTAGT GTTGG R7 AACCATAGGCGAGATGAGAAAT	60	112	ITS2	Scorpion	*Schena* et al. 2002
Rosellinia necatrix	R15fw R18rev	CCATAGGCGAGATGAGAAATC CAGCCCCTCGAAGTCAGT	58	71	ITS2	Scorpion	*Schena & Ippolito* 2003
Rosellinia necatrix	R2 fw R5rev TR2-5 probe	CAAAACCCATGTGAACATACCA CAATGCTAAACAGAGTTTCGTG 6-FAM-GTTAGGGCCTACCCGGTGGG-TAMRA	60	133	ITS1	TaqMan	*Shishido* et al. 2012*
Rosellinia necatrix	R10 fw R7rev TR10-7 probe	CCCCTG TTG CTTAGT GTTGG AACCATAGGCGAGATGAGAAAT 6-FAM-AGTCAGTGGCGGAGTCGGTC-TAMRA	60	112	ITS2	TaqMan	*Shishido* et al. 2012*

*forward and reverse primers are from Shena et al. 2002

Acknowledgements

This work was supported by the Hungarian Ministry of Agriculture and the KTIA_AIK_12-1-2013-0001 project. N. Czotter participates in the Program for Reinforcement of Scientists of Hungarian Ministry of Agriculture.

References

Albertazzi G, Caffagni A, Milc JA, Francia E, Roncaglia E, Ferrari F, Tagliafico E, Stefani E & **Pecchioni N (2009):** Gene expression in grapevine cultivars in response to Bois Noir phytoplasma infection. *Plant Science*. 176: 792–804.

Al Rwahnih M, Daubert S, Golino D & **Rowhani A (2009):** Deep sequencing analysis of RNAs from a grapevine showing Syrah decline symptoms reveals a multiple virus infection that includes a novel virus. *Virology*. 387: 395–401.

Al Rwahnih M, Sudarshana MR, Uyemoto JK & **Rowhani A (2012):** Complete genome sequence of a novel vitivirus isolated from grapevine. *Journal of Virology.* 86: 9545.

Al Rwahnih M, Dave A, Anderson MM, Rowhani A, Uyemoto JK & **Sudarshana MR (2013):** Association of a DNA virus with grapevines affected by red blotch disease in California. *Phytopathology*. 103: 1069–1076.

Angelini E, Bianchi GL, Filippin L, Morassutti C & **Borgo M (2007**): A new TaqMan method for the identification of phytoplasmas associated with grapevine yellows by real-time PCR assay. *Journal of Microbiological Methods*. 68: 613–622.

Aroca A, Raposo R & **Lunello P (2008):** A biomarker for the identification of four *Phaeoacremonium* species using the β-tubulin gene as the target sequence. *Applied Microbiology & Biotechnology*. 80: 1131–1140.

Berger J, Dalla Via J & **Baric S (2009):** Development of a TaqMan allelic discrimination assay for the distinction of two major subtypes of the grapevine yellows phytoplasma Bois noir. *European Journal of Plant Pathology*. 124: 521–526.

Bertolini E, Olmos A, Lopez MM & **Cambra M (2003):** Multiplex nested reverse transcription-polymerase chain reaction in a single tube for sensitive and simultaneous detection of four RNA viruses and *Pseudomonas savastanoi* pv. *savastanoi* in olive trees. *Phytopathology*. 93: 286–292.

Bertolini E, Garcia J, Yuste A & **Olmos A (2010):** High prevalence of viruses in table grape from Spain detected by real-time RT-PCR. *European Journal of Plant Pathology*. 128: 283–287.

Bester R, Pepler PT, Burger JT & **Maree HJ (2014):** Relative quantitation goes viral: An RT-qPCR assay for a grapevine virus. *Journal of Virological Methods.* 210C: 67–75.

Beuve M, Sempe L & **Lemaire O (2007):** A sensitive one-step real-time RT-PCR method for detecting Grapevine leafroll-associated virus 2 variants in grapevine. *Journal of Virological Methods*. 141: 117–124.

Bianco PA, Casati P & **Marziliano N (2004**): Detection of phytoplasmas associated with grapevine Flavescence dorée disease using real-time PCR. *Journal of Plant Pathology*. 86: 259–264.

Bini F, Geider K & **Bazzi C (2008):** Detection of *Agrobacterium vitis* by PCR using novel *virD2* gene-specific primers that discriminate two subgroups. *European Journal of Plant Pathology.* 122: 403–411.

Bisztray GyD, Civerolo EL, Dula T, Kölber M, Lázár J, Mugnai L, Szegedi E & **Savka M A (2012):** Grapevine pathogens spreading with propagating plant stock: detection and methods for elimination. In: Szabó PV & Shojania J, eds, *Grapevines: Varieties, Cultivation and Management*, Nova Science Publishers, Inc., 1–86.

Blasioli S, Biondi E, Braschi I, Mazzucchi U, Bazzi C & **Gessa CE (2010):** Electronic nose as an innovative tool for the diagnosis of grapevine crown gall. *Analytica Chimica Acta*. 672: 20–24.

Bonants P, Groenewald E, Rasplus JY et al. (2010): QBOL: a new EU project focusing on DNA barcoding of quarantine organisms. *OEEP/EPPO Bulletin*. 40: 30–33.

Boonham N, Kreuze J, Winter S, van der Vlugt R, Bergervoet J, Tomlinson J & **Mumford R (2014):** Methods in virus diagnostics: From ELISA to next generation sequencing. *Virus Research*. 186: 20–31.

Camps C, Kappel C, Lecomte P, Léon C, Coutos-Trévenot P, Delrot S & **Gomés E (2014):** Identification of grapevine marker genes for early, non-destructive *Eutypa lata* infection diagnosis. *Plant Pathology*. 63: 323–333.

Čepin U, Gutiérrez-Aguirre I, Balažic L, Pompe-Novak M, Gruden K & **Ravnikar M (2010):** A one-step reverse transcription real-time PCR assay for the detection and quantitation of *Grapevine fanleaf virus*. *Journal of Virological Methods*. 170: 47–56.

Choi H-K, Goes da Silva F, Lim H-J, Iandolino A, Seo Y-S, Lee S-W & **Cook DR (2010a):** Diagnosis of Pierce's disease using biomarkers specific to *Xylella fastidiosa* rRNA and *Vitis vinifera* gene expression. *Phytopathology*. 100: 1089–1099.

Choi YJ, Yun HK, Park KS, Noh JH, Heo YY, Kim SH et al. (2010b): Transcriptional profiling of ESTs responsive to *Rhizobium vitis* from 'Tanmara' grapevines (*Vitis* sp.), *Journal of Plant Physiology*. 167: 1084–1092.

Christensen NM, Nicolaisen M, Hansen M & **Schulz A (2004):** Distribution of phytoplasmas in infected plants as revealed by real-time PCR and bioimaging. *Molecular Plant-Microbe Interactions*. 17: 1175–1184.

Christensen NM, Nyskjold H & **Nicolaisen M (2013):** A real-time PCR detection system for the Bois noir and Flavescence dorée phytoplasmas and quantification of the target DNA. In: Dickinson M & Hodgetts J, eds, *Phytoplasma Methods and Protocols*, Humana Press, 245–253.

Coetzee B, Freeborough MJ, Maree HJ, Celton JM, Rees DJ & **Burger JT (2010):**Deep sequencing analysis of viruses infecting grapevines: Virome of a vineyard. *Virology.* 400: 157–163.

Daire X, Clair D, Larrue J & **Boudon-Padieu E (1997):** A survey of grapevine yellows in diverse European countries and Israel. *Vitis.* 36: 53–54.

Donaire L, Wang Y, Gonzalez-Ibeas D, Mayer KF, Aranda MA & **Llave C (2009):** Deep-sequencing of plant viral small RNAs reveals effective and widespread targeting of viral genomes. *Virology.* 392: 203–214.

Dreo T, Gruden K, Manceau C, Janse JD & **Ravnikar M (2007):** Development of a real-time PCR-based method for detection of *Xylophilus ampelinus. Plant Pathology*. 56: 9–16.

Engel EA, Escobar PF, Rojas LA, Rivera PA, Fiore N & **Valenzuela PDT (2010):** A diagnostic oligonucleotide microarray for simultaneous detection of grapevine viruses. *Journal of Virological Methods*. 163: 445–451.

Espinoza C, Vega A, Medina C, Schlauch K, Cramer G & **Arce-Johnson P (2007):** Gene expression associated with compatible viral diseases in grapevine cultivars. *Functional and Integrated Genomics*. 7: 95–110.

Fahrentrapp J, Michl G & **Breuer M (2013):** Quantitative PCR assay for detection of Bois noir phytoplasmas in grape and insect tissue. *Vitis.* 52: 85–89.

Francis M, Lin H, Cabrera-La Rosa J, Doddapaneni H & **Civerolo EL (2006):** Genome-based PCR primers for specific and sensitive detection and quantification of *Xylella fastidiosa*. *European Journal of Plant Pathology*. 115: 203–213.

Fung RW, Gonzalo M, Fekete C, Kovacs LG, He Y, Marsh E, McIntyre LM, Schachtman DP & **Qiu W (2008):** Powdery mildew induces defense-oriented reprogramming of the transcriptome in a susceptible but not in a resistant grapevine. *Plant Physiology*.146: 236–249.

Giampetruzzi A, Roumi V, Roberto R, Malossini U, Yoshikawa N, La Notte P, Terlizzi F, Credi R & **Saldarelli P (2012):** A new grapevine virus discovered by deep sequencing of virus- and viroid-derived small RNAs in cv Pinot gris. *Virus Research*. 163: 262–268.

Goto M, Honda E, Ogura A, Nomoto A & **Hanaki K-I (2009):** Colorimetric detection of loop-mediated isothermal amplification by using hydroxy naphtol blue. *BioTechniques*. 46: 167–172.

Harper SJ, Ward LI & **Clover GRG (2010)**: Development of LAMP and real-time PCR methods for the rapid detection of *Xylella fastidiosa* for quarantine and field applications. *Phytopathology*. 100: 1282–1288.

Harper SJ, Delmiglio C, Ward LI & **Clover GRG (2011):** Detection of *Tomato black ring virus* by real-time one-step RT–PCR. *Journal of Virological Methods*. 171: 190-194.

Ho T & **Tzanetakis IE (2014):** Developement of a virus detection and discovery pipeline using next generation sequencing. *Virology.* 471–473: 54–60.

Hren M, Boben J, Rotter A, Kralj P, Gruden K & **Ravnikar M (2007):** Real-time PCR detection systems for Flavescence dorée and Bois noir phytoplasmas in grapevine: comparison with conventional PCR detection and application in diagnostics. *Plant Pathology*. 56: 785–796.

Hren M, Nicolic P, Rotter A, Blejec A, Terrier N, Ravnikar M, Dermastia M & **Gruden K (2009):** 'Bois noir' phytoplasma induces significant reprogramming of the leaf transcriptome in the field gown grapevine. *BMC Genomics*. 10: 460.

Hren M, Dreo T, Erjavec J, Nikolic, Boben J, Gruden K, Dermastia M, Camloh M & **Ravnikar M (2010):** Real-time PCR detection methods for economicaly important grapevine related bacteria. pp.: 229–246 Delrot S et al. (eds.) *Methodologies and Results in Grapevine Research*, Springer Science+Business Media B. V.

Johnson KL, Zheng D, Kaewnum S, Reid CL & **Burr TJ (2013):** Development of a magnetic capture hybridization real-time PCR assay for detection of tumorigenic *Agrobacterium vitis* in grapevines. *Phytopathology.* 103: 633–640.

Kogovšek P et al. (2015): LAMP assay and rapid sample preparation method for on-site detection of flavescence dorée phytoplasma in grapevine. Plant Pathology. 64: 286–296.

Kreuze JF, Perez A, Untiveros M, Quispe D, Fuentes S, Barker I & **Simon R (2009):** Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: a generic method for diagnosis, discovery and sequencing of viruses. *Virology.* 388: 1–7.

Lebas B & **Ward L (2012):** *Vitis* (grapevine) post-entry quarantine testing manual. http://www.biosecurity.govt.nz/regs/imports/plants/high-value-crops.

Lopez-Fabuel I, Wetzel T, Bertolini E, Bassler A, Vidal E, Torres LB, Yuste A & **Olmos A (2013):** Real-time multiplex RT-PCR for the simultaneous detection of the five main grapevine viruses. *Journal of Virological Methods*. 188: 21–24.

Louws FJ, Rademaker JLW & **de Bruijn FJ (1999):** The three ds of PCR-based genomic analysis of phytobacteria: diversity, detection, and disease diagnosis. *Annual Review of Phytopathology*. 37: 81–125.

Luigi M & **Faggioli F (2013):** Development of a quantitative real-time RT-PCR (qRT-PCR) for the detection of hop stunt viroid. *European Journal of Plant Pathology.* 137: 231–235.

Makarova O, Contaldo N, Paltrinieri S, Kawube G, Bertaccini A & **Nicolaisen M (2012):** DNA barcoding for identification of '*Candidatus Phytoplasmas*' using a fragment of the elongation factor Tu gene. *PLOS ONE* 7(12): e52092.

Manduláné Farkas E, Czotter N, Lózsa R, Dula T, Ember I, Várallyay É & **Szegedi E (2014):** Conventional PCR primers for the detection of grapevine pathogens disseminated by propagating material. *International Journal of Horticultural Science.* 20: 69–80.

Margaria P, Turina M & **Palmano S (2009):** Detection of Flavescence dorée and Bois noir phytoplasmas, Grapevine leafroll associated virus-1 and -3 and Grapevine virus A from the same crude extract by reverse transcription-Real Time TaqMan assays. *Plant Pathology*. 58: 838–845.

Martín MT, Cobos R, Martín L & **López-Enríquez L (2012):** Real-Time PCR Detection of *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum. Applied and Environmental Microbiology*. 78: 3985–3991.

Martos S, Torres E, El Bakali MA, Raposo R, Gramaje D, Armengol J & **Luque J (2011):** Co-operational PCR coupled with dot blot hybridization for the detection of *Phaeomoniella chlamydospora* on infected grappevine wood. *Journal of Phytopathology*. 159: 247–254.

Navarro B, Pantaleo V, Gisel A, Moxon S, Dalmay T, Bisztray Gy, Di Serio F & **Burgyán J (2009):** Deep sequencing of viroid-derived small RNAs from grapevine provides new insight on the role of RNA silencing in plant-viroid interaction. *PLOS ONE* 4(11):e7686

Notomi T, Okayama H, Masuvbuchi H, Yonekawa T, Watanabe K Amino N & **Hase T (2000):** Loop-mediated isothermal amplification of DNA. *Nucleic Acids Research.* 28(12): e63.

Osman F & **Rowhani A (2006):** Application of a spotting sample preparation technique for the detection of pathogens in woody plants by RT-PCR and real-time PCR (TaqMan). *Journal of Virological Methods*.133: 130–136.

Osman F & **Rowhani A (2008):** Real-time RT-PCR (TaqMan®) assays for the detection of viruses associated with Rugose wood complex of grapevine. *Journal of Virological Methods*. 154: 69–75.

Osman F, Leutenegger C, Golino D & **Rowhani A (2007):** Real-time RT-PCR (TaqMan®) assays for the detection of Grapevine Leafroll associated viruses 1–5 and 9. *Journal of Virological Methods*. 141: 22–29.

Osman F, Leutenegger C, Golino D & **Rowhani A (2008):** Comparison of low-density arrays, RT-PCR and real-time TaqMan® RT-PCR in detection of grapevine viruses. *Journal of Virological Methods*. 149: 292–299.

Osman F, Hodzic E, Omanska-Klusek A, Olineka T & **Rowhani A (2013):** Development and validation of a multiplex quantitative PCR assay for the rapid detection of Grapevine virus A, B and D. *Journal of Virological Methods*. 194: 138–145.

Overton BE, Stewart EL, Qu X, Wenner NG & **Chryst BJ (2004):** Qualitative real-time PCR SYBR® Green detection of Petri disease fungi. *Phytopathologia Mediterranea*. 43: 403–410.

Pacificio D, Caciagli P, Palmano S, Mannini F & **Marzachi C (2011)**: Quantitation of *Grapevine leafroll associated virus-1* and *-3*, *Grapevine virus A*, *Grapevine fanleaf virus* and *Grapevine fleck virus* in field-collected *Vitis vinifera* L. 'Nebbiolo' by real-time reverse transcription-PCR. *Journal of Virological Methods*. 171: 190–194.

Pantaleo V, Saldarelli P, Miozzi L, Giampetruzzi A, Gisel A, Moxon S, Dalmay T, Bisztray G & **Burgyan J (2010):** Deep sequencing analysis of viral short RNAs from an infected Pinot Noir grapevine. *Virology* 408: 49–56.

Papayiannis LC (2014): Diagnostic real-time RT-PCR for the simultaneous detection of Citrus exocortis viroid and Hop stunt viroid. *Journal of Virological Methods*. 196: 93–99.

Pelletier C, Salar P, Gillet J, Cloquemin G, Very P, Foissac X & **Malembic-Maher S (2009):** Triplex real-time PCR assay for sensitive and simultaneous detection of grapevine phytoplasmas of the 16SrV and 16SrXII-A groups with an endogenous analytical control. *Vitis*. 48: 87–95.

Santi S, De Marco F, Polizzotto R, Grisan S & **Musetti R (2013):** Recovery from stolbur disease in grapevine involves changes in sugar transport and metabolism. *Frontiers in Plant Science*. 4: 171.

Schaad NW, Opgenorth D & **Gaush P (2002):** Real-time polymerase chain reaction for one-hour on-site diagnosis of Pierce's disease of grape in early season asymptomatic vines. *Phytopathology*. 92: 721–728.

Schena L & **Ippolito A (2003):** Rapid and sensitive detection of *Rosellinia necatrix* in roots and soils by real-time Scorpion PCR. *Journal of Plant Pathology*. 85: 15–25.

Schena L, Nigro F & **Ippolito A (2002):** Identification and detection of *Rosellinia necatrix* by conventional and real-time Scorpion PCR. *European Journal of Plant Pathology*. 108: 355–366.

Schena L, Nigro F, Ippolito A & **Gallitelli D (2004):** Real-time quantitative PCR: a new technology to detect and study phytopathogenic and antagonistic fungi. *European Journal of Plant Pathology*. 110: 893–908.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL et al. (2012): Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Sciences of the United States of America.* 109: 6241–6246.

Shishido M, Kubota I & **Nakamura H (2012):** Development of real-time PCR assay using TaqMan probe for detection and quantification of *Rosellinia necatrix* in plant and soil. *Journal of General Plant Pathology*. 78: 115–120.

Sun N, Deng C, Zhao X, Zhou Q, Ge G, Liu Y, Yan W & **Xia Q (2014):** Extraction of total nucleic acid based on silica-coated magnetic particles for RT-qPCR detection of plant RNA virus/viroid. *Journal of Virological Methods*. 196: 204–211.

Szegedi E, Ember I, Bisztray Gy, Dula B-né, Hajdu E, Kölber M, Lázár J, Nagy B & **Szűcsné Varga G (2012):** Integration of elimination and diagnostic methods for the producion of healthy grapevine propagating material (in Hungarian with English summary). *Növényvédelem.* 48: 469–480.

Thompson JR, Fuchs M, McLane H, Celebi-Toprak F, Fischer KF, Potte JL & **Perry KL (2014):** Profiling viral infections in grapevine using a randomly primed reverse transcription-polymerase chain reaction/macroarray multiplex platform. *Phytopathology.* 104: 211–219.

Tomlinson JA, Boonham N & **Dickinson M (2010)**: Development and evaluation of a one-hour DNA extraction and loop-mediated isothermal amplification assay for rapid detection of phytoplasmas. *Plant Pathology*. 59: 465–471.

Walsh HA & **Pietersen G (2013**): Rapid detection of Grapevine leafroll-associated virus type 3 using a reverse transcription loop-mediated amplification method. *Journal of Virological Methods.* 194: 308–316.

Wu Q, Wang Y, Cao M, Pantaleo V, Burgyan J, Li W-X & **Ding S-W (2012):** Homology-independent discovery of replicating pathogenic circular RNAs by deep sequencing and a new computational algorithm. *Proceedings of the National Academy of Sciences of the United States of America.* 109: 3938–3943.

Zhang Y, Singh K, Kaur R & **Qiu W (2011**): Association of a novel DNA virus with the grapevine vein-clearing and vine decline syndrom**e**. *Phytopathology*. 101: 1081–1090.

Zhang Z, Peng S, Jiang D, Pan S, Wang H & **Li S (2012):** Development of a polyprobe for the simultaneous detection of four grapevine viroids in grapevine plants. *European Journal of Plant Pathology*. 132: 9–16.

Zhao F, Chen L, Perl A, Chen S & **Ma H (2011):** Proteomic changes in grape embryogenic callus in response to *Agrobacterium tumefaciens*-mediated transformation. *Plant Science*. 181: 485–495.