

International Journal of Horticultural Science 2014, 20 (3–4): 69–80.

Agroinform Publishing House, Budapest, Printed in Hungary

ISSN 1585-0404

Conventional PCR primers for the detection of grapevine pathogens disseminated by propagating material

Manduláné Farkas¹, E., Czotter², N., Lózsa³, R., Dula⁴, T., Ember³, I.,
Várallyay², É. & Szegedi^{1#}, E.

¹National Agricultural Research and Innovation Centre, Research Institute for Viticulture and Enology, Experimental Station of Kecskemét, 6000 Kecskemét-Katonatelep, Katona Zsigmond út 5., Hungary

²National Agricultural Research and Innovation Centre, Agricultural Biotechnology Institute, 2100 Gödöllő, Szent-Györgyi Albert út 4., Hungary

³Corvinus University of Budapest, Faculty of Horticulture, Department of Viticulture, 1118 Budapest, Villányi út 29-43., Hungary

⁴DULA Grape & Wine Advisory Ltd., 3300 Eger, Eszterházy tér 9., Hungary; szegedi.erno@naik.hu

Summary: Polymerase chain reaction driven by sequence specific primers has become the most widely used diagnostic method to detect and identify plant pathogens. The sensitive and cost-effective pathogen detection is exceptionally important in the production of propagating material. In this paper we have collected primer sequence data from the literature for the detection of the most important grapevine pathogens disseminated by propagating stocks by conventional polymerase chain reaction. Basic protocols to obtain template nucleic acids have also been briefly reviewed.

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

Grapevine cultivation is endangered by several pathogens disseminated in latent form by propagating material. These pathogens include several viroids, viruses, phytoplasmas, bacteria and fungi (Bisztray et al. 2012, Martelli 2014). Their presence in the plant frequently cannot be recognized by symptoms in the plantations, but disease outbreak may take place in the later stage of infection or in the new plantations under favourable environmental conditions. Therefore the use of healthy planting material has a vital importance. Pathogen-free grapevine stocks can be selected from the existing plantations by various diagnostic protocols or produced by various curative (e. g., hot water) treatments and *in vitro* shoot tip and/or apical meristem cultures (Bisztray et al. 2012). The pathogen-free status of hot water treated and micropropagated plants should also be tested by appropriate diagnostic methods.

During the recent decades the polymerase chain reaction (PCR) has become the most common diagnostic protocol in plant pathology. The introduction of the conventional PCR (including multiplex, nested and reverse transcription PCR) has opened wide application possibilities due to its simplicity and cost effectiveness. Later, it was followed by the more sensitive, but also more costly, quantitative real-time PCR (qRT-PCR). Quantitative real-time PCR protocols have already been developed for several viroids (Papayiannis 2014, Sun et al. 2014), viruses (Harper et al. 2011, Osman & Rowhani 2008, Pacificio et al. 2011), phytoplasmas (Angelini

2010, Fahrenttrapp et al. 2013, Pelletier et al. 2009), bacteria (Dreo et al. 2007, Harper et al. 2010, Johnson et al. 2013) and fungi (Martin et al. 2012, Schena et al. 2004).

These protocols are based on the amplification of given nucleic acid sequences derived from plants or isolated microbes. Since viroids, viruses and phytoplasmas are closely associated with plant cells they are detected from total plant nucleic acid (RNA or DNA) preparations. Bacteria and fungi can be recovered from grapevine bleeding sap or from plant tissues on appropriate culture media thus they can be detected and identified both from total plant DNA and pure cultures. Recently, a medium for recovery of axenic phytoplasma cultures has also been developed (Contaldo et al. 2012) that will allow the identification of phytoplasmas from isolated colonies as well in the future.

For nucleic acid (RNA or DNA) isolation several protocols have been developed during the last decades. For rapid field sampling the use of FTA cards (Whatman) can be proposed that allows a simple collection, transport and storage of nucleic acids derived from tissue sap or from homogenized tissues. Such samples then can be processed in PCR (Grund et al. 2010) to detect plant pathogens. Kits specifically developed for plant nucleic acids isolation (e. g., Spectrum™ Plant Total RNA Kit produced by Sigma-Aldrich and RNeasy Plant RNA Mini Kit produced by Qiagen) are also widely used for diagnostic purposes (Cseh 2012, Gambino & Gribaudo 2006, Li et al. 2011, Nassuth et

al. 2000, Ragozzino et al. 2004). Conventional nucleic acid isolation protocols include the use of SDS/phenol extraction (Franke et al. 1995, Ragozzino et al. 2004), CTAB (Gambino et al. 2008, Li et al. 2008, Lodhi et al. 1994, Steenkamp et al. 1994, Zhang et al. 2012), guanidine isothiocyanate (Gambino et al. 2006, MacKenzie 1997, Vasanthaiah et al. 2008), and silica-based methods (Digiario 2007, Gambino 2006, Hajizadeh et al. 2012, Sun et al. 2014). These protocols are also suitable to isolate nucleic acids from bacteria or fungi (Aroca & Raposo 2007, Botha et al. 2001, Hamelin et al. 1996, Neuhauser et al. 2009, Rodrigues et al. 2003, Szegedi 2003). For PCR analysis of pure bacterial cultures a simple triton/sodium-azide lysis (Abolmaaty et al. 2002) was found highly appropriate (Szegedi & Botka 2002). Specific nucleic acid sequences can be enriched by magnetic capture hybridization for subsequent PCR analysis (Johnson et al. 2013).

Primer sequence data for a given pathogen group have already been published by several authors (e. g., Angelini 2010, Constable et al. 2010, Ghignone & Migheli 2005,

see also: <http://www.sppadbase.ipp.cnr.it>, OEEP/EPPO 2005, Palacio-Bielsa et al. 2009, Thompson et al. 2014). In the recent paper we collected a dataset of primers for conventional PCR (and reverse-transcription PCR for viroids and viruses) from the literature for the detection of various grapevine pathogens which have or may have significant importance in the production of propagating stocks. These groups of pathogens include viroids (Table 1.), the most common viruses including the newly described ones that may have potential importance in the future (Table 2.), phytoplasmas (Table 3.), bacteria (Table 4.) and fungi causing wooden infection frequently followed by trunk death (Table 5.). For the direct practical use of data described in this paper we list the primer sequences and the gene they are specific for, their published annealing temperatures and the length of the amplified fragments. The aim of our work is to provide comprehensive data for grapevine research and quarantine laboratories that work in studying or controlling grapevine pathogens and the plant health status of the propagation material stocks.

Table 1. Viroid-specific primers

Viroid	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Reference
AGVd	c2-h2+	TTCGGTGAGTACCACAGGAAC ACTCGTCCCAGCGGTCCCAAC	55	130	Wan Chow Wah & Symons, 1997
AGVd	Fw Rev	GTCGACGAAGGGTCCTCAGCAGAGCACC GTCGACGACGAGTCGCCAGGTGAGTCTT	60	369	Eiras et al. 2006
AGVd	AGVd-P7 AGVd-P8	ACCTGCAGGGAAGCTAGCTGGGTC CCCTGCAGGTTTCGCCAGCAAGCGC	56	369	Jiang et al. 2009b
AGVd	AGVd-mF AGVd-mR	GTCCTCAGGAGAGCACC GG GAAAACCTGGTTGGGACCGCTG	60	195	Hajizadeh et al. 2012
CEVd	CEVd-Fw CEVd-Rev	GGAAACCTGGAGGAAGTTCG CCGGGGATCCCTGAAGGA	60	375 (fl)	Eiras et al. 2006
CEVd	CEVd-mF CEVd-mR	GTGTCCTTCCTTTGGCTGCTG TGGCCCGGAGAACAGTGAAG	51	153	Hajizadeh et al. 2012
GHVd	F1/R1	TTACGGAATTCTACCCCTCCCCAGCAGATGAGATCTTTAAGTTTCGTCC TTTTGGACTCGTCAGGAGCACCACA	nd	377	Wu et al. 2012
GHVd	F2/R2	CACGAAGTTTTAACCTTCGTAAGTCGGGCACTGTGTGGTG ACTTCGTGGGAAAAGGTTACCGGCTAAGGCTTGACCGG	nd	382	Wu et al. 2012
GHVd	F3/R3	CTCTGGCAGATTTCGCTCCTAGGCTAGAACCGGTCCA CAGAGCTCCAACCTCAAGAATGGCAGCTAACCTTCCCC	nd	379	Wu et al. 2012
GYSVd-1	GV1M GV2P	GCGGGGGTTCCGGGGATTGC TAAGAGGTCTCCGGATCTTCTTGC	66	365-370 (fl)	Polivka et al. 1996
GYSVd-1	PBCVd100C PBCVd194H	AGACCCCTTCGTCGACGACGA TGTCCTCCCTAGTCGAGCGGA	56	220	Nakaune & Nakano 2006
GYSVd-1	Fw Rev	GAGGTCCTCGGATCAC AGAGCGCAATGCTGAATAGGC	60	222	Eiras et al. 2006
GYSVd-1	GYSVd-PF GYSVd-PR	TTGGATCCCACCTCGGAAGGCCGC TTGGATCCTAACACAGGAACCACA	56	365-370 (fl)	Jiang et al. 2009a
GYSVd-1	GYSVd-1-mF GYSVd-1-mR1	CAAAGCCCTTTTCTTTCAACTGAG CCCAGAGCAGCGTGGTCC	52	249	Hajizadeh et al. 2012
GYSVd-2	cl-h3+	ACCGGCTTCGGAGATAGAAG AATGAGCCTCGTCGTCGA	56	123	Wan Chow Wah & Symons, 1997

Viroid	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Reference
GYSVd-2	c2- hl+	CCGAGGTGTAACACAGGAACC TTGAGGCCCGGCGAAACGC	55	194	Wan Chow Wah & Symons, 1997
GYSVd-2	Fw Rev	TTGAGGCCCGGCGAAACGC ACCGGCTTCGGAGATAGAAG	60	363 (fl)	Eiras et al. 2006
GYSVd-2	GYSVd-2-P1 GYSVd-2-P2	ACTAGTACTTTCTTCTATCTCCGAAGC ACTAGTCCGAGGACCTTTTCTAGCGCTC	56	363 (fl)	Jiang et al. 2009c
GYSVd-3 (=CGVd)	GYSVd-PF GYSVd-PR	TTGGATCCCACCTCGGAAGGCCGCC TTGGATCCTAACACAGGAACCACA	56	366 (fl)	Jiang et al. 2009a
HSVd	HV1M HV2P	GGCTCAAGAGAGGATCCGCG CCTCTGGGGAATTCTCGAGTTGC	65	295-300 (fl)	Polivka et al. 1996
HSVd	F R	CTGGGGAATTCTCGAGTTGCC AGGGGCTCAAGAGAGGATCCG	50	297 (fl)	Farkas et al. 1999
HSVd	HSV-83M HSV-78P	AACCCGGGGCTCCTTTCTCA AACCCGGGGCAACTTCTC	50 56	~300 (fl)	Sano et al. 2001
HSVd	Fw Rev	ACTTCTCAGAATCCAGCGAG TGCCCCGGGGCTCCTTTCTCAGGT	60	297-302 (fl)	Eiras et al. 2006

AGVd = Australian grapevine viroid, CEVd = Citrus exocortis viroid, CGVd = Chinese grapevine viroid, HSVd = Hop stunt viroid, GYSVd-1, -2 and -3 = Grapevine yellow speckle viroid-1, -2 and -3, and GHVd = Grapevine hammerhead viroid, nd = no data, fl = proper full length viroid could be amplified

*published annealing temperatures

Table 2. Virus-specific primers

Virus*	Primer name	Primer sequence (5'-3')	At/Tm (°C)**	Fragment length (nt)	Gene	Reference
GFLV	GFLV H2999 GFLV C3310	TCGGGTGAGACTGCGCAACTTCCTA GATGGTAACGCTCCCGCTGCTCTT	52	311	RNA2-polyprotein	MacKenzie et al. 1997
GFLV	GFLVFw GFLVRev	ATGCTGGATATCGTGACCTGT GAAGGTATGCCTGCTTCAGTGG	56	118	RNA1-polyprotein	Gambino & Gribaudo 2006
GFLV	F CPS R EV00N1	TTGTGCGCCCAGATCTCTCTTTACCA GACTACACATATACACTTGGGTCTTTTAA	55	555	RNA2-polyprotein	Demangeat et al. 2004
GFLV	GFLV V1/F GFLV C1/R	ACCGGATTGACGTGGGTGAT CCAAAGTGTGGTTCCCAAGA	nd	322	RNA2-polyprotein	Sanchez et al. 1991
GFLV	GFLVpoly5238FwGFLV poly6048Rev	ATCGATACTCCTGGTACG ATAAAGCTCGACAAGTGC	52	828	RNA1-polyprotein	Pacifico et al. 2011
GFLV	GFLV CP 433V GFLV CP 912C	GAAGTGGCAAGCTGTCGTAGAAC GCTCATGTCTCTGACTTTGACC	58	711	RNA2-polyprotein	Osman & Rowhani 2006
GFLV	GFLV-sp s Nepo-A a	TCAGATTTTAAAGGGCGTCCA TGDCASWVARYTCYCCATA	50	260	RNA2-polyprotein	Digiario et al. 2007
ArMV	ArMV Fw ArMV Rev	TGACAACATGGTATGAAGCACA TATAGGGCCTTTCATCACGAAT	56	402	RNA1-polyprotein	Gambino & Gribaudo 2006
ArMV	H1124 C1642	CAGCGGATTGGGAGTTCGT TTGGCCCAGATATAGCGTAAAAAT	50	517	RNA2-polyprotein	MacKenzie et al. 1997
ArMV	H428 C867	GCGGCGGATTGGGAGTT CGATGGTAGGGGGAGCGTATT	54	440	RNA2-polyprotein	Nassuth et al. 2000
ArMV	ArMV-sp s Nepo-A a	GCGGGAATATATCTGAAA TGDCASWVARYTCYCCATA	60	301	RNA2-polyprotein	Digiario et al. 2007
ArMV	Upper Lower	CGGATTGGGAGTTTCGTTGTCG CCGTTCCATTCATAACAACCTC	50	340	RNA2-polyprotein	Bertolini et al. 2001
ArMV	ArMV-CP1202F ArMV CP1313R	CTGTGCCATCCTTCCCAATGAT GAGATGCTCCATCCATGCCAGT	58	294	RNA2-polyprotein	Osman & Rowhani 2006
ToRSV	TRSV-sp s Nepo-A a	CAGTGAGGATGCACGCCCC TGDCASWVARYTCYCCATA	50	202	RNA2-polyprotein	Digiario et al. 2007
ToRSV	ToRSV5(fw) ToRSV6(rev)	AGGTAGGACGCYATTGTTCCAGG AGTCTCAACTTAACATACCACTAC	54	428	RNA1-polyprotein 3'UTR	Li et al. 2011

Virus*	Primer name	Primer sequence (5'-3')	At/Tm (°C)**	Fragment length (nt)	Gene	Reference
ToRSV	ToRSV g-V/F ToRSV g-C/R	GTCACTACTCTTAACGCTAACCC CCACTCATACTCCAGTCATC	58	444	RNA2-polyprotein	Osman et al. 2008
NepoA	Nepo-A s Nepo-A a	GGHDTBCAKTMYARRARTGG TGDCCASWVARYTCYCCATA	50	256	RNA2-polyprotein	Digiario et al. 2007
GCMV	GCMV 5'3140 GCMV 3'3831	CATGGTCTAGCCACTAGGAG GTAGTGGCACACATGATGGC	54	692	RNA2-polyprotein	Brandt & Himmler 1995
GCMV	Nepo-B s GCMV-sp-a	ATGTGYGCHACYACWGGHATGCA CTGCAAGGGAAGTTGATAAGGG	50	187	RNA2-polyprotein	Digiario et al. 2007
TBRV	Nepo-B s TBRV-sp-a	ATGTGYGCHACYACWGGHATGCA ATGACACTCTAGAAGAAAGTTG	50	486	RNA2-polyprotein	Digiario et al. 2007
NepoB	Nepo-B s Nepo_B a	ATGTGYGCHACYACWGGHATGCA TTCTCTDHAAGAAATGCCTAAGA	50	391	RNA2-polyprotein	Digiario et al. 2007
GBLV	GBLV-CPs GBLV-CPa	AGTGCCCTTTTAGCCGATACCAG TCACTTAAGTGCGCTTACGCT	58	1565	RNA2-polyprotein	Elbeaino et al. 2011
GLRaV-1	LR1CPF1 LR1CPR1	CTAGCGTTATATCTCAAAATGA CCCATCACTTCAGCACATAAA	nd	502	coat protein	Engel et al. 2010
GLRaV-1	GLRaV-1Fw GLRaV-1Rev	TCTTTACCAACCCCGAGATGAA GTGTCTGGTGACGTGCTAAACG	56	232	coat protein	Gambino & Gribaudo 2006
GLRaV-1	GLRaV1poly230Fw GLRaV1poly1227Rev	CCACGATAAGYGACAACCTCCCGA CGTTAACTTGAGRTCGAACCCTAA	50	998	RNA dependent RNA polymerase	Pacifico et al. 2011
GLRaV-1	HSP70-417 F HSP70-737 R	GAGCGACTTGGCGACTTATCGA GGTAAACGGGTGTTCTTCAATTCT	58	320	HSP70-like protein	Osman et al. 2007
GLRaV-1	LQV1-H47 LEV1-C447	GTTACGGCCCTTTGTTTATTATGG CGACCCCTTTATTGTTTGAGTATG	60	401	coat protein	Osman & Rowhani, 2006
GLRaV-1	LR1 HSP70-149 f LR1 HSP70-293 r	ACCTGGTTGAACGAGATCGCTT GTAAACGGGTGTTCTTCAATTCTCT	60	145	HSP70-like protein	Osman et al. 2007
GLRaV-1	LR1-9727U LR1-10019D	TCGTAACGGCCGCTTCAGTA GTTCGTAACGTGCACGGAAG	56	303		Nakaune & Nakano 2006
GLRaV-2	LR2 12474U LR2 12806D	TTGACAGCAGCCGATTAAGCG CTGACATTATTGGTGGCAGCGG	56	333	HSP90-like protein	Nakaune & Nakano 2006
GLRaV-2	LR2 V2dCPf2 LR2 V2CPr1	ACGGTGTGCTATAGTGCGTG GCAGCTAAGTACGAATCTTC	nd	514	minor and major coat protein	Bertazzon & Angelini 2004
GLRaV-2	GLRaV-2F GLRaV-2R	GGTGATAACCGACGCCTCTA CCTAGCTGACGCAGATTGCT	56	543	major coat protein	Gambino & Gribaudo 2006
GLRaV-2	LR2-U2 LR2-L2	ATAATTCGGCGTACATCCCCACTT GCCCTCCGCGCAACTAATGACAG	52	331	HSP70-like protein	Bertazzon & Angelini 2004
GLRaV-2	CPV F CPC R	TGGAGTTGATGTCGACAGC ACGACCGAACGTTCTAAGTT	58	338	major coat protein	Osman et al. 2007
GLRaV-2	RGHSP227V/F RGHSP777C/R	GCGACTCCAGCAACTTTAGTGA GTCTAACGAAAGATCGGGTTCTAAG	58	551	HSP70-like protein	Osman et al. 2008
GLRaV-3	LR3 LC1F LR3 LC2R	CGCTAGGGCTGTGGAAGTATT GTTGTCCTCCGGTACCAGATAT	52	546	HSP70-like protein	Turturo et al. 2005
GLRaV-3	GLRaV-3F GLRaV-3R	TACGTAAAGGACGGGACACAGG TGCGGCATTAATCTTCATTG	56	336	coat protein	Gambino & Gribaudo 2006
GLRaV-3	LR3-H330 LR3-C629	GATGCTTTCGCGTATTTCTTG CGGCACGATCGTACTTTCTAA	54	300	HSP90-like protein	Mackenzie et al. 1997

Virus*	Primer name	Primer sequence (5'-3')	At/Tm (°C)**	Fragment length (nt)	Gene	Reference
GLRaV-3	CP-111 F CP-722 R	AAAGTAGGTTAAGGACGGGACACA AGGGTCGCCGTGATGAAG	58	612	coat protein	Osman et al. 2007
GLRaV-3	GLRaV3-56f GLRaV3-285r	AAGTGCTCTAGTTAAGGTCAGGAGTGA GTATTGGACTACCTTTCGGGAAAAT	60	230	HSP70-like protein	Bertolini et al. 2010
GLRaV-3	GLRaV3poly7209Fw GLRaV3poly8145Rev	GGGTTGTGACGACTCTGTGGCGCAT GATATCTGAAGTTTTGGAAGCT	52	958	RNA-dependent RNA polymerase	Pacifico et al. 2011
GLRaV-3	LR3-8033U LR3-8408D	TTACGGCACAAACGCTACCAG CTGGTGTGGTAGAGTAGTTCC	56	376	HSP90-like protein	Nakaune & Nakano 2006
GLRaV-3	H587(Fw) C547 (Rev)	ATAAGCATTCCGGATGGACC ATTAACCTTGACGGATGGCACGC	62	340	RNA-dependent RNA polymerase	Minafra & Hadidi 1994
GLRaV4	LR4 HSPV-F LR4 HSPV-R	ACATTCTCCACCTTGTGCTTTT CATACAAGCGAGTGCAATTACA	58	321	HSP70-like protein	Osman et al. 2007
GLRaV5	HPPV F HSPV R	AACACTCTGCTTTTCTGCTGGCA TCTCCAGAAGACGGACCAATGTAA	58	273	HSP70-like protein	Osman & Rowhani 2006
GLRaV5	HSP70-29F HSP70-634R	CACTCTGCTTTTCTGCTGGCA CGACGCACAGACGTAGGATGA	58	600	HSP70-like protein	Osman et al. 2007
GLRaV4-5	HSP45A HSP45B	GTATCTYATGTACCAACAGAT GGTATGAACAARTTCAATGC	53	370	HSP70-like protein	Routh et al. 1998
GLRaV7	LR7F LR7R	TATATCCCAACGGAGATGGC ATGTTCTCCACCAAAATCG	nd	502	HSP70-like protein	Engel et al. 2008
GLRaV9	LR9F LR9R	CGGCATAAGAAAAGATGGCAC TCATTCACCACTGCTTGAAC	58	393	HSP70-like protein	Alkowni et al. 2004
GVA	GVA Fw GVA Rev	GAGGTAGATATAGTAGGACCTA TCGAACATAACCTGTGGCTC	56	272	coat protein	Gambino & Gribaudo 2006
GVA	GVA 6540U GVA 6880D	TTTGGGTACATCGCGTTGGT TCTAAGCCCCACGCGAAGT	56	341	coat protein	Nakaune & Nakano 2006
GVA	GVAH587 V1/F GVAC995 C1/R	GACAAATGGCACACTACG AAGCCTGACCTAGTCATCTTGG	62	430	coat protein	Minafra & Hadidi 1994
GVA	GVAdeg7Fw GVAdeg4Rev	MRNCCMGARTAYGATGC TRTARAABGCYACCTC	52	652	replicase	Pacifico et al. 2011
GVA	GVA-H7038 GVA-C7273	AGGTCCACGTTTGTCTAAG CATCGTCTGAGGTTTCTACTAT	54	240	putative RNA binding protein	MacKenzie 1997
GVB	GVB H28 GVB C410	GTGCTAAGAACGCTTTCACAGC ATCAGCAAAACGCTTGAACCG	56	460	putative RNA binding protein	Minafra & Hadidi 1994
GVD	GVDORF2-F1 GVDORF2-R1	TAATAGGGCCTAAGTC GGGCGTTGAATACACCTTTAGC	50	371	coat protein	Lebas & Ward 2012
GVD	D_ORF/F D_ORF/R	CTTAGGACGCTTTCGGGTACA CTGCTCTCCAACCGACGACT	58	465	coat protein	Abou-Ghanem et al. 1997
GVD	GVDmu-554f GVDmu-661r	AGGTGTATTCAACGCCAGTC GCCCTACGCTTCTTAGCATAACTAC	57	108	coat protein and RNA binding protein	Osman et al. 2013
GVA, GVB, GVD	VT-165 F1 VT-594 R1	ACYCTCTTYGGGTACATHGC GCBCCYTCHGTVCGAAAGAG	55	429	coat protein	Osman et al. 2013
GVE	GVE-1For GVERev	AATGGAGTCAAAAGCGATCC GTAGGGTCAATCAACCAACA	55	992	coat protein	Coetzee et al. 2010
GfKv	GfKvF GfKvR	TGACCAGCCTGCTGTCTCTA TGGACAGGGAGGTGTAGGAG	56	179	coat protein	Gambino & Gribaudo 2006

Virus*	Primer name	Primer sequence (5'-3')	At/Tm (°C)**	Fragment length (nt)	Gene	Reference
GFkV	RD1 RD2	CYCARCAYAARGTVAACGA GCGCATGCABGTSAGRGGG	56	386	replicase	Nakaune & Nakano 2006
GFkV	GFk V1/F GFk C1/R	GGTCCTCGGCCAGTGAAAAAGTA GGCCAGGTTGTAGTCGGTGTGTC	58	352	replicase	Osman et al. 2008
GFkV	FL CP V FL CP C	CCTCGTGTAAGCATCCATCT CCGAAGACGGAGAGGATCTC	58	260	coat protein	Osman & Rowhani, 2006
GFkV	GFkV OB F GFkV OB R	CGAGAACTCTCTTTTACCTC CCGGCGTGGATGTAGAG	60	147	replicase	Bertolini et al. 2010
GFkV	GFkVrep4944Fw GFkC1m/R	GCGYATCTYACRGCCG GCCAGGYTGTAGTCGGTGTGTT	52	324	replicase	Pacífico et al. 2011
GRSPaV	RSP-up1 RSP-do2	TGAGATGGTYGCTAATATCG CTATTAGTACGGTATTCCAG	56	242	coat protein + 3'UTR	Nakaune & Nakano 2006
GRSPaV	RSPaVFw RSPaVRev	GGGTGGGATGTAGTAACTTTTGA GCAAGTGAATGAAAGCATCACT	56	155	replicase	Gambino & Gribaudo 2006
GRSPaV	48V 49C	AGCTGGGATTATAAGGGAGGT CCAGCCGTTCCACCACTAAT	52	330	coat protein	Lima et al. 2006
GRSPaV	RSP-H5638 RSP-C5992	AGGGATTGGCTGTAGATGTT CTCAGGCAACCCCAAAAA	54	355	replicase	MacKenzie, 1997
GPGV	GPgV5619f GPgV6668r	TGGGAGGATTGCAACAGGGGCT ATTGCCTCGTCAACTTTGCGA	nd	1049	movement protein	Giampetruzzi et al. 2012
GPGV	GPGVFw GPGVRev	ATGTCGATTCTCAGGAGCTG CTACATACTAAATGCACTC	nd	588	coat protein	Cho et al. 2013
GPGV	GPG-5637F GPG5939R	ATTGCGGAGTTGCCTTCAAAG CTGAGAAGCATTTGCCCA	56	303	movement protein	Glasa et al. 2014
GPGV	GPG6609F GPG7020R	GAGATCAACAGTCAGGAGAG GACTTCTGGTGCCTTATCAC	56	412	coat protein	Glasa et al. 2014
GRVfV	GVFVC105F1 GVFVC105R2	CCTGTCGCTTCTTCTCATCT CATCTTCCATGCCATTTCTTG	nd	323	polyprotein1	Al Rwahnih et al. 2009
GSyV-1	Det-F Det-R	CAAGCCATCCGTGCATCTGG GCCGATTTGGAACCCGATGG	nd	296	putative movement protein	Al Rwahnih et al. 2009
GAMaV	GAMaVF3 GAMaVR2	GCATCTCCAATCTGTCCCAT AGTACGAGGTCTCGGTGGTG	nd	479	replicase-associated polyprotein	Al Rwahnih et al. 2009
GVCV#	GVCV-F1 GVCV-R1	CACGTTTCAAAGAAAGATGGAC ATCCKTCCATGCAWCCGTCAG	52	526	polyprotein ORF3	Zhang et al. 2011
GRBaV*	GRBaVF1 GRBaVR1	CTCGTCGATTTGTAAGA ACTGACAAGGCCTACTACG	60	557	V2 protein	Al Rwahnih et al. 2013

*ArMV: Arabis mosaic virus, GAMaV: Grapevine asteroid mosaic-associated virus, GBLV: Grapevine Bulgarian latent virus, GCMV: Grapevine chrome mosaic virus, GFLV: Grapevine fanleaf virus, GFkV: Grapevine fleck virus, GLRaV-1, -2, -3, -4, -5, -7 and -9: Grapevine leafroll-associated virus-1, -2, -3, -4, -5, -7 and -9, respectively, GPGV: Grapevine pinot gris virus, GRBaV: Grapevine red blotch-associated virus, GRSPaV: Grapevine rupestris stem pitting-associated virus, GRVfV Grapevine rupestris vein-feathering virus, GSyV-1: Grapevine Syrah virus-1, GVCV: Grapevine vein clearing virus, GVA: Grapevine virus A, GVB: Grapevine virus B, GVD: Grapevine virus D, GVE: Grapevine virus E, Nepo A: Nepovirus subgroup A, Nepo B: Nepovirus subgroup B, TBRV: Tomato black ring virus, ToRSV: Tomato ringspot virus

**published annealing temperatures, nd = no data

#: GRBaV and GVCV are DNA viruses.

Table 3. Phytoplasma specific primers

Phytoplasma	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Target gene	Reference
Universal	P1 P7	AAGAGTTTGTATCCTGGCTCAGGATT CGTCCTTCATCGGCTCTT	50	1850	16S and intergenic 16S–23S and a small part of 23S rRNA gene	Deng and Hiruki 1991, Schneider et al. 1995
Universal	fU5 rU3	CGGCAATGGAGGAAACT TTCAGCTACTCTTTGTAACA	55	880	16S rRNA gene	Lorenz et al., 1995

Phytoplasma	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Target gene	Reference
Universal	R16F2n R16R2	GAAACGACTGCTAAGACTGG TGACGGGGCGGTGTGTACAAACCCCG	55	1250	16S rRNA gene	Lee et al., 1998
Flavescence dorée (FD) (16SrV-C, D)	FD9f1 FD9r1	GAATTAGAACTGTTTGAAGACG TTTGCTTTCATATCTGTATCG	55	1300	Non ribosomal DNA	OEEP/EPPO 2007**, Daire et al., 1997
Flavescence dorée (16SrV-C, D)	FD9f3b FD9r2	TAATAAGGTAGTTTTATATGACAAG GACTAGTCCCGCCAAAAG	55	1150	Non ribosomal DNA	OEEP/EPPO 2007**, Clair et al., 2003a, 2003b, Angelini et al., 2001
Bois noir (BN, Stolbur) (16SrXII-A)	STOL11f2 STOL11r1	TATTTTCCTAAAATTGATTGGC TGTTTTTGCACCGTTAAAGC	55	830	Non ribosomal DNA	OEEP/EPPO 2007**, Daire et al., 1997, Clair et al. 2003a, 2003b
Bois noir	STOL11f3 STOL11r2	ACGAGTTTTGATTATGTTCAC GATGAATGATAACTTCAACTG	55	720	Non ribosomal DNA	OEEP/EPPO 2007**, Clair et al. 2003b
16S group I,II,XII,XV specific	R16(I)F1 R16(I)R1	TAAAAGACCTAGCAATAGG CAATCCGAACTAAGACTCT	50	1100	16S rRNA gene	Lee et al., 1994
16S group III specific	R16(III)F1 R16(III)R1	AAGAGTGAAAAAACTCCC TTCGAACTGAGATTGA	50	800	16S rRNA gene	Lee et al., 1994
16S group V specific	R16(V)F1 R16(V)R1	TAAAAGACCTTCTTCGG TTCAATCCGTACTGAGACTACC	50	1100	16S rRNA gene	Lee et al., 1994

*published annealing temperatures, **see also papers cited therein

Table 4. Bacterium specific primers

Species	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Target gene	Reference
<i>Agrobacterium spp.</i>	UF f (fw) B1R r (rev) B2R r (rev) AvR r (rev) ArR r (rev)	GTAAGAAGCGAACGCAGGGA ACTGACAATGACTGTTCTACGCGTAA TCCGATACCTCCAGGGCCCCTCAC AACTAACTCAATCGCGCTATTAAC AAAACAGCCACTACGACTGTCTT	67	184, 1066, 478, 1006	23S RNA gene	Puławska et al. 2006
<i>Agrobacterium tumefaciens</i>	VCF (fw) VCR (rev)	ATCATTGTAGCGACT AGCTCAAACCTGCTTC	55	730	<i>virC</i> gene	Sawada et al. 1995
<i>A. tumefaciens</i>	virD2A (fw) virD2C (rev) virD2E (rev)	ATGCCCGATCGAGCTCAAGT TCGTCTGGCTGACTTTCGTGCATAA CCTGACCCAAACATCTCGGCTGCCCA	50	224, 338	<i>virD2</i> gene	Haas et al. 1995
<i>Agrobacterium spp.</i>	VCF3 (fw) VCR3 (rev)	GGCGGGCGYGCYGAAAGRAARACYT AAGAACGYGGNATGTTGCATCTYAC	60	414	<i>virC1-virC2</i>	Kawaguchi et al. 2005
<i>Agrobacterium vitis</i>	PGF (fw) PGR (rev)	GGGGCAGGATGCGTTTTTGTGAG GACGGCACTGGGGCTAAGGAT	54-58	466	poligalacturo- nase gene	Szegedi & Bottka 2002
<i>A. vitis</i>	Ab3-F3 (fw) Ab3-R4 (rev)	ATGACGGTAGTCGGAGAAGAAGCC CTGTCTCTGTGTCCCGAAAAGG	62	570	16S rDNA gene	Kawaguchi et al. 2005
<i>A. tumefaciens</i> , <i>A. vitis</i>	iaaH-F10 (fw) iaaH-R10 (rev)	GGAAACATGCATGAGTTATCGTT CCACATCAGCATCAAGTTCATC	54	424	oct, nop pTi- <i>iaaH</i>	Bini et al. 2008
<i>A. vitis</i>	S4iaaM5 (fw), S4iaaM3 (rev)	CGCGTCCCCGTTTACTACTA CGAGATCGCGCTTCAAGAT	54	800	vitopin pTi <i>iaaM</i>	Bini et al. 2008
<i>Xylophilus ampelinus</i>	S3 (fw) S4 (rev)	GGTGTTAGGCCGAGTAGTGAG GGTCTTTCACCTGACCGCTTA	55	277	ITS	Botha et al. 2001
<i>X. ampelinus</i>	Xamp1.27A (fw) Xamp1.27B (REV)	GATCGCAAGAAATCCCGATG AAATCCCTTCGTTGTCTG	60	310	nd**	Manceau et al. 2000
<i>Xylella fastidiosa</i>	RST31 (fw), RST33 (rev)	GCGTTAATTTTCGAAGTGATTGATTGCG CACCATTTCGATCCCGGTG	55	733	Specific EcoRI fragment	OEEP/EPPO 2004

Species	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Target gene	Reference
<i>X. fastidiosa</i>	S19 (fw) S21 (fw) A19 (rew) A21 (rew)	CGGCAGCACATTGGTAGTA GCAAATTGGCACTCAGTATCG CTCCTCGCGGTTAAGCTAC CGATACTGAGTGCCAATTTGC	55	600-1350	<i>16S rDNA</i>	Rodrigues et al. 2003
<i>X. fastidiosa</i>	FXY _{gyr499} (fw) RXY _{gyr907} (rev)	CAGTTAGGGGTGTCAGCG CTCAATGTAATTACCCAAGGT	54	429	<i>gyrase b</i>	Rodrigues et al. 2003

*published annealing temperatures, **nd: no data

Table 5. Fungus specific primers

Species	Primer	Primer sequence (5'-3')	At/Tm (°C)*	Fragment length (nt)	Target gene	Reference
Universal	ITS1F(fw) ITS5 (fw), ITS4(rev)	CTTGGTCATTTAGAGGAAGTAA GGAAGTAAAAGTCGTAACAAAGG TCCTCCGCTTATTGATATGC	55, 50	584, 620	ITS (5.8S-28S rDNA)	Hamelin et al. 1996 Tegli et al. 2000
<i>Phaeomoniella chlamydospora</i>	MONO1475 (fw) MONO1962 (rev)	GATCAAACGCCTTGGTGGTCC ATTGCATCTTGCAAAGGGAC	52	489	SCAR	Rigdway et al. 2005
<i>Phaeoacremonium chlamydosporum</i> (<i>Pch</i>)	Pal1N(fw), Pal2 (rev)	AGGTCGGGGGCCAAC AGGTGTAACACTACTGCCG	50	400	ITS	Tegli et al. 2000
<i>Phaeoacremonium aleophilum</i> (<i>Pal</i>)	Pch1 (fw), Pch2 (rev)	CTCCAACCCTTTGTTTATC TGAAAGTTGATATGGACCC	50	360	ITS	Tegli et al. 2000
<i>Phaeoacremonium</i> spp.	Pm1 (fw) Pm2 (rev)	CTCCAACCCTTTGTGAACAT CGAGCCCGCCACTGACTT	52	415	ITS	Aroca & Raposo 2007
<i>Botryosphaeria</i> spp.	ITS1 NL4	TCCGTAGGTGAACCTGCGG GGTCCGTGTTTCAAGACGG	50	1200	ITS and rDNA regions	Alves et al. 2005
<i>Cylindrocarpon destructans</i>	Dest1(fw) Dest4 (rev)	TTGTTGCCTCGGCGGTGCCTG GGTTTAAACGGCGTGGCCGCGCTGTT	60	400	ITS1-ITS2	Hamelin et al. 1996 Nascimento et al. 2001
<i>Rosellinia necatrix</i>	R1(fw) R2(fw) R3(fw) R5(fw) R10(fw), and R7(rev) R8(rev) R11(rev)	ATAACTCCCAAAACCCATGTGA CAAAACCCATGTGAACATACCA CGAAGTGCCCTACCCTGTTA CACGAAACTCTGTTTAGCATTGA CCCCTGTTGCTTAGTGTGG AACCATAGGCGAGATGAGAAAT CCGAGGTCAACCTTTGGTATAG CACAACCATAGGCGAGATGA	60	112-500	ITS1-ITS2	Schena et al. 2002
<i>Roesleria subterranea</i>	Rs1R(fw) Rs2F(rev)	TCCGGAACGTCTATAGCGAGGAGA TCGCGGGCAACCGGCTCACGC	60	360	ITS1-ITS2	Neuhauser et al. 2009

*published annealing temperatures

Acknowledgements

This work was supported by the Hungarian Ministry of Agriculture/Rural Development and the KTIA_AIK_12 -1-2013-0001 project. N. Czotter participates in the Program for Reinforcement of Scientists of Hungarian Ministry of Agriculture. We apologize to those authors who were not cited due to space limit.

References

Abolmaaty A, Vu C, Oliver J & Levin, RE (2000): Development of a new lysis solution for releasing genomic DNA from bacterial cells for DNA amplification by polymerase chain reaction. *Microbios*. 101: 181-189.

Abou-Ghanem N, Saldarelli P, Minafra A, Buzkan N, Castellano MA & Martelli GP (1997): Properties of Grapevine virus D, a novel putative Trichovirus. *Journal of Plant Pathology*. 78:15–25.

Alkowni R, Rowhani A, Daubert S & Golino D (2004): Partial characterization of a new ampelovirus associated with grapevine leafroll disease. *Journal of Plant Pathology*. 86: 123-133.

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, 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.

- Alves A., Phillips AJL, Henriques I & Correia A (2005):** Evaluation of amplified ribosomal DNA restriction analysis as a method for the identification of *Botryosphaeria* species. *FEMS Microbiology Letters*. 245: 221-229.
- Angelini E (2010):** Field assessment and diagnostic methods for detection of grapevine phytoplasmas. [In: S. Delrot S et al., eds., *Methodologies and results in grapevine research.*] Springer Science+Business Media BV, 247-258.
- Angelini E, Clair D, Borgo M, Bertaccini A & Boudon-Padieu E (2001):** Flavescence dorée in France and Italy - Occurrence of closely related phytoplasma isolates and their near relationships to Palatinate grapevine yellows and an alder phytoplasma. *Vitis*. 40: 79-86.
- Aroca A & Raposo R (2007):** PCR-based strategy to detect and identify species of *Phaeoacremonium* causing grapevine diseases. *Applied and Environmental Microbiology*. 73: 2911-2918.
- Bertazzon N & Angelini E (2004):** Advances in the detection of Grapevine leafroll-associated virus 2 variants. *Journal of Plant Pathology*. 86: 283-290.
- Bertolini E, Olmos A, Martinez MC, Gorris MT & Cambra M (2001):** Single-step multiplex RT-PCR for simultaneous and colourimetric detection of six RNA viruses in olive trees. *Journal of Virological Methods*. 96: 33-41.
- 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.
- Bini F, Kuczmozg A, Putnoky P, Otten L, Bazzi C, Burr TJ & Szegedi E (2008):** Novel pathogen-specific primers for the detection of *Agrobacterium vitis* and *Agrobacterium tumefaciens*. *Vitis*. 47: 181-189.
- Bisztray GyD, Civerolo EL, Dula T, Kölber M, Lázár J, Mugnai L, Szegedi E & Savka MA (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.
- Botha WJ, Serfonstein S, Greyling MM & Berger DK (2001):** Detection of *Xylophilus ampelinus* in grapevine cuttings using a nested polymerase chain reaction. *Plant Pathology*. 50: 515-526.
- Brandt S & Himmler G (1995):** Detection of nepoviruses in ligneous grapevine material by using IC/PCR. *Vitis*. 34: 127-128.
- Cho IS, Jung SM, Cho JD, Choi GS & Lim HS (2013):** First report of grapevine 'Pinot gris' virus infecting grapevine in Korea. *New Disease Reports*. 27: 10.
- Clair D, Larrue J, Aubert G, Gillet J, Cloquemin G & Boudon-Padieu E (2003a):** Direct sensitive diagnosis of Flavescence dorée and Bois noir using a multiplex nested-PCR assay and its use in field surveys, 82-83. 14th ICVG Conference, Locorotondo, 12-17th September.
- Clair D, Larrue J, Aubert G, Gillet J, Cloquemin G & Boudon-Padieu E (2003b):** A multiplex nested-PCR assay for sensitive and simultaneous detection and direct identification of phytoplasma in the Elm yellows group and Stolbur group and its use in survey of grapevine yellows in France. *Vitis*. 42: 151-157.
- Coetzee B, Maree HJ, Stephan D, Freeborough MJ & Burger JT (2010):** The first complete nucleotide sequence of a grapevine virus E variant. *Archives of Virology*. 155: 1357-1360.
- Cseh E (2012):** Occurrence of grapevine viruses in Hungary and molecular studies of some Hungarian Grapevine leafroll-associated virus 1 and 3 isolates. Pannon University, Georgikon Faculty, Keszthely (Hungary), http://konyvtar.uni-pannon.hu/doktori/2013/Cseh_Eszter_dissertation.pdf (in Hungarian with English summary). PhD thesis.
- Constable FE, Nicholas P & Brendan CR (2010):** Development and validation of diagnostic protocols for the detection of endemic and exotic pathogens of grapevines. Final Report DPI 05/04, <http://www.gwrdc.com.au>.
- Contaldo N, Bertaccini A, Paltrinieri S, Windsor HM & Windsor GD (2012):** Axenic culture of plant pathogenic phytoplasmas. *Phytopathologica Mediterranea*. 51: 607-617.
- Daire X, Clair D, Reinert W & Boudon-Padieu E (1997):** Detection and differentiation of grapevine yellows phytoplasmas belonging to the elm yellows group and to the stolbur subgroup by PCR amplification of non-ribosomal DNA. *European Journal of Plant Pathology*. 103: 507-514.
- Demangeat G, Komar V, Cornuet P, Esmenjaud D & Fuchs M (2004):** Sensitive and reliable detection of grapevine fanleaf virus in a single *Xiphinema index* nematode vector. *Journal of Virological Methods*. 122: 79-86.
- Deng S & Hiruki C (1991):** Localisation of pathogenic mycoplasmalike organisms in plant tissue using in situ hybridization. *Proceedings of Japan Academy, Series B., Physical and Biological Sciences*. 67: 197-202.
- Digiario M, Elbeaino T & Martelli GP (2007):** Development of degenerate and species-specific primers for the differential and simultaneous RT-PCR detection of grapevine-infecting nepoviruses of subgroups A, B and C. *Journal of Virological Methods*. 141: 34-40.
- 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.
- Eiras M, Targon MLPN, Fajardo TVM, Flores R & Kitajima EW (2006):** *Citrus exocortis viroid* and *Hop Stunt viroid* doubly infecting grapevines in Brasil. *Phytopathologia Brasileira*. 31: 440-446.
- Elbeaino T, Digiario M, Fallanaj F, Kuzmanovic S & Martelli GP (2011):** Complete nucleotide sequence and genome organisation of grapevine Bulgarian latent virus. *Archives of Virology*. 156: 875-879.
- Engel EA, Escobar P, Montt C, Gomez-Talquencia S & Valenzuela PDT (2008):** First report on the occurrence of Grapevine leafroll-associated virus 7 and 9 in Chilean grapevines. *Plant Disease*. 92: 1252-1253.
- 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.
- 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.
- Farkas E, Palkovics L, Mikulás J & Balázs E (1999):** High incidence of *Hop Stunt viroid* in Hungarian grapevines. *Acta Phytopathologica et Entomologica Hungarica*. 34: 7-11.
- Franke KE, Liu Y & Adams DO (1995):** Yield and quality of RNA from grape berries at different developmental stages. *American Journal of Enology and Viticulture*. 46: 315-318.
- Gambino G & Gribaudo I (2006):** Simultaneous detection of nine grapevine viruses by multiplex reverse transcription-polymerase chain reaction with coamplification of a plant RNA as internal control. *Phytopathology*. 96: 1223-1229.
- Gambino G, Bondaz J & Gribaudo I (2006):** Detection and elimination of viruses in callus, somatic embryos and regenerated

plantlets of grapevine. *European Journal of Plant Pathology*. 114: 397-404.

Gambino G, Perrone I & Gribaudo I (2008): A rapid and effective method for RNA extraction from different tissues of grapevine and other woody plants. *Phytochemical Analysis*. 19: 520-525.

Giampetruzzi A, Roumi V, Roberto R, Malossini U, Yoshikawa N, La Notte P, Telizzi 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.

Whignone S & Migheli Q (2005): The database of PCR primers for phytopathogenic fungi. *European Journal of Plant Pathology*. 113: 107-109.

Glasa M, Predajna L, Kominek P, Nagyova A, Candresse T & Olmos A (2014): Molecular characterization of divergent grapevine Pinot gris virus isolates and their detection in Slovak and Czech grapevines. *Archives of Virology*.

Grund E, Darissa O & Adam G (2010): Application of FTA cards to sample microbial plant pathogens for PCR and RT-PCR. *Journal of Phytopathology*. 158: 750-757.

Guo R, Sano T, Cheng Z & Li SF (2007): Detection of Australian grapevine viroid in a grapevine more than 100 years old in Xinjiang, China. *Plant Pathology*. 56: 339-339.

Haas JH, Moore LW, Ream W & Manulis S (1995): Universal PCR primers for detection of phytopathogenic *Agrobacterium* strains. *Applied and Environmental Microbiology*. 61: 2879-2884.

Hajizadeh M, Navarro B, Bashir NS, Torchetti EM & Di Serio F (2012): Development and validation of a multiplex RT-PCR method for the simultaneous detection of five grapevine viroids. *Journal of Virological Methods*. 179: 62-69.

Hamelin RC, Bérubé P, Gignac M & Bourassa M (1996): Identification of root rot fungi in nursery seedlings by nested multiplex PCR. *Applied and Environmental Microbiology*. 62: 4026-4031.

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.

Jiang D, Guo R, Wu Z, Wang H & Li S (2009a): Molecular characterization of a member of a new species of grapevine viroid. *Archives of Virology*. 154: 1563-1566.

Jiang D, Peng S, Wu Z, Cheng Z & Li S (2009b): Genetic diversity and phylogenetic analysis of Australian Grapevine Viroid (AGVd) isolated from different grapevines in China. *Virus Genes*. 38: 178-183.

Jiang D, Zhang Z, Wu Z, Guo R, Wang H, Fan P & Li S (2009c): Molecular characterization of grapevine yellow speckle viroid-2 (GYSVd-2). *Virus Genes*. 38: 515-520.

Jiang D, Sano T, Tsuji M, Araki H, Sagawa K, Purushothama CRA, Zhang Z, Guo R, Xie L, Wu Z, Wang H & Li S (2012): Comprehensive diversity analysis of viroids infecting grapevine in China and Japan. *Virus Research*. 169: 237-245.

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.

Kawaguchi A, Sawada H, Inoue K & Nasu H (2005): Multiplex PCR for the identification of *Agrobacterium* biovar 3 strains. *Journal of General Plant Pathology*. 71: 54-59.

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

Lee I-M, Gundersen DE, Hammond RW & Davis RE (1994): Use of mycoplasma-like organism (MLO) group-specific oligonucleotide primers for nested-PCR assays to detect mixed-MLO infections in a single host plant. *Phytopathology*. 84: 559-566.

Lee I-M, Gundersen-Rindal DE, Davis RE & Bartoszyk IM (1998): Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. *International Journal of Systematic Bacteriology*. 48: 1153-1169.

Li R, Mock R, Fuchs M, Halbrendt J, Howell B & Liu Z (2011): Characterization of the partial RNA1 and RNA2 3'untranslated region of Tomato ringspot virus isolates from North America. *Canadian Journal of Plant Pathology*. 33: 94-99.

Li R, Mock R, Huang Q, Abad J, Hartung J & Kinard G (2008): A reliable and inexpensive method of nucleic acid extraction for the PCR-based detection of diverse plant pathogens. *Journal of Virological Methods*. 154: 48-55.

Lima MF, Alkowni R, Uyemoto JK, Golino D, Osman F & Rowhani A (2006): Molecular analysis of a California strain of *Rupestris* stem pitting-associated virus isolated from declining Syrah grapevines. *Archives of Virology*. 151: 1889-1894.

Lodhi MA, Ye G-N, Weeden NF & Reisch BI (1994): A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. *Plant Molecular Biology Reporter*. 12: 6-13.

Lorenz KH, Schneider B, Ahrens U & Seemüller E (1995): Detection of the apple proliferation and pear decline phytoplasmas by PCR amplification of ribosomal and nonribosomal DNA. *Phytopathology*. 85: 771-776.

MacKenzie DJ (1997): A standard protocol for the detection of viruses and viroids using a reverse transcription-polymerase chain reaction technique. Document CPHBT-RT-PCR 1.000 The Canadian Food Inspection Agency.

MacKenzie DJ, McLean MA, Murkerji S & Green M (1997): Improved RNA extraction from woody plants for the detection of viral pathogens by reverse transcription polymerase chain reaction. *Plant Disease*. 81: 222-226.

Manceau C, Coutaud M-G & Guyon R (2000): Assessment of subtractive hybridization to select species and subspecies specific DNA fragments for the identification of *Xylophilus ampelinus* by polymerase chain reaction (PCR). *European Journal of Plant Pathology*. 106: 243-253.

Martelli GP (2014): Directory of virus and virus-like diseases of the grapevine and their agents. *Journal of Plant Pathology*. (Rivista di Patologia Vegetale) Vol. 96, No 1 sup.

Martin MT, Cobos R, Martin L & López-Enriquez L (2012): Real-time PCR Detection of *Phaeoconiella chlamydospora* and *Phaeoacremonium aleophilum*. *Applied and Environmental Microbiology*. 78: 3985-3991.

Martinelli L, Candioli E, Costa D & Minafra A (2002): Stable insertion and expression of the movement protein gene of Grapevine Virus A (GVA) in grape (*Vitis rupestris* S.). *Vitis*. 41: 189-193.

Minafra A & Hadidi A (1994): Sensitive detection of grapevine virus A, B, or leafroll associated III from viruliferous mealybugs

- and infected tissue by cDNA amplification. *Journal of Virological Methods*. 47: 175-188.
- Nakaune R & Nakano M (2006):** Efficient methods for sample processing and cDNA synthesis by RT-PCR for the detection of grapevine viruses and viroids. *Journal of Virological Methods*. 134: 244-249.
- Nascimento T, Rego C & Oliveira H (2001):** Detection of *Cylindrocarpon* black-foot pathogens in grapevine by nested PCR. *Phytopathologia Mediterranea*. 40: S357-S361.
- Nassuth A, Pollari E, Helmecky K, Stewart S & Kőfalvi SA (2000):** Improved RNA extraction and one-tube RT-PCR assay for simultaneous detection of control plant RNA plus several viruses in plant extracts. *Journal of Virological Methods*. 90: 37-49.
- Neuhauser S, Huber L & Kirschnair M (2009):** A DNA based method to detect the grapevine root-rotting fungus *Roesleria subterranea* in soil and root samples. *Phytopathologia Mediterranea*. 48: 59-72.
- OEEP/EPPO (2004):** Diagnostic protocols for regulated pests: *Xylella fastidiosa*. *OEEP/EPPO Bulletin*. 34: 155-157.
- OEEP/EPPO (2007):** Grapevine flavescence dorée phytoplasma. *OEEP/EPPO Bulletin*. 37: 536-542.
- 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.
- Pacifico 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 time reverse transcription-PCR. *Journal of Virological Methods*. 171: 190-194.
- Pacifico 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*. 172: 1-7.
- Palacio-Bielsa A, Cambra MA & López MM (2009):** PCR detection and identification of plant-pathogenic bacteria: updated review of protocols (1989-2007). *Journal of Plant Pathology*. 91: 249-297.
- 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, Vry P, Foissac X & Malembic-Maher S (2009):** Triplex real-time PCR assay for sensitive and simultaneous detection of phytoplasmas of the 16SrV and 16SrXII-A groups with an endogenous analytical control. *Vitis*. 48: 87-95.
- Polivka H, Staub U & Gross HJ (1996):** Variation of viroid profiles in individual grapevine plants: novel grapevine yellow speckle viroid-1 mutants show alteration of hairpin I. *Journal of General Virology*. 77: 155-161.
- Puławska J, Willems A & Sobiczewski P (2006):** Rapid and specific identification of four *Agrobacterium* species and bivars using multiplex PCR. *Systematic and Applied Microbiology*. 29: 470-479.
- Ragozzino E, Faggioli F & Barba M (2004):** Development of a one-tube one-step RT-PCR protocol for the detection of seven viroids in four genera: Apscaviroid, Hostuviroid, Pelamoviroid and Pospiviroid. *Journal of Virological Methods*. 121: 25-29.
- Rezaian MA, Krake LR & Golino DA (1992):** Common identity of grapevine viroids from USA and Australia revealed by PCR analysis. *Intervirology*. 34: 38-43.
- Ridgway HJ, Steyaert JM, Pottinger BM, Carpenter M, Nicol D & Stewart A (2005):** Development of an isolate-specific marker for tracking *Phaeoemoniella chlamydospora* infection in grapevines. *Mycologia*. 97: 1093-1101.
- Rodrigues JLM, Silva-Stenico ME, Gomes JE, Lopes RS & Tsai SM (2003):** Detection and diversity assessment of *Xylella fastidiosa* in field-collected plant and insect samples by using 16S RNA and *gyrB* sequences. *Applied and Environmental Microbiology*. 69: 4249-4255.
- Routh, G., Zhang, Y. P., Saldarelli, P. & Rowhani, A. (1998):** Use of degenerate primers for partial sequencing and RT-PCR-based assays of grapevine leafroll-associated viruses 4 and 5. *Phytopathology*. 88: 1238-1243.
- Sanchez F, Chay C, Borja MJ, Rowhani A, Romero J, Bruening G & Ponz F (1991):** cDNA sequence of the capsid protein gene and 3' untranslated region of a fanleaf isolate of grapevine fanleaf virus. *Nucleic Acids Research*. 19: 5440.
- Sano T, Mimura R & Ohsima K (2001):** Phylogenetic analysis of hop and grapevine isolates of Hop Stunt Viroid supports a grapevine origin for hop stunt disease. *Virus Genes*. 22: 53-59.
- Sawada H, Ieki H & Matsuda I (1995):** PCR detection of Ti and Ri plasmids from phytopathogenic *Agrobacterium* strains. *Applied and Environmental Microbiology*. 61: 828-831.
- 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 (2004):** Real-time PCR detection and quantification of soilborne fungal pathogens: the case of *Rosellinia necatrix*, *Phytophthora nicotianae*, *P. citrophthora*, and *Verticillium dahliae*. *Phytopathologia Mediterranea*. 43: 273-280.
- Schneider B & Seemüller E (1996):** Sequence and RFLP analysis of the gene coding for the elongation factor TU of several phytoplasma strains for differentiation and classification of phytoplasmas. *IOM Letters*. 4: 281.
- Steenkamp J, Wiid I, Lourens A & van Helden P (1994):** Improved method for DNA extraction from *Vitis vinifera*. *American Journal of Enology and Viticulture*. 45: 102-106
- 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 (2003): Opines in naturally infected grapevine crown gall tumors. *Vitis*. 42: 39-41.

Szegedi E & Bottka S (2002): Detection of *Agrobacterium vitis* by polymerase chain reaction in grapevine bleeding sap after isolation on a semiselective medium. *Vitis*. 41: 37-42.

Tegli S, Bertelli E & Surico G (2000): Sequence analysis of ITS ribosomal DNA in five *Phaeoacremonium* species and development of a PCR-based assay for the detection of *P. chlamydosporum* and *P. aleophilum* in grapevine tissue. *Phytopathologia Mediterranea*. 39: 134-149.

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/microarray multiplex platform. *Phytopathology*. 104: 211-219.

Turturo C, Saldarelli P, Yafeng D, Digiario M, Minafra A, Savino V & Martelli GP (2005): Genetic variability and population structure of Grapevine leafroll-associated virus 3 isolates. *Journal of General Virology*. 86: 217-224.

Vasanthaiiah HKN, Katam R & Sheikh MB (2008): Efficient protocol for isolation of functional RNA from different grape tissue rich in polyphenols and polysaccharides for gene expression studies. *Electronic Journal of Biotechnology*. 11: 1-8.

Wan Chou Wah YF & Symons RH (1997): A high sensitivity RT-PCR assay for the diagnosis of grapevine viroids in field and tissue culture samples. *Journal of Virological Methods*. 63: 57-69.

Wu Q, Wang Y, Cao M, Pantaleo V, Burgyán 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 National Academy of Sciences of the United States of America*. 109: 3938-3943.

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

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