Plant Viruses ©2007 Global Science Books

Grapevine Leafroll-Associated Virus 1 as a Common Grapevine Pathogen

Nuredin Habili[1] • Petr Komínek[2*] • Alan Little[1]

[1] School of Agriculture, Food & Wine, Waite Campus, The University of Adelaide, PMB1, Glen Osmond, South Australia 5064
[2] Crop Research Institute, Drnovská 507, 161 06, Prague 6 – Ruzyně, Czech Republic

Corresponding author: * **kominek@vurv.cz**

ABSTRACT

Grapevine leafroll-associated virus 1 (GLRaV-1) is a member of the genus *Ampelovirus*, family *Closteroviridae*. GLRaV-1 is one of the at least ten viruses causing grapevine leafroll disease worldwide, characterized by a typical downrolling of the leaf and its premature discoloration. It can cause delayed fruit maturity and reduced sugar content of the berries. GLRaV-1 naturally infects plants of the *Vitis* genus only. It is transmitted by infected propagation material and by the scale insects *Neopulvinaria innumerabilis* and *Parthenolecanium corni* and by the mealybugs *Heliococcus bohemicus* and *Phenacoccus aceris*. Virions are filamentous in shape, containing a coat protein of 35 kDa size. Within the virion the virus has a positive sense, single stranded RNA molecule of 17,647 nt in length and lacking a poly (A) tail. The genome contains 10 major ORFs encoding a putative RNA helicase, an RNA-dependent RNA polymerase and a homologue of the HSP70 family of heat shock proteins, which is a unique feature among plant viruses, being present only in closteroviruses. Further ORFs encode the viral coat protein and two diverged copies of the coat protein. Serology and molecular techniques such as RT-PCR and molecular hybridization are useful for the specific and sensitive detection of GLRaV-1.

Keywords: ampelovirus, closterovirus, detection, taxonomy, vector
Abbreviations: BYV, Beet yellows virus; **ELISA**, enzyme-linked immunosorbent assay; **GLRaV-1**, Grapevine leafroll-associated virus 1; **GLRaV-3**, Grapevine leafroll-associated virus 3; **GVA**, Grapevine virus A; **nt**, nucleotide; **ORF**, open reading frame

CONTENTS

BACKGROUND

Leafroll is a major disease of grapevine, which can cause yield loss, reduced sugar in berries and diminished wine quality. *Grapevine leafroll-associated virus 1* (GLRaV-1) is one of the at least 10 viruses of the family *Closteroviridae* known to be associated with leafroll disease in grapevines. The long filamentous virions of GLRaV-1 were first characterized over two decades ago (Gugerli *et al*. 1984). Leafroll viruses of the grapevine are assigned into at least two genera of the family *Closteroviridae*. One genus, *Ampelovirus*, contains most of the leafroll virus types, while *Grapevine leafroll-associated virus 2* is the only leafroll virus belonging to the *Closterovirus* genus (Martelli *et al*. 2002). GLRaV-1 can only infect *Vitis vinifera* as well as a number of *Vitis* spp., in which it is usually symptomless.

Before we describe the GLRaV-1 in detail, it is worth presenting a background on its debilitating effect on the most commonly grown grape variety in Australia. Sultana (synonymous: Thompson Seedless, Sultana) is an economically important grapevine variety in the dried fruit industry in Australia. Since its early introduction, a number of Sultana clones had shown severe leafroll symptoms with low yields. Woodham *et al*. (1984) found that in those Sultana clones with severe virus symptoms, the mean yield was reduced by 35% over 6 seasons. They attributed the low yield in Sultana to a graft-transmissible leafroll virus. At that time the various types of leafroll viruses were not yet described. Now we know that the main culprit in the reduction of yield in Australian Sultana was GLRaV-1 (Habili *et al*. 1997). The virus genome in a Sultana clone (E1) has been totally sequenced for the first time (Little 2004).

SYMPTOMS

Most of the leafroll symptoms appear late in summer or early in autumn, when the nights get cooler. However, in Thailand, where the vines do not senesce leaves (no dormancy), the virus symptoms are more pronounced just after the harvest which is around February (Habili, pers. obs., 2007). These include reddening of leaf blades in red varieties while the main veins remain green (**Fig. 1**). This is accompanied

Received: 27 February, 2007. Accepted: 9 April, 2007.

Invited Mini-Review

Fig. 1 Symptoms of GLRaV-1 on young self-rooted plant of grapevine cv. 'Blauer Portugieser' (Czech Republic) (**A**) and on grapevine cv. 'Merlot' (Australia) (**B**).

by downward rolling and thickening of leaves. The colour of leaves in white varieties turns yellow, which could easily be confused with the natural yellowing in autumn. Most white varieties do not show any clear symptoms, and hence could act as a major source for virus spread via propagating material or by insect vectors. Although both GLRaV-1 and GLRaV-3 induce typical leafroll symptoms, type 1 is a milder virus especially in the sensitive variety Pinot Noir (Habili, unpublished observations). Reduced wine quality in leafroll-affected vines may result from delayed fruit maturity, the reduction in sugar by 25-50% and poorly coloured fruit.

VIRUS VECTORS

Two published reports, both from Europe, are available on the natural transmission of GLRaV-1 either by scale insects or mealybugs (**Table 1**). In 1997, Fortusini *et al*. (1997) from Italy reported that the scale insect *Neopulvinaria innumerabilis* could transmit GLRaV-1 only when the inoculum source was doubly infected with *Grapevine virus A* (GVA), but failed to do so when the source was apparently infected with GLRaV-1 alone. On the other hand, the scale insect *Parthenolecanium corni* transmitted the virus when GVA was not present in the donor host. Sforza *et al*. (2003) reported that two mealybug species, *Heliococcus bohemicus* and *Phenacoccus aceris* and one soft scale insect species, *P. corni* were able to transmit the virus under experimental conditions in mixed infections with GLRaV-3. It is worth noting that not all mealybugs can transmit GLRaV-1. For example, in New Zealand two potentially active mealybug vectors of GLRaV-3, *Pseudococcus longispinus* and *P. calceolariae*, failed to transmit GLRaV-1 to recipient grapevines (Petersen and Charles 1997).

Table 1 Scale insects and mealybug species known to transmit GLRaV-1.

Insect vector	Family	Reference
Scale insect: *Neopulvinaria innumerabilis*	Coccidae	Fortusini *et al*. 1997
Scale insect: *Parthenolecanium corni*	Coccidae	Fortusini *et al*. 1997
Scale insect: *Parthenolecanium corni*	Coccidae	Sforza *et al*. 2003
Mealybug: *Heliococcus bohemicus*	Pseudococcidae	Sforza *et al*. 2003
Mealybug: *Phenacoccus aceris*	Pseudococcidae	Sforza *et al*. 2003

DISTRIBUTION OF GLRaV-1

GLRaV-1 occurs worldwide and its origin could have been paralleled with that of *Vitis vinifera*. We have detected GLRaV-1 in samples of own-rooted local table grapes grown in phylloxera-free regions of Iran (Habili *et al*. 2003). It is interesting to note that in most vines GVA, a *Vitivirus*, was also present. Therefore, the observation by Fortusini *et al*. (1997) that the scale insect *Neopulvinaria innumerabilis* could transmit GLRaV-1 only in the presence of GVA is interesting. In Australia, the incidence of GLRaV-1 was 3.7% in 2479 vine samples tested (Habili and Symons 2000). **Table 2** lists the incidence of GLRaV-1 and GLRaV-3 in a number of countries. In most of these countries, the incidence of GLRaV-3 was higher, indicating that this virus may have a more efficient vector.

In the Czech Republic, during several surveys done by ELISA, the GLRaV-1 incidence reached 15.4%. GLRaV-1 occurred either by itself or as a mixed infection with viruses like GVA, GLRaV-3 or GFkV (Komínek 2006). Using RT-PCR analysis the incidence of GLRaV-1 reached more than 90%, in a survey done on a small scale, which showed a higher distribution of GLRaV-1 than previously revealed by a large-scale ELISA. However, a similar result was obtained in randomly collected samples from Turkey: RT-PCR detected GLRaV-1 in 80% of the samples (Bryxiová *et al*. 2006), while only 8% of these tested positive using ELISA (Akbas *et al*. 2007). These results confirm the higher sensitivity of RT-PCR versus ELISA in the detection of GLRaV-1.

ANALYSIS OF THE GLRaV-1 GENOME

The first GLRaV-1 isolate, which has been fully sequenced, was from a low-yielding Sultana clone (E1) grown in Australia. We first detected GLRaV-1 in this grapevine clone in 1996 (Habili *et al*. 1996). Our initial approach to the molecular characterization of this leafroll virus isolate was via dsRNA analysis following cloning and sequencing a fragment of the GLRaV-1 genome (Habili *et al*. 1997). This work led to a complete sequence of the viral genome (Fazeli and Rezaian 2000; Little 2004).

The genome of viruses from the family *Closteroviridae* is divided into two blocks. The first block contains the leader proteinase (PRO), methyltransferase (MTR), helicase (HEL), and RNA polymerase (POL). These 4 domains form the viral RNA replicase gene block. The second block consists of five genes, which is also called quintuple gene block (QGB). The QGB which is unique to the family *Closteroviridae* includes genes coding for a small hydrophobic protein, a homolog of 70-kDa heat shock proteins (HSP-70h), a ~60-kDa protein (p60), a major capsid protein and two duplicated capsid proteins (CP, CPd1 and CPd2, respectively). Functions of these proteins were identified using two representatives of the genus *Closterovirus*. The QGB proteins maybe engaged in cell-to-cell movement (Peremyslov *et al*. 1999; Alzhanova *et al*. 2000). In addition, CPd, HSP-70h, and p60 assemble short virion tails, whereas CP forms a long filamentous virion body (Agranovsky *et al*. 1995; Peremyslov *et al*. 2004; Satyanarayana *et al*. 2004).

The genome of the GLRaV-1 consists of 17,647 nucleotides (Fazeli and Rezaian 2000; Little 2004) with 10 major ORFs and small non-coding regions at the 5′ and 3′-terminus with no poly(A) tract. The scheme of the genome is presented in **Fig. 2**, functional domains and their positions in the genome are listed in **Table 3**. The first open reading frame (ORF 1a) encodes for a putative papain-like protease (P-PRO), methyltransferase (MTR) and RNA helicase (HEL) proteins, which are located at the C-terminal of a larger protein. The downstream ORF (1b) overlaps with ORF 1a and lacks an initiation codon. This ORF probably expressed via a +1 frameshift and encodes for the RNA polymerase (POL) gene with a size of 59.2 kDa. ORF 2 encodes for a small hydrophobic protein of 6.7 kDa with an unknown function. ORF 3 encodes a homologue of the HSP70 family of heat shock proteins of 59.5 kDa in size. ORF 4 codes a 54.6 kDa protein and ORF 5 is the coat protein with a Mr of 35 kDa. ORFs 6 and 7 code for two minor proteins which are the diverged forms of the coat protein. These are called coat protein duplicate (CPd1 and CPd2, respectively). This is the only *Ampelovirus* found so far to carry two CPd proteins. Members of the genus *Ampelovirus* has the major coat protein located upstream from the duplicate CP, while in members of the genus *Closterovirus*, the major CP is located downstream from CPd.

Gene expression strategies in these viruses involve proteolytic processing and translational frame shift, both at the 5′-terminal. However, the genes at the 3′-end are expressed

Table 2 Incidence (% infection) of GLRaV-1 and GLRaV-3 in different countries.

Country	Total no. tested	GLRaV-1 (%)	GLRaV-3 (%)	Reference
Australia	2479	3.7	4.2	Habili and Symons 2000
Austria	?	40[1]	not tested	Leonhardt *et al*. 1998
Brazil	650	6.9	14.7	Fajardo *et al*. 2002
Canada	11,417	1.7	10.8	MacKenzie *et al*. 1996
Croatia	300	72.3	24.3	Poljuha *et al*. 2004
Czech rep.	488	15.4	8.2	Komínek 2006
France	?	8[2]	15	Greif *et al*. 1997
Greece	494	41	46	Avgelis *et al*. 1997
South Africa	407 [3]	1	49.8	Pietersen 2006 (pers. comm. with N. Habili)
Thailand	12	83.3	33.3	N. Habili (unpublished, 2007)
Tunisia	510	8	58	Mahfoudhi, Chabbouh, Habili, 2007, unpublished results
Turkey	10	80	not tested	Bryxiová *et al*. 2006
USA	221	4	26	Rowhani *et al*. 1997

[1] Data from cv. 'Grüner Veltliner' grown in Niederösterreich, Austria.
[2] Average for 8 regions.
[3] Collected from 70 vineyards in the Western Cape, South Africa.

Table 3 Scheme of the GLRaV-1 genome.

ORF/region	Functional domain/protein	Position (nt)*
5′ non-coding	32 nt	1-32
1a and 1b	P-Pro, MTR, HEL, POL	33-7983
2	p7 hydrophobilc protein	8778-8957
3	p60, HSP70-like	8963-10594
4	p55, HSP90-like	10591-12039
5	p35, coat protein	12143-13111
6	p55, CP duplicate 1 (CPd1)	13134-14636
7	p50, CP duplicate 2 (CPd2)	14640-15962
8	p22	16042-16611
9	p24	16655-17287
3′ non-coding	360 nt	17288-17647

* Based on the partial known sequence of 12,394 nt in the GenBank (Accession number AF195822) and completed sequence (Little 2004). See also the relevant CDS in Genbank.

Table 4 Primers and cycling conditions used by different authors for the RT-PCR detection of GLRaV-1.

Forward primer	Reverse primer	Target gene, position in AF195822	Expected product size (bp)	PCR cycling condition	Reference
5′ AAT CCT ATG CGT CAG TAT GC 3′	5′ TGG CAT CGT TGC TAA ATT GAG 3′	CP 7589-7763	175	35× (94°C/30 s, 56°C 30 s, 72°C 60 s)	Habili *et al*. 2003
5′ TCT TTA CCA ACC CCG AGA TGA A 3′	5′ GTG TCT GGT GAC GTG CTA AAC G 3′	CP 7245-7476	232	35× (94°C/30 s, 50°C 45 s, 72°C 60 s)	Gambino and Gribaudo 2006
5′ GAG CGA CTT GCG ACT TAT CGA 3′	5′ GGT AAA CGG GTG TTC TTC AAT TCT 3′	HSP 70 4822-5142	320	35× (94°C/30 s, 58°C 45 s, 72°C 60 s)	Osman *et al*. 2007
5′ GCA ACT GCA ATT TCC ACA GA 3′	5′ CTT TCT CGT TCG GCT TCA AC 3′	CP 7346-7673	327	35× (94°C/45 s, 58°C 45 s, 72°C 60 s)	Gambino *et al*. 2006
5′ CAG GCG TCG TTT GTA CTG TG 3′	5′ TCG GAC AGC GTT TAA GTT CC 3′	HSP 70 4125-4664	540	40× (94°C/60 s, 50°C 60 s, 72°C 60 s)	Komínek *et al*. 2005
5′ GGC TCG AGA TGG CGT CAC TTA TAC CTA 3′	5′ CCT CTA GAC ACC AAA TTG CTA GCG A 3′	ORF9 (p24) 11401-12033	633	40× (94°C/15 s, 55°C 30 s, 68°C 60 s)	Little and Rezaian 2006

via subgenomic RNA transcripts. Three subgenomic RNAs were identified in GLRaV-1 (**Fig. 2**) (Fazeli and Rezaian 2000). The largest of which carries the CP gene and is around 6 kp. Two smaller species of 1.7 and 1.1 kb were also detected. All these three and possibly more subgenomic RNAs are 3′ co-terminal.

Limited functional analysis on ORFs 2, 3, 6 and 9 assigned their expressed proteins to GLRaV-1 movement and maintenance in the cells (Little 2004). Little (2004) suggested that the ORF 2 encodes a vesicle inducing protein that is targeted to the endoplasmic reticulum. Transient expression of the ORF 2 protein lead to the formation of multivesicular bodies similar in appearance to the previously reported BYV-type vesicles, named after the *Closterovirus* type member *Beet Yellows Virus* (Agranovsky 1995). Transient expression of ORF 3 and 6 resulted in their targeting to the cell periphery. This is consistent with the homologous ORF 3 of BYV, which is localised to the plasmodesmata (Medina *et al*. 1999) and functions as one of the viral movement proteins (Peremyslov *et al*. 1999).

ORF 3 encodes a 70-kDa heat shock protein homolog. The *Closteroviridae* is the only virus family that encodes such a protein with similarity to cellular chaperones (Agranovsky *et al*. 1991). Proteins of HSP family are normally found in all types of cellular organisms, where they protect the cell against heat shocks and assist in protein folding, assembly and disassembly of multi-subunit complexes and translocation of proteins between cell compartments (Bukau and Horwich 1998). These proteins share the same type of structural organization with a conserved N-terminal ATPase domain (Flaherty *et al*. 1990) and a more variable C-terminal domain (Zhu *et al*. 1996). Furthermore, the HSP-70s share the same basic mechanism involving repeated cycles of binding and releasing from target proteins as regulated by ATP hydrolysis (Bukau and Horwich 1998). HSP70 homologs encoded by closteroviruses appear to retain all functional domains of cellular HSP proteins (Agranovsky *et al*. 1997).

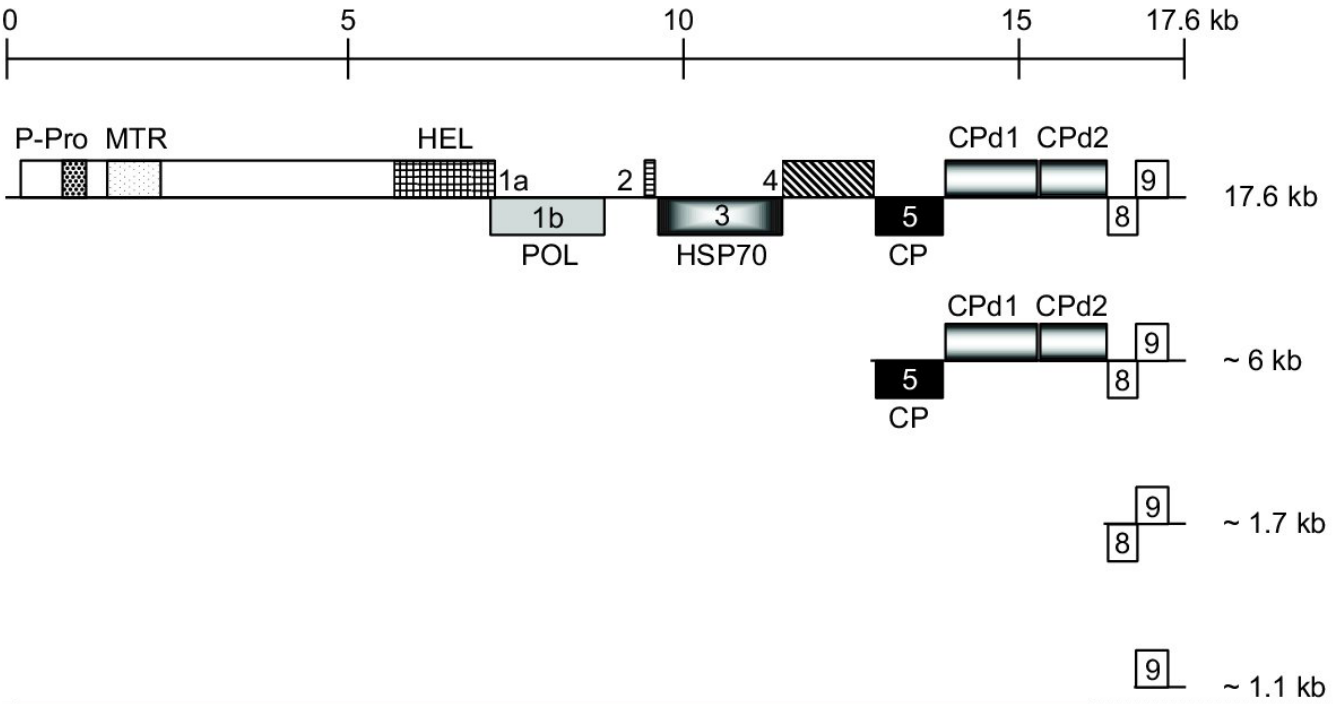

Fig. 2 Scheme of GLRaV-1 genome and subgenomic RNAs.

```
     1  Phosphate 1
LR1  MEVGLDFGTTFSTSCFSIPTQDDSGCVSLVNSPFVPTQIFI-GSDMTYSIGHRAYSDFVAGKPGGLYINPKRWVGVDSFNFHAVKRRLNPEYEVKI
     ...G.D.GTT.S-------------.V..V.S....T....-G.....S..H..Y.................VG.D.....A.....N....VK.
EC   LAAGIDLGTTNS-------------LVATVRSGQAETLADHEGRHLLPSVVH--YQ------------QQGHSVGYDART-NAALDTANTISSVKR
     20

     96
LR1  NNGEI--------------------SIGSVGNTNAPLMRVVDLVFLFVKGILLET-EEAVGKAVTGVVCTVPAEYNSFKRSFLGVA--LEGLGKPLR
     ..G..--------------------.........A.L...V.......K..L...-.EA......GVV.TVPA......R.....A--L.GL-..LR
EC   LMGRSLADIQQRYPHLPYQFQASENGLPMIETAAGLLNPVRVSADILKA-LAARATEALAGELDGVVITVPAYFDDAQRQGTKDAARLAGL-HVLR
     88

     170 Connect 1               Phosphate 2
LR1  ALINEPTSAAL-YGAVKGGSLRETYAVFDFGGGTLDISFISRFNNVVSVLFSKGDNFLGGRDIDRAIVNFLRKEKRIKGNIDAGILSVMIADLKEK
     -L.NEPT.AA.-YG...G.--....AV.D.GGGT.DIS.......V..VL...GD..LGG.D.D.......R....I.---D....-.....L...
EC   -LLNEPTAAAIAYGLDSGQ--EGVIAVYDLGGGTFDISILRLSRGVFEVLATGGDSALGGDDFDHLLADYIREQAGIP---DRSDN-RVQRELLDA
     182

     265                                                                    Adenosine
LR1  ICTNGGTQQTQVKTSNGLETLSMSVDELNAVSEPFIDRAVKIFAEGAEDL-KRCPIVC-----------------VLTGGSVALPLVRPKLENL--
     -.........------.......V..--...E--I.R..--F.E....L-KR....C-----------------V..GGS...PLVR.......--
EC   -AIAAKIALSD------ADSVTVNVAG--WQGE--ISREQ--FNELIAPLVKRTLLACRRALKDAGVEADEVLEVVMVGGSTRVPLVRERVGEFFG
     271

     341               Connect 2
LR1  -PYVSSVAYDSQTFRLSVAIGAKIYGDILTGQSDLR---LIDTVSQTLSDELSG-FTELVIFPKGHPVPSVYETSFQVSG---STMEYGIFEGESN
     -P...S...D.------.VAIGA.I..DIL.G.........---L.D.....L..E..G-..E.VI-P.....P......F.........M......GE..
EC   RPPLTSIDPDK-----VVAIGAAIQADILVGNKPDSEMLLLDVIPLSLGLETMGGLVEKVI-PRNTTIPVARAQDFTTFKDGQTAMSIHVMQGERE
     354

     429
LR1  RTWMNEI--AFKGTDYRPSNERKND--KVKYEISVDGKLKLS
     ........--.F..............--.V......DG.L...
EC   LVQDCRSLARFALRGIPAL-PAGGAHIRVTFQVDADGLLSVT
     444
```

Fig. 3 A pairwise comparison of aminoacid sequence of HSP70 protein of GLRaV-1 (GenBank accession number AAF22740) and hscA chaperone protein from HSP70 family of *Escherichia coli* (Genbank accession number P36541) using BLAST (Alstschul *et al.* 1997) and CDD (Marchler-Bauer *et al.* 2005) programs. LR1, GLRaV-1; EC, *Escherichia coli*; - gap (missing amino acid); identical amino acids are marked in a middle row; five conserved motifs for ATPase activity according to Bork *et al*. (1992) are marked above the sequences; ultra-conserved residues are marked in red. Identities 127/522 (24.3%).

Here, we show a 24.3% amino acid identity between the N-terminal region of the GLRaV-1 HSP70 and that of *Escherichia coli* (**Fig. 3**). Most of these identities fell into five conserved ATPase domains described by Bork *et al*. (1992).

The HSP70 gene was used as a basic target for initial molecular characterisation of a number of viruses from the family *Closteroviridae* and of their genetic diversity (Karasev *et al*. 1994; Tian *et al*. 1996; Alkowni *et al*. 2004). In a number of closteroviruses, the HSP70 gene is the only se-

quenced part of the viral genome.

Sequence variability in the HSP-70 gene of GLRaV-1 was observed by Komínek *et al*. (2005). This enabled them to describe two groups of GLRaV-1 isolates, tentatively named A and E, often occurring as a mixed infection in a single grapevine plant.

Little *et al*. (2001) reported that sequence diversity exists in the GLRaV-1 genome and this is mainly present in ORFs 3, 6 and 7. ORF7, which is the CPd2, shows the highest sequence variability, with a mutation occurring in 60% of the nucleotide positions. These variations do not change either the size of the ORFs or the behaviour of their relevant translation products. However, this finding has an important practical message, which should be taken into account when designing primers for the RT-PCR detection of GLRaV-1. Little (2004) has found that by designing PCR primers from sequences on ORF 9, the ambiguities associated with sequence variability in GLRaV-1 PCR could be avoided (see also **Table 4**).

DETECTION

GLRaV-1 has not yet been transmitted to any herbaceous indicator plant. The only possible use of the biologically based detection method is via grafting to *Vitis* woody indicators - for example to *Vitis vinifera* cv. 'Cabernet Franc' (Pathirana and McKenzie 2005). However, woody indicators are not virus specific, and they can detect leafroll as a disease, but not any specific virus. Serology is commonly used for specific detection of the virus using both polyclonal and monoclonal antibodies (Seddas *et al*. 2000). Molecular techniques such as RT-PCR and molecular hybridization using RNA probes are useful for the detection of GLRaV-1 as they seem to be more sensitive and highly specific.

RT-PCR with degenerated primers showing generic specificity is widely used (Tian *et al*. 1996). However their use for direct detection of leafroll viruses from total RNA extracts has limitations in PCR. The condition of a low stringency (eg a lower annealing temperature) can result in the amplification of non-specific fragments other than that of the targeted virus (ribosomal RNA or the universal *Rupestris stem pitting-associated virus*) as reported by Komínek *et al*. (2004). This problem may be overcome by the use of degenerated primers containing inosine used in a set of nested PCR reactions (Dovas and Katis 2003).

Another approach to molecular detection of grapevine viruses including GLRaV-1 is designing PCR primers for the simultaneous detection of more than one virus in a single reaction. This is called multiplex RT-PCR, which can detect up to nine viruses saving both time and money (Gambino and Gribaudo 2006). However, at our hands the sensitivity of detection of each individual virus in the multiplex system was greatly reduced as compared to a "monoplex" PCR system.

The success of GLRaV-1 detection using molecular techniques is limited by two factors, sequence variation in the viral RNA and a low template concentration. One way to overcome this problem, as shown by Little and Rezaian (2006), is the use of magnetic capture hybridisation to allow the removal of RT-PCR inhibitors and the addition of 100-fold excess template RNA to a single reaction.

Alternatively, for virus detection molecular hybridization with RNA probes, which may overcome minor sequence variability in a target sequence, could be used (Komínek and Bryxiová 2005).

GLRaV-1 was detected by single tube RT-PCR in the presence of Sybr Green G using a real-time approach coupled with melting temperature analysis of the generated amplicons. A pair of coat protein specific primers was used which enabled the routine detection of the virus and its variants from the same plant (Habili *et al*. 2003).

Real-time approach was similarly reported as a highly sensitive method enabling the detection of more virus variants than conventional RT-PCR (Osman *et al*. 2007).

An overerview of the primers and PCR conditions used by different authors for the GLRaV-1 detection is given in **Table 4**.

SANITATION

Successfull elimination of GLRaV-1, as well as other grapevine viruses can be achieved by thermotherapy of shoot tips, a practice routinely applied for grapevines (R. Davies, pers. comm.; Leonhardt *et al*. 1998; Valero *et al*. 2003) or by a somatic embryogenesis using anthers and ovaries as a starting material (Gambino *et al*. 2006). Somatic embryogenesis can be used for the elimination of phloem-inhabiting viruses, while it seems not to be effective in the case of *Grapevine fanleaf virus* (Goussard and Wiid 1992).

Mannini and Credi (2003) evaluated the effect of heat-treatment on the elimination of GLRaV-1 in a mixed infection with GVA from grapevines cv. 'Nebbiolo' and 'Grignolino'. The virus elimination resulted in higher vine vigour and an increased vegetative growth. The yield increased by 30% due to a larger bunch size and an increase in shoot growth. However, juice composition (soluble solids and titrable acidity) was not affected as a result of virus elimination.

CONCLUSIONS

GLRaV-1 is the first *Ampelovirus* detected in grapevines. The virus is second after GLRaV-3 both in its severity and its rate of infection in *Vitis vinifera*. The viral genome has been fully sequenced and its 10 open reading frames have been described. However, the function of only a few of these ORFs has been studied. The HSP70 gene has been sequenced in most isolates from around the world and its application in understanding of the viral genome diversity and the virus distribution has been described. RT-PCR remains one of the most sensitive and reliable methods for the GLRaV-1 identification provided an efficient method of RNA isolation and an inhibitor-free procedure is followed. RT-PCR remains the method of choice for virus detection following a virus elimination program using meristem tip culture coupled with high temperature incubation of the explants (pers. comm. of N. Habili with Rodney Davies of the University of Adelaide, South Australia).

Finally, it is recommended that a vigorous virus testing protocol should be adopted, especially by the European grapevine nurseries, before exporting planting material to newly established vineyards in countries like Thailand (see also **Table 3**), where the environmental conditions may augment the viral debilitating effects.

ACKNOWLEDGEMENTS

The experiments on GLRaV-1 in the Czech Republic and publishing of the paper are supported by the project MZE0002700603 of the Czech Ministry of Agriculture.

REFERENCES

Agranovsky AA (1995) Structure and expression of RNA genomes of closteroviruses. *Molecular Biology* **29**, 751-764

Agranovsky AA, Boyko VP, Karasev AV, Koonin EV, Dolja VV (1991) Putative 65 kDa protein of Beet yellows closterovirus is a homolog of HSP70 heat-shock proteins. *Journal of Molecular Biology* **217**, 603-610

Agranovsky AA, Lesemann DE, Maiss E, Hull R, Atabekov JG (1995) "Rattlesnake" structure of a filamentous plant RNA virus built of two capsid proteins. *Proceedings of the National Academy of Sciences USA* **92**, 2470-2473

Akbas B, Kunter B, Ilhan D (2007) Occurrence and distribution of grapevine leafroll-associated viruses 1, 2, 3 and 7 in Turkey. *Journal of Phytopathology* **155**, 122-124

Agranovsky AA, Folimonova SY, Folimonov AS, Denisenko ON, Zinovkin RA (1997) The beet yellows closterovirus p65 homologue of HSP70 chaperones has ATPase activity associated with its conserved N-terminal domain but does not interact with unfolded protein chains. *Journal of General Virology* **78**, 535-542

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

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**, 3389-3402

Alzhanova DV, Hagiwara Y, Peremyslov VV, Dolja VV (2000) Genetic analysis of the cell-to-cell movement of beet yellows closterovirus. *Virology* **267**, 192-200

Avgelis A, Rumbos I, Katis N, Rumbou A, Nikolaou N, Dimou D (1997) Association of closteroviruses GLRaV 1 and GLRaV 3 with leafroll symptoms in Greek vineyards. *Extended Abstracts 12th Meeting ICVG*, *Lisbon*, *Portugal*, *29 Sept.-2 Oct. 1997*, pp 117-118

Bork P, Sander C, Valencia A (1992) An ATPase domain common to prokaryotic cell cycle proteins, sugar kinases, actin, and hsp70 heat shock proteins. *Proceedings of the National Academy of Sciences USA* **89**, 7290-7294

Bryxiová M, Komínek P, Glasa M, Ercisli S, Hizarci Y (2006) Distribution of grapevine viruses in the Czech Republic. *15th Meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG)*, *Extended Abstracts*, *3-7 April 2006*, *Stellenbosch*, *South Africa*, pp 176-177

Bukau B, Horwich AL (1998) The Hsp70 and Hsp60 chaperone machines. *Cell* **92**, 351-366

Dovas CI, Katis NI (2003) A spot multiplex nested RT-PCR for the simultaneous and generic detection of viruses involved in the aetiology of grapevine leafroll and rugose wood of grapevine. *Journal of Virological Methods* **109**, 217-226

Fajardo TVM, Kuhn GB, Eiras M, Nickel O (2002) Detection of Closteroviruses in grapes and partial characterization of an isolate of Grapevine leafroll-associated virus 3. *Fitopatologia Brasileira* **27**, 58-64

Fazeli FC, Rezaian MA (2000) Nucleotide sequence and organization of ten open reading frames of the grapevine leafroll-associated virus 1 genome and identification of three subgenomic RNAs. *Journal of General Virology* **81**, 605-615

Flaherty KM, DeLuca-Flaherty C, McKay DB (1990) Three dimensional structure of the ATPase fragment of a 70K heat shock cognate protein. *Nature* **346**, 623-628

Fortusini A, Scattini G, Prati S, Cinquanta S, Belli G (1997) Transmission of grapevine leafroll virus 1 (GLRV-1) and grapevine virus A (GVA) by scale insects. *Extended Aabstracts 12th Meeting ICVG*, *Lisbon*, *Portugal*, *29 Sept.-2 Oct. 1997*, pp 121-122

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, Gribaudo I (2006) Simultaneous detection of nine grapevine viruses by multiplex reverse transcription-polymerase chain reaction with co-amplification of a plant RNA as internal control. *Phytopathology* **96**, 1223-1229

Goussard PG, Wiid J (1992) The elimination of fanleaf virus from grapevines using *in vitro* somatic embryogenesis combined with heat therapy. *South African Journal of Enology and Viticulture* **13**, 81-83

Greif C, Cloquemin G, Blaszczyk G, Gillet J, Perrot-Minnot MJ, Grenan S, Walter B (1997) Epidemiological survey of the grapevine leafroll disease in French wine growing regions. *Extended Abstracts 12th Meeting ICVG*, *Lisbon*, *Portugal*, *29 Sept.-2 Oct. 1997*, pp 119-120

Gugerli P, Brugger JJ, Bovey R (1984) L'enroulement de la vigne: mise en évidence de particules virales et développement d'une méthode immuno-enzymatique pour le diagnostic rapide. *Revue Suisse de Viticulture Arboriculture et Horticulture* **16**, 299-304

Habili N, Ewart AJW, Fazeli CF, Scott NS, Krake LR, Rezaian MA (1996) Virus types associated with grapevine leafroll disease in Australia. *Australian Grapegrower and Winemaker* **390a**, 25-28

Habili N, Fazeli CF, Rezaian MA (1997) Identification of a cDNA clone specific to grapevine leafroll-associated virus 1 and occurrence of the virus in Australia. *Plant Pathology* **46**, 516-522

Habili N, Symons RH (2000) Grapevine viruses detected by Waite Diagnostics in Australia. *13th Meeting of the International Council for the Study of Viruses and Virus-like Diseases of the Grapevine (ICVG). March 12-17 2000*, *Adelaide*, *Australia*, pp 124-126

Habili N, Afsharifar A, Symons RH (2003) First detection of an *Ampelovirus*, a *Maculavirus* and two vitiviruses in Iranian table grapes. *14th ICVG Meeting*, *Locorotondo (Bari)*, *Italy*, pp 162-163

Karasev AV, Nikolaeva OV, Koonin EV, Gumpf DJ, Garnsey SM (1994) Screening of the closterovirus genome by degenerate primer-mediated polymerase chain reaction. *Journal of General Virology* **75**, 1415-1422

Komínek P (2006) Distribution of grapevine viruses in the Czech Republic. *15th Meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG)*, *Extended Abstracts*, *3-7 April 2006*, *Stellenbosch*, *South Africa*, pp 212-213

Komínek P, Bryxiová M, Glasa M (2004) Partial molecular characterization of a Czech isolate of Grapevine leafroll-associated virus 3. *Journal of Phytopathology* **152**, 427-431

Komínek P, Bryxiová M (2005) Comparison of three techniques for the detection of Grapevine leafroll-associated virus 1. *Acta Virologica* **49**, 37-43

Komínek P, Glasa M, Bryxiová M (2005) Analysis of the molecular variability of Grapevine leafroll-associated virus 1 reveals the presence of two distinct virus groups and their mixed occurrence in grapevines. *Virus Genes* **31**, 247-255

Leonhardt W, Wawrosch C, Auer A, Kopp B (1998) Monitoring of virus diseases in Austrian grapevine varieties and virus elimination using *in vitro* thermotherapy. *Plant Cell*, *Tissue and Organ Culture* **52**, 71-74

Little A (2004) Complete sequence, improved detection and functional analysis of *Grapevine leafroll-assicaited virus 1* (GLRaV-1). PhD Thesis, The University of Adelaide, Australia, 101 pp

Little A, Fazeli CF, Rezaian MA (2001) Hypervariable genes in Grapevine leafroll associated virus 1. *Virus Research* **80**, 109-116

Little A, Rezaian MA (2006) Improved detection of grapevine leafroll-associated virus 1 by magnetic capture hybridisation RT-PCR on a conserved region of viral RNA. *Archives of Virology* **151**, 753-761

MacKenzie DJ, Johnson RC, Warner C (1996) Incidence of four important viral pathogens in Canadian vineyards. *Plant Disease* **80**, 955-958

Mannini F, Credi R (2003) Appraisal of agronomic and enological modification in the performances of grapevine clones after virus eradication. *Extended Abstracts, 13th Meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine*, *March 12-17*, *2000*, *Adelaide*, *Australia*, pp 151-154

Marchler-Bauer A, Anderson JB, Cherukuri PF, DeWeese-Scott C, Geer LY, Gwadz M, He S, Hurwitz DI, Jackson JD, Ke Z, Lanczycki CJ, Liebert CA, Liu C, Lu F, Marchler GH, Mullokandov M, Shoemaker BA, Simonyan V, Song JS, Thiessen PA, Yamashita RA, Yin JJ, Zhang D, Bryant SH (2005) CDD: a conserved domain database for protein classification. *Nucleic Acids Research* **33**, D192-D196

Martelli GP, Agranovsky AA, Bar-Joseph M, Boscia D, Candresse T, Coutts RHA, Dolja VV, Falk BW, Gonsalves D, Jelkmann W, Karasev AV, Minafra A, Namba S, Vetten HJ, Wisler GC, Yoshikawa N (2002) The family Closteroviridae revised. *Archives of Virology* **147**, 2039-2044

Medina V, Peremyslov VV, Hagiwara Y, Dolja VV (1999) Subcellular localization of the HSP70-homolog encoded by beet yellows closterovirus. *Virology* **260**, 173-181

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

Pathirana R, McKenzie MJ (2005) A modified green-grafting technique for large-scale virus indexing of grapevine (*Vitis vinifera* L.). *Scientia Horticulturae* **107**, 97-102

Peremyslov VV, Hagiwara Y, Dolja VV (1999) HSP70 homolog functions in cell-to-cell movement of a plant virus. *Proceedings of the National Academy of Sciences USA* **96**, 14771-14776

Peremyslov VV, Andreev IA, Prokhnevsky AI, Duncan GH, Taliansky ME, Dolja VV (2004) Complex molecular architecture of beet yellows virus particles. *Proceedings of the National Academy of Sciences USA* **101**, 5030-5035

Petersen CL, Charles JG (1997) Transmission of grapevine leafroll-associated closteroviruses by *Pseudococcus longispinus* and *P. calceolariae*. *Plant Pathology* **46**, 509-515

Poljuha D, Sladonja B, Peršuriæ D (2004) Survey of five indigenous Istrian cultivars for the presence of six grape viruses. *American Journal of Enology and Viticulture* **55**, 286-287

Rowhani A, Uyemoto JK, Golino DA (1997) A comparison between serological and biological assays in detecting grapevine leafroll associated viruses. *Plant Disease* **81**, 799-801

Satyanarayana T, Gowda S, Ayllón MA, Dawson WO (2004) Closterovirus bipolar virion: Evidence for initiation of assembly by minor coat protein and its restriction to the genomic RNA 5′ region. *Proceedings of the National Academy of Sciences USA* **101**, 799-804

Seddas A, Haidar MM, Greif C, Jacquet C, Cloquemin G, Walter B (2000) Establishment of a relationship between grapevine leafroll closteroviruses 1 and 3 by use of monoclonal antibodies. *Plant Pathology* **49**, 80-85

Sforza R, Boudon-Padieu E, Greif C (2003) New mealybug species vectoring Grapevine leafroll-associated viruses-1 and -3 (GLRaV-1 and -3). *European Journal of Plant Pathology* **109**, 975-981

Tian T, Klaassen VA, Soong J, Wisler G, Duffus JE, Falk BW (1996) Generation of cDNAs specific to lettuce infectious yellows closterovirus and other whitefly-transmitted viruses by RT-PCR and degenerate oligonucleotide primers to the closterovirus gene encoding the HSP70 homologue. *Phytopathology* **86**, 1167-1173

Valero M, Ibanez A, Morte A (2003) Effects of high vineyard temperatures on the grapevine leafroll associated virus elimination from *Vitis vinifera* L. cv. Napoleon tissue cultures. *Scientia Horticulturae* **97**, 289-296

Woodham RC, Antcliff AJ, Krake LR, Taylor RH (1984) Yield differences between Sultana clones related to virus status and genetic factors. *Vitis* **23**, 73-83

Zhu X, Zhao X, Burkholder WF, Gragerov A, Ogata CM, Gottesman ME, Hendrikson WA (1996) Structural analysis of substrate binding by the molecular chaperone DnaK. *Science* **272**, 1606-1614