

DETECTION AND COAT PROTEIN GENE CHARACTERIZATION OF AN ISOLATE OF *Grapevine virus B* FROM CORKY BARK-AFFECTED GRAPEVINES IN SOUTHERN BRAZIL

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ABSTRACT

An isolate of *Grapevine virus B* (GVB), obtained by indexing *Vitis labrusca* and *V. vinifera* grapevines on the indicator LN33, was transmitted mechanically to several *Nicotiana* species. The virus was partially purified from *N. cavicola* and the coat protein estimated at 23 kDa by SDS-PAGE. In negatively stained leaf extracts of experimentally inoculated *N. cavicola* and *N. occidentalis*, flexuous particles with cross banding were observed, predominantly measuring 750-770 x 12 nm, with a modal length of 760 nm. Decoration indicated a clear, positive reaction against AS-GVB. In DAS-ELISA, GVB was detected in *N. cavicola* and grapevine

extracts, and Western blots showed homologous and cross reaction of GVB and GVA antisera with GVB coat protein. Using specific primers for GVB, a fragment of 594 bp, comprising the coat protein gene coding for 197 amino acids, was amplified by RT-PCR with viral RNA extracted from GVB-infected *N. occidentalis*. The nucleotide and the deduced amino acid sequences of the coat protein gene showed high identities with Italian and Japanese isolates of GVB.

Additional keywords: GVB, *Vitis* spp., *Vitivirus*, electron microscopy, ELISA, Western blot, GVA.

RESUMO

Detecção e caracterização do gene da proteína capsidial de um isolado de *Grapevine virus B* de videiras do Sul do Brasil com intumescimento dos ramos

Um isolado de *Grapevine virus B* (GVB), obtido por indexação de *Vitis labrusca* e *V. vinifera* na indicadora LN33, foi transmitido mecanicamente para várias espécies do gênero *Nicotiana*. O vírus foi parcialmente purificado de *N. cavicola* e a proteína capsídica foi estimada em 23 kDa por SDS-PAGE. Ao microscópio eletrônico, observou-se, em extrato foliar de *N. occidentalis* e *N. cavicola*, partículas flexuosas com estriações transversais, predominantemente medindo 750-770 x 12 nm, com comprimento modal de 760 nm. A decoração das partículas indicou reação clara e positiva com anti-soro contra GVB. Em

DAS-ELISA, GVB foi detectado em extratos de *Nicotiana* spp. e de videira (*Vitis* spp.). Em Western blots foram observadas reações específicas e cruzadas, da proteína capsídica de GVB com os anti-soros contra GVB e GVA, respectivamente. A partir do RNA viral extraído de *N. occidentalis*, foi amplificado por RT-PCR um fragmento de 594 bp específico de GVB, que codificou 197 aminoácidos do gene da proteína capsídica. As seqüências de nucleotídeos e de aminoácidos deduzidos do gene da proteína capsidial apresentaram alta identidade com isolados italianos e japoneses de GVB.

INTRODUCTION

Corky bark, a component of the grapevine (*Vitis* spp.) rugose wood complex, is caused by *Grapevine virus B* (GVB), genus *Vitivirus* (Regenmortel *et al.*, 2000), which is widespread worldwide in grape growing regions (Martelli, 1993). The virus has filamentous particles of ca. 800 nm length, and a single type of coat protein (CP) subunit with a molecular mass of 21.6-23 kDa, according to whether estimates are made from the amino acid sequence of the CP cistron (Minafra *et al.*, 1994) or from the electrophoretic mobility of purified virus preparations (Boscia *et al.*, 1993).

The genome is a positive, single-stranded RNA of about 7,600 nucleotides in size (Boscia *et al.*, 1993). The viral genome has been sequenced (Minafra *et al.*, 1994; Saldarelli *et al.*, 1996). Although GVB has been recently reported in the Brazilian States of São Paulo (Kuniyuki *et al.*, 2000) and Rio Grande do Sul (Nickel *et al.*, 2000), the corky bark-affected grapevines have been indexed earlier (Kuniyuki & Costa, 1987; Kuhn, 1992). With an incidence of up to 60% in some vineyards in southern Brazil, it is highly relevant economically. Corky bark causes decreased production, incomplete ripening of grapes and progressive decline and death of *Vitis labrusca* L. and *V. vinifera* grape cultivars

(Kuhn, 1992). Cultivars and rootstocks differ in their susceptibility to the disease. Some are symptomless carriers or exhibit only mild symptoms, while others suffer rapid decline. Typically swelling and longitudinal bark cracks in young branches and the early drying of branches occur in *V. labrusca*, while the reaction of *V. vinifera* cultivars is restricted to swelling at the graft region and/or precocious reddening of leaves. There is evidence of the natural spread of this disease worldwide, including the Serra Gaúcha, Rio Grande do Sul. There is experimental proof of controlled transmission of GVB by several species of the pseudococcid mealybug genera *Pseudococcus* and *Planococcus* (Engelbrecht & Kasdorf, 1990; Boscia *et al.*, 1993; Garau *et al.*, 1995; Golino *et al.*, 1995), which is consistent with the distribution pattern of the disease in vineyards indicating the involvement of a vector. Since symptoms are not always expressed in affected seedlings, precise and efficient diagnostic methods are relevant.

Here we report on the diagnosis and the characterization of the coat protein gene of a Brazilian isolate of GVB (named GVB BR1), as well as the involvement of this virus in corky bark grapevine disease in Southern Brazil and the biological reaction it induces in infected herbaceous indicator plants.

MATERIAL AND METHODS

Virus sources and mechanical inoculation of herbaceous hosts

The isolate GVB BR1 was obtained by indexing *V. labrusca* cvs. Isabel and Niagara, and *V. vinifera* grapevines cvs. Gewürztraminer and Cabernet Sauvignon on the indicator LN33 (Couderc 1613 x Thompson Seedless) during a survey carried out in the counties of Bento Gonçalves and Farroupilha, Brazil (Kuhn, 1992). The virus was recovered from corky bark-affected LN33 by grinding mature leaves (or main veins and petioles) in the presence of 50 mM potassium phosphate buffer pH 7.5, containing 2.5% nicotine and mechanically inoculating it on to carborundum-dusted leaves of *Chenopodium amaranticolor* Coste et Reyn., *C. quinoa* Willd., *Nicotiana occidentalis* Wheeler, *N. occidentalis* sp. *obliqua*, *N. occidentalis* sp. *obliqua* P1, *N. occidentalis* 37B, *N. benthamiana* Domin., and *N. cavicola*. Latent infections were ascertained by back inoculating on *N. occidentalis* and by electron microscopy.

Virus purification, serology and electron microscopy

GVB was partially purified from *N. cavicola* or *N. occidentalis* adopting the procedure of Boscia *et al.* (1993) with minor modifications. Briefly, systemically infected leaves were powdered in the presence of liquid nitrogen, extracted in buffer, clarified, concentrated by cycles of differential centrifugation and submitted to one cycle of ultracentrifugation in a CsSO₄ gradient. The virus bands were extracted with hypodermic needles, dialysed, given a 12.000 g centrifugation and stored at -20 °C.

ELISA and Western blots were carried out using policlonal GVB antiserum against an Italian isolate of GVB (kindly supplied by G.P. Martelli, Bari, Italy), a policlonal *Grapevine virus A* (GVA) antiserum (kindly supplied by Istituto de Fitovirologia Applicata, Turin, Italy) and commercial policlonal antisera against GVB and GVA (Sanofi, France or Agritest, Italy) with extracts of GVB-affected *Nicotiana* spp. cultivated in a glasshouse and of corky bark-infected grapes (veins and petioles of mature leaves). Samples for ELISA (Clark & Adams, 1977) were diluted 1:5 (w/v) and analysed according to standard methods. Aliquots of the virus suspension were denatured with an equal volume of SDS-PAGE sample buffer (Sambrook *et al.*, 1989) and electrophoresed in a discontinuous polyacrylamide gel system at 120 V for 2 h in running buffer (Laemmli, 1970) in a vertical mini-PAGE unit. The molecular weight of protein subunits was determined by comparison with molecular weight standards. Gels were stained with silver nitrate (Blum *et al.*, 1987). Electrotransfer of proteins to nitrocellulose membranes for Western blots was done from unstained gels at 100 V for 1 h in transfer buffer (Towbin *et al.*, 1979). Besides partially purified GVB suspension, crude extracts of petioles and veins of GVB infected plants were used for Western blot analysis.

For virus particle observations, foliar extracts of infected *N. cavicola* and *N. occidentalis* were negatively stained with 2% uranyl acetate, mounted on carbon-stabilized collodion-coated grids and examined in a Philips EM 208 electron microscope. One hundred-seven particles were measured and grouped at 10 nm class intervals. Decoration tests were performed according to Milne & Lesemann (1984), using the antisera against GVB and GVA mentioned above.

Synthesis of cDNA, RT-PCR amplification, cloning and sequencing

Viral RNA was extracted by adsorbing total nucleic acids on silica particles as described previously (Nickel *et al.*, 1999). Preparation of silicon dioxide was done according to Boom *et al.* (1990). Complementary GVB7038 (5' ACTCGTCAGACAACTCTATATC 3') and homologous primers GVB6445 (5' ATGGAAAATATATCCCGGATGG 3') were synthesized according to published sequences (Saldarelli *et al.*, 1996; GenBank data, access code X75448). Alternatively, the same reaction was carried out with primers GVB H28 (5'GTGCTAAGAACGTCTTCACAGC3') and C410 (5'ATCAGCAAACACGCTTGAACCG3') (Minafra & Hadidi, 1994). For reverse transcription 5 µl of viral RNA template were mixed with 0.5 µl complementary primer GVB 7038 (1 µg/µl), 4.5 µl deionized water, and incubated 2 min at 90 °C. After primer annealing at room temperature for 3 min, 15 µl of reaction mixture [containing 5 µl M-MLV 5x buffer, 0.7 µl RNase inhibitor (40 U/µl), 1 µl 10 mM dNTP (2.5 mM each), 1 µl M-MLV reverse transcriptase (200 U/µl) and 7.3 µl water] were added and the mixture was incubated at 37 °C for 1 h. Aliquots of 40 µl of the amplification mixture [5.0 µl 10x Taq DNA polymerase buffer,

5.0 µl 10 µM dNTPs (2.5 µM each), 1 µl of each primer, 0.5 µl (0.5 unit) of *Taq* DNA polymerase and 27.5 µl water] were mixed with a 10 µl aliquot of the cDNA reaction. The PCR amplification profile was 30 cycles of 30 sec at 94 °C, 30 sec at 62 °C, and 45 sec at 72 °C (denaturing, annealing and extension, respectively) and a final elongation step of 5 min at 72 °C. An aliquot of the PCR amplification reaction was run in a 1.5% agarose gel for 2 h at 70 V. Molecular weight marker Lambda DNA cut with *Pst* I was used to determine the size of amplification products, which were stained with ethidium bromide and visualized over UV light. Viral bands were immediately cut out of the gel and DNA was eluted for cloning procedures.

The amplified fragment with 594 bp was cloned into a pGEM-T Easy vector (Promega) and used to transform competent JM 109 *Escherichia coli* cells according to the manufacturer's instructions. Ampicillin-resistant transformants were selected and recombinant plasmids were isolated from overnight cultures by cell lysis (Sambrook *et al.*, 1989). Purified plasmid DNA was sequenced on an automatic sequencer. Sequence data were analysed using the Blast resources through the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/>). Search and alignment of the sequenced clone were done within the GenBank database.

RESULTS

Virus sources and mechanical inoculation of herbaceous hosts

GVB isolate BR1 was successfully transmitted mechanically from LN33 to all *Nicotiana* species mentioned, except *N. benthamiana*, *C. quinoa* and *C. amaranticolor*, which were not infected. Symptoms appeared at seven to nine days post-inoculation.

Nicotiana occidentalis 37B and *N. occidentalis* spp. *obliqua* P1 are severely affected by the virus, initially showing necrotic spotting and necrotic areas on the stems, and later a systemic necrosis, leaf deformation and size reduction, dwarfing and a rosette-like growth. In the late stages of infection, leaves and stems turn lilac. Sometimes infection was lethal to these species.

Nicotiana occidentalis, *N. occidentalis* spp. *obliqua* and *N. cavicola* initially show chlorotic spotting and vein clearing that turn necrotic, forming a necrotic net over the leaf surface. Leaves with symptoms are wrinkled, and their size is gradually reduced. In late infection stages plant habitus is rosette-like. Infection is heavy but not lethal.

Partial purification, serology and electron microscopy/ISEM

Coat protein subunits were estimated at 23 kDa by SDS-PAGE in partially purified virus suspensions and by Western blot in GVB-infected grapevine leaf extracts (data not shown). Western blots revealed homologous and heterologous reactions (data not shown) of GVB BR1 coat

protein respectively with a GVB antiserum (Agritest, Italy) and GVA antiserum (Turin, Italy). In ELISA, reactions were homologous only (Table 1).

In negatively stained leaf extracts of experimentally inoculated *N. cavicola* and *N. occidentalis*, flexuous particles, with the cross banding typical of Vitivirus were observed, predominantly measuring 750-770 x 12 nm with modal length of 760 nm (Figure 1A). In ISEM GVB BR1 particles were heavily decorated, indicating a strong positive reaction against GVB antiserum (Figure 1B). Decoration with GVA antiserum was negative.

PCR, cloning, sequence determination and homology analysis

An expected 594 bp DNA fragment comprising the complete coat protein gene of GVB BR1 was amplified (Figure 2). The open reading frame (ORF) representing the GVB coat protein gene contained 591 nucleotides, coding for a protein of 197 amino acids with a predicted M_r of about 23 kDa. The nucleotide and the deduced amino acid sequences of GVB BR1 coat protein gene (Figure 3) are accessible in the GenBank database under number AF438410.

The nucleotide and deduced amino acid sequences of the coat protein gene showed high identities with other two isolates of GVB: 81.1% and 97.4% with an Italian isolate (X75448), and 81.4% and 94.9% with a Japanese isolate (AB039842), respectively (Table 2).

Comparison of the amino acid sequence of GVB BR1 coat protein gene with related virus species and viral proteins revealed extensive homologies with Vitivirus such as *Heracleum latent virus* (HLV), *Grapevine virus D* (GVD) and GVA, and lower homologies with virus from the genera *Trichovirus* and *Capillovirus* (Table 3).

DISCUSSION

Mechanical transmission, directly from field (or glasshouse-) grown vines, was successful though not easily performed. As reported for other phloem-limited grapevine viruses, transmission of GVB is more likely to succeed when in vitro-grown explants are used as the inoculum source

TABLE 1 - ELISA of *Nicotiana* spp. and grapevine (*Vitis* spp.) cv. LN33 grown under greenhouse conditions performed using a polyclonal antiserum against *Grapevine virus B* (GVB). ELISA values at 405 nm were an average of two samples

SAMPLE	ELISA VALUE
Grapevine cv. LN33 healthy	0.243
Grapevine cv. LN33 infected	2.224
<i>N. cavicola</i> healthy	0.242
<i>N. cavicola</i> infected	0.554
Negative control of ELISA test	0.234
GVB positive control of ELISA test	1.764

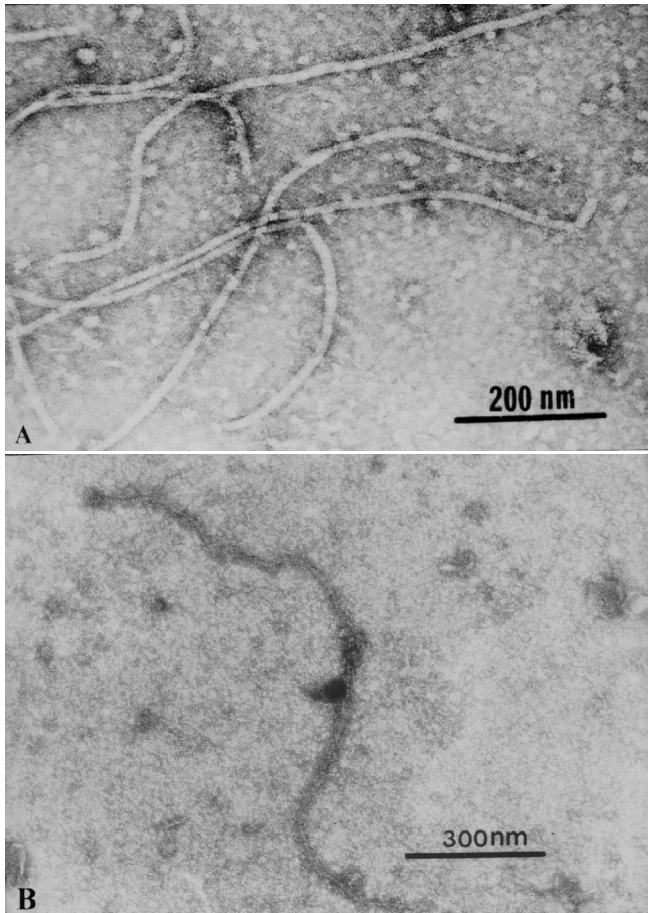


FIG. 1 - A. Electron micrographs of *Grapevine virus B* (GVB) BR1 particles from a leaf extract of *Nicotiana occidentalis*; **B.** Two GVB BR1 particles, joined by their extremities, from a leaf extract of *N. cavicola* decorated by an antiserum against GVB, both negatively stained in 2% uranile acetate.

(Bonavia *et al.*, 1996). Our attempts were unsuccessful to *in vitro* grow GVB-affected LN33 in Galzy medium (Galzy, 1964), in which we do routine vegetative propagation of grapevines. Growth of these cultures was extremely poor; most explants did not produce any shoots. Among species that were not infected, *N. benthamiana* is known as a difficult host (with Italian isolates), that could eventually be infected using viral RNA (A. Minafra, Italy, personal communication) although it is easily infected by South African isolates (Goszczyński *et al.*, 1996). The existence of biologically different strains, based on differential reaction of *N. occidentalis*, has been reported (Boscia *et al.*, 1993). By the same logic, non-infection of *N. benthamiana* by GVB BR1 characterizes it as a biologically different strain. It can not be excluded that the inoculum used here may contain more than one strain. This view is consistent with the different reactions produced occasionally on the same indicator by the same inoculum (vein chlorosis or necrosis of *N. cavicola*, not shown). It should be stressed that GVB BR1

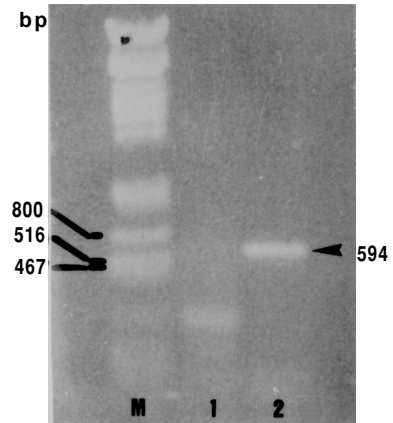


FIG. 2 - Electrophoretic analysis of PCR amplification products from total RNA of *Nicotiana cavicola* inoculated with *Grapevine virus B* (GVB) BR1, agarose 1.2%. Lanes (1) negative reaction with GVA primers (Minafra & Hadidi, 1994), (2) amplified fragment of GVB BR1 of 594 bp, (M) molecular weight marker, *Pst*I-cut Lambda DNA.

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1  ATGGAAAATATATCCCGGATGGCAAAAATAAGGGCGAATATCTCCGAG
   M E N I S R M A K I R A N I S E
49  CTCCTGTGCGCGGGAGTACATTTGTAACTGACGCCCGTGAAACTGGG
   L L C A G V T F V T D A R E T G
97  TTTGATCGTCCCATGTACTTCAGGACCCTATTCGGGTACATTGCATTA
   F D R P M Y F R T L F G Y I A L
145  ACTGGACATCAGCAAAAGCACAGCACTACGAGAATGTAGATATTATA
   T G T S A K A Q H Y E N V D I I
193  GGTGATAAAGTAGGAGCAGAGGGTCTAGATAGTAGGGAACTGTCAAT
   G D K V G A E G L D S R G T V N
241  ATCTCGGAACAGGTGAAGAAGATGATGGTACTCTCGAGCAGTACCA
   I S E Q V K K M M G Y S R A V P
289  TCTGGGGTGTGTAAGGGCCTTACTCTGAGGCAAATGTGTGAACCTTTC
   S G V C K G L T L R Q M C E P F
337  GCAGAGGAAGCTAGGGACTGCTTACTATCTGGCAACCTGAGGGTA
   A E E A R D C L T I L A T L R V
385  TACAGCAGGCTCGCACTCAAGATGGCCAAACTGGCCAAAAGAACC
   Y S R L A L K M A K L G Q K E P
433  CAAGTTATGTTTCGATTTTAACTCTGGTCTTAACTGCTCGCTCTATCC
   Q V M F D F N S G L N L L A L S
481  GCTACCGAGGCATCTGCAATAAATCTCTGAACTCAAGGCTCTTTCGA
   A T E A S A I Q S L N S R L F R
529  ACTGAGGGTGCAAAGAACGTATTACAGCACAAGCCAGCGTGGGTGAG
   T E G A K N V F T A Q A S V G E
577  CAGTCTGTTCGAGATATAG 594
      Q S V E I *
    
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FIG. 3 - Nucleotide sequence of the coat protein gene of *Grapevine virus B* (GVB) BR1. The deduced amino acid sequence of coat protein gene is shown below the nucleotide sequence. Asterisk indicates the coat protein termination codon; GVB 6445 and GVB 7038 are the 5' forward and the 3' reverse primers, respectively (underlined). This sequence is accessible at GenBank under number AF438410.

TABLE 2 - Pairwise percent identities among coat protein amino acid sequences (above diagonal) and coat protein nucleotide sequences (below diagonal) of *Grapevine virus B* Brazilian BR1 isolate (GenBank access AF438410) and other isolates

	GVB BR1 594 bp/197 aa	GVB ⁽¹⁾ X75448	GVB ⁽²⁾ AB039842	GVB ⁽³⁾ 2022155E
GVB BR1 594 bp/197 aa	—	97.4	94.9	96.8 ^(*)
GVB⁽¹⁾ X75448	81.1	—	94.9	99.4 ^(*)
GVB⁽²⁾ AB039842	81.4	81.9	—	93.7 ^(*)
GVB⁽³⁾ 2022155E	n.d.	n.d.	n.d.	—

n.d. - not determined

^(*) - To 2022155E GVB access only 191 amino acids sequence is available. Access: GVB BR1: Brazilian isolate of *Grapevine virus B* (GVB) (GenBank access AF438410), (1) Saldarelli *et al.* (1996), (2) Coat protein gene of *Grapevine virus B* (GVB) isolated in Japan (In press, 2000), (3) Minafra *et al.* (1994). GenBank database access code closed to virus name.

inoculum often induced a systemic necrotic reaction on *N. occidentalis* and occasionally a lethal reaction on *N. occidentalis* spp. *obliqua* P1. The virus could be detected in several *Vitis* spp. cultivars. As more sensitive diagnostic methods are used, besides making diagnosis of non-symptomatic infections in nuclear mother stock and in nurseries more efficient and faster, the already high incidence of GVB (Kuhn, 1992) may turn out to be higher.

Usually commercial GVA antisera did not cross-react

in ELISA with GVB BR1 or other GVB-positive tissues tested by ELISA. On the other hand, GVB BR1 reacted positively to the GVA antisera used in Western blots, which confirmed a previous report, demonstrating that both viruses share antigenic determinants (Goszczynski *et al.*, 1996). It was shown that the reaction of antisera against GVB and GVA is virus-specific in ELISA and IEM, indicating that the antigenic determinants responsible for cross reactions in Western blots are mainly cryptotopes (Goszczynski *et al.*, 1996), which are not accessible for antibodies when non-denatured samples are used in ELISA and electron microscopy. The intensity of the heterologous reaction observed in this work (data not shown) is surprising, despite a distant serological relationship due to a few common internal antigenic determinants (Regenmortel *et al.*, 2000).

The particle modal length of 760 nm of GVB BR1 fits the size range of the viruses for the genus *Vitivirus* (725-825 nm) (Regenmortel *et al.*, 2000) and agrees quite closely with values of American (800 nm) and Canadian (775 nm) GVB isolates (Boscia *et al.*, 1993).

Alignment of the coat protein gene sequences of GVB BR1 with sequences of GVA, GVD and HLV, the other members of the newly created genus *Vitivirus*, showed extensive homologies at the amino acid level, confirming a similar report by Goszczynski *et al.* (1996) based on Western blot results and Regenmortel *et al.* (2000).

The unequivocal identification of GVB in corky bark-affected LN33 in connection with the non-amplification of GVA by RT-PCR (Figure 2) and the non-decoration of particles in IEM with GVA-AS, excludes the hypothesis of GVA presence in these samples and underlines the association of GVB with corky bark etiology.

The obtained sequence data show a small variability

TABLE 3 - Comparison (in percentage of identity) between deduced amino acid of coat protein genes of *Grapevine virus B* (GVB) BR1 (AF438410) and viruses from related genera

VIRUS	GENUS	GENBANK ACCESS	HOMOLOGIES*
1. <i>Heracleum latent virus</i> (HLV)	<i>Vitivirus</i>	X79270	66.4 (131/197)
2. <i>Grapevine virus D</i> (GVD)	<i>Vitivirus</i>	Y07764	63.9 (103/161)
3. <i>Grapevine virus A</i> (GVA)	<i>Vitivirus</i>	AF007415	57.0 (113/198)
4. <i>Grapevine virus A</i> (GVA)	<i>Vitivirus</i>	AB039841	57.0 (113/198)
5. <i>Grapevine virus A</i> (GVA)	<i>Vitivirus</i>	X75433	56.0 (111/198)
6. <i>Citrus tatter leaf virus</i> (CTLV)	<i>Capillovirus</i>	AF233298	36.4 (39/107)
7. <i>Apple chlorotic leaf spot virus</i> (ACLSV)	<i>Trichovirus</i>	X99752	33.7 (54/160)
8. <i>Cherry virus A</i> (ChVA)	<i>Capillovirus</i>	X82547	33.5 (54/161)
9. <i>Cherry mottle leaf virus</i> (ChMLV)	<i>Trichovirus</i>	AF170028	33.3 (52/156)
10. <i>Apple chlorotic leaf spot virus</i> (ACLSV)	<i>Trichovirus</i>	C45353	31.8 (51/160)
11. <i>Apple chlorotic leaf spot virus</i> (ACLSV)	<i>Trichovirus</i>	M58152	31.8 (51/160)
12. <i>Apple chlorotic leaf spot virus</i> (ACLSV)	<i>Trichovirus</i>	AF251275	31.8 (51/160)
13. <i>Apple chlorotic leaf spot virus</i> (ACLSV)	<i>Trichovirus</i>	D14996	31.2 (50/160)
14. <i>Apple chlorotic leaf spot virus</i> (ACLSV)	<i>Trichovirus</i>	AJ243438	30.6 (50/163)
15. <i>Apple stem grooving virus</i> (ASGV)	<i>Capillovirus</i>	AB004063	30.5 (52/170)
16. <i>Citrus tatter leaf virus</i> (CTLV)	<i>Capillovirus</i>	2121406A	30.5 (52/170)
17. <i>Citrus tatter leaf virus</i> (CTLV)	<i>Capillovirus</i>	D14455	30.5 (52/170)
18. <i>Citrus tatter leaf virus</i> (CTLV)	<i>Capillovirus</i>	D16368	30.5 (52/170)
19. <i>Apple stem grooving virus</i> (ASGV)	<i>Capillovirus</i>	D14995	30.0 (51/170)

^(*) Identical amino acids/aligned amino acids

of coat protein genes among three GVB isolates from distinct geographical regions. However GVB BR1 is less close to GVA (56-57% homology) than Italian isolates of GVB (59%) (Saldarelli *et al.*, 1996). Our data, agreeing with Minafra *et al.* (1994), reveal some homology of GVB with coat protein genes of *Apple chlorotic leaf spot virus* (ACLSV) genus *Trichoviruses* and *Apple stem grooving virus* (ASGV) genus *Capillovirus* (Table 3).

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