

Nomenclature of grapevine leafroll-associated putative closteroviruses

by

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S u m m a r y: Comparative immunoenzymatic (ELISA), immunoelectron microscopic (IEM) and immunoblotting tests were carried out with antisera produced in different laboratories and commercial diagnostic kits on closterovirus-like viruses reported in the literature under the name of grapevine corky bark-associated virus (GCBaV) and grapevine leafroll-associated viruses IIa and IIb (GLRaV IIa and GLRaV IIb). The results of these studies have established that GCBaV is the same as GLRaV IIb and that both viruses are apparently identical to an isolate of GLRaV-2 identified in France, whose designation as the authentic GLRaV 2 is proposed. GLRaV IIa is serologically distinct from all known closterovirus-like viruses of the grapevine and, therefore, the provisional name of grapevine leafroll-associated virus 6 (GLRaV-6) is suggested for it.

K e y w o r d s: closterovirus, leafroll, corky bark, serology, immunoblotting, immunoelectron microscopy.

Introduction

Leafroll is a long known graft-transmissible disease of *Vitis* that induces distinct symptoms in most European grape varieties (i.e., reddish or yellowish discolorations and rolling of the leaves) (BOVEY *et al.* 1980; MARTELLI 1993), but not in American *Vitis* species and their hybrids, with the exception of *Vitis riparia* Gloire (VUITTENEZ 1985; GREIF *et al.* 1993). The breakthrough in the etiology of this disorder, which had remained undetermined for many years, came in the late 1970s when filamentous particles with the typical structure and outward appearance of closterovirus virions were recovered from infected vines in Japan (NAMBA *et al.* 1979). In the years that followed, studies carried out primarily in USA (HU *et al.* 1990 a, b) and Europe (GUGERLI *et al.* 1984; ROSCIGLIONE and GUGERLI 1986; ZIMMERMANN *et al.* 1990 a, b), demonstrated that at least five different closterovirus-like viruses, all serologically unrelated to one another, were associated with the disease (for a historical review, see BOVEY and MARTELLI 1992). In the literature, these viruses were referred to as "grapevine leafroll-associated viruses (GLRaV)".

In the course of its 10th Meeting (Volos, Greece, 1990), the International Council for the Study of Viruses and Virus Diseases of the Grapevine (ICVG) addressed the issue of the nature and nomenclature of closterovirus-like grapevine viruses, confirming that there were five separate such viral species apparently involved in the induction of red leaf syndromes, for which the name grapevine leafroll-associated viruses was to be retained, followed by Roman numerals I to V (i.e., GLRaV I - V). However, more recently,

the International Committee on Taxonomy of Viruses (ICTV) has determined that virus acronyms that have numbers in their name are to be written with Arabic numerals separated by a hyphen from the letters (FAUQUET and MARTELLI 1995; MURPHY *et al.* 1995). Therefore, the correct name and relative abbreviation of the closterovirus-like grapevine viruses now is "grapevine leafroll-associated viruses 1 to 5" (GLRaV -1 to -5).

The lack of mechanical transmissibility of GLRaVs has impaired their physico-chemical and molecular characterization. The scanty information available indicates that GLRaV-3 has a gene coding for a HS70-related protein (LING *et al.* 1994), which is specific to all closterovirus species sequenced so far, and induces a cytopathology comparable to that of true closteroviruses (FAORO *et al.* 1992). However, the same GLRaV-3 has coat protein (CP) subunits with a M_r of ca. 43 kDa (ZIMMERMANN *et al.* 1990 a; LING *et al.* 1994) which is about the double of the size of CP subunits of definitive closteroviruses (CANDRESSE and MARTELLI 1995). GLRaV-1, -4, and -5 have similarly large CP subunits, ranging in size from ca. 35 to 39 kDa (GUGERLI and RAMEL 1993).

For GLRaV-2, two different M_r of CP subunits, i.e. 36 kDa and 26 kDa, were reported from USA (BOSCIA *et al.* 1990) and France (ZIMMERMANN *et al.* 1990 b), respectively. This confusing situation was clarified in part by GUGERLI and RAMEL (1993) who, using a monoclonal antibody denoted MCA 29-1, proved that the original Swiss source of GLRaV-2 (cv. Chasselas 8/22) contained two different closterovirus-like viruses. Of these, the one recognized by MCA 29-1 had particles differing slightly in outward appearance

from those of the other virus and CP subunits with a M_r of 26.5 kDa, a value in line with the French estimate. This virus, provisionally called GLRaV IIB, reacted also with a French antiserum to GLRaV-2. The other still uncharacterized component of the virus mixture of Chasselas 8/22 was given the name of GLRaV IIA (GUGERLI and RAMEL 1993).

Grapevine corky bark-associated virus (GCBaV) is a clostero-like virus recovered from a Californian accession of cv. Semillon affected by corky bark, described and partially characterized by NAMBA *et al.* (1991). This virus, which will be referred to as GCBaV-NY, was reported to be non mechanically transmissible and serologically unrelated to the five known GLRaVs. It was therefore regarded as a distinct viral species differing from GLRaVs also because of the lower M_r (24 kDa) of its CP subunits (NAMBA *et al.* 1991).

Recently, at Bari, a clostero-like virus was transmitted to *Nicotiana benthamiana* by inoculation of sap expressed from *in vitro*-grown explants of the same Californian source of GCBaV (Semillon VCA3v7) used by NAMBA *et al.* (1991), obtained some years ago from Dr. A.C. GOHEEN (D. BOSCIA and N. ABOU-GHANEM in CASTELLANO *et al.* 1995). This virus induced intracellular modifications typical of clostero-virus infections (CASTELLANO *et al.* 1995), was heavily decorated by the American antiserum As-CB100 to GCBaV-NY and proved to possess CP subunits with M_r of ca. 22 kDa (N. ABOU-GHANEM and D. BOSCIA, unpublished information). It was therefore identified as an isolate of GCBaV, which will be referred to as GCBaV-BA.

The availability of what appeared to be a genuine culture of GCBaV and the puzzling occurrence of a GLRaV-2 component (GLRaV IIB) with similarly low M_r CP subunits (GUGERLI and RAMEL 1993), suggested to investigate whether there was any relationship between these two viruses.

Materials and methods

The studies carried out were based on comparative immunoenzymatic (DAS-ELISA; CLARK and ADAMS 1977) and immunoelectron microscopy (IEM; MILNE and LUISONI 1977) tests, and Western blotting (HU *et al.* 1990 a). In these studies the following antisera were used:

- (i) A polyclonal antiserum to GLRaV-2 raised at Colmar (ZIMMERMANN *et al.* 1990 a) or its commercial version (Sanofi Sant Animale, France)
- (ii) A polyclonal antiserum to GLRaV-2 raised at Geneva (BOSCIA *et al.* 1990)
- (iii) The monoclonal antibody MCA 29-1 specific to the IIB component of GLRaV-2 raised at Nyon (GUGERLI and RAMEL 1993)
- (iv) The polyclonal antiserum As-CB100 to GCBaV raised at Geneva (NAMBA *et al.* 1991)
- (v) A polyclonal antiserum to GLRaV-1 raised at Bari
- (vi) A polyclonal antiserum to GLRaV-1 raised at Colmar

- (vii) A commercial monoclonal antiserum to GLRaV-1 (Bioreba, Switzerland)

- (viii) The monoclonal antibody GB1E2G to grapevine trichovirus B (GVB) raised at Bari (BOSCIA *et al.* 1994).

In Italy, tissue extracts were prepared as described by NAMBA *et al.* (1991) from leaves and petioles of: (i) five grapevine accessions from the diseased grapevine collection of the University of Bari, none of which had indexed positive for corky bark on LN 33, but were known to be infected either by GLRaV-1 (cvs Catarratto/PA24 and Taifi/LE15) or GCBaV (cvs Waltham Cross/AU2 and Muscat de Vratz/BG44) alone, or by a mixture of the two viruses (LN33/F7); (ii) the cv. Semillon source of GCBaV-BA (VCA3v7); (iii) a healthy LN 33 vine (control); (iv) tissues from GCBaV-BA-infected and healthy (control) *N. benthamiana* plants.

In France, biotin-streptavidin-amplified DAS-ELISA tests were done on petioles and main vein leaf extracts of: (i) healthy cv. Pinot noir (control); (ii) four grapevine accessions that had indexed positive for corky bark, three of which were also infected by GLRaV-1 or GLRaV-2; (iii) sixteen grapevine accessions that had indexed positive for leafroll and were infected by GLRaV-2. Ten of these accessions induced also graft incompatibility on Kober 5BB; (iv) tissues from GCBaV-BA-infected and healthy (control) *N. benthamiana* plants. In these tests, the French antiserum to GLRaV-2 or the antiserum As-CB100 were used for trapping whereas biotinylated IgGs to GLRaV-2 were used for antigen detection. All accessions were also tested with a monoclonal antibody to GBV.

In Switzerland, extracts of GCBaV-BA-infected *N. benthamiana* were tested in DAS-ELISA with the monoclonal antibody MCA 29-1. Moreover, with immunoprecipitation electron microscopy (IPEM) tests partially purified and enriched leaf extracts from infected *N. benthamiana* were precipitated with MCA 29-1, negatively stained and viewed under the electron microscope.

Results and discussion

ELISA tests done in Italy (Tab. 1) clearly indicated that the antisera to GLRaV-2 (Sanofi) and to GCBaV-NY (As-CB100) recognized equally well, and without apparent differences, GLRaV-2 in grapevine accessions AU2 and BG44 and GCBaV-BA in cv. Semillon and infected *N. benthamiana*.

In France, antisera to GLRaV-2 and GCBaV-NY (As-CB100) recognized GCBaV-BA in *N. benthamiana* plants and GLRaV-2 in all grapevine accessions infected either by GLRaV-2 alone or in mixture with GLRaV-1 or GVB, but gave no signal with extracts from the corky bark-affected grapevine accessions *V. rupestris* N40, Chardonnay T145 and T222 (Tab. 2).

Very strong positive ELISA reactions were also obtained in the Swiss tests where extracts from *N. benthamiana* infected by GCBaV-BA were assayed with the monoclonal antibody MCA 29-1.

Table 1

Results of ELISA tests carried out at Bari with grapevine accessions and *N. benthamiana* plants infected by GLRaV-1, GLRaV-2 or GCBaV-BA

Sample	Status ^(a)	ELISA reactions to antisera prepared to:			
		GLRaV-1 Bari	GLRaV-1 Bioreba	GLRaV-2 Sanofi	GCBaV As-CB100
LN 33/F7	Leafroll	+(b)	+	+	+
Taifi /LE15	Leafroll	+	+	-	-
Catarratto/PA24	Leafroll	+	+	-	-
Waltham Cross/AU2	Leafroll	-	-	+	+
Muscat de Vratz/BG44	Leafroll	-	-	+	+
Semillon	Corky bark	-	-	+	+
LN33	Healthy	-	-	-	-
<i>N. benthamiana</i>	GCBaV	-	-	+	+
<i>N. benthamiana</i>	Healthy	-	-	-	-

(a) Status refers to disease condition as ascertained either by reactions of woody indicators or symptoms shown by the vines themselves.

(b) + = positive reaction (A_{405} range with different antisera = 0.430-1.920); - = negative reaction (A_{405} range with different antisera = 0.050-0.160)

In Western blot assays carried out at Bari, dissociated CP preparations of GCBaV-BA gave a strong reaction with the antiserum As-CB100 to GCBaV-NY and the French antiserum to GLRaV-2 (Fig. 1), indicating that these antisera contain common antibodies. These results confirmed similar tests made at Nyon, in which the antiserum As-CB100 to GCBaV-NY had reacted with the I1b component of cv. Chasselas 8/22. Always at Nyon, French commercial (Sanofi) and non commercial antisera to GLRaV-2 reacted in Western blots equally well with the I1b component.

In IEM tests carried out at Bari GCBaV-BA particles were strongly decorated by the American and French antisera to GLRaV-2 and the antiserum As-CB100 to GCBaV-NY (Fig. 2 A,B). Similar results were obtained at Nyon in IPEM tests with the monoclonal antibody MAB 29-1, specific to the I1b component of cv. Chasselas 8/22 (Fig. 2 C).

Concentrated extracts from accession LE15 affected by GLRaV-1 alone, as confirmed by ELISA with the Bioreba kit (Tab. 1), were decorated equally well by the antisera to GLRaV-1 raised at Bari and to GLRaV-2 raised at Geneva, but not by the French antiserum to GLRaV-2, nor by the antiserum As-CB100 to GCBaV-NY.

Table 2

Results of ELISA testing of grapevine accessions and *N. benthamiana* plants made at Colmar, using antisera specific to GLRaV-1, GLRaV-2, GCBaV and GVB

Sample	Status ^(a)	ELISA reactions to antisera prepared to:			
		GLRaV-1 Colmar	GLRaV-2 Colmar	GCBaV As-CB100	GVB Bari
Pinot noir	Healthy	-(b)	-	-	-
<i>V. rupestris</i> /N40	CB	-	-	-	+
Chardonnay/T45	LR+CB	+	-	-	+
Chardonnay/T222	LR+CB	+	-	-	+
Bourboulenc/Z122	LR+CB	nt ^(c)	+	+	+
Plant de Treffort/T76	LR+GI	-	+	+	-
Alphonse Lavallée/T93	LR+GI	+	+	+	-
Gros vert/T94	LR+GI	+	+	+	-
Chatus/T113	LR+GI	-	+	+	-
Grosse Rousette/T24	LR+GI	+	+	+	-
Onchette/T130	LR+GI	-	+	+	-
Chardonnay/T132	LR+GI	-	+	+	-
Chardonnay/V38	LR+GI	-	+	+	-
Madeleine angevine/Y131	LR	nt	+	+	-
Cape currant/Y204	LR	+	+	+	-
Chaouch rose/Y206	LR	nt	+	+	-
Henab Turkey/Y229	LR	nt	+	+	-
Counoise/Z127	LR	nt	+	+	-
Ruby Cabernet/Z170	LR	nt	+	+	-
Perle de Csaba/Z225	LR+GI	-	+	+	-
St Pierre doré/Z241	LR+GI	-	+	+	-
<i>N. benthamiana</i>	GCBaV	-	+	+	-
<i>N. benthamiana</i>	Healthy	-	-	-	-

(a) Status refers to disease condition as ascertained either by reactions of woody indicators or symptoms shown by the vines themselves. CB = corky bark; LR = leafroll; GI = graft incompatibility on Kober 5BB.

(b) + = positive reaction (A_{405} range with different antisera = 0.350-1.550); - = negative reaction (A_{405} range with different antisera = 0.035-0.150) (c) nt = not tested.

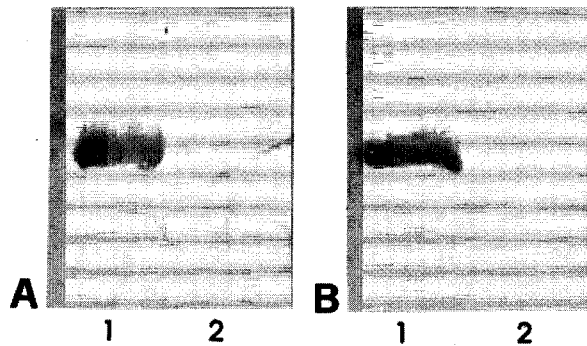


Fig. 1: Western blots showing the positive reaction of dissociated GCBaV-BA coat protein with an antiserum to GCBaV-NY (A, lane 1) and a French antiserum to authentic GLRaV-2 (B, lane 1). Lanes 2 in A and B panels contain dissociated coat protein of grapevine trichovirus A, used as control.

Based on these results, the following conclusions were drawn:

(i) The antiserum to GLRaV-2 raised at Geneva (BOSCIA *et al.* 1990) contains antibodies to both GLRaV-2 and GLRaV-1, with a prevalence of the latter, especially clear in ELISA reactions. This is likely to have caused the wrong M_r determination reported by BOSCIA *et al.* (1990) and the lack of recognition of GCBaV-NY as an isolate of GLRaV-2.

(ii) GCBaV-BA is serologically very close to, if not the same as, GCBaV-NY. However, GCBaV-BA is also serologically very similar to, if not the same as, GLRaV-2, this notion being supported by evidence from ELISA, IEM and Western blots using antisera specific to GLRaV-2. In this connection it is worth pointing out that the possible serological relatedness of GLRaV-2 with the putative agent of corky bark had been observed by PIETERSEN and KASDORF (1993) based on the response of IEM tests in which the French antiserum to GLRaV-2 and an antiserum raised in Canada to a virus from a corky bark-affected cv. Semillon accession from California were used.

(iii) The IIb component of cv. Chasselas 8/22 is the

same as the GLRaV-2 isolate from France (ZIMMERMANN *et al.* 1990 b) and GCBaV-BA.

(iv) The IIa component of Chasselas 8/22 is a virus serologically different from GLRaV-2 and, apparently, from all other known GLRaVs.

From all the above it seems plausible to suggest that:

a. There is no reason for retaining the name "grapevine corky bark-associated virus" for the clostero-like virus described by NAMBA *et al.* (1991), nor the name "grapevine leafroll-associated virus IIb" (*sensu* GUGERLI and RAMEL, 1993), both these viruses being the same as GLRaV-2.

b. The GLRaV-2 isolate from France (*sensu* ZIMMERMANN *et al.* 1990 b) which, contrary to Swiss GLRaV-2 reported earlier (ROSCIGLIONE and GUGERLI 1986), appears to be a pure isolate of this virus, be regarded as the authentic GLRaV-2. As to the naming of GLRaV-2, the retention of the words "leafroll-associated" may be justified by its occurrence in cv. Gamay Rouge de la Loire with red leaf symptoms (GUGERLI *et al.* 1990). This, although recently an intriguing association was detected in France between GLRaV-2 and a graft incompatibility condition of Kober 5BB (GREIF *et al.* 1993; see also Tab. 2), confirmed by similar observations made in Southern Italy (R. GARAU, U. PROTA and D. BOSCIA, unpublished information).

c. The IIa component of cv. Chasselas 8/22 be given the provisional name of grapevine leafroll-associated virus 6 (GLRaV-6).

In conclusion, it seems that none of the clostero-like grapevine viruses known so far has a clear-cut association with corky bark. If it is true that such association was reported by various authors (GUGERLI and RAMEL 1993; TANNE *et al.* 1993), data from surveys recently carried out in Southern Italy (M. DIGIARO and D. BOSCIA, unpublished information) and France (C. GREIF and B. WALTER, unpublished information) do not support these observations. Instead, the results of French ELISA tests (Tab. 2) confirmed the previously reported very close association of GVB with corky bark (BOSCIA *et al.* 1993)

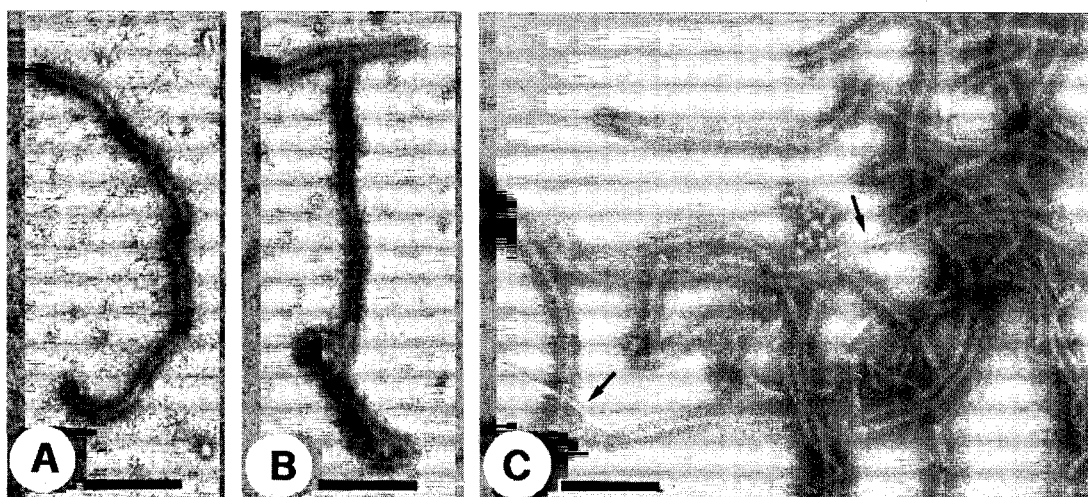


Fig. 2: Individual GCBaV-BA particles decorated by antisera to GCBaV-NY (A) and to the authentic French isolate of GLRaV-2 (B) (Bari). C: A group of GCBaV-BA particles clumped and decorated by the monoclonal antibody MCA 29-1 to GLRaV IIb (Nyon). Non decorated filaments (arrows) are fragments of grapevine virus C particles that contaminated the virus culture. Bars = 250 nm.

Thus, the current status of grapevine clostero-like viruses can be summarized as follows :

(i) GLRaV-1 and GLRaV-3 are by far the most widespread among the grapevine clostero-like viruses and are both so consistently and intimately connected with leafroll disease, including the leafrolling syndrome shown by *V. riparia* (GLRaV-1), that they can be regarded as genuine agents of leafroll. The word "associated" may soon be dropped from their name.

(ii) GLRaV-2 seems less commonly encountered than the above viruses, from which it differs because of the outward appearance of the particles and the lower M_r of CP subunits. It may be associated both with leafroll syndromes and graft incompatibility disorders.

(iii) GLRaV-4 and GLRaV-5 are apparently much less represented in nature and less known than the other viruses. Their association with leafroll rests largely on circumstantial evidence.

(iv) GLRaV-6 is the least known among the clostero-like grapevine viruses. Whether it is associated, and to what extent, with leafroll symptoms remains to be established.

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