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Production and use of an antiserum to grapevine virus B capsid protein purified from SDS-polyacrylamide gels

by

D. E. Goszczynski, G. G. F. Kasdorf and G. Pietersen

Agricultural Research Council, Plant Protection Research Institute, Pretoria, Republic of South Africa

Summary: Antiserum to electrophoretically-separated capsid protein of grapevine virus B (GVB) was produced. After easy and effective elimination of antibodies cross-reactive with grapevine virus A (GVA), the antiserum was successfully used in ELISA for the detection of GVB in grapevines.

Key words: grapevine virus B, antisera, ELISA.

Introduction

Grapevine virus B (GVB) is a trichovirus (Martelli *et al.* 1994) involved in the aetiology of the worldwide-spread and economically important corky bark disease of grapevines (Bovey *et al.* 1980; Bovey and Martelli 1992; Boscia *et al.* 1993; Bonavia *et al.* 1996).

Physico-chemical and serological properties of GVB were determined following the isolation of the virus by mechanical transmission from grapevines to herbaceous species (Boscia *et al.* 1993 and 1994; Goszczynski *et al.* 1996). However, antiserum to GVB which could be used in ELISA was not available because of the poor immunogenicity of the virus and the difficulty in its separation from plant proteins using standard purifications methods (Boscia *et al.* 1994; Goszczynski *et al.* 1996). Recently, monoclonal antibodies to GVB were produced and a sensitive ELISA was developed (Bonavia *et al.* 1996). However, production of monoclonal antibodies is expensive, labor intensive and requires specially equipped laboratories and trained personnel. In addition, the high specificity of monoclonal antibodies can be disadvantageous as viruses which differ even in a single amino acid in the capsid protein may escape detection (Van Regenmortel 1982; Pappu *et al.* 1995).

In this paper we report a simple, cheap and quick method to prepare antiserum to GVB which can be used for the specific detection of this virus by ELISA.

Materials and methods

Viruses and their purification: GVB isolate 94/971 and grapevine virus A (GVA) isolate 92/778 and their purification procedures from *Nicotiana benthamiana* were as described by Goszczynski *et al.* (1996). Low speed centrifugation of preparations of viruses at the final purification step was omitted. Viruses prepared this way are referred to as partially purified.

SDS-PAGE, Western Blot and IEM were done as described by Goszczynski *et al.* (1996). A Dual-mini vertical unit (American Bionetics Inc., USA) was used for electrophoresis, unless otherwise stated. Leaf extracts from systemically virus-infected or healthy *N. benthamiana* were used in Western blots.

Electrophoretic separation of capsid proteins of GVB: Preparations of partially purified GVB, concentrated about 6 times by freeze-drying were subjected to overnight electrophoresis in 13 cm SDS-polyacrylamide gels at 60 V. The gels were stained with 0.05 % Coomassie brilliant blue R-250 (prepared in water) for 10-15 min as described by Harlow and Lane (1988). Following destaining of gels in water for 10-30 min, the GVB capsid protein band, unlike standard method of Laemmli (1970), is visible as a less intensively stained band on a stained gel background. The band was cut out, freeze-dried and pulverised in a mortar.

Production of antisera: Antiserum to electrophoretically separated capsid protein of GVB was prepared by 4 subcutaneous injections of rabbit (spaced 2, 4 and 4 weeks apart) with polyacrylamide-containing virus protein (resuspended in distilled water) mixed with complete (first injection) and incomplete (subsequent injections) Freund's adjuvant. Blood was collected 3 weeks after the last injection.

Antiserum to glutaraldehyde-fixed GVB was prepared by 3 intramuscular injections, 2 weeks apart, of goat or rabbit with preparations of fixed virus mixed with complete (first injection) or incomplete (subsequent injections) Freund's adjuvant. Blood was collected 2 weeks after the last injection. Fixed GVB was prepared as described by Bem and Murant (1979) from partially purified preparations of the virus. After final dialysis, precipitated virus was pelleted by low-speed centrifugation and resuspended in 1/10 volume of buffer.

Correspondence to: Dr. D. E. Goszczynski, Agricultural Research Council, Plant Protection Research Institute, Private Bag X134, Pretoria 0001, Republic of South Africa. Fax: +27-12-8081489. E-mail: nipbdg@plant1.agric.za

Cross-adsorption of antiserum to capsid protein of GVB with GVA: The preparation of partially purified GVA was incubated in a boiling water bath for ca. 6 min to precipitate proteins. After centrifugation at 10,000 *g* for 10 min, the pellet was resuspended in 9 ml of PBS (pH 7.4) and mixed with 1 ml of antiserum. The suspension was incubated overnight at 4 °C with gentle shaking and then centrifuged at 10,000 *g* for 20 min. The supernatant was mixed 1:1 with glycerol and stored at -20 °C. The preparation, referred to here as "adsorbed antiserum" was used at a dilution of 200 times, and was equivalent to a dilution of 4000 times the original crude antiserum.

ELISA: Plate-trapped antigen (PTA) and antibody-trapped antigen (ATA) ELISAs were as described by TORRANCE (1992). In ATA-ELISA, immunoglobulins (0.5 μg ml^{-1}) from goat antiserum to glutaraldehyde-fixed GVB were used for coating the plates. Monoclonal antibody (MAB1) to GVB (BONAVIA *et al.* 1996), kindly donated by D. BOSCIA (Universita Degli Studi di Bari, Dipartimento di Protezione delle Piante Dalle Malattie, Italy), was diluted 4000 times. Goat anti-rabbit (GAR) and goat anti-mouse (GAM) alkaline phosphatase conjugates (Sigma, St. Louis, USA) were diluted 1000 times. Absorbance (A_{405}) values were measured following 50 min of substrate incubation unless otherwise stated. The samples were considered virus-infected if absorbance values were twice that of the mean of the healthy controls.

Grapevines and their preparation for ELISA: Corky bark positive and healthy grapevines were obtained from Plant Quarantine Station, Stellenbosch and KWV (Kooperatiewe Wynboversvereniging), Paarl, respectively. Dormant grapevine canes for testing by ELISA were collected in August 1996 and March 1997. Extracts from grapevine cane phloem were prepared as described by GOSZCZYNSKI *et al.* (1995) by using 1 g of cane shavings with 5 ml of standard extraction/conjugate buffer (CLARK and ADAMS 1977).

Results and Discussion

SDS-PAGE analysis of concentrated preparations of partially purified GVB showed amongst other bands an intensively stained band of viral capsid protein (Fig. 1). Antiserum to this band used in Western blot of GVB-infected *N. benthamiana* strongly reacted not only with capsid protein of the virus but also with antigens of different electrophoretic mobility (Fig. 2). These were apparently different forms of virus capsid proteins, as no bands, except minor upper one were observed on Western blots in reaction of the antiserum with sap of healthy *N. benthamiana* (Fig. 2).

Despite a strong reaction in Western blots, the antiserum to capsid protein of GVB, unlike analogous antisera to GLRaV-1 and GLRaV-3 (GOSZCZYNSKI *et al.* 1995), did not decorate the virus particles in IEM. This indicates that the

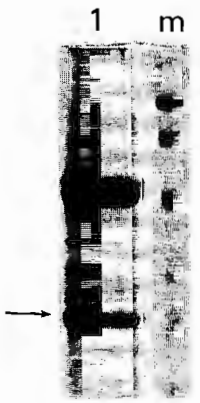

Fig. 1: SDS-PAGE analysis of a concentrated preparation of partially purified GVB (1). m = prestained SDS-PAGE standards (Bio Rad, low range). The arrow indicates position of the capsid protein of GVB. The gel was stained as described by LAEMMLI (1970).

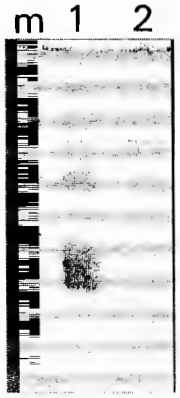

Fig. 2: Western blot analysis of antibody activity of antiserum to electrophoretically-separated GVB. Extracts of virus-infected (1) and healthy (2) *N. benthamiana* were used. m = prestained SDS-PAGE standards.

antiserum contains antibodies mainly against cryptotopes of GVB.

The antiserum clearly cross-reacted with GVA in Western blot. This was reported also for other antisera to GVB (GOSZCZYNSKI *et al.* 1996). The cross-reactivity was also observed in ELISA (Tab. 1). Overnight incubation of the antiserum with a heat-denaturated preparation of GVA followed by low-speed centrifugation effectively eliminated antibodies reactive with this virus.

Among a number of forms of ELISA tested using cross-adsorbed antiserum to capsid protein of GVB, indirect ATA-ELISA applying immunoglobulins from goat antiserum to glutaraldehyde-fixed virus for coating the plates was the most sensitive. This ELISA was used for the detection of GVB in 10 different grapevines cultivars all indexed CB positive and 3 healthy grapevines collected in two successive seasons. The samples were also tested by ATA-ELISA using monoclonal antibody (MAb1) to GVB as second antibodies. The ELISA tests were repeated 4 times. The results of ELISA using monoclonal antibody showed that all 10 CB-positive grapevines were infected with GVB confirming the association of GVB with this disease (BOSCIA *et al.* 1993; BONAVIA *et al.* 1996). Only 9 of them were infected according to ELISA based on polyclonal antibodies. The ELISA using monoclonal antibody was likely to be more

Table 1

Detection of GVB by PTA-ELISA using unadsorbed or cross-adsorbed antiserum to capsid protein of the virus

Antigen (*N. benthamiana* leaf extracts)	Antiserum	
	Unadsorbed	Cross-adsorbed
GVB	2.520[1]	1.184
GVA	0.850	0.026
Healthy	0.085	0.027

[1] Absorbance values (A_{405}) obtained after 25 min of substrate incubation.

sensitive as absorbance values obtained for GVB isolates in this ELISA were usually more than two times higher than in ELISA based on polyclonal antibodies (Tab. 2). Interestingly, this was not the case for the isolate of GVB from grapevine cv. Shiraz, which consistently yielded similar absorbance values by these two ELISAs. The most probable reason for this result is that GVB isolate from cv. Shiraz is serologically different from other GVB isolates under study. The reaction of antisera used in ELISA with GVB isolate from cv. Shiraz can be regarded as homologous as GVB was mechanically transmitted to *N. benthamiana* from this grapevine, and named GVB isolate 94/971 (Goszczynski *et al.* 1996), to which the antisera applied in this study were produced. The existence of GVB isolates with different antigenic structure has been reported earlier by Bonavia *et al.* (1996).

Although our ELISA based on polyclonal antibodies showed lower sensitivity of detection of GVB than ELISA using monoclonal antibody, the results presented in this work show that the antiserum to electrophoretically separated capsid protein of GVB has diagnostic value and can be used in ELISA. This antiserum can be prepared in standardly equipped laboratories in a relatively short time and at low cost, which is a clear advantage over production of monoclonal antibodies. It is necessary to point out that in the present study only one animal was immunized with the capsid protein of GVB. As immunological responses to injected antigen can be very variable amongst outbred animals like rabbits (Van Regenmortel 1982), it is possible that a better antiserum than the one described here can be obtained.

Table 2

Detection of GVB in various grapevines by ATA-ELISA based on only polyclonal (PAb) or polyclonal and monoclonal (MAb) antibodies

Grapevines	Absorbance (A_{405}) PAb	MAb
Corky bark positive:		
Shiraz	1.544[1]	1.863
	1.396[2]	1.528
Cabernet Sauvignon	2.671	3.872
	0.626	1.563
Riparia Gloire de Montpellier	0.264	0.599
	0.122	0.267
Colombard	0.150	0.559
	0.209	0.838
Cape Riesling	0.509	1.441
	0.367	0.912
Pais	0.075	0.169
	0.156	0.459
Queen of the Vineyard	0.275	0.647
	0.406	0.930
Alphonse Lavallée	0.338	1.041
	0.311	0.815
Jubily	0.626	1.455
	0.256	0.562
LN33	0.104	0.307
	0.097	0.263
Healthy:		
Cabernet Sauvignon	0.062	0.076
	0.052	0.069
Cabernet Franc	0.063	0.080
	0.058	0.070
LN33	0.064	0.072
	0.058	0.073

[1,2] Absorbance values obtained for grapevines collected in August 1996 (1) and March 1977 (2).

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References

Bem, F.; Murant, A. F.; 1979: Host range, purification and serological properties of heracleum latent virus. Ann. Appl. Biol. **92**, 243-256.

Bonavia, M.; Digiaro, M; Boscia, D; Boari, A; Bottalico, G; Savino, V; Martelli, G. P.; 1996: Studies on "corky rugose wood" of grapevine and on the diagnosis of grapevine virus B. Vitis **35**, 53-58.

Boscia, D.; Abu-Ghanem, N.; Saldarelli, P.; Minafra, A.; Castellano, M. A.; Garau, R.; Savino, V.; Martelli, G. P.; 1994: A comparative study of grapevine virus B isolates. Riv. Pathol. Vege. Ser. V. **4**, 11-24.

--; Savino, V.; Minafra, A.; Namba, S.; Elicio, V.; Castellano, M. A.; Gonsalves, D.; Martelli, G. P.; 1993: Properties of a filamentous virus isolated from grapevines affected by corky bark. Arch. Virol. **130**, 109-120.

Bovey, R.; Martelli, G. P.; 1992: Directory of Major Virus and Virus-like Diseases of Grapevines. Mediterranean Fruit Crop Improvement Council and International Council for the Study of Viruses and Virus Diseases of the Grapevine, Bari.

- -; Gärtel, W.; Hewitt, W. B.; Martelli, G. P.; Vuittenez, A.; 1980: Virus and Virus-like Diseases of Grapevine. Payot Editions, Lausanne.

Clark, M. F.; Adams, A. N.; 1977: Characteristics of the micro-plate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. **34**, 475-483.

Goszczynski, D. E.; Kasdorf, G. G. F.; Pietersen, G.; 1995: Production and use of antisera specific to grapevine leafroll-associated viruses following electrophoretic separation of their proteins and transfer to nitrocellulose. Afr. Plant. Prot. **1**, 1-8.

- -; - -; - -; 1996: Western blots reveal that grapevine virus A and B are serologically related. J. Phytopathol. **144**, 581-583.

HARLOW, E.; LANE; D.; 1988: Antibodies, a Laboratory Manual. Cold Spring Harbor Laboratory Publications, Cold Spring Harbor, NY.

LAEMMLI, U. K.; 1970: Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature **277**, 680-685.

MARTELLI, G. P.; CANDRESSE, T.; NAMBA, S.; 1994: Trichovirus, a new genus of plant viruses. Arch. Virol. **134**, 451-455.

PAPPU, H. R.; PAPPU, S. S.; KANO, T.; KOIZUMI, M.; CAMBRA, M.; MORENO, P.; SU, H. J.; GARNSEY, S. M.; LEE, R. F.; NIBLETT, C. L.; 1995: Mutagenic analysis and localization of a highly conserved epitope near the amino terminal end of the citrus tristeza closterovirus capsid protein. Phytopathology **85**, 1311-1315.

TORRANCE, L.; 1992: Serological methods to detect plant viruses: production and use of monoclonal antibodies. In: DUNCAN, L.M.; TORRANCE, L. (Eds.): Techniques for the Rapid Detection of Plant Pathogens, 7-33. Blackwell Scientific Publications, Oxford.

VAN REGENMORTEL, M. H. V.; 1982: Serology and Immunochemistry of Plant Viruses. Academic Press, New York.

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