

SHORT COMMUNICATION  
IMMUNODETECTION OF THE 20 kDa PROTEIN ENCODED  
BY ORF 2 OF *GRAPEVINE VIRUS B*

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SUMMARY

A polyclonal antiserum was raised to the 20 kDa product encoded by ORF2 of *Grapevine virus B* (GVB) using a recombinant protein. A rabbit was immunized with the polypeptide expressed in *Escherichia coli* fused with glutathione-S-transferase. The antiserum successfully detected the 20 kDa protein in *Nicotiana occidentalis* plants in the late stages of infection, an accumulation pattern that differs substantially from that of GVB movement protein.

*Key words:* grapevine, polyclonal antiserum, virus, GVB, recombinant protein, immunodetection.

One of the differential traits that prompted the establishment of *Vitivirus* as a genus separate from *Trichovirus* was the presence of an extra gene (ORF2) coding for a 20 kDa protein, located between the genes encoding replication-associated and movement proteins, respectively (Martelli *et al.*, 1997). ORF2 was detected in the genome of *Grapevine virus A* (GVA) (Minafra *et al.*, 1997), *Grapevine virus B* (GVB) (Saldarelli *et al.*, 1996) and *Heracleum latent virus* (HLV) (unpublished information), all definitive species of the genus. The ORF2-encoded proteins of different GVB isolates have a highly conserved sequence (unpublished information) but show a lower similarity (about 44%) with that of the comparable product of GVA, and no significant sequence homology with available protein sequences from databases (Saldarelli *et al.*, 1996; Minafra *et al.*, 1997). Thus, computer-assisted sequence comparisons had given no hints on their possible function, which is still undetermined. Although the GVB 20 kDa protein was successfully expressed as a glutathione-S-transferase (GST) fusion protein in *Escherichia coli* and antisera were raised to the recombinant protein, all

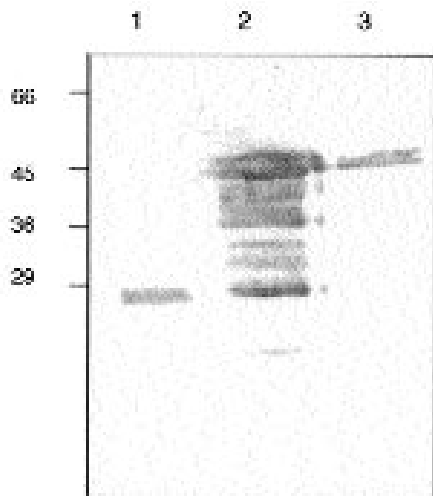
attempts to detect it serologically in total or fractionated protein extracts from GVB-infected *Nicotiana occidentalis* failed (Saldarelli *et al.*, 1996). A new set of experiments was therefore devised in which a newly produced polyclonal antiserum was used on total protein extracts from infected hosts up to 22 days post inoculation (d.p.i.).

The same *E. coli* clone of previous studies containing the pGEX 3X-ORF2 plasmid was induced with 0.1 mM IPTG (pyranoside) for 6 h and the resulting fusion protein was purified from total bacterial proteins by SDS-PAGE and recovered as previously described (Saldarelli *et al.*, 1996). Refolding of the eluted protein was favoured by dialysis for two days against phosphate buffered saline (PBS). Rabbit immunization and bleeding was as previously reported (Saldarelli *et al.*, 1996). The ORF2-encoded 20 kDa product in infected *N. occidentalis* was detected in total protein extracts from 200 mg of plant tissues homogenized in 1 vol. of Laemmli buffer (Deom *et al.*, 1990). SDS-PAGE and Western blot analysis were done out according to Sambrook *et al.* (1989), using antiserum cross-absorbed with total proteins from healthy plants.

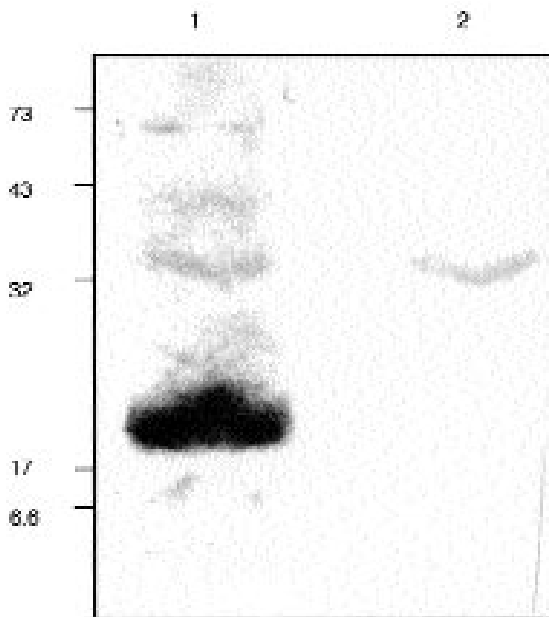
The antiserum raised proved to be highly specific recognizing both the ca 45 kDa ORF2-GST fusion product in total protein extracts of the *E. coli* clone carrying the recombinant plasmid (Fig. 1, lane 2) and the 27 kDa GST expressed by pGEX 3X recombinant bacteria (Fig. 1, lane 1). The reaction with eluted fusion protein used as injected antigen was also clear-cut detecting a single product about 45 kDa in size (Fig. 1, lane 3).

This antiserum was used for monitoring the accumulation pattern of the GVB 20 kDa protein in total protein extracts from top leaves of *N. occidentalis* inoculated at the 6 true leaf stage and collected from 3 to 22 d.p.i. at 2 or 3 day intervals. No positive Western blot reactions were observed in two separate experiments up to the last sampling (22 d.p.i.) when a product of the expected size was clearly recognized by the antiserum (Fig. 2, lane 1). No comparable product was present in extracts from healthy leaf tissues of the same age (Fig. 2, lane 2).

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**Fig. 1.** Immunodetection of GVB ORF 2 in *E. coli*. Total protein extracts from bacteria containing plasmid pGEX 3X (lane 1) and GVB ORF2-pGEX 3X recombinant plasmid (lane 2) after IPTG induction. Purified GVB ORF2-GST fusion protein is in lane 3. Molecular size markers on the left of the panel.



**Fig. 2.** Western blot detection of GVB 20 kDa protein. Total protein extracts from GVB-infected (lane 1) and healthy (lane 2) *N. occidentalis* plants 22 days post inoculation. Molecular size markers on the left of the panel.

Previous unsuccessful attempts to detect GVB 20 kDa protein in infected hosts monitored up to 12 d.p.i. were explained admitting that either this protein was expressed transiently, or in quantities below the level detectable with the extraction technique used (Saldarelli *et al.*, 1996). The quality of the antisera used for immunodetection was also questioned. The present results indicate that previous failures were due simply to the accumulation of this product in detectable amounts in very late stages of infection.

Movement proteins of the same virus appear as early as 3 to 6 d.p.i. in certain cell compartments (*i.e.* crude membrane and cell wall fractions) with progressive enrichment till the late stages of infection (*i.e.* 20 d.p.i.) (Saldarelli *et al.*, 2000a). The accumulation pattern of GVB 20 kDa protein clearly differs but still gives no hints on its biological function. To unravel this, mutagenesis experiments with infectious cDNA GVB clones (Saldarelli *et al.*, 2000b) are now underway.

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