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Some properties of a hitherto undescribed filamentous virus of the grapevine

by

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S u m m a r y : An apparently new, non mechanically-transmissible clostero-like virus, for which the name "grapevine leafroll-associated virus 7" (GLRaV-7) is proposed, was found in Albanian grapevine accessions. Virus particles were filamentous, had conspicuous cross banding and a length of 1500-1700 nm. Virions had coat protein subunits with an estimated M_r of ca. 37 kDa and a ssRNA genome with size of ca. 19.5 kb as deduced from the estimate of dsRNA (ca. 19.5 kbp) extracted from grapevine tissues. A virus-specific antiserum was raised, which decorated virions at a dilution of 1:1000. This antiserum did not recognize particles of any of the six grapevine leafroll-associated clostero-like viruses (GLRaV-1 to -6) known to date, nor of grapevine trichovirus A (GVA) and B (GVB). Grapevine indicators graft-inoculated with material from accessions containing GLRaV-7 reacted with mild leafroll-like symptoms. In a survey in which 2226 vines from 30 different countries were examined by ELISA, GLRaV-7 was found in 141 plants from Albania, Greece, Hungary, Egypt, and Italy.

K e y w o r d s : closterovirus, serology, ELISA, leafroll.

Introduction

Among the material collected in Albania in the course of repeated surveys for virus diseases of the grapevine (MERKURI *et al.* 1994), an unidentified white-berried cultivar denoted AA42 that did not show any symptoms, was found to contain filamentous virus-like particles. These particles were not recognized by antisera raised to five of the grapevine leafroll-associated viruses (GLRaV-1 to -5) and to the virus then known as grapevine corky bark-associated (GCBaV), now synonimized with GLRaV-2 (BOSCIA *et al.* 1995). This virus, referred to as V-AA42, was therefore investigated in more detail and, as reported in the present paper, proved to be a novel member of the large family of grapevine clostero-like viruses.

Materials and methods

Mechanical transmission of V-AA42 to herbaceous plants (*Nicotiana benthamiana, N. clevelandii, N. occidentalis, N. cavicola, N. rotundifolia, Chenopodium quinoa, Gomphrena globosa*) was attempted using the following inocula: (i) sap from grapevine tissues of different organs (leaves, petioles, roots) of glasshouse-grown, rooted cuttings or *in vitro*-grown explants (MONETTE and GODKIN 1993) extracted with 0.01M phosphate buffer pH 7.0, with the addition or not of 3 % nicotine or 0.01 M cysteine HCl; (ii) micropurified extracts from cortical scrapings of mature canes (GUGERLI *et al.* 1984); (iii) total nucleic acid extracts (WHITE and KAPER 1989).

V-AA42 was purified from batches of at least 60 g of cortical scrapings as described by NAMBA *et al.* (1991), with two final cycles of sucrose Cs_2SO_4 cushion-step gradient centrifugation. Virus-containing bands were collected with a syringe and dialyzed against 0.1M Tris-HCl buffer pH 7.5. Purified virus suspensions were mixed with an equal volume of 0.1M Tris-HCl buffer pH 6.8 containing 5 % sucrose, 2.5 % SDS and 5 % 2-mercaptoethanol, and dissociated by boiling for 2 min. Coat protein (CP) preparations thus obtained were analysed in discontinuous polyacrylamide gel electrophoresis and Western blotting, as described by LAEMMLI (1970) and HU *et al.* (1990). Their molecular weight was calculated using a commercial kit of pre-stained markers (MW-SDS-Blue, Sigma Chemical Co., St. Louis). After overnight transfer, Western blot membranes were blocked with 5 % non fat milk powder dissolved in phosphate-buffered saline (PBS) and incubated overnight at 4 °C with the virus-specific antiserum obtained as specified below. Membranes were then washed in PBS with 0.05 % Tween 20 and incubated with a Bio Rad Gold Enhancement Kit.

A polyclonal antiserum was raised in a rabbit immunized with two subcutaneous injections of Cs_2SO_4-purified preparations emulsified in incomplete Freund's adjuvant, given at weekly intervals and followed by a boosting intravenous injection. Antiserum collection began one week after the last injection.

Double-stranded RNA (dsRNA) was recovered by phenol extraction from batches of 20 g of cortical scrapings and purified by chromatography on cellulose CF11 columns (DODDS 1993). After enzymatic digestion with DNase and RNase as described by SALDARELLI *et al.* (1994), preparations were analysed in 6 % polyacrylamide gel electrophoresis and stained with silver nitrate. Markers for size estimation were dsRNAs from grapevine trichovirus A

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(GVA)-infected *N. benthamiana* plants and a GLRaV-3-infected grapevine.

Particle length was determined on micropurified virus preparations obtained either from leaf petioles or cortical scrapings according to GUGERLI *et al.* (1984). Preparations were mounted on carbonated grids pre-coated with V-AA42 antiserum diluted 1:500, stained with 2 % aqueous uranyl acetate and viewed with a Philips 201C electron microscope. TMV particles were used as internal calibration standard. Immune electron microscopy (IEM and ISEM) was used according to MILNE (1993) and ELISA according to CLARK and ADAMS (1977).

In spring 1995 virus-free *Vitis rupestris*, LN 33, Kober 5 BB, 110 R and *V. vinifera* cv. Cabernet Sauvignon were graft-inoculated with material from three white-berried grapevine accessions two of which, as indicated by ELISA testing of crude and micropurified cortical scraping extracts, seemed to contain only V-AA42, and one contained V-AA42 and GVA.

Results and discussion

All attempts to transmit V-AA42 to herbaceous plants failed. Therefore we characterized virus extracted and purified from tissues of a couple of grapevine accessions in which neither trichoviruses nor clostero-like viruses other than V-AA42 were apparently present.

Virus particles were filamentous (Fig. 1 A), exhibited the conspicuous cross banding and the open structure (Fig. 1 B) typical of species of the *Closterovirus, Trichovirus*, and *Capillovirus* genera, and a length within the range reported for closteroviruses (CANDRESSE and MARTELLI 1995). The 110 particles that were individually measured spanned 600–2100 nm in length. Of these, 71 (65 %) were 1500 to 1700 nm long, with a peak of 46 (42 %) measuring 1500 nm. These values may be biased by the fact that measurements were performed on virus preparations that had undergone treatments (i.e., micro-purification) likely to damage particle integrity. On the other hand, the virus concentration in infected grapevines was such that only an exceedingly low number of virions (often fragmented anyhow) could be observed in leaf dip ISEM.

Low virus concentration and recovery impaired all attempts to obtain a direct estimate of the molecular weight of coat protein and nucleic acid from dissociated viral preparations resolved by gel electrophoresis. However, Western blots in which a V-AA42-specific antiserum was used, indicated that viral coat protein subunits migrated as a major band (Fig. 2) with an estimated M_r of ca. 37 kDa, thus within the range of values reported for all GLRaVs, except for GLRaV-2 (ZIMMERMANN *et al.* 1990; GUGERLI and RAMEL 1993; BOSCIA *et al.* 1995).

The V-AA42 dsRNA electrophoretic pattern was as complex as with other closteroviruses (DODDS and BAR-JOSEPH 1983), including those infecting grapevines (REZAIAN *et al.* 1991; HABILI and REZAIAN 1995), and exhibited multiple bands (not shown). The largest dsRNA species, interpreted as the full-genome replicative form, migrated at the same rate as the largest dsRNA of GLRaV-3, reported to have a size of ca. 19.5 kbp (HABILI *et al.* 1995).

The antiserum to V-AA42 was virus-specific, but prior to use in ELISA it was pre-absorbed with healthy plant antigens. In IEM tests carried out at Bari all particles in preparations from vines used for micropurification were decorated. The antiserum decorated homologous virions (Fig. 1 C) at a dilution of 1:1000, but not particles of GVA, grapevine trichovirus B (GVB), GLRaV-1, GLRaV-2 and GLRaV-3. Similar results were obtained with ELISA tests in which in addition to the above, sources infected by GLRaV-5 were also tested. These findings were complemented by the results of ELISA and IEM tests conducted by C. GREIF at Colmar (France) and by P. GUGERLI at Nyon (Switzerland) where no reaction was observed with GLRaV-4, GLRaV-5 and GLRaV-6 (= GLRaV-IIa, BOSCIA *et al.* 1995).

In autumn 1995, some vines of LN 33 and Cabernet Sauvignon graft-inoculated with material from grapes infected with V-AA42 showed mild leafroll symptoms (i.e., reddening and rolling of the leaves).

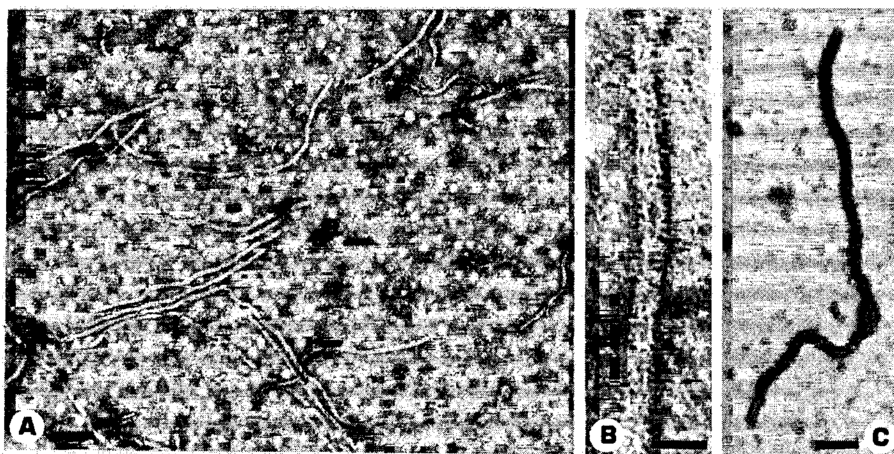

Fig. 1: **A** Purified preparation of GLRaV-7 mounted in uranyl acetate; bar = 100 nm. **B** Detail of a virus particle showing cross banding; bar = 30 nm. **C** Particle of GLRaV-7 heavily decorated by the homologous antiserum; bar = 100 nm.

This fact, and the negative serological reactions obtained in our and foreign laboratories, make it plausible to conclude that V-AA42 is a putative closterovirus capable of eliciting a red leaf syndrome. This virus appears to belong to the family of comparable grapevine viruses endowed with CP subunits of M_r much higher (GUGERLI and RAMEL 1993) than that of other species of the *Closterovirus* genus (CANDRESSE and MARTELLI 1995) but differs serologically from all of them. Therefore, the provisional name of grapevine leafroll-associated virus 7 (GLRaV-7) is proposed.

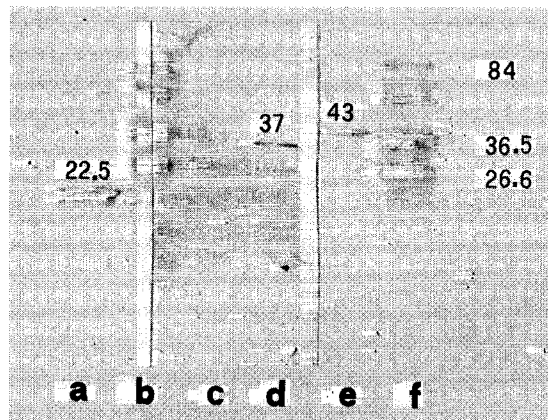

Fig. 2: Western blot of dissociated coat protein of GVA (lane a), GLRaV-7 (lane d) and GLRaV-3 (lane e). Pre-stained mol. wt. markers in lane b and f. Healthy grapevine extract (control) in lane c. Figures are the M_r of viral coat protein subunits and of markers expressed as kDa.

A survey in which grapevine accessions from 30 different countries were investigated for the presence of GLRaV-7, showed that it occurred in slightly more than 6 % of the samples (141 infected vines out of 2226). It was found occasionally in accessions from Egypt, Hungary and Greece but it was much more frequent in Albania (34 % of 41 samples). GLRaV-7 was not detected in any of 272 vines of different cultivars native to the southern Italian mainland but, intriguingly, it was rather frequent (20 % of 54 samples) in the cultivars of the island of Ischia (Southwestern Italy). In Apulia (Southeastern Italy), GLRaV-7 was only found in the variety Victoria, a white-berried table grape of Bulgarian origin introduced a few years ago via Greece. Six vineyards planted with Victoria, originating directly from different Greek sources, were surveyed by ELISA (ca. 150 vines sampled at random in each planting.) Three of these vineyards were found to be GLRaV-7-free, whereas three had GLRaV-7 infection rates ranging from 14 to 37 %.

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