Association of Maize Rough Dwarf Virus with Mal de Rio Cuarto in Argentina

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ABSTRACT

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Mal de Rio Cuarto (MRC) is a severe disease of maize in Argentina. Its symptoms are like those caused by maize rough dwarf virus (MRDV), which has hitherto been reported only from the Mediterranean and surrounding regions. Samples of plants affected with MRC were examined by immunoelectron microscopy and by polyacrylamide gel electrophoretic analysis of double-stranded RNA. The tests showed the presence in diseased plants of MRDV-like particles that reacted with MRDV antisera, and of double-stranded RNA species corresponding in electrophoretic pattern with MRDV RNA. MRC is, therefore, associated with and likely to be caused by a strain of MRDV.

Additional key words: double-stranded RNA, fijivirus, reoviridae.

Maize rough dwarf virus (MRDV), a member of the fijivirus group within the Reoviridae, causes severe disease in maize (*Zea mays* L.), particularly in modern hybrids of North American origin. The virus has been reported only from the Mediterranean basin and surrounding countries (12). The isometric virus particles, 65–70 nm in diameter, easily degrade to form subviral particles (SVPs) 50 nm in diameter, bearing elements designated as B spikes (4–6,10,12). Recently, very serious losses to maize grown in Argentina have been caused by a disease named Mal de Rio Cuarto (MRC), which produces symptoms like those of MRDV (15,16).

The purpose of the research reported here was to isolate and describe the viruslike SVPs from MRC-affected plants and to compare them morphologically and serologically to MRDV.

MATERIALS AND METHODS

Leaf samples from MRC-diseased and healthy maize plants, separately excised leaf enations from diseased maize plants, and samples of the graminaceous weed *Digitaria sanguinalis* (L.) Scop., also bearing MRC symptoms, were collected in the field in Argentina, air-dried, and sent to Italy for examination. Samples of MRDV-infected maize, maintained in the glasshouse in Italy, were also air-dried and used for comparison.

Subsamples of the material were crushed in a small volume of water, negatively stained in uranyl acetate, and examined by transmission electron microscopy. Other subsamples were tested with various antisera by using immunosorbent electron microscopy (ISEM) and the decoration technique (11,13). The ISEM technique was applied at 22 C, using antiserum dilutions of 1/1,000 in 0.1 M phosphate buffer (pH 7), serum coating times of 5 min, and particle-trapping times of 15 min. The antisera used were: a serum (A50) reacting with MRDV SVPs (9); a serum (A166) reacting with both SVPs and virions of MRDV (2); a serum reacting with pangola stunt virus (PaSV) SVPs (3); a serum reacting with oat sterile dwarf virus (OSDV) SVPs (7); and a serum reacting with rice ragged stunt virus (RRSV) particles (14). The decoration technique was applied to particles already trapped by using antiserum A50. The decorating antiserum was applied for 15 min at 22 C, at dilutions from 1/2 to 1/1,280 in 0.1 M phosphate buffer, pH 7.

Samples of enations and of healthy leaves were processed to extract any high-molecular-weight dsRNA (7) and this was analyzed by electrophoresis in 5% polyacrylamide gels (PAGE) (1). RNA from MRDV and PaSV were used as standards.

RESULTS

Samples of MRC-infected maize leaves, maize leaf enations, and leaves of *D. sanguinalis* each contained particles (~50 nm in diameter) that were indistinguishable from the B-spiked SVPs of MRDV (Fig. 1A). Approximately three particles per 1,000 μm^2 of support film were seen in the enation material, compared with an approximately fivefold higher number in air-dried MRDV leaf enation material. In the MRC-infected samples, no particles corresponding to intact fijivirus particles were seen, whereas in MRDV-infected air-dried samples, about one particle in 10 was intact. No viruslike particles were seen in samples of healthy leaves of maize or of *D. sanguinalis* from Argentina.

In ISEM experiments with MRC maize leaf enation material, the anti-MRDV serum A50, the anti-RRSV serum or buffer alone trapped about 20, one and two SVPs per 1,000 μm^2, respectively. Both MRDV antisera A50 and A166 and the PaSV antiserum strongly decorated the MRC-associated SVPs, whereas the OSDV and RRSV antisera gave no decoration at any dilution (Fig. 1B–D). The decoration titers (highest dilution giving a detectable antibody halo) of the two MRDV antisera were compared with MRC-associated or MRDV SVPs. The MRC-associated SVPs gave titers one or two twofold steps lower than did the homologous MRDV SVPs (Table 1).

The PAGE analysis of the RNAs extracted from MRC leaf enations showed the presence of fijiviruslike dsRNA. In the system used, the RNAs of MRDV-, PaSV-, and MRC-affected tissue yielded very similar patterns (Fig. 2). The 10 genome segments of MRDV RNA separated into eight bands, with segments 2, 3, and 4 forming two bands. PaSV RNA gave a slightly different pattern, with the same segments running as one band. RNA from MRC-affected tissue resembled MRDV rather than PaSV RNA in this respect.

DISCUSSION

Our results show that maize plants affected with MRC contain particles indistinguishable from the B-spiked SVPs of fijiviruses. The particles are closely related serologically to MRDV and PaSV SVPs, which are themselves closely related serologically (3). The slight lowering of titer shown by both the MRDV antisera when reacting with MRC-associated SVPs could mean that these are not

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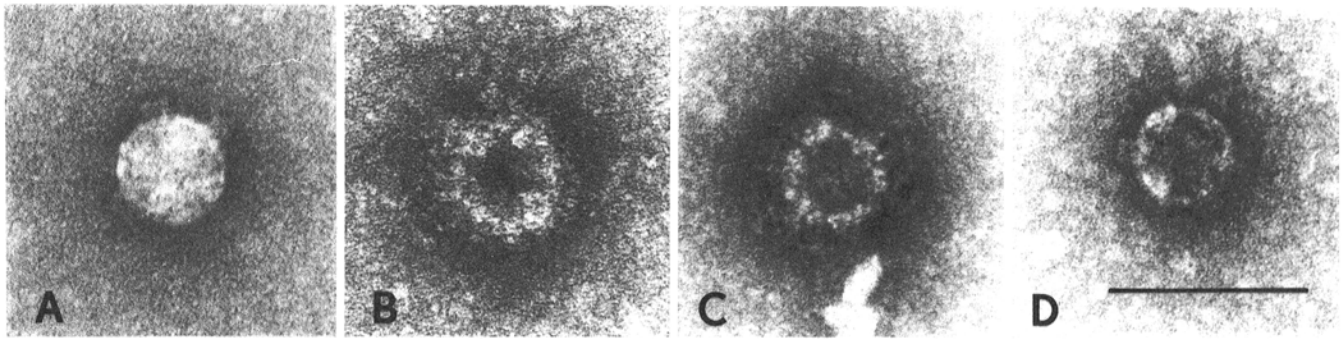

Fig. 1. Electron micrographs of subviral particles from Argentine maize plants affected with Mal de Rio Cuarto. Negatively stained with uranyl acetate. The bar represents 100 nm. **A,** A particle without antiserum treatment; **B and C,** particles incubated with maize rough dwarf virus antisera A50 at dilution 1/10 and A166 at dilution 1/40, respectively; **D,** a particle incubated with oat sterile dwarf antiserum at dilution 1/10. Note the antibody halos in B and C; these are absent in A and D.

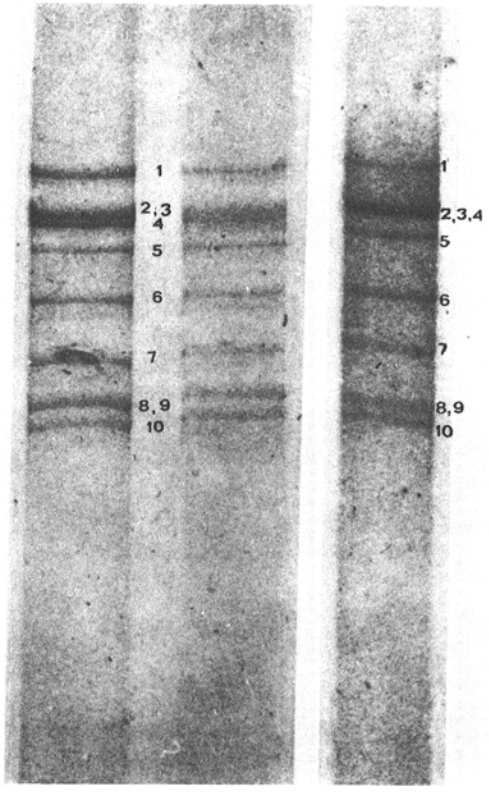

Fig. 2. Polyacrylamide (5%) slab gels showing co-electrophoresis of RNAs from Mal de Rio Cuarto-affected maize (left), maize rough dwarf virus (center), compared with RNA from pangola stunt virus (right). Numbers indicate the genome segments.

serologically identical to the MRDV SVPs, or that the MRC antigens were slightly damaged in transit. At present, we cannot distinguish between these alternatives.

The PAGE results support the morphological and serological evidence for the association of MRC with a fijivirus. The bands obtained showed that the material contained a virus very similar to MRDV, less similar to PaSV, and quite distinct from other Reoviridae. It appears that, weight for weight, MRC material contained less viral RNA than is generally found with MRDV. With MRDV, 1 g (fresh weight) of enations provides enough dsRNA to load three to six gels, whereas 0.2 g of air-dried MRC enations was insufficient for only one gel.

The low dsRNA yield from MRC-diseased maize correlated with other findings. Thus, in crude electron microscope preparations we found about five times fewer particles than in similarly prepared material from MRDV-infected maize; yields of purified MRC-associated SVPs were also lower than expected for MRDV (F. Nome and E. Dal Bó, *unpublished*).

TABLE 1. Titers[a] of two maize rough dwarf virus (MRDV) antisera reacted with MRDV or Mal de Rio Cuarto (MRC)-associated subviral particles

Antigen	Antiserum titer	
	MRDV antiserum A50	MRDV antiserum A166
MRDV	1/640	1/1,280
MRC	1/160	1/640

[a]Estimated by immunoelectron microscopy.

The diseased plants of *D. sanguinalis* also appeared to contain the same particles as MRC-affected maize. In Argentina, this weed host may therefore prove to be an important reservoir of the virus, as it is in Northern Italy (8).

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