Serological Relationships Among Four Australian Strains of Sugarcane Mosaic Virus as Determined by Immune Electron Microscopy

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ABSTRACT

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Serological relationships among the four Australian strains of sugarcane mosaic virus, johnsongrass (JG), sugarcane (SC), Queensland blue couch grass (BC), and sabi grass (Sabi), were compared using two variations of immune electron microscopy. Particles of each strain were counted after being trapped on grids coated with protein A and homologous and heterologous antisera (differential trapping) or were trapped with homologous antisera, coated with homologous and heterologous antisera (decoration), and photographed. Results obtained from the differential trapping and decoration methods both led to the conclusion that the JG strain is not related serologically to the other three strains, SC is closely related to BC, and Sabi but is distinct from both, whereas BC and Sabi are very closely related to each other. Results are discussed in light of previous reports that show that JG is related serologically to other strains of the virus.

From 23 naturally infected species of Gramineae in three states of Australia, Queensland, New South Wales, and Northern Territory, Teakle and Grylls (17) identified four strains of sugarcane mosaic virus (SCMV) based on natural and experimental host ranges, symptoms in certain differential plants, and antigenic properties. These strains were designated johnsongrass (JG), sugarcane (SC), Queensland blue couch grass (BC) and sabi grass (Sabi), each named after an important perennial host. Using microprecipitin serological tests, Teakle and Grylls (17) found that the JG strain was distantly related to the SC, BC, and Sabi strains but the latter three were very closely related to each other. Sabi was the only strain that infected a dicotyledonous plant species, namely French bean (17). In this paper, we report immune electron microscopic (IEM) investigations of the same four SCMV strains, demonstrating that 1) the JG strain is not related serologically to the other three strains, 2) the SC strain is related to the BC and Sabi strains but is distinct from both, and 3) the BC and Sabi strains are very closely related to each other.

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MATERIALS AND METHODS

The four SCMV strains were originally obtained from D. S. Teakle, University of Queensland, and maintained in their perennial hosts (4). For this investigation, they were transferred to sweet corn cultivar Iochief by mechanical inoculation. Systemically infected leaves were harvested 3 wk after inoculation, ground in a mortar with 10 ml/g of 0.1 M phosphate buffer, pH 7.0, and the extracts filtered through a nylon cloth.

Antisera to the four strains were the same as described previously (8,12) and had homologous titers of 1:512 (JG), 1:256 (SC and Sabi), and 1:32 (BC) in slide precipitin and microprecipitin tests.

The immunosorbent electron microscope technique (ISEM) used for trapping virus particles was one in which the copper-coated, formvar-filmed specimen grids are first coated with protein A (a wall protein of *Staphylococcus aureus*) and then with the specific antiserum (PA-ISEM) (3,12). The methods of preparing and processing the grids were essentially the same as described previously (12). The protein A (Pharmacia Fine Chemicals, Uppsala, Sweden) concentration (0.01 mg/ml) and antiserum dilution (in homologous combinations) for coating grids (1:50) were those recently found optimal for the technique (13). An antiserum dilution of 1:50 was also used for antibody coating of the virus particles (decoration) in each case. Dilutions of the antisera and protein A solution were prepared in the phosphate buffer. Particles of the four SCMV strains were trapped on grids and decorated by antisera to the four strains in all possible combinations; preimmune serum (dilution 1:50) was used as a control. Two percent aqueous solutions of phosphotungstic acid (pH 7.0) or uranyl acetate (pH 4.5) were used as stains. Particles were counted in a Hitachi HS8 electron microscope at ×15,000 using five fields of view on randomly selected squares on each of the two grids per treatment. The mean number of particles was calculated and multiplied by a relative area factor calculated for the electron microscope used to obtain the mean number of particles per 1,000-μm^2 area (10). To evaluate the decorating

ability of the antisera to the four strains in homologous and heterologous situations, particles were photographed at ×54,000.

RESULTS

When particles of each of the four SCMV strains were applied to grids coated with either homologous or heterologous antisera, the homologous combinations always trapped the maximum number of particles (Table 1). Antiserum to the JG strain did not selectively trap SC, BC, and Sabi strains; the number of particles counted was very similar to or less than that obtained using preimmune serum. Similarly, antisera to SC, BC, and Sabi strains did not selectively trap particles of the JG strain. There was very little difference between the number of particles of BC and Sabi strains trapped when either BC or Sabi antiserum was used, indicating that they are very closely related. The SC antiserum trapped a significant number of BC and Sabi particles. Similarly, BC and Sabi antisera trapped a larger number of particles of the SC strain than that obtained with this strain using preimmune serum, indicating a partial identity of SC to BC and Sabi strains.

When each of the four strains were trapped on grids using homologous antiserum, then decorated with homologous and heterologous antisera, the homologous antisera decorated the particles of their respective strains completely. One example is shown in Figure 1A. BC or Sabi particles were decorated to the same extent when either BC or Sabi antiserum was used. The BC and Sabi antisera decorated the SC particles only partially (Fig. 1B,C); however, the JG antiserum did not decorate the particles of SC (Fig. 1D), BC, or Sabi strains. Similarly, particles of the JG strain were not decorated by antisera to SC, BC, and Sabi strains.

Use of different dilutions of the JG antiserum for trapping (undiluted, 1:10, and 1:100) and for decorating (1:10, 1:100, and 1:500) particles of the SC, BC, and Sabi strains did not alter the results obtained at 1:50 dilution of this antiserum (Table 1, Fig. 1).

DISCUSSION

Our IEM investigation of the four SCMV strains has demonstrated that the BC and Sabi strains are very closely related serologically, the SC strain is related to BC and Sabi but is distinct, whereas the JG strain is not related to any of the other three. Similar interpretations were made when the four strains were used to test a highly sensitive new technique, the electroblot radioimmunoassay (EBRIA) (8,14). These interpretations contrast with those of Teakle and Grylls (17), who reported a distant serological relationship between the JG and the other three SCMV strains using microprecipitin tests. One explanation for the divergence of interpretations may be that PA-ISEM and EBRIA are highly specific techniques and are, therefore, incapable of detecting distant serological relationships between virus strains, although few reports published so far on the specificity of PA-ISEM do not support this view (5,9,16). It is possible, however, that PA-ISEM has a narrower specificity than microprecipitin tests.

Our IEM investigations did not reveal any serological relationship between the JG and the other Australian SCMV strains. The microprecipitin tests of Teakle and Grylls (17) indicated only a distant serological relationship between the former and the latter strains. In contrast, close serological relationships have been reported between the JG and non-JG strains of SCMV occurring in the United States (2,11,15). This indicates that the JG strain and its equivalent (maize dwarf mosaic virus-A) SCMV strains occurring in Australia and in the United States may be different. Observations of Snazelle et al (15) support this view. They found no reaction between antisera to the Australian JG isolates and antigens of several SCMV strains from the United States, including maize dwarf mosaic virus-A. Whatever the degree of serological relationship, it appears certain that the Australian JG is a strain of SCMV not only because of its similar biological properties (17) but also on the basis of structural properties of its coat protein (D. D. Shukla and K. H. Gough, *unpublished*).

The two variations of IEM used in this work, namely differential trapping and decoration, led to the same conclusion regarding the relationships among the four SCMV strains, indicating that either of the two methods can be used to differentiate strains of plant viruses. Our results show that the particle-trapping ability of an antiserum is similar to its ability to decorate particles of a given strain (Table 1, Fig. 1). Partial identity of

Table 1. Number of particles of johnsongrass (JG), sugarcane (SC), Queensland blue couch grass (BC), and sabi grass (Sabi) strains of sugarcane mosaic virus (SCMV) trapped per 1,000-μm^2 area of electron microscope grids coated with protein A plus antisera to each of the four strains

Antiserum	Number of particles of SCMV strains[a]			
	JG	SC	BC	Sabi
JG	5,320 ± 975	155 ± 183	89 ± 115	68 ± 106
SC	200 ± 195	3,205 ± 586	1,661 ± 318	1,424 ± 372
BC	178 ± 141	1,135 ± 221	2,648 ± 917	2,626 ± 1,048
Sabi	155 ± 150	1,135 ± 296	3,026 ± 435	3,294 ± 619
Preimmune serum	311 ± 215	244 ± 245	178 ± 140	111 ± 157

[a] Mean and standard deviation of five areas on each of two grids per treatment.

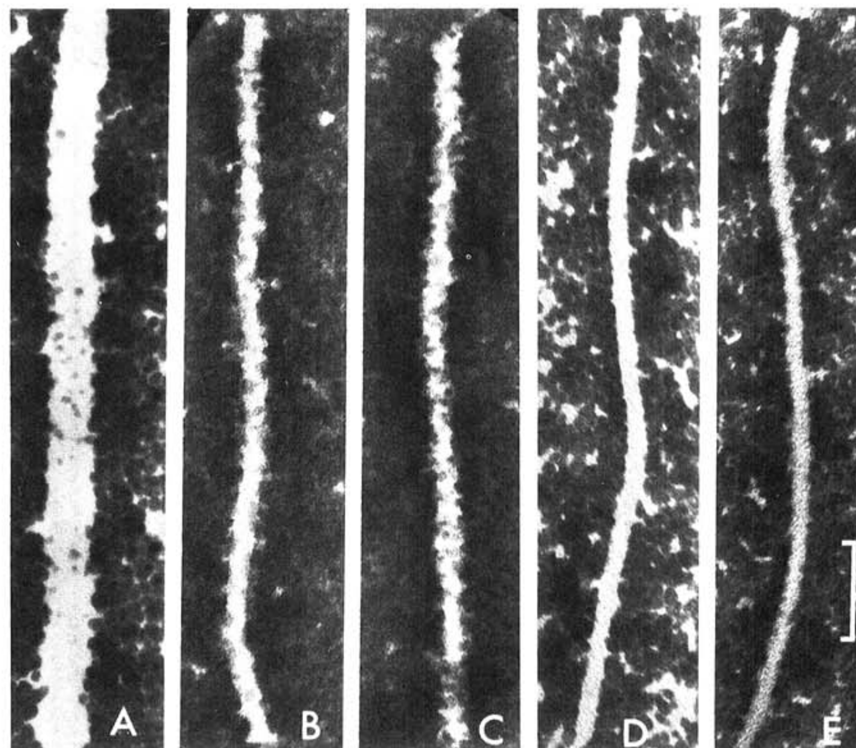

Fig. 1. Particles of the sugarcane (SC) strain of sugarcane mosaic virus trapped on grids coated with protein A + SC antiserum and decorated by antisera to **(A)** SC, **(B)** Queensland blue couch grass, **(C)** sabi grass, and **(D)** johnsongrass strains and **(E)** by a preimmune serum. The stain used was a 2% aqueous solution of phosphotungstic acid, pH 7.0. Scale bar = 100 nm.

a strain is revealed by partial decoration of the particles by antiserum of a related strain as was found among the SC, BC, and Sabi strains (Fig. 1B,C). Similarly, the number of particles trapped on grids from heterologous and homologous virus-antisera combinations (differential trapping) can be used to demonstrate the extent of relationships among strains of a plant virus. However, we have found differential trapping much simpler to use than the decoration method because with the former, the particles can be counted directly from the images on the electron microscope screen. In the latter case, it is difficult to determine, even at high magnification, the relative amount of particle decoration by antisera to different strains and it is necessary that the images of the particles be photographed (13). Moreover, the differential trapping method alone reveals relationships among strains quantitatively, whereas the decoration method gives only a qualitative assessment. The few comparisons available (5,6,16) indicate that the PA-ISEM used in this work may be more suitable for differential trapping experiments than the conventional Derrick-ISEM, where grids are coated with antiserum only (1,7). Furthermore, PA-ISEM is known to possess higher sensitivity and broader specificity than the Derrick-ISEM (3,5,12,16).

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