Occurrence in Sugarcane of a Bacilliform Virus Related Serologically to Banana Streak Virus

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ABSTRACT

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A virus with bacilliform particles measuring 131 × 31 nm was found in the sugarcane (*Saccharum officinarum*) clones Mex. 57-473 and CP 44-101 grown in Morocco. This virus was also present in a sample of Mex. 57-473 from Hawaii. No foliar symptoms were associated with sugarcane bacilliform virus (SCBV) infection. The virus was transmitted mechanically to healthy sugarcane, and infection was detected reliably by enzyme immunoassay. SCBV was not transmitted by sap inoculation to banana or to any test plants other than sugarcane. Banana streak virus (BSV) was transmitted by mechanical inoculation to sugarcane but induced no foliar symptoms. No serological differences were detected between SCBV and BSV, but lack of information on additional biological and other properties of the two viruses precludes the assumption they are identical. No serological relationship was detected between SCBV or BSV and the bacilliform viruses occurring in cacao, rice, *Canna indica*, yam, *Commelina diffusa*, and *Kalanchoë blossfeldiana*.

During electron microscopic examination of leaf dip preparations of clones of sugarcane (*Saccharum officinarum* L.) showing foliar symptoms of infection by sugarcane mosaic virus (SCMV), several collections of the clone Mex. 57-473 growing in field plots in northwestern Morocco were found to contain bacilliform viruslike particles. The particles resembled those of the group of viruses that includes banana streak (BSV) (8), cacao swollen shoot (CSSV) (1), rice tungro bacilliform (RTBV) (1), and rubus yellow net (RYNV) (7) viruses, as well as similar viruses associated with yam internal brown spot (4), Dendrobium leaf spot (10), "Alomae" disease of *Colocasia esculenta* (L.) Schott (6), top spotting of *Kalanchoë blossfeldiana* Poelln. (5), yellow mottle of canna (13), and symptoms in *Commelina diffusa* Burm. f. (9). This report describes the transmission, purification, and serological relationships of the bacilliform virus, tentatively named sugarcane bacilliform virus (SCBV), occurring in Morocco in the sugarcane clones Mex. 57-473 and CP 44-101.

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MATERIALS AND METHODS

Virus source. An isolate of SCBV was obtained from the clone Mex. 57-473 collected at Sidi Bouknadel, Morocco. The virus isolate was maintained in vegetatively propagated sugarcane and was also transferred by mechanical inoculation to virus-free plants of sugarcane clone CP 44-101. An isolate of BSV (8) used in these studies was obtained from field-infected Dwarf Cavendish banana (*Musa* sp.) free from infection by cucumber mosaic (CMV) and banana bunchy top (BBTV) viruses and was maintained by vegetative propagation.

Mechanical inoculation and host range. Mechanical inoculation was done using crude extracts obtained by grinding infected sugarcane or banana leaf tissue in 1% (w/v) K_2HPO_4 containing 0.2% (w/v) Na_2SO_3. Carborundum (600 mesh) was added to the crude extract, and this mixture was used to inoculate healthy test plants. Test plants and buffer-inoculated checks were kept in the greenhouse at 25–30 C for 3–18 mo after inoculation. Virus transmission was monitored by symptom expression, electron microscopy, and enzyme immunoassay (EIA).

Virus purification. SCBV was purified by a modification of the method described for BSV (8). Infected sugarcane leaf tissue, minus midrib, was finely chopped and homogenized in 2 vol (w/v) of 0.1 M Tris-citrate buffer, pH 7.4, containing 0.4% (w/v) Na_2SO_3 and 0.1% (v/v) Triton X-100. After a single cycle of differential centrifugation (12,000 *g* for 15 min, 185,000 *g* for 30 min), the unclarified pellets were resuspended overnight at 5 C in 0.1 M phosphate buffer, pH 6.3, containing 1.5 L of 2% (v/v) Celluclast (Novo Industri A/S, Bagsvaerd, Denmark) (12). The suspension was clarified by stirring with 30% (v/v) $CHCl_3$, followed by low-speed centrifugation (10,000 *g* for 10 min). Virus was precipitated from the clarified extract by ultracentrifugation (185,000 *g* for 30 min) through a 20% (w/v) sucrose cushion, then was purified further by centrifugation on a sucrose-CsCl gradient (3,8). Final virus preparations were resuspended in 0.01 M phosphate buffer, pH 7.2.

Antiserum preparation and serological tests. A rabbit anti-SCBV serum was prepared by intramuscular and subcutaneous injection of purified virus emulsified in Freund's incomplete adjuvant. Four series of injections were administered at 14-day intervals, and blood was collected by sacrificing the animal 1 mo after the final immunization. Antisera to BSV were prepared as described previously (8).

EIA and double-immunodiffusion tests in aqueous agarose gels were done as described for BSV (8). Partially purified preparations (i.e., after a single cycle of differential centrifugation) of SCBV and BSV were used as antigens in all cases. Controls consisted of similarly prepared extracts of uninfected sugarcane and banana leaf tissue. Extracts of healthy and bacilliform virus-infected *Canna indica* L. (13), *K. blossfeldiana* (5), and *C. diffusa* (9) were tested against BSV and SCBV antisera by EIA and immunodiffusion. SCBV and BSV antigens were tested by immunodiffusion and immunoelectron microscopy (IEM) against antisera to CSSV, yam (*Dioscorea bulbifera* L.) bacilliform virus, and RTBV. Antisera against the *Canna*, *Kalanchoë*, and *Commelina* viruses and antigens of the cacao, yam, and rice viruses were unavailable for reciprocal tests with BSV and SCBV.

Electron microscopy and IEM. Crude leaf extracts and purified virus preparations were negatively stained with 2% sodium phosphotungstate (PTA), pH 6.8. For virus particle measurements, microscope magnification was calibrated using a grating replica. IEM was done as described for BSV (8) except that relative efficiency of trapping of SCBV particles by homologous or heterologous (BSV) antiserum was expessed as total particle count in 40 random fields of view at a

microscope screen magnification of 35,000×.

RESULTS

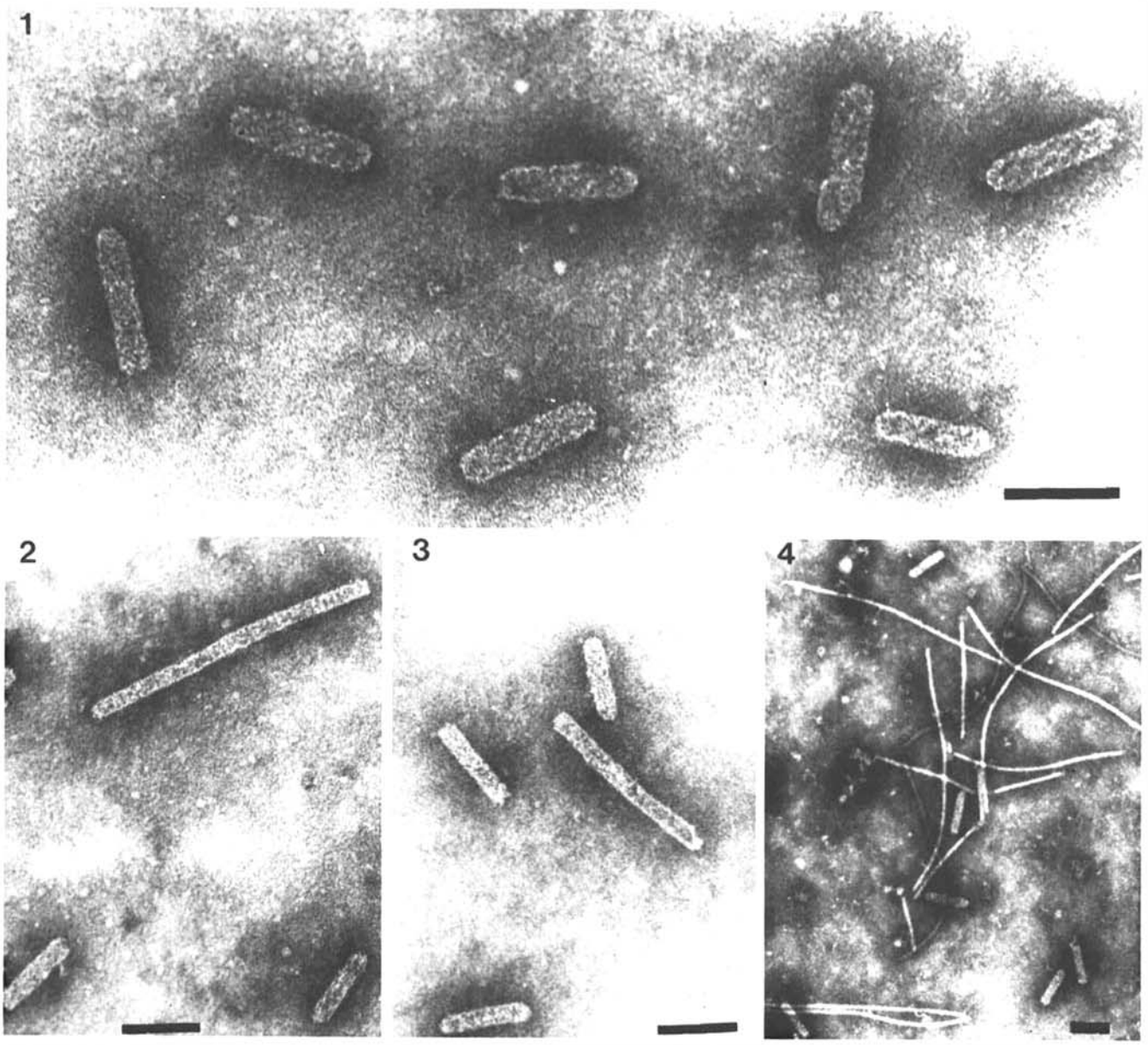
Virus occurrence. SCBV was detected by electron microscopy and EIA in all collections of the clone Mex. 57-473 in Morocco and in one of 17 samples of CP 44-101 collected in four separate sugarcane areas of northern Morocco. CP 44-101 is the predominant sugarcane cultivar grown in Morocco. The virus was also found in a sample of Mex. 57-473 provided by the Hawaiian Sugar Planters' Association, Aiea, Hawaii.

Mechanical transmission, host range, and symptoms. SCBV was transmitted by mechanical inoculation from infected Mex. 57-473 to healthy CP 44-101. No foliar symptoms were observed on infected CP 44-101, but virus transmission was verified by electron microscopy and EIA. SCBV was not transmitted to any of the following: Giant Cavendish banana (*Musa* sp.)., maize (*Zea mays* L. 'Earliking' and 'NK 199'), barley (*Hordeum vulgare* L. 'NK 38' and 'Capri'), wheat (*Triticum aestivum* L. 'Siete Cerros'), oats (*Avena sativa* L. 'Clintland 64'), johnsongrass (*Sorghum halepense* (L.) Pers.), *Chenopodium quinoa* Willd., *Nicotiana benthamiana* Domin, and *N. glutinosa* L. No symptoms were visible on these test plants, and SCBV was not detected by electron microscopic examination of negatively stained leaf dip preparations or by EIA in either inoculated leaves or uninoculated new growth.

BSV, which was not transmitted mechanically to banana or any other test plants (8), was transmitted mechanically from infected banana to healthy sugarcane CP 44-101. No symptoms were produced in sugarcane, but BSV was detected by electron microscopy, IEM, and EIA.

Virus purification, particle morphology, and particle dimensions. From the relatively low numbers of particles visible in leaf dip preparations and the relatively low concentration of particles in purified extracts, it appeared that SCBV, like BSV (8), occurs in low concentrations in infected plant tissue. As with BSV (8), cellulolytic enzyme treatment did not increase the amount of virus obtained from infected tissue but did free virus particles trapped in aggregates with host material during ultracentrifugation.

Measurements were made of 150 particles of SCBV in purified preparations negatively stained with PTA. These particles had a mean length of 131 ± 7 nm and a mean width of 31 ± 2 nm (Fig. 1). Elongated (300–500 nm) particles were observed occasionally (Figs. 2 and 3).



Figs. 1–4. Purified preparations of sugarcane bacilliform virus (SCBV) stained with 2% sodium phosphotungstate, pH 6.8. **(1)** Typical bacilliform particles, **(2 and 3)** elongated particles, and **(4)** negatively stained mixture of SCBV (bacilliform particles) and sugarcane mosaic virus (flexuous rods). Partially purified from a mixed infection in sugarcane (*Saccharum officinarum* L.) clone CP 44-101. Scale bar = 100 nm.

Serological tests and serological relationship of SCBV to BSV. In double-immunodiffusion tests in 0.9% aqueous agarose gels, SCBV and BSV reacted with each other's antiserum and produced precipitin lines that fused in both cases (Figs. 5 and 7). The homologous and heterologous antiserum titers were identical (1:64) for all four antigen-antiserum combinations. In intragel adsorption tests in which the center well was precharged with the heterologous antigen, no precipitin lines occurred between either SCBV or BSV antiserum and either antigen (Figs. 6 and 8).

In an IEM test, SCBV antiserum trapped 301 and BSV antiserum trapped 328 SCBV particles from a diluted partially purified suspension of SCBV. On a grid coated with normal serum, three SCBV particles were trapped. Reciprocal trapping of BSV on grids coated with SCBV antiserum was not done.

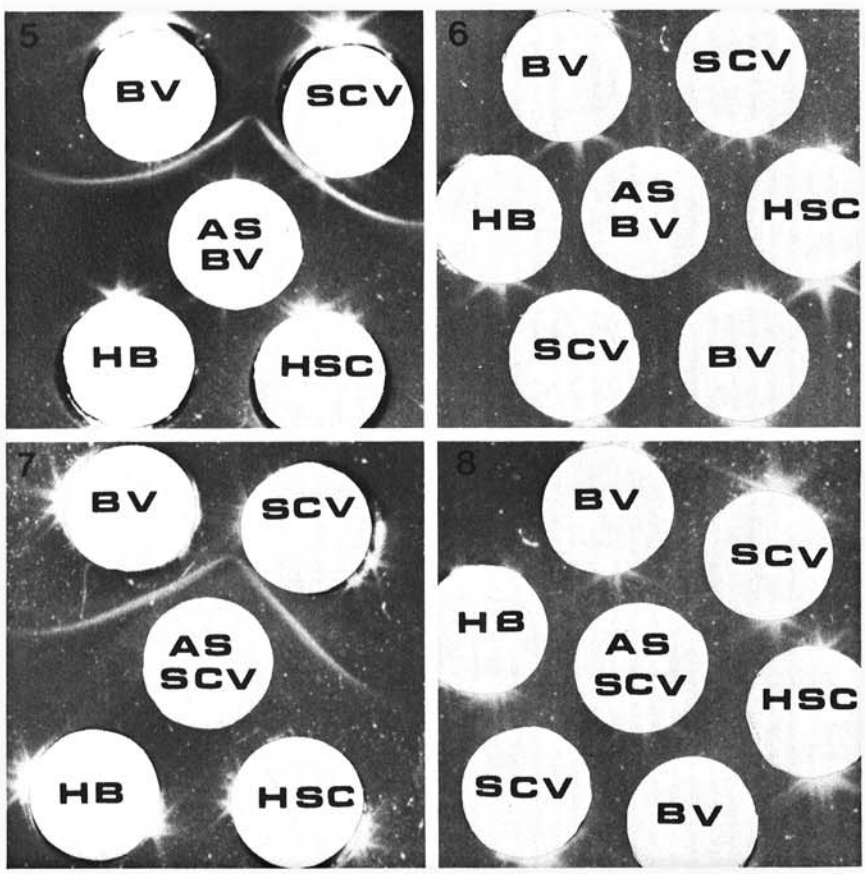
Reliable detection of SCBV by EIA in sugarcane leaf tissue was obtained with sample dilutions of 1:2 (w/v) in PBST-PVP (2) and an alkaline phosphatase-IgG conjugate dilution of 1:1,000. Under these conditions, SCBV-infected leaf tissue gave spectrophotometric OD_{405} readings of 0.50–0.70, compared with healthy control readings of 0.04–0.06. In the immunodiffusion, EIA, and IEM tests, BSV and SCBV were found to be unrelated serologically to the small nonenveloped bacilliform viruses occurring in cacao (1), *C. diffusa* (9), *K. blossfeldiana* (5), yam (4), rice (11), and canna (13).

DISCUSSION

The bacilliform virus occurring in sugarcane in Morocco and tentatively named SCBV is closely related serologically to BSV (8), which also occurs in Morocco. The particle dimensions of SCBV (131 × 31 nm) are slightly larger than those reported for BSV (119 × 27 nm) (8) but are not sufficiently different to suppose a lack of identity between the two viruses. The relationship between the two viruses can be resolved only when additional biological, physical, and chemical properties of both are determined. Should a vector or other means be found to transmit either BSV or SCBV to healthy banana test plants, further light will be shed on the relationship between the two viruses.

Yield data on Mex. 57-473 obtained in cultivar trials in Morocco during the past 10 yr suggest that SCBV infection may exert a deleterious effect on the productivity of this clone. This possibility, as well as the effect of SCBV infection on the field performance of other sugarcane clones, needs to be verified by adequately designed field or greenhouse tests. Greenhouse trials showed that SCMV symptoms were more pronounced and SCBV particle concentration was markedly greater in CP 44-101 and Mex. 57-473 infected simultaneously with both viruses (Fig. 4). The possible result of this apparent synergistic effect on plant growth and yield would be of interest for further study.

The occurrence of SCBV in Mex. 57-473 from Hawaii and in all Moroccan collections of Mex. 57-473 suggests that SCBV was introduced into Morocco in this clone. The detection of SCBV in field-grown CP 44-101 also suggests the occurrence in Morocco of an insect vector(s) capable of transmitting the virus. Field observations of BSV infection in banana in southern Morocco also suggest the possible existence of an insect vector (8). It would also be of interest to investigate the possibility of pollen transmission of these viruses, in the light of reported pollen transmission of the bacilliform particles associated with top spotting of *Kalanchoë* (5).



Figs. 5–8. Homologous and heterologous double-immunodiffusion reactions between the bacilliform viruses occurring naturally in sugarcane (SCV) and banana (BV) and their antisera (AS SCV and AS BV, respectively). Controls consist of partially purified extracts from healthy sugarcane (HSC) and healthy banana (HB). **(5 and 7)** Antigen-antibody precipitin lines in agarose gels not cross-adsorbed with either antigen. **(6 and 8)** Lack of precipitin line formation in gels in which the center antiserum depot was charged previously with **(6)** SCV antigen or **(8)** BV antigen.

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