Detection of Sweet Potato Virus Disease–Associated Closterovirus in a Sweet Potato Accession in the United States

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ABSTRACT

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A closterovirus (sweet potato virus disease–associated closterovirus [SPVD-aC]) that, in association with sweet potato feathery mottle potyvirus (SPFMV), causes the Nigerian sweet potato virus disease (SPVD), was detected in sweet potato (*Ipomoea setosa*) following grafting of infected scions of sweet potato cv. White Bunch, an accession in the USDA sweet potato repository. Confirmation of infection with SPVD-aC was by a riboprobe hybridization assay specific for the viral RNA coupled with chemiluminescent detection of the bound RNA, and SPFMV was detected by enzyme-linked immunosorbent assay (ELISA) using a specific antiserum. This is the first report of SPVD-aC and of SPVD in sweet potato in North America. Use of the riboprobe hybridization assay should facilitate a more rapid detection of SPVD-aC infected sweet potato and subsequent selection of virus-free germ plasm for international exchange.

The USDA Plant Genetics Resources Unit at Griffin, GA, is the repository for sweet potato (*Ipomoea batatas* (L.) Lam.) germ plasm in the United States. The U.S. sweet potato collection contains approximately 400 land races and cultivars introduced into the United States from 26 countries. Sweet potato germ plasm from sources outside the United States is routinely screened for virus infection by serology and by graft-transmission to *I. setosa* before it is introduced into the collection. The collection also contains about 200 U.S. sweet potato breeding lines and heirloom cultivars (very old cultivars usually introduced from elsewhere) not previously screened for virus infection. All sweet potato germ plasm is maintained in vitro and made available for distribution to breeding programs and for research purposes.

Between 1988 and 1990, about 100 U.S. cultivars and breeding lines were selected at random from the collection and cultured from meristems. Recently, plants derived from these meristem cultures were screened for the presence of sweet potato feathery mottle potyvirus (SPFMV), sweet potato mild mottle virus, (SPMMV), sweet potato latent virus (SPLV), and a recently described (13) closterovirus (sweet potato virus disease–associated closterovirus [SPVD-aC]) containing a bipartite genome (2; S. Winter, *unpublished*) which is associated with the sweet potato virus disease complex (SPVD) in Nigeria. The latter virus is consistently associated with SPFMV in causing SPVD, a debilitating disease of sweet potato first described in Nigeria (10) nearly two decades ago. SPVD-aC is also of special significance to virus-indexing in quarantine stations and in germ plasm collections, because plants infected with it are essentially symptomless (13), and indexing for the virus by graft-transmission to indicator plants is tedious, often requiring several weeks for a reliable diagnosis. A detection procedure for SPVD-aC has been developed, based on a nucleic acid hybridization assay using a cloned cDNA of viral RNA as a detecting probe (12). This paper reports the presence of SPVD-aC in a sweet potato accession in the repository and summarizes the results of applying a riboprobe hybridization assay to detect SPVD-aC in sweet potato.

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MATERIALS AND METHODS

Index grafting. In vitro plantlets of sweet potato were transplanted into soil and grown under greenhouse conditions at 26 to 30°C until about 10 nodes had developed. Three one-node cuttings were taken from the basal portion of a vine of each plant to be tested and wedge-grafted onto young (10 to 14 days old) seedlings of *I. setosa* Kerr. Some of the test plants developed symptoms typical of SPVD 2 to 3 weeks after grafting, indicating the presence of both SPVD-aC and SPFMV in the corresponding sweet potato plantlets. For test plants with inconclusive symptom expression, or for those that did not express any symptoms (suggesting the possibility of infection only with SPVD-aC), one-node cuttings were again taken from the corresponding sweet potato plantlets and grafted to seedlings of *I. setosa* that had been previously infected with SPFMV by mechanical inoculation of fully expanded primary leaves. The presence of SPVD-aC was then indicated by pronounced SPVD symptoms typically expressed by plants following dual infection by SPVD-aC and SPFMV. Symptoms of SPVD infection in *I. setosa* developed about 3 weeks after graft-inoculation.

Isolation of dsRNA, Northern and dot blotting. DsRNA comparisons and subsequent Northern analysis were conducted with sweet potato samples from the following sources: International Institute of Tropical Agriculture, Ibadan, Nigeria; a field isolate collected from the Meru region in Kenya (courtesy A. A. Brunt, Horticulture International, Littlehampton, U.K.); and a Brazilian isolate from the cultivar Pedra 2, obtained at Recife (9). An isolate of the whitefly (*Bemisia tabaci*)-transmitted lettuce infectious yellows virus (LIYV) (3) maintained in *Nicotiana clevelandii* A. Gray was obtained from James E. Duffus, (USDA/ARS, Salinas, CA) for comparison with SPVD-aC. These two viruses were compared because they share some common properties, including a bipartite genome (in contrast to all other characterized closteroviruses, whose genomes are monopartite) and have been proposed as members of the new genus *Biclovirus* of the *Closteroviridae* (2). DsRNA preparations were made by using standard procedures (7,11) followed by treatment of nucleic acid preparations with DNase I, RNase T1, and proteinase K. DsRNA species in extracts were separated electrophoretically in TAE-buffered 0.6% agarose

gels, and the gels were subsequently stained with ethidium bromide to show the dsRNA bands. After examination, the dsRNA was denatured in situ under mild alkaline conditions (50 mM NaOH) and transferred to nylon membranes (Zeta Probe, Bio-Rad Laboratories, Ltd., Mississauga, Ontario) in vacuo at 90 mm of H_20 for 60 min, incubated in 0.4 N NaOH for 10 min, neutralized in 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and baked in vacuo at 80°C. For dot blot hybridization, leaf samples of virus-infected and healthy *I. batatas* and *I. setosa* were homogenized in 10× SSC (1/10 wt/vol) containing 6% formaldehyde and 0.1% 2-mercaptoethanol, and serial dilutions were spotted onto nylon membranes.

Probe preparation. Strand specific (–) sense SPVD-aC RNA probes were labeled by incorporating digoxigenin-UTP (Boehringer Mannheim Canada, Laval, Québec) during transcription from the linearized recombinant Bluescript plasmid pRCL84 (insert size 1.0 kb) using T7-RNA polymerase (4). pCL84 was derived from nucleotide sequences near the 5′ end of SPVD-aC RNA-1 while pCL12 originated from the 5′ end of RNA-2 in a region containing the conserved heat shock (HSP 70) protein homologue common to the closteroviruses.

Hybridization with labeled RNA probes. Digoxigenin (DIG)-UTP-labeled RNA probes were used at 50 ng/ml of hybridization solution (50% formamide, 5% SSC, 0.01 M potassium phosphate, pH 7.2, 2% casein, 0.2% sodium dodecyl sulfate (SDS), 0.1% sodium lauryl sarcosine, 100 µg/ml yeast tRNA). RNA probes in hybridization buffer were stored at –20°C so that the same source could be used several times. Hybridization was for 16 h at 68°C, and the membranes were subsequently washed under high stringency conditions (0.1× SSC, 0.1% SDS) at 68 to 72°C. The bound DIG-labeled riboprobe was detected with anti-DIG (alkaline phosphatase-conjugated sheep-anti-digoxigenin antibody [Fab-fragment, Boehringer] diluted to 1:10,000) and reported by the signal generated by the chemiluminescent substrate 3-(2′-spiro-adamantane)-4-methoxy-4-(3′-phosphoryloxy)phenyl-1,-2-dioxetane (AMPPD) on X-ray film (5).

RESULTS AND DISCUSSION

No meristem-derived plantlets showed any conspicuous leaf symptoms when transplanted to soil. Of 25 sweet potato accessions, previously shown by testing to be free of SPFMV, SPMMV, and SPLV, all but White Bunch tested negative for SPVD-aC in grafting assays on SPFMV-infected *I. setosa* and in dot blot hybridization with SPVD-aC probes using samples from graft-inoculated *I. setosa*. Plantlets of White Bunch, when grown under greenhouse conditions, developed shorter internodes reflecting a bushy type of growth, but no unusual leaf symptoms were observed. However, SPFMV-infected *I. setosa* test plants developed strong symptoms of SPVD within 2 to 3 weeks after grafting with White Bunch scions (Fig. 1), and the presence of SPFMV was confirmed by enzyme-linked immunosorbent assay (ELISA) using a SPFMV antiserum prepared in our laboratory.

Nucleic acid hybridization assays using (–) sense RNA probes to SPVD-aC sequences provided evidence that the closterovirus was present in the sweet potato cultivar White Bunch and in SPFMV-infected *I. setosa* grafted with scions from White Bunch (Fig. 2). DsRNA profiles of all plant extracts infected with SPVD-aC revealed a pattern similar to that prepared from sweet potato infected with the Nigerian isolate of SPVD-aC (Fig. 3). A similar pattern was also observed by others (1) in sweet potato infected with the whitefly-transmitted component of SPVD. The distinct doublet of dsRNA in the 7.5- and 9-kbp size range reflects the bipartite nature of the SPVD-aC genome, which appears similar to that of lettuce infectious yellows virus (6). This doublet was not resolved in our earlier experiments (13) using polyacrylamide gels, thus leading to the erroneous interpretation and positioning of the cDNA clones on the genome of SPVD-aC. The additional, less intense band that migrated more slowly than the 9.0-kbp SPVD-aC band in samples 4, 6, and 7 (Fig. 3) is probably the 11.0-kbp dsRNA intermediate of SPFMV (8) in the dual infection caused by SPFMV and SPVD-aC.

The RNA probe pRCL84 hybridized to the large 9.0-kbp, but not to the 7.5-kbp, dsRNA in extracts from plants infected with SPVD-aC (Fig. 4). When a stripped and washed membrane was again sub-

Fig. 1. Symptoms in sweet potato feathery mottle potyvirus (SPFMV)-infected *Ipomoea setosa* following grafting of a White Bunch scion infected with sweet potato virus disease (SPVD) (left) compared to *I. setosa* grafted with a scion from virus-free Jewel (right). Note chlorosis and leaf deformation in *I. setosa* (left), which is typical of the interaction between SPVD-associated closterovirus and SPFMV.

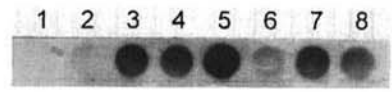

Fig. 2. Dot blot hybridization of the cRNA transcript pRCL84 to sap extracts from *Ipomoea* spp.: (1) healthy *I. setosa*; (2) healthy *I. batatas*; (3) sweet potato virus disease (SPVD)-White Bunch, USA-infected *I. setosa*; (4) SPVD-infected *I. batatas* White Bunch; (5) SPVD-Nigeria-infected *I. setosa*; (6) SPVD-aC-Nigeria-infected *I. batatas*; (7) SPVD-Pedra 2, Brazil-infected *I. setosa*; and (8) SPVD-aC-infected *I. batatas* Pedra 2, Brazil.

jected to hybridization conditions (using the pRCL12 RNA probe), hybridization signals to the 7.5-kbp dsRNA and to the subgenomic dsRNA bands (4.4, 2.5, and 2.0 kbp) were observed (data not shown). Neither of the RNA probes hybridized with dsRNA extracts from virus-free plants or to those from plants infected with LIYV or SPFMV.

The dsRNA patterns obtained with the different SPVD-aC samples appear to be very similar, and differences are most probably due to the concentration of total dsRNA in the samples. The 4.4-kbp dsRNA, often appearing as a doublet, was always observed; whereas the two dsRNA species of 2.5 kbp and 2.0 kbp were not always observed.

We have shown that the sweet potato accession White Bunch in the U.S. sweet potato repository is mixedly infected with SPFMV and SPVD-aC. This is the first report of the occurrence of SPVD and of SPVD-aC in North America. Accessions of American origin in the repository, including White Bunch, originated from breeding programs in the southeastern states. White Bunch is an heirloom sweet potato cultivar that has been maintained by breeders for its bunchy phenotype, and it may have originated by selection from cv. Bunch Porto Rico or cv. White Triumph, both of which are also heirloom cultivars. White Bunch has probably been infected for decades, but it is not known how it became infected with SPVD-aC. About one-half of the repository accessions, but none of the 25 samples tested in this work, have been subjected to viral therapy, and no systematic surveys for sweet potato viruses have been conducted in the United States using newer technologies.

DsRNA profiles and Northern analyses confirmed that the isolate of SPVD-aC in White Bunch is similar to other isolates of the virus present in Nigeria, Brazil, and East Africa. The fact that similar isolates of the closterovirus have been detected in sweet potato production areas in Africa (13) and in repositories in the Americas (9; this report) suggests that SPVD-aC has been distributed in infected plant material, very likely via international exchange of germ plasm.

The *I. setosa* graft-indicator host has proved to be very useful to detect SPVD-aC when other means of detection were not available. Moreover, the synergistic interaction between SPFMV and SPVD-aC readily occurs even in the presence of other viruses in *I. setosa*. We believe a test based on *I. setosa* is better than the current indexing system, which uses the *I. batatas* tester clone No. 8 (TIb-8) (developed by the International Institute of Agriculture), because it takes less time and symptoms are more severe in *I. setosa*. Moreover, symptoms are not masked, as often occurs in TIb-8. Grafting scions infected with SPVD-aC to SPFMV-infected *I. setosa* results in unmistakable symptoms of SPVD. However, it must be emphasized that only *I. setosa* preinfected with SPFMV will express good symptoms of SPVD, indicating the presence of SPVD-aC in the grafted scion. The use of the RNA hybridization assay method described here will facilitate more rapid testing with greater sensitivity and provide a sound basis for selection of virus-free stock for international exchange of germ plasm.

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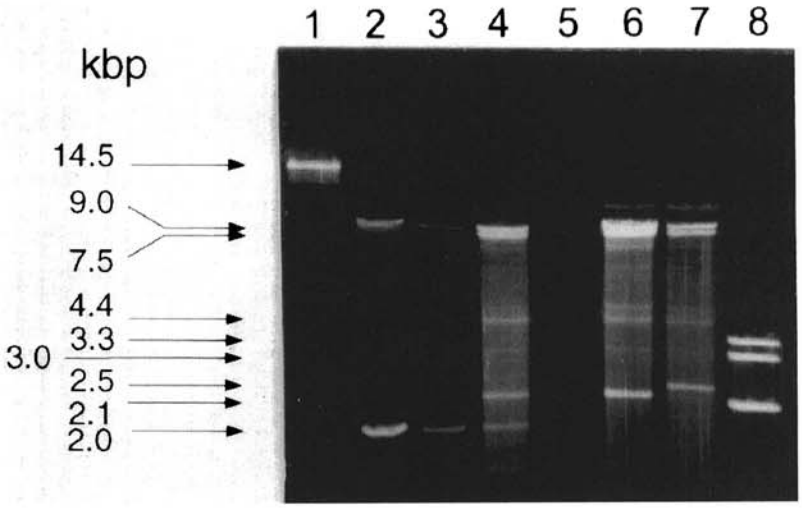

Fig. 3. Agarose gel electrophoresis of dsRNA extracts from infected *Ipomoea batatas* cultivars: (1) marker dsRNA (14.5 kbp) from beet yellows closterovirus (BYV)-infected *Sonchus*; (2) sweet potato virus disease–associated closterovirus (SPVD-aC)-infected Nigeria; (3) SPVD-aC-infected Pedra 2, Brazil; (4) SPVD-infected White Bunch, USA; (5) SPVD-infected Meru, Kenya; (6) SPVD-infected Pedra 2, Brazil; (7) SPVD-infected Nigeria; and (8) marker dsRNAs (3.3, 3.0, 2.1 kbp) from cucumber mosaic virus (CMV)-infected tobacco. Approximate sizes (kbp) of the dsRNAs are indicated on the left side of the gel. Weak resolution of lanes 2, 3, and 5 is due to low concentrations of dsRNA.

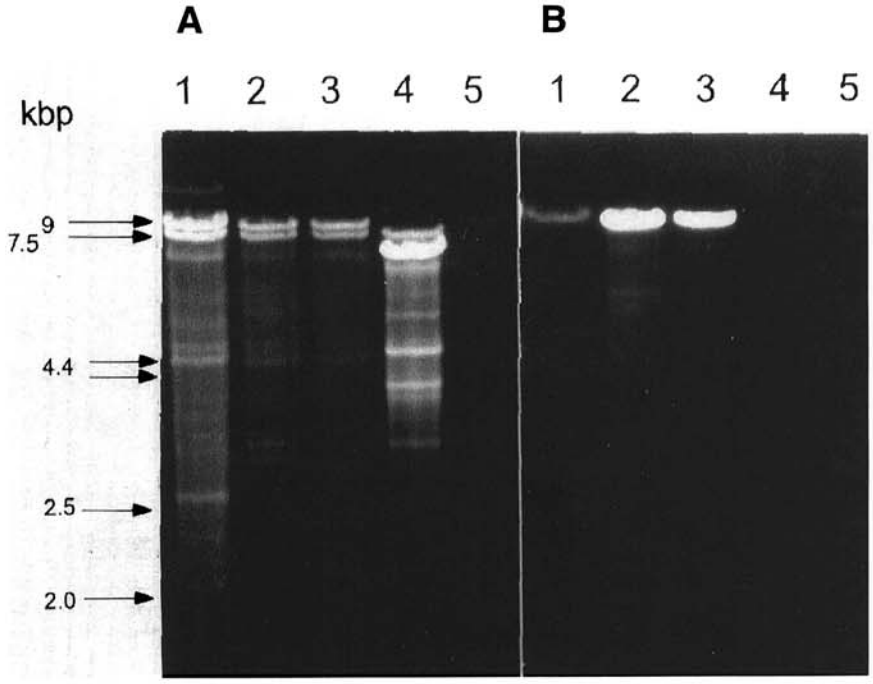

Fig. 4. Agarose gel electrophoresis **(A)** and Northern blot hybridization **(B)** of dsRNA extracts from infected *Ipomoea batatas* cultivars: (1) sweet potato virus disease (SPVD)-infected Nigeria; (2) SPVD-infected White Bunch, USA; (3) SPVD-infected Pedra 2, Brazil; (4) marker dsRNAs from lettuce infectious yellows closterovirus (LIYV); and (5) SPVD-associated closterovirus (aC)-infected Nigeria. Approximate sizes (kbp) of dsRNAs are indicated on the left side of the gel. Weak hybridization in (1) is due to excessively high concentrations of nucleic acid, which prevented antibody reaction. Missing hybridization in (5) is due to extremely low concentration of target RNA sequence, which is typical of *I. batatas* infected only with SPVD-aC.

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