Identification of the Coat Protein Gene of a Sweet Potato Sunken Vein Closterovirus Isolate from Kenya and Evidence for a Serological Relationship Among Geographically Diverse Closterovirus Isolates from Sweet Potato

Ute Hoyer, Edgar Maiss, Wilhelm Jelkmann, Dietrich-E. Lesemann, and H. Josef Vetten

First, fourth, and fifth authors: Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA), Institut für Biochemie und Pflanzenvirologie, Messeweg 11/12, D-38104 Braunschweig, Germany; second author: Universität Hannover, Institut für Pflanzenkrankheiten und Pflanzenschutz, Herrenhäuser Str. 2, D-30419 Hannover, Germany; third author: BBA, Institut für Pflanzenschutz im Obstbau, Schwabenheimer Str. 101, D-69221 Dossenheim, Germany.

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The nucleotide sequence of the coat protein gene of the Kenyan isolate of SPSVV was submitted to the EMBL, GenBank, and DDBJ nucleotide sequence databases and assigned the accession number X80995.

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ABSTRACT

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A Kenyan isolate of sweet potato sunken vein virus (SPSVV-Ke), a tentative member of the genus *Closterovirus*, was transmitted to *Ipomoea setosa* by the whitefly *Bemisia tabaci*. Cross-banded filamentous particles about 850 nm in length were detected in infected plants by immunoelectron microscopy (IEM) with an antiserum to virions of an Israeli isolate of SPSVV (SPSVV-Is). Viral double-stranded RNA species of about 10 and 9 kbp were extracted from infected *I. setosa* and used as templates for complementary DNA (cDNA) synthesis. Sequencing of selected cDNA clones revealed an open reading frame of 774 nucleotides that encodes a protein with an estimated molecular mass of 29,028 Da. Computer analysis of the deduced amino acid sequence of this protein indicated a distinct affinity to the coat protein (CP) of lettuce infectious yellows closterovirus (LIYV) and a lesser similarity to the CPs of beet yellows and citrus tristeza closteroviruses, suggesting that it is the CP of SPSVV-Ke. After expression of the CP gene of SPSVV-Ke in *Escherichia coli*, its identity as the viral CP was confirmed by Western blot analysis with the SPSVV-Is antiserum. This antiserum and a rabbit antiserum raised against the bacterially expressed CP of SPSVV-Ke were used in Western blot and IEM experiments for assessing the serological relationships among SPSVV-Ke, SPSVV-Is, and sweet potato virus disease-associated closterovirus isolates from Nigeria and the United States. Results showed that SPSVV-Ke is closely related serologically to similar closterovirus isolates infecting sweet potato in Israel, Nigeria, and the United States but differs from them in reacting weakly with an antiserum to LIYV in IEM and Western blots.

Additional keywords: coat protein expression, dsRNA.

Definite and tentative members of the genus *Closterovirus*, such as beet yellows virus (BYV) and citrus tristeza virus (CTV), are typically aphid-transmitted, and some can be transmitted, with difficulty, by mechanical inoculation (8,10). In addition, many closteroviruses are not sap-transmissible and are vectored by whiteflies (*Trialeurodes* spp. and *Bemisia tabaci* Gennadius) and pseudococcid mealybugs, and others have unknown vectors (10). The best characterized whitefly-transmitted closterovirus is lettuce infectious yellows virus (LIYV), which has been reported only from the United States and is semipersistently transmitted by *B. tabaci* (15). The 3′ region of the closterovirus genome has a characteristic gene array that includes open reading frames (ORFs) for a small membrane protein, a homologue of the HSP70 family of chaperone proteins, a protein of unknown function, a diverged duplicate of the coat protein (CP), and the CP (2,3,4,9,27). LIYV differs from the monopartite closteroviruses BYV and CTV (2,4, 18,27) in several respects: the LIYV genome is bipartite, contains ORFs that are unrelated to ORFs of other closteroviruses, and the positions of the CP gene and its diverged CP duplicate are reversed (19,20). Therefore, LIYV will probably be assigned to a separate taxon for which the name *Biclovirus* has been proposed (14).

Symptoms such as vein clearing, leaf strapping, chlorosis, and stunting were associated with the sweet potato virus disease (SPVD) first described in Nigeria (32). Diseases similar to SPVD were later reported for sweet potato (*Ipomoea batatas* (L.) Lam.) from many African countries, Israel, and the United States (26), suggesting that the causal agent has a wide distribution. Some of these diseases are due to the synergism between sweet potato

Corresponding author: H. J. Vetten; E-mail address: h.j.vetten@bba.d400.de

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feathery mottle potyvirus (SPFMV) and a whitefly-transmitted agent (11,26,29). Recent investigations (11,35) have demonstrated that the latter is a closterovirus that is transmitted semipersistently by the whitefly *B. tabaci* but not by mechanical means, has flexuous filamentous particles that are about 900 nm in length, with a characteristic cross-banding substructure, and induces closterovirus-specific vesicle clusters in phloem cells of infected plants. Because of the characteristic symptoms induced in several sweet potato genotypes the name "sweet potato sunken vein virus" (SPSVV) has been proposed for the sweet potato closterovirus from Israel (11) (SPSVV-Is). The only other viruses resembling SPSVV-Is are SPVD-associated closteroviruses (SPVD-AC) from Nigeria (22,35) and the United States (1) as well as LIYV (15).

Little information is available on the relationships among LIYV, SPSVV-Is, and SPVD-AC from various countries. Because sweet potato is immune to LIYV (15) and SPSVV-Is did not react with an antiserum to LIYV (11), these two viruses seem to be unrelated. Apart from a report that the double-stranded (ds) RNA patterns from SPVD-affected Brazilian and Nigerian sweet potatoes are similar and that the Brazilian SPVD-AC was detected by dot blot and Northern blot hybridization with RNA probes complementary to RNA of the Nigerian SPVD-AC (28), there is no published information on the affinities among SPSVV-Is and SPVD-AC from various countries. When analyzing SPVD-affected sweet potato plants collected during recent virus surveys in Kenya, our initial immunoelectron microscopic (IEM) examination of such samples indicated that they contained closterovirus-like particles that were specifically trapped and strongly decorated by an antiserum to SPSVV-Is. Similar observations also were made with samples from SPVD-affected sweet potato plants originating from Nigeria and the United States. These observations suggested that the closterovirus-like particles in SPVD-affected sweet potato from various countries are serologically related to SPSVV-Is and should be regarded as SPSVV isolates. These results prompted us to produce an antiserum to a Kenyan isolate of SPSVV (SPSVV-Ke) for studying the serological relationship among various SPVD-AC isolates. However, due to difficulties in obtaining sufficient yields of purified SPSVV-Ke preparations for production of a high-quality antiserum, we cloned and sequenced parts of the SPSVV-Ke genome to identify the CP gene of SPSVV-Ke for expression in bacterial cells and subsequent production of antibodies.

In this paper, we describe the identification of the CP gene of SPSVV-Ke and the production of a high-titered antiserum to bacterially expressed CP of SPSVV-Ke. This antiserum and the SPSVV-Is antiserum were used to study the serological relationship among geographically diverse isolates of SPVD-AC.

MATERIALS AND METHODS

Origin, isolation, and maintenance of virus isolates. An isolate of LIYV was provided in lettuce plants by B. W. Falk, University of California, Davis, and maintained in *Chenopodium murale* L. and *Nicotiana clevelandii* Gray by whitefly transmission (*B. tabaci*). Plants of *I. setosa* Ker. and *I. batatas* 'Georgia Jet' containing a SPSVV-Is isolate (11) were provided by J. Cohen, Volcani Center, Bet Dagan, Israel. SPVD-affected sweet potato plants originating from Nigeria (35) and the United States were obtained from S. Winter and R. I. Hamilton, Pacific Agricultural Research Center, Vancouver, BC, Canada, and shoots of sweet potato plants showing severe SPVD symptoms were collected near Kagamega in western Kenya. All isolates were maintained as rooted stem cuttings under greenhouse conditions at 25°C and above. Scions of the SPVD-affected sweet potato plants from Kenya, Nigeria, and the United States were grafted onto 2- to 3-week-old seedlings of *I. setosa*. Upon symptom development in *I. setosa*, about 50 adults of *B. tabaci* reared on cotton seedlings were caged on each symptomatic plant for an acquisition access feeding period of 48 h and were transferred to another set of young *I. setosa* seedlings for an inoculation access feeding period of 48 h (11). Three to five weeks after inoculation, these *I. setosa* seedlings were tested by enzyme-linked immunosorbent assay and IEM for possible virus infections with antisera to SPFMV, sweet potato mild mottle (SPMMV), and sweet potato latent potyviruses as well as to SPSVV-Is. Of the viruses tested for, only closteroviruses reacting in IEM with SPSVV-Is antiserum were detected. Subsequently, SPSVV-Is as well as the SPVD-AC from Nigeria, the United States, and Kenya were maintained in *I. setosa* by graft inoculation or whitefly transmission (*B. tabaci*). Because the SPVD-AC from Kenya, Nigeria, and the United States could be strongly decorated with antiserum to SPSVV-Is, they were considered isolates of SPSVV and are referred to as SPSVV-Ke, SPSVV-N, and SPSVV-USA, respectively.

dsRNA isolation, complementary DNA (cDNA) synthesis, cloning, and sequence analysis. Unless otherwise stated, standard molecular biology methods were used (30). Total dsRNA was isolated from SPSVV-Ke–infected *I. setosa* plants as previously described (11,25). After analysis by nondenaturing agarose gel electrophoresis, two dsRNA species, estimated to be about 10 and 9 kbp, were excised from the gel and served as templates for synthesis of cDNA, with random hexamers as primers. cDNA was subsequently cloned into the *Eco*RV site of the Bluescript M13$^+$ vector (17), and the resulting clones were sequenced by the dideoxynucleotide chain termination method (31) by the ^{T7}Sequencing kit (Pharmacia, Uppsala, Sweden). Sequences of internal regions of several cDNA inserts were obtained from deletion clones generated by restriction enzyme and exonuclease III digestion. The nucleotide sequence of the putative CP gene of SPSVV-Ke was determined from both strands of four independent cDNA clones (p11, p28, p70, and p102). Sequencing data were collected, assembled, and analyzed by the Genetics Computer Group sequence software (12) and the databases of the Heidelberg Unix Sequence Analysis Resources (HUSAR 3.0, German Cancer Research Centre Heidelberg). Multiple alignments of amino acid (aa) sequences were generated by CLUSTAL (16). Database searches were performed by BLASTP (6).

Expression of the CP gene. For the expression of the SPSVV-Ke CP in *Escherichia coli*, a 950-bp DNA fragment containing the entire putative CP ORF of SPSVV-Ke was synthesized by polymerase chain reaction (PCR) with a 27-mer oligonucleotide (5′-GGGGATCC**ATG**GATACTGACAAAGTAA-3′) comprising the first 19 nucleotides of the CP ORF and 8 additional nucleotides upstream of the start codon ATG (bold) that contained a *Bam*HI site (underlined). The second PCR primer of 17 nucleotides was specific to the Bluescript M13$^+$ vector. A deletion clone of cDNA clone p70 containing the complete CP ORF was linearized with *Xba*I and served as template for PCR amplification. The PCR product obtained was digested with *Bam*HI and *Sal*I (a restriction site 138 bp downstream from the stop codon of the CP ORF) and ligated into the *Bam*HI- and *Sal*I-digested expression plasmid pQE-30 (Qiagen Ltd., Chatsworth, CA), which was then used to transform *E. coli* M15 (pREP4). Two clones were used for expression in *E. coli* induced by 2 mM isopropyl-β-D-thiogalactopyranoside. In all cases, induced and noninduced recombinant *E. coli* cultures as well as *E. coli* cultures transformed with the expression plasmid alone were tested. The bacterially expressed protein was purified to near homogeneity from cell lysates on a nickel-charged nitrolotriacetic acid (Ni-NTA) resin (Qiagen) essentially following the manufacturer's instructions.

Antiserum production and antisera used. Polyclonal antibodies were raised in a rabbit by administering at weekly intervals a total of four intramuscular injections each containing 200 μg of purified bacterially expressed protein emulsified with an equal volume of Freund's adjuvant. After the final injection, the rabbit was bled every fortnight. Rabbit antisera to CTV, LIYV, SPFMV, SPMMV, SPSVV-Is, and bacterially expressed CP of BYV were donated by R. Casper, BBA, Braunschweig, Germany; J. E. Duffus, Agricultural

Research Station, Salinas, CA; J. W. Moyer, North Carolina State University, Raleigh; J. Richter, Bundesanstalt für Züchtungsforschung, Aschersleben, Germany; J. Cohen; and A. A. Agranovsky, Belozersky Institute, Moscow, respectively.

Analysis of partially purified virus preparations by Western blotting. From 5 g each of leaf tissue infected with SPSVV-Ke, SPSVV-Is, and SPSVV-N as well as noninfected leaves, partially purified virion preparations were obtained by previously described procedures (19). Pellets obtained from virus-infected tissue after the first high-speed centrifugation step contained numerous closterovirus-like particles that were resuspended in 200 μl of 1× loading buffer (21) but without 2-mercaptoethanol (ME) and bromophenol blue (BPB). After determination of protein concentrations (Pierce BCA protein assay reagent, Pierce Ltd., Rockford, IL), appropriate amounts of ME and BPB were added to the preparations, which were then heated for 5 min at 95°C prior to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (21).

Appropriate concentrations of the expressed CP and the partially purified preparations were first assessed by SDS-PAGE and Coomassie brilliant blue staining prior to use in Western blot analyses. Western blots were conducted essentially as previously described (34), using a transfer buffer containing 25 mM Tris, 190 mM glycine, and 20% methanol. After blocking membranes with 3% (wt/vol) gelatin (Bio-Rad Laboratories, Munich) for 1 h, immunoblots were reacted with appropriate dilutions of rabbit antisera and purified rabbit immunoglobulin G (IgG) followed by alkaline phosphatase (AP)-labeled goat anti-rabbit IgG (Dianova, Hamburg, Germany) at a dilution of 1:2,500. Biotinylated marker proteins (Boehringer GmbH, Mannheim Germany) were used and incubated with streptavidin-AP complexes (Dako Ltd., Hamburg, Germany) at a final dilution of 1:3,000. Nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphate, toluidine salt (Boehringer Mannheim) was used as AP substrate for visualizing antibody-specific proteins and marker proteins.

Electron microscopy. For IEM examination of virus-infected plants and virion preparations, antisera were routinely diluted 1:1,000 and 1:50 for trapping and decoration, respectively, unless otherwise stated. To determine the decoration titers of the antisera (23), antibody-coated grids were floated overnight on homogenates of excised midrib tissue, washed, and incubated for 15 min with a series of twofold dilutions of the antisera. To determine the particle trapping efficiency of the antisera, immunosorbent electron microscopy (ISEM) was carried out essentially as described (24), but a trapping time of 48 h was applied. Particle-length measurements were done on grids coated with SPSVV-Is antiserum, incubated overnight on extracts from SPSVV-Ke–infected *I. setosa*, and stained with 1% (wt/vol) uranyl acetate. At least 200 particles were measured in a Zeiss EM10C electron microscope (Zeiss, Oberkochen, Germany) at a magnification of 50,000 with a Morphomat 30 image analyzing system.

RESULTS AND DISCUSSION

Virus isolation and viral dsRNA analysis. After whitefly transmission from *I. setosa* previously graft-inoculated with SPVD-affected sweet potato scions from Kenya, *I. setosa* seedlings became stunted and had smaller, brittle, yellow, and downward rolled leaves. These symptoms resembled those described previously for SPSVV-Is (11) and SPSVV-N (35). When these seedlings were

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          ::::   :   : :  :    :              ::                :
SPSVV-Ke  MDTDKVNDDKNFDSDSDVGFGDRNVNDNLRRRSDTIDNENVNNVGRRDMELTSGILTSEQ
LIYV      MDTD--GDNDVFGSGNDTRNNDDKKKEEMKQ--NISDNSQIISTRDHEADIIGSISKED-
BYV       MG----------------------------------------------SAEPISAIATFEN
CTV       MDDET--------------------KKLKNKNKET---------KEGDDVVAAESSFSS
          *.                                             . . ...  . .

               : :: :     :  :      :   :  : :                      :
SPSVV-Ke  LALARLGKIQVYSNSPDIMSKSQEDEFKRHMESFVRQITGETTLSPE-IFASFYASLFQA
LIYV      -----LSKIVVRVDRHDALSANDVQSFREAMINFMRDKDPNRNQPSDKLIIAMEVGVYQM
BYV       VSLADQTCLHG-EDCDK-LRKNFEECLK--------LKG----VPEDNLGIALGLCLYSC
CTV       VNLHIDPTLITMNDVRQ-LSTQQNAALNRDL--FLTLKGKHPNLPDKDKDFRIAMMLYRL
                  .    .  . ..    . ..          .     . .     .   ..

            :  ::  :     :::                 : : :::   :    :  :  :::
SPSVV-Ke  WANQSTS-EKNASNANLENVFMV-NGKEYSWRTHSFINHMQSN--MPDVKNAARKWARAH
LIYV      VINLGTS-AKLGNANNLEFTIAY-DQETRTYKVADFVNYMQSR--MRNSPNVVRQYARAM
BYV       ATI-GTSNKVNVQPTSTFIKASFGGGKELYLTHGELNSFLGSQKLLEGKPNKLRCFCRTF
CTV       AVK-SSSLQSDDDATGITYTRE---GVEVDLSDKLWTDVVFNSKGIGNRTNALRVWGRTN
              ..*       ..           .         .. .    ..   *  * . *.

                    ::       : :: :::  :     ::  :    ::   :         :
SPSVV-Ke  ANDYKVLVGLGIVK-PDYHLQAKQGVLPEYWHLATDFMRGNDMATTADGLAATLMMKRNA
LIYV      EKTINNIRSAGIIN-SNGVLAAKHGVLASYRNSYSDFAVGFGNDTTDAQLTSLMLARKQA
BYV       QKDYISLRKEYRGKLPPIARANRHGLPAEDHYLAADFI-STSTELTDLQQSRLLLARENA
CTV       DALYLAFCRQNR-NLSYGGRPLDAGIPAGYHYLCADFL--TGAGLTDLECAVYIQAKEQL
          .     .      . .    .   *. ..     .**    . . *.   .  .  . .

          ::         ::  ::       :
SPSVV-Ke  LCNKDNKN-SIYNVTQLT-GTGLHC
LIYV      LCKGEGGSVEHYNTMQLA-NLKHPC
BYV       THTEFSSESPVTSLKQLGRGLGTGR
CTV       LKKRGADDVVVTNVRQLGK--FNTR
            . .  .    .  **.
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Fig. 1. Alignment of the complete coat protein (CP) amino acid (aa) sequences of sweet potato sunken vein virus from Kenya (SPSVV-Ke) (257 aa), lettuce infectious yellows virus (LIYV) (249 aa), beet yellows virus (BYV) (204 aa), and citrus tristeza virus (CTV) (223 aa) generated by CLUSTAL software with minor modifications. According to the standard run parameters of CLUSTAL (16), identical and well-conserved residues in the closterovirus CPs are indicated by asterisks and dots, respectively, below the sequences. Gaps within the sequences are indicated by dashes. Residues identical for SPSVV-Ke and LIYV are denoted by double dots above the sequences. The conserved Arg (R) and Asp (D) residues that putatively form a salt bridge are shaded.

analyzed by IEM, only cross-banded filamentous particles reacting only with the SPSVV-Is antiserum and measuring about 850 nm in length were detected. The particle morphology of SPSVV-Ke is consistent with the length measurements of 850 and 950 nm determined for SPSVV-Is (11) and SPSVV-N (35) particles, respectively, and agrees well with a particle length of about 900 nm for LIYV (D.-E. Lesemann, and H. J. Vetten, *unpublished data*, in contrast to 1,800 to 2,000 nm [15]). dsRNA analysis yielded two major dsRNA species of about 10 and 9 kbp (data not shown) similar in size to those observed for SPSVV-Is (11).

Sequence analysis. To identify the CP gene of SPSVV-Ke, the sequences of several cDNA clones were compared to available CP sequences of filamentous plant viruses. Computer analysis of the sequences of four clones (p11, p28, p70, and p102) that provided unambiguous sequence information revealed one ORF of 774 nucleotides potentially encoding a protein with a deduced molecular mass of 29,028 Da. Because this ORF had a low but significant similarity with CP sequences of the closteroviruses BYV, CTV, and LIYV (2,19,27), it was suspected that it codes for the CP of SPSVV-Ke, although the predicted molecular mass of 29 kDa is lower than 34 kDa, the size estimate reported for the CP of SPSVV-Is (11). When the deduced aa sequence (257 aa) of the 29-kDa ORF was compared with the CP sequences of other filamentous plant viruses, it contained the invariant Arg (R) and Asp (D) residues conserved in the CPs of all filamentous plant viruses and possibly involved in salt bridge formation (13). Moreover, the CLUSTAL alignment (16) of the CP aa sequences of SPSVV-Ke, LIYV, BYV, and CTV revealed 11 perfect matches and 55 highly conserved positions (Fig. 1). There were 64 perfect matches between the CPs of SPSVV-Ke and LIYV, resulting in an aa sequence identity of approximately 25%. The same alignment was used to generate a cluster dendrogram illustrating the tentative phylogenetic relationships between the closterovirus CPs. The resulting dendrogram (data not shown) showed one group consisting of the two aphid-transmitted closteroviruses, BYV and CTV, which have CP sizes of 22 (2) and 25 kDa (7,33), respectively. The whitefly-transmitted SPSVV-Ke and LIYV, which have CP sizes of 29 (current study) and 28 kDa (19), respectively, formed a second group, indicating that the whitefly-transmitted closteroviruses are phylogenetically closely related to each other and code for a larger CP than the aphid-transmitted ones.

Expression of the CP gene. SDS-PAGE analysis of the expression products of two clones revealed a major product of about 30 kDa and several minor products of smaller size in induced, but not in noninduced, *E. coli* cells (data not shown). The size of the major expression product, about 30 kDa, was in good agreement with the expected size of a protein that contains the 29-kDa viral CP plus 10 vector-derived aa residues, including a tag of 6 histidine residues required for protein purification plus 2 primer-derived residues at its N terminus.

Western blot analysis of the expressed CP. When the purified bacterially expressed protein was reacted with an antiserum to SPSVV-Is in Western blots, one major specific protein band of 30 kDa and one very faint protein band of approximately 25 kDa were revealed (Fig. 2, lane 4), suggesting that the 30-kDa band contains the intact CP of SPSVV-Ke and that the other band probably is a degradation product of this protein. The very weak reactions of the SPSVV-Is antiserum with a protein of about 30 kDa in crude preparations from the noninduced and induced expression vector pQE-30 and from the noninduced clone pCP5 (Fig. 2, lanes 1, 2, and 3) appeared to be nonspecific reactions with an *E. coli* protein, although the weak band in lane 3 also may indicate a low level of protein expression that is probably due to incomplete promoter repression of the expression vector pQE-30.

No specific reactions were observed when the expressed protein was incubated with preimmune serum (data not shown) as well as with antisera to LIYV, BYV-CP, CTV, SPFMV, and SPMMV (Fig. 2, lanes 5 through 9). The very faint bands of approximately 30 and 42 kDa revealed with the BYV-CP and CTV antisera, respectively (Fig. 2, lanes 6 and 7), were considered nonspecific reactions because BYV-CP antiserum did not decorate SPSVV-Ke particles (decoration tests are described below), and we were unable to detect a 42-kDa protein after SDS-PAGE analysis of the purified bacterially expressed CP of SPSVV-Ke.

To provide evidence that the *E. coli*-expressed protein and the viral CP in SPSVV-infected plants are of similar size, further Western blot experiments were conducted by incubating IgG to SPSVV-Is with SDS-PAGE–resolved proteins in partially purified virion preparations from SPSVV-Ke, SPSVV-N, and SPSVV-Is infected and noninoculated control leaves. While no reaction was observed with the preparation from noninoculated plants (Fig. 3, lane 5), strong reactions were obtained with the bacterially expressed 30-kDa protein and with a 30-kDa protein present in each of the partially purified virion preparations of the individual SPSVV isolates (Fig. 3, lanes 1 through 4). This confirmed the expected size of the expressed protein and indicated that the viral CPs of all SPSVV isolates studied have a similar size and are serologically closely related. Although a CP size of 34 kDa has been reported previously for SPSVV-Is (11), the size of the viral CP of SPSVV-Is was about 30 kDa in the current study and was indistinguishable in size from that of the two other SPSVV isolates tested. The additional protein band of 26 kDa that was present only in the partially purified virion preparations of SPSVV-N and SPSVV-Is (Fig. 3, lanes 3 and 4) is regarded as a degradation product of the viral CP, whereas the absence of any degradation product in the SPSVV-Ke preparation (Fig. 3, lane 2) suggests a higher proteolytic stability of the SPSVV-Ke CP, as confirmed by the SPSVV-Ke antiserum reaction in Figure 4. The 60-kDa band of the SPSVV-N isolate (Fig. 3, lane 3) might be a dimeric form of the viral CP. In view of the recent report that the aberrant CP gene product of LIYV has a predicted size of 52 kDa (20), the 60-kDa protein in the SPSVV-N preparation also might represent a diverged CP (3,5, 9,27). There is no direct evidence, however, for a second structural protein in LIYV and SPSVV particles.

Properties of the antiserum to the bacterially expressed CP gene. Identification of the CP gene of SPSVV-Ke was further substantiated with the rabbit antiserum to the bacterially expressed protein of SPSVV-Ke in IEM and Western blot analyses. This antiserum not only specifically trapped and decorated the particles

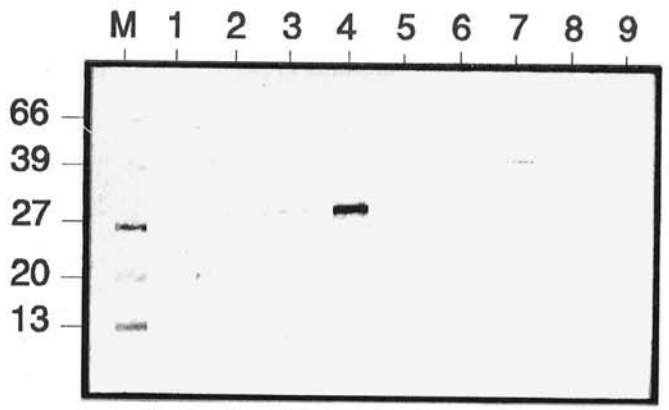

Fig. 2. Western blot analysis of the nickel-charged nitrolotriacetic acid (Ni-NTA) resin-purified expression protein with rabbit immunoglobulin G (IgG) to sweet potato sunken vein virus from Israel (lanes 1 through 4, 4 µg/ml), sweet potato feathery mottle virus (lane 8, 1 µg/ml), and sweet potato mild mottle vrius (lane 9, 1 µg/ml) as well as rabbit antisera to lettuce infectious yellows virus (lane 5, diluted 1:400), beet yellows virus coat protein (lane 6, diluted 1:400), and citrus tristeza virus (lane 7, diluted 1:400) followed by alkaline phosphatase-labeled goat anti-rabbit IgG. Lanes 1, 2, 3, and 4 through 9 contain proteins of the bacterial pellet of noninduced pQE-30, induced pQE-30, noninduced pCP5, and Ni-NTA resin-purified protein expressed by the induced pCP5 clone, respectively. Two micrograms of protein was used for each of lanes 4 through 9. Lane M contains biotinylated marker proteins. Numbers on the left are the molecular mass values (in kilodaltons) of marker proteins.

of SPSVV-Ke but also those of SPSVV-Is, SPSVV-N, and SPSVV-USA, whereas no virions were trapped and decorated with preimmune serum (data not shown). In Western blots, the antiserum strongly reacted with its approximately 30-kDa homologous protein used as positive control (Fig. 4, lane 1) and with a 30-kDa protein in the partially purified virion preparations of SPSVV-Ke, SPSVV-N, and SPSVV-Is (Fig. 4, lanes 2 through 4). The Western blot reactions of the SPSVV-Ke antiserum with the bacterially expressed protein and the viral CPs were similar to those of the SPSVV-Is antiserum (Fig. 3). However, the SPSVV-Ke antiserum detected several additional smaller proteins in the Ni-NTA resin-purified expression protein (Fig. 4, lane 1). These minor proteins may be incomplete or degraded expression products that were detected only very weakly by the SPSVV-Is antiserum at about eight times higher concentrations of the expressed CP (Fig. 2, lane 4) than those used in Figure 3, lane 1.

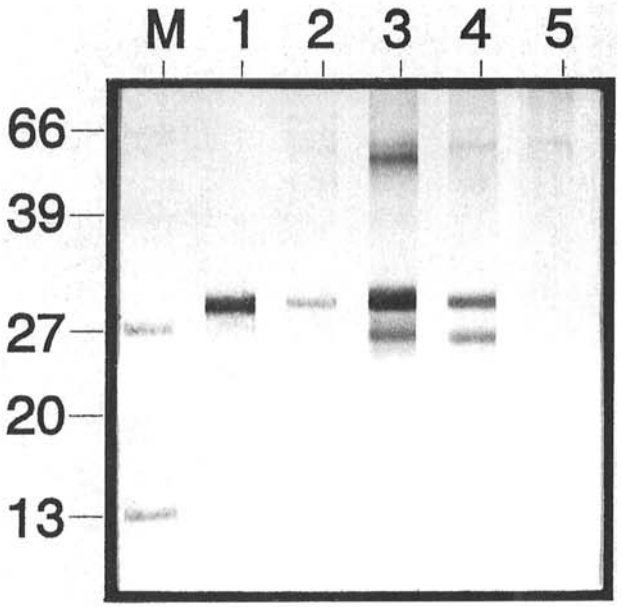

Fig. 3. Western blot analysis of nickel-charged nitrolotriacetic acid resin-purified coat protein expressed in *Escherichia coli* (lane 1, 0.25 µg) and proteins (25 µg of total protein per lane) in partially purified virion preparations of sweet potato sunken vein virus from Kenya (SPSVV-Ke) (lane 2), SPSVV from Nigeria (SPSVV-N) (lane 3), and SPSVV from Israel (SPSVV-Is) (lane 4), and from noninoculated *Ipomoea setosa* plants (lane 5), with 4 µg of rabbit immunoglobulin G (IgG) to SPSVV-Is virions per ml as detecting antibody followed by alkaline phosphatase-labeled goat anti-rabbit IgG. Lane M contains biotinylated marker proteins. Numbers on the left are the molecular mass values (in kilodaltons) of marker proteins.

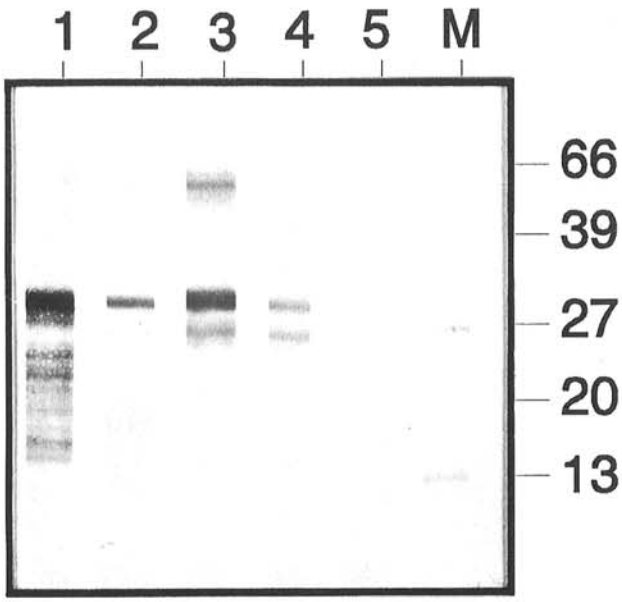

Fig. 4. Western blot analysis of nickel-charged nitrolotriacetic acid resin-purified coat protein (CP) expressed in *Escherichia coli* (lane 1, 0.25 µg) and proteins (25 µg of total protein per lane) in partially purified virion preparations of sweet potato sunken vein virus from Kenya (SPSVV-Ke) (lane 2), SPSVV from Nigeria (SPSVV-N) (lane 3), SPSVV from Israel (SPSVV-Is) (lane 4), and from noninoculated *Ipomoea setosa* plants (lane 5), with 4 µg of rabbit immunoglobulin G (IgG) to the bacterially expressed CP of SPSVV-Ke per ml followed by alkaline phosphatase-labeled goat anti-rabbit IgG. Lane M contains biotinylated marker proteins. Numbers on the right are the molecular mass values (in kilodaltons) of marker proteins.

The difference in the reaction of the minor proteins with the two antisera is explained by the fact that the SPSVV-Ke antiserum was raised against the expressed CP and partially degraded expression products and, hence, contains a considerable amount of antibodies directed not only to metatopes but also to a range of internal epitopes, whereas the SPSVV-Is antiserum was raised against SPSVV virions (11) and, thus, predominantly contains antibodies to epitopes on the particle surface. Therefore, the two antisera differ in their relative content of antibodies to degraded CP as well as to cryptotopes and metatopes.

IEM studies of the serological relationships among SPSVV isolates. To determine the serological relationships between SPSVV-Ke, SPSVV-Is, SPSVV-N, and SPSVV-USA, decoration titer experiments were conducted with antisera to SPSVV-Ke, SPSVV-Is, LIYV, and BYV CP in homologous and heterologous combinations. The results summarized in Table 1 showed that the reciprocal decoration titers of the antisera to SPSVV-Ke and SPSVV-Is with particles of the four SPSVV isolates from Kenya, Israel, Nigeria, and the United States ranged from 3,200 to 12,800 but differed only by one to three twofold-dilution steps, suggesting a close serological relationship among the geographically diverse closterovirus isolates from sweet potato. The BYV-CP antiserum had a homologous decoration titer of only 1:200 and did not decorate particles of any of the SPSVV isolates, confirming the nonspecificity of the weak reaction of this antiserum with the expressed protein in Western blots (Fig. 2, lane 6).

Serological relationships between SPSVV isolates and LIYV. Interestingly, LIYV antiserum that had a homologous decoration titer of 1:12,800 gave a decoration titer of 1:800 only with SPSVV-Ke particles and did not decorate particles of the three other SPSVV isolates (Table 1), even at an antiserum dilution of 1:3. Decoration of SPSVV particles with LIYV antiserum also was demonstrated for eight other SPSVV isolates originating from various locations in Kenya. The weak heterologous IEM reaction of the LIYV antiserum with SPSVV-Ke was corroborated by a Western blot experiment in which the LIYV antiserum weakly

TABLE 1. Homologous and heterologous decoration titers of antisera to lettuce infectious yellows virus (LIYV), beet yellows virus (BYV) coat protein, virions of the sweet potato sunken vein virus isolate from Israel (SPSVV-Is), and the expressed coat protein of SPSVV-Ke (Kenya) with particles of LIYV, BYV, SPSVV-Ke, SPSVV-Is, SPSVV-N (Nigeria), and SPSVV-USA

Virus isolates[a]	Antiserum[b]			
	SPSVV-Is	SPSVV-Ke	LIYV	BYV
SPSVV-Is	12,800	12,800	...[c]	...
SPSVV-N	6,400	6,400	...	...
SPSVV-USA	12,800	3,200	...	...
SPSVV-Ke	12,800	12,800	800	...
LIYV	...	...	12,800	nt[d]
BYV	...	...	...	200

[a] Trapping of particles of SPSVV-Is, SPSVV-Ke, SPSVV-N, and SPSVV-USA was done for 24 h on grids coated with a 1:1,000 dilution of the SPSVV-Is antiserum. Using similar conditions, LIYV and BYV particles were trapped by their homologous antisera.

[b] Reciprocal values of decoration titers.

[c] No decoration visible at an antiserum dilution of 1:3.

[d] Not tested.

reacted only with the viral CP of SPSVV-Ke but not with the SPSVV-Ke expressed CP or with the viral CP of SPSVV-N and SPSVV-USA (data not shown).

In reciprocal tests, neither the antiserum to SPSVV-Ke nor the antiserum to SPSVV-Is decorated LIYV particles. This was confirmed by ISEM experiments in which both the SPSVV-Ke and SPSVV-Is antisera also failed to trap any LIYV particles after incubation of extracts from virus-infected leaves for 48 h, whereas 448 particles of SPSVV-Ke and 200 particles of SPSVV-Is were trapped per standard area (approximately 1,000 μm^2) in homologous combinations. Under these conditions 338, 41, and no particles of LIYV, SPSVV-Ke, and SPSVV-Is, respectively, were trapped by the LIYV antiserum. Together with the Western blot results, the IEM data suggest that SPSVV-Ke shares at least one antigenic determinant with LIYV and, thus, deviates slightly serologically from the three other SPSVV isolates, which did not react with LIYV antiserum.

The reaction of the LIYV antiserum with SPSVV-Ke particles differed from its homologous decoration titer by more than four twofold-dilution steps, and thus, the serological relationship between LIYV and SPSVV-Ke seems to be weak. In reciprocal tests, the SPSVV-Ke antiserum did not decorate or trap LIYV particles, indicating a distant one-way relationship between SPSVV-Ke and LIYV. This distinctness is supported further by an aa sequence similarity of only 25% between the CPs of SPSVV-Ke and LIYV (current study), although there are stretches of identical aa residues in the CPs of SPSVV-Ke and LIYV (Fig. 1), which could result in common antigenic determinants. The reason for the one-way relationship between SPSVV-Ke and LIYV might be the fact that the SPSVV-Ke and LIYV antisera were raised against expressed CP and predominantly intact virions (15), respectively. Unlike the SPSVV-Ke antiserum, the LIYV antiserum seems to contain a certain amount of antibodies capable of detecting external epitopes that are shared by LIYV and SPSVV-Ke particles. In addition, the expressed CP may differ in conformation from viral CP monomers and, hence, have different epitopes, because the LIYV antiserum reacted in Western blots only with the viral CP of SPSVV-Ke, not with its expressed CP.

In conclusion, we identified the CP gene of SPSVV-Ke based on sequence analyses, Western blot analysis, and IEM. The finding of low but significant CP aa sequence similarity between SPSVV-Ke and monopartite and bipartite closteroviruses supports the earlier assignment of SPSVV isolates to the genus *Closterovirus*. Based on a CP aa sequence identity of about 25% and a weak serological relationship between SPSVV-Ke and LIYV, SPSVV-Ke has distinct CP affinities to LIYV, a bipartite closterovirus (20). In addition to these CP properties, LIYV and SPSVV isolates share particle morphology (current study), vector specificity (11,15,22), and similar dsRNA sizes (11,19, current study), suggesting a bipartite single-stranded RNA genome also for SPSVV. Our data provide the first report of a close serological relationship among geographically diverse closterovirus isolates from sweet potato and suggest that SPSVV has a worldwide distribution. However, SPSVV-Ke appears to be a serologically distinct strain of SPSVV, because it differed from the other SPSVV isolates in the epitopes recognized by the LIYV antiserum.

LITERATURE CITED

1. Abad, J. A., French, R. D., and Moyer, J. W. 1992. Double stranded (DS) RNA analysis of the white-fly component of the sweetpotato virus disease (SPVD-WF) of sweetpotato. (Abstr.) Phytopathology 82:1070.
2. Agranovsky, A. A., Boyko, V. P., Karasev, A. V., Lunina, N. A., Koonin, E. V., and Dolja, V. V. 1991. Nucleotide sequence of the 3′-terminal half of the beet yellows closterovirus RNA genome: Unique arrangement of eight virus genes. J. Gen. Virol. 72:15-23.
3. Agranovsky, A. A., Koenig, R., Maiss, E., Boyko, V. P., Casper, R., and Atabekov, J. G. 1994. Expression of the beet yellows closterovirus capsid protein and p24, a capsid protein homologue, *in vitro* and *in vivo*. J. Gen. Virol. 75:1431-1439.
4. Agranovsky, A. A., Koonin, E. V., Boyko, V. P., Maiss, E., Frötschl, R., Lunina, N. A., and Atabekov, J. G. 1994. Beet yellows closterovirus: Complete genome structure and identification of a leader papain-like thiol protease. Virology 198:311-324.
5. Agranovsky, A. A., Lesemann, D.-E., Maiss, E., Hull, R., and Atabekov, J. G. 1995. "Rattlesnake" structure of a filamentous plant RNA virus built of two capsid proteins. Proc. Natl. Acad. Sci. USA 92:2470-2473.
6. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. J. Mol. Biol. 215:403-410.
7. Bar-Joseph, M., and Lee, R. F. 1989. Citrus tristeza virus. CMI/AAB Descriptions of Plant Viruses, No. 383. Commonwealth Mycological Institute and the Association of Applied Biologists, Kew, Surrey, England.
8. Bar-Joseph, M., and Murant, A. F. 1982. Closterovirus group. CMI/AAB Descriptions of Plant Viruses, No. 260. Commonwealth Mycological Institute and the Association of Applied Biologists, Kew, Surrey, England.
9. Boyko, V. P., Karasev, A. V., Agranovsky, A. A., Koonin, E. V., and Dolja, V. V. 1992. Coat protein gene duplication in a filamentous RNA virus of plants. Proc. Natl. Acad. Sci. USA 89:9156-9160.
10. Candresse, T., and Martelli, G. P. 1995. Genus *Closterovirus*. Pages 461-464 in: Virus Taxonomy. F. A. Murphy, C. M. Fauquet, D. H. L. Bishop, S. A. Ghabrial, A. W. Jarvis, G. P. Martelli, M. A. Mayo, and M. D, Summers. 6th Report of the International Committee on Taxonomy of Viruses. Arch. Virol. Suppl. 10.
11. Cohen, J., Franck, A., Vetten, H. J., Lesemann, D. E., and Loebenstein, G. 1992. Purification and properties of closterovirus-like particles associated with a whitefly-transmitted disease of sweet potato. Ann. Appl. Biol. 121:257-268.
12. Devereux, J., Haeberli, P., and Smithies, O. 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. 12:387-395.
13. Dolja, V. V., Boyko, V. P., Agranovsky, A. A., and Koonin, E. V. 1991. Phylogeny of capsid proteins of rod-shaped and filamentous RNA plant viruses: Two families with distinct patterns of sequence and probably structure conservation. Virology 184:79-86.
14. Dolja, V. V., Karasev, A. V., and Koonin, E. V. 1994. Molecular biology and evolution of closteroviruses: Sophisticated build-up of large RNA genomes. Annu. Rev. Phytopathol. 32:261-285.
15. Duffus, J. E., Larsen, R. C., and Liu, H. Y. 1986. Lettuce infectious yellows virus—A new type of whitefly-transmitted virus. Phytopathology 76:97-100.
16. Higgins, D. G., and Sharp, P. M. 1988. CLUSTAL: A package for performing multiple sequence alignment on a microcomputer. Gene 73:237-244.
17. Jelkmann, W., Martin, R. R., and Maiss, E. 1989. Cloning of four plant viruses from small quantities of double-stranded RNA. Phytopathology 79:1250-1253.
18. Karasev, A. V., Boyko, V. P., Gowda, S., Nikolaeva, O. V., Hilf, M. E., Koonin, E. V., Niblett, C. L., Cline, K., Gumpf, D. J., Lee, R. F., Garnsey, S. M., Lewandowski, D. J., and Dawson, W. O. 1995. Complete sequence of the citrus tristeza virus RNA genome. Virology 208:511-520.
19. Klaassen, V. A., Boeshore, M., Dolja, V. V., and Falk, B. W. 1994. Partial characterization of the lettuce infectious yellows virus genomic RNAs, identification of the coat protein gene, and comparison of its amino acid sequence with those of other filamentous RNA plant viruses. J. Gen. Virol. 75:1525-1533.
20. Klaassen, V. A., Boeshore, M. L., Koonin, E. V., Tian, T., and Falk, B. W. 1995. Genome structure and phylogenetic analysis of lettuce infectious yellows virus, a whitefly-transmitted, bipartite closterovirus. Virology 208:99-110.
21. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.) 227:680-685.
22. Larsen, R. C., Laakso, M., and Moyer, J. W. 1991. Isolation and vector relations of a whitefly-transmitted component of the sweet potato virus disease (SPVD) complex from Nigeria. (Abstr.) Phytopathology 81: 1157.
23. Milne, R. G. 1984. Electron microscopy for the identification of plant viruses in *in vitro* preparations. Methods Virol. 7:87-120.
24. Milne, R. G., and Lesemann, D.-E. 1984. Immunosorbent electron microscopy in plant virus studies. Methods Virol. 8:85-101.
25. Morris, T. J., and Dodds, J. A. 1979. Isolation and analysis of double-stranded RNA from virus-infected plant and fungal tissue. Phytopathology 69:854-858.
26. Moyer, J. W., and Salazar, L. F. 1989. Viruses and viruslike diseases of sweet potato. Plant Dis. 73:451-455.
27. Pappu, H. R., Karasev, A. V., Anderson, E. J., Pappu, S. S., Hilf, M. E., Febres, V. J., Eckloff, R. M. G., McCaffery, M., Boyko, V., Gowda, S., Dolja, V. V., Koonin, E. V., Gumpf, D. J., Cline, K. C., Garnsey, S. M., Dawson, W. O., Lee, R. F., and Niblett, C. L. 1994. Nucleotide sequence and organization of eight 3′ open reading frames of the citrus tristeza

closterovirus genome. Virology 199:35-46.
28. Pio-Ribeiro, G., Winter, S., Hamilton, R. I., de Assis Filho, F. M., and da Paz, C. D. 1994. First report of sweet potato virus disease-associated closterovirus in Brazil. Plant Dis. 78:1122.
29. Rossel, H. W., and Thottappilly, G. 1985. Sweet potato. Pages 10-13 in: Virus Diseases of Important Food Crops in Tropical Africa. International Institute of Tropical Agriculture, Ibadan, Nigeria.
30. Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
31. Sanger, F., Hicklen, S., and Coulson, A. R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA. 74:5463-5467.
32. Schaefers, G. A., and Terry, E. R. 1976. Insect transmission of sweet potato disease agents in Nigeria. Phytopathology 66:642-645.
33. Sekiya, M. E., Lawrence, S. D., McCaffery, M., and Cline, K. 1991. Molecular cloning and nucleotide sequencing of the coat protein gene of citrus tristeza virus. J. Gen. Virol. 72:1013-1020.
34. Towbin, H., Staehelin, T., and Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. Proc. Natl. Acad. Sci. USA 76:4350-4354.
35. Winter, S., Purac, A., Leggett, F., Frison, E. A., Rossel, H. W., and Hamilton, R. I. 1992. Partial characterization and molecular cloning of a closterovirus from sweet potato infected with the sweet potato virus disease complex from Nigeria. Phytopathology 82:869-875.