Isolation, Molecular Cloning, and Detection of Strawberry Vein Banding Virus DNA

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ABSTRACT

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DNA with caulimovirus properties was isolated from strawberry vein banding virus (SVBV). Native viral DNA of 7.8 kbp was circular and double-stranded. Each DNA strand contained one discontinuity positioned at either 0.0 or 0.5 map units on the circular molecule. *Eco*RI-digested SVBV DNA was cloned into the *Escherichia coli* plasmid pUC8. A recombinant plasmid (pSVBV-E3) containing a 7.8-kbp *Eco*RI insert hybridized to SVBV DNA but not to cauliflower mosaic virus DNA and had a restriction map identical to that of SVBV DNA. Dot hybridization tests using pSVBV-E3 as a probe indicated SVBV DNA titer varied greatly between leaflets sampled from the same plant.

Strawberry vein banding virus (SVBV) was originally described as a distinct virus infecting strawberry by Frazier in 1955 (4). Although vigor may be reduced in commercial strawberry cultivars infected with SVBV, the symptoms produced are not sufficient for diagnosis (6,14,15). Detection of SVBV in strawberry is currently performed by graft transmission to indicator species of *Fragaria*. In indicator species, symptom expression is variable and depends upon the isolate tested (5) and the presence of other viruses (15) as well as environmental and cultural factors (5).

SVBV is a member of the caulimovirus group of plant viruses (3,6). SVBV-infected cells contain cytoplasmic inclusion bodies, typical of those produced by other caulimoviruses, composed of 40- to 45-nm spherical virions embedded in a proteinaceous matrix (10). Further evidence of the caulimovirus nature of SVBV was provided by Morris et al (16), who established a distant serological relationship between SVBV and cauliflower mosaic virus (CaMV), using CaMV antiserum.

Although SVBV is presumed to have a double-stranded DNA genome (3,6), the nucleic acid of SVBV has not previously been isolated or analyzed. We describe the isolation and molecular cloning of SVBV DNA. Our analysis and preliminary physical mapping of the SVBV genome provide additional evidence for the inclusion of SVBV in the caulimovirus group. We also demonstrate the utility of cloned viral DNA sequences for the detection of SVBV in infected plants.

MATERIALS AND METHODS

Virus isolates and maintenance. The SVBV isolate used in this study was initially obtained from N. W. Frazier ca. 1978 and is the same isolate used in experiments described previously (16). SVBV was maintained by vegetative propagation of runners of an infected indicator species, *F. vesca* L. clone UC 6. The CaMV isolate used was an undesignated isolate obtained from R. J. Shepherd and propagated in *Brassica campestris* L. 'Just Right.' The identification of the isolate as CaMV was verified by host responses (systemic infection of turnip and local lesion production on *Datura stramonium* L.); the presence of cytoplasmic inclusions, as detected by light microscopy of epidermal strips stained with phloxine; reactivity with CaMV-cabbage B isolate antiserum; and semipersistent transmission by the cabbage aphid, *Brevicoryne brassicae* (L.).

Sources of enzymes and other materials. Restriction endonucleases were obtained from either Bethesda Research Laboratories (BRL, Gaithersburg, MD) or International Biotechnologies, Inc. (New Haven, CT) and used essentially as described by Maniatis et al (12). DNA polymerase I and T_4 DNA ligase were obtained from BRL. DNase I, RNase A, yeast tRNA, and salmon testes DNA were purchased from Sigma (St. Louis, MO). Proteinase K was purchased from Beckman (Palo Alto, CA). The nitrocellulose used was BA-85 (Schleicher & Schuell, Keene, NH).

Virion DNA isolation. CaMV DNA was isolated by the method of Gardner and Shepherd (7). SVBV DNA was isolated by adapting the procedure of R. D. Richins and R. J. Shepherd (*personal communication*) for the isolation of cassava vein mosaic virus DNA. Symptomatic leaves (300 g, processed in 75-g batches) of chronically infected UC 6 were ground to a fine powder in liquid N_2 and homogenized for 1 min in a blender with five volumes of extraction buffer containing 0.2 M TRIS-Cl (pH 7.5), 50 mM sodium benzoate, 20 mM ethylenediaminetetraacetic acid (EDTA), 2% polyvinylpyrrolidone-10, 0.2% 2-mercaptoethanol, and 1.5 M urea. Triton X-100 was added to 2%, and the homogenate stirred on ice overnight. The following day, the homogenate was centrifuged for 20 min at 6,000*g*, and the supernatant filtered through Miracloth (Cal Biochem, La Jolla, CA). Virions were then concentrated from the supernatant by centrifugation through a 5-ml layer of 30% sucrose for 90 min at 63,000*g*. The virion-enriched pellets were washed three times with distilled H_2O, drained, and resuspended in TE (10 mM TRIS-Cl and 1 mM EDTA), pH 7.6 (9 ml per 75 g tissue extracted).

Unencapsidated nucleic acids present in enriched virion preparations were digested by incubation for 2 hr at 37 C with DNase I and RNase A (each at 20 $\mu g \cdot ml^{-1}$) after the addition of NaCl to 50 mM and $MgCl_2$ to 10 mM. Following nuclease digestion, Mg^{2+} was chelated by adding EDTA to 50 mM. Virions were then disrupted by adding sodium dodecyl sulfate (SDS) to 1% and proteinase K to 10 $\mu g \cdot ml^{-1}$. After incubation for 2 hr at 50 C, the mixture was chilled to 0 C and centrifuged at 12,000*g* for 10 min. The supernatant was extracted once with phenol, once with phenol/chloroform (1:1, by volume), twice with chloroform/isoamyl alcohol (24:1), and twice with water-saturated ether. DNA was concentrated from the aqueous phase by precipitation in 70% ethanol containing 0.1 M sodium acetate overnight at −20 C. The precipitate was recovered by centrifugation for 10 min at 8,000*g*, and the pellets were drained, vacuum dried, and resuspended in TE, pH 7.6. Most contaminants were removed by chromatography on Whatman DE 52 cellulose. Virion DNA was bound to the DE 52 column in TNE (TE with 100 mM NaCl), pH 7.6, and washed extensively with TNE, and the DNA was eluted from the column in TE, pH 7.6, containing 0.5 M NaCl. Yeast tRNA (1 $\mu g \cdot ml^{-1}$) was added to the eluate as a carrier, and the DNA concentrated by

ethanol precipitation. Further purification of virion DNA was accomplished by rate-zonal sedimentation in linear-log sucrose gradients.

Molecular cloning of caulimovirus DNA. Purified SVBV DNA was digested with the restriction endonuclease *Eco*RI and ligated to *Eco*RI-digested *Escherichia coli* plasmid pUC8 (20-to-1 mass excess of virion DNA to plasmid). CaMV DNA was digested with *Pst*I and ligated to *Pst*I-digested pUC8. Recombinant plasmids were used to transform *E. coli* strain JM 83 made competent by the $CaCl_2$ procedure as modified by Carrington and Morris (1), and the transformants plated onto Luria agar containing 50 $\mu g \cdot ml^{-1}$ ampicillin, 40 μM isopropylthio-β-D-galactoside, and 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (20 $\mu g \cdot ml^{-1}$) (20). After 20 hr of incubation at 37 C, ampicillin-resistant, white-pigmented colonies (containing plasmid with no β-galactosidase activity) were selected and screened for insert size by electrophoresis of the recombinant plasmids after digestion with restriction endonucleases. Recombinant plasmids containing insert DNA that comigrated with full-length linear virion DNA were further screened by Southern hybridization of ^{32}P-labeled plasmids with virion DNA.

Southern hybridization. Recombinant plasmids were purified from *E. coli* and labeled with ^{32}P by nick translation (12). Virion DNA samples were electrophoresed in 1% agarose using TRIS-phosphate-EDTA buffer (11). DNA was transferred to nitrocellulose by the Southern blotting procedure (12). Following transfer, the nitrocellulose filters were air dried and then baked under vacuum at 80 C for 2 hr. Prehybridization was accomplished at 42 C for 12–18 hr in 50% deionized formamide, 5× SSPE (1× = 150 mM NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA, pH 7.4), 5× Denhardt's solution (1× = 0.02% each ficoll, polyvinyl-pyrrolidone-10, and bovine serum albumin), and autoclaved salmon testes DNA at 250 $\mu g \cdot ml^{-1}$. Hybridization conditions were the same as for prehybridization, except that Denhardt's solution was adjusted to 2× and ^{32}P-labeled alkali-denatured plasmid DNA was added (specific activity 1×10^7 to 7×10^7 cpm $\cdot \mu g^{-1}$ DNA, 5×10^5 to 1×10^6 cpm $\cdot ml^{-1}$). Unhybridized probe was removed by washing the filters twice for 30 min each in 2× SSC (1× = 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) and 0.1% SDS at room temperature and twice for 30 min each in 0.1× SSC and 0.1% SDS at 65 C. Autoradiograms were prepared by exposing the dried filters to X-Omat X-ray film (Kodak) with the aid of a Du Pont Cronex Lightning Plus intensifier screen at −70 C for 24–72 hr.

Dot hybridization. Dot hybridization tests were performed as described for caulimoviruses by Maule et al (13) with the following modifications. Leaf tissue was first ground in liquid N_2 and then resuspended in 10 volumes of TE, pH 7.6. NaOH was added to 0.5 M, and the samples incubated at room temperature for 10 min. Aliquots were then spotted onto nitrocellulose (presoaked in 6× SSC), using a BRL Hybridot manifold with suction. The filter was then neutralized, chloroform-treated, and baked as described by Maule et al (13). Prehybridization, hybridization, washing, and autoradiography were as described for Southern hybridization.

Physical mapping of SVBV DNA. Restriction endonuclease sites were mapped by single and double digests of virion or cloned SVBV DNA. Single-stranded discontinuities present in virion DNA were mapped by restriction endonuclease digestion followed by alkali denaturation of virion DNA (9), and the products analyzed by Southern hybridization.

Infectivity assays of cloned DNA. Apparent full-length cloned DNAs were excised from recombinant plasmids with *Eco*RI (SVBV) or *Pst*I (CaMV) and then mechanically inoculated to the appropriate plant host in TE, pH 7.6, using Celite as an abrasive. In some experiments, the excised viral inserts were circularized by ligation with T_4 DNA ligase under conditions favoring the production of circular monomers (12). Infectivity of cloned DNAs was determined by symptom expression and, in the absence of symptoms, by dot hybridization.

RESULTS

Virion DNA isolation. Preparations of SVBV DNA recovered from DE 52 cellulose contained species with electrophoretic mobilities similar to those of circular and linear forms of CaMV DNA (Fig. 1). SVBV linear DNA was estimated to be slightly smaller (7.8 kbp) than CaMV linear DNA (8.0 kbp). SVBV DNA purified on DE 52 was often brown and still contained impurities that inhibited most restriction endonucleases. Further purification of SVBV DNA by rate-zonal sedimentation in sucrose gradients separated virion DNA from most of the brown material, which remained near the top of the gradient. Gradient fractions containing circular SVBV DNA (19S) were selected for further analysis. The yield of SVBV DNA was low relative to that of CaMV, with less than 1 μg recovered from 1 kg of tissue.

Molecular cloning of SVBV DNA. The circular nature of gradient-purified 19S SVBV DNA was confirmed by analysis with restriction endonucleases. When digested with *Eco*RI, *Bam*HI, *Kpn*I, or *Pst*I, 19S SVBV virion DNA migrated in agarose gels with the linear form of CaMV DNA, indicating that the native molecule was circular and that each restriction endonuclease cleaved circular SVBV DNA at a single site. CaMV virion DNA contained a single *Pst*I site. Two recombinant plasmids, designated pSVBV-E3 and pCaMV-1, containing apparent full-length caulimovirus inserts (Fig. 2), were analyzed by Southern hybridization with virion DNAs (Fig. 3). Each recombinant plasmid hybridized to only the homologous virion DNA. Minor bands in SVBV DNA preparations, not evident by ethidium bromide staining, were detected by Southern hybridization. The nature of these bands is unknown, although they appear to be

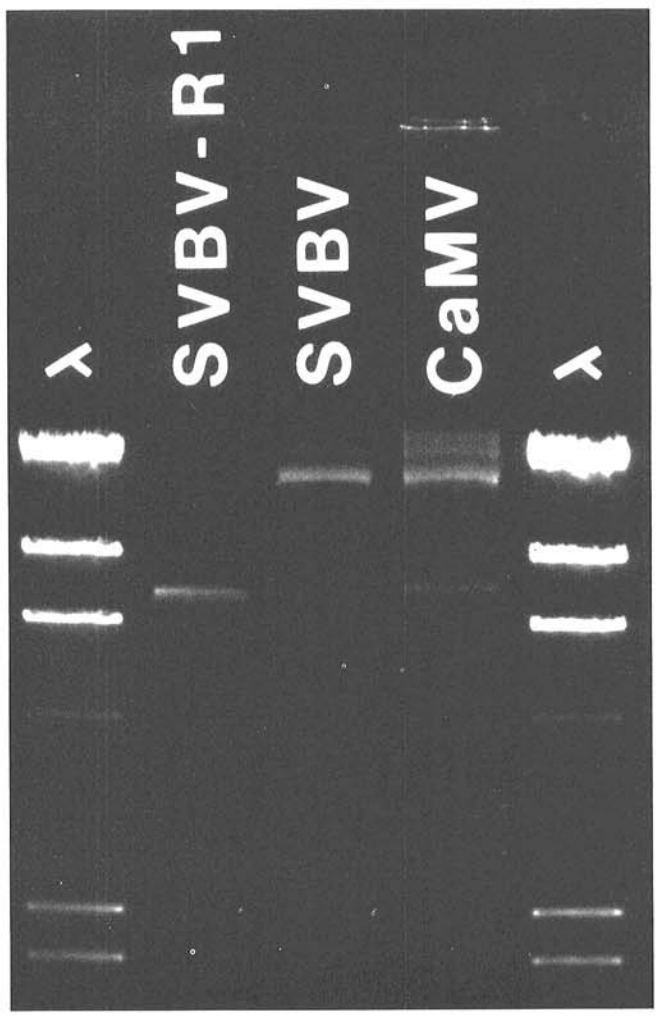

Fig. 1. Electrophoresis of native virion DNA (SVBV) and *Eco*RI-digested virion DNA (SVBV-R1) of strawberry vein banding virus, in 1% agarose, relative to DNA of cauliflower mosaic virus (CaMV), with its circular forms (upper three bands) and linear form (lower band). The outer lanes (λ) are lambda *Hin*dIII fragments, used as size standards.

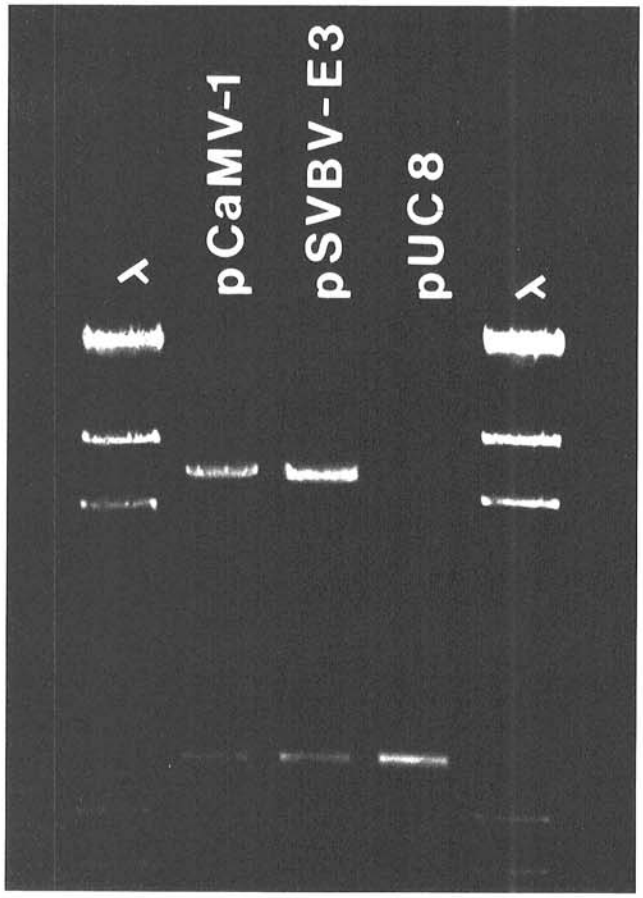

Fig. 2. Electrophoresis of the recombinant plasmids pSVBV-E3 and pCaMV-1, containing caulimovirus DNA inserts, in 1% agarose. pSVBV-E3 was digested with *Eco*RI, and pCaMV-1 digested with *Pst*I. The *Escherichia coli* plasmid pUC8, containing no insert, was digested with *Eco*RI. The outer lanes (λ) are lambda *Hin*dIII size standards.

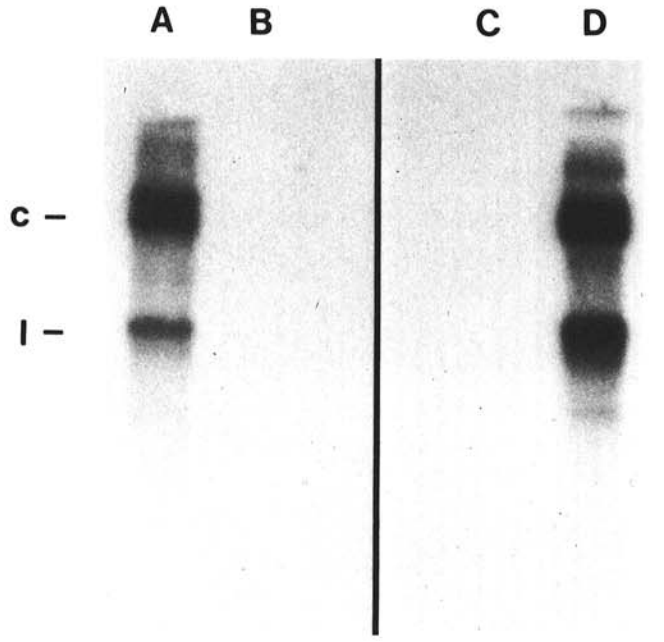

Fig. 3. Southern hybridization of virion DNAs (50 ng) of cauliflower mosaic virus (A and C) and strawberry vein banding virus (B and D) probed with nick-translated pCaMV-1 (A and B) and pSVBV-E3 (C and D). Letters at left denote the mobility of circular (c) and linear (l) forms present in native virion DNA preparations.

virus-related, because they hybridized specifically with pSVBV-E3.

Physical mapping of SVBV DNA. An endonuclease restriction map of SVBV DNA was constructed using pSVBV-E3 (Fig. 4). The 7.8-kbp *Eco*RI insert of pSVBV-E3 contained no internal *Eco*RI sites and a single site each for *Bam*HI, *Pst*I, and *Kpn*I. Both the number and the position of the *Eco*RI, *Bam*HI, *Pst*I, and *Kpn*I sites present in pSVBV-E3 were consistent with endonuclease restriction sites mapped on circular SVBV DNA isolated from virions.

Single-stranded discontinuities present in circular SVBV DNA were mapped by alkali denaturation with or without prior endonuclease digestion (Fig. 5). When circular SVBV DNA was alkali-denatured, only a single major band was observed, which comigrated with the α strand of alkali-denatured CaMV DNA. This result suggested SVBV circular DNA contained only two single-stranded discontinuities, with each strand containing a

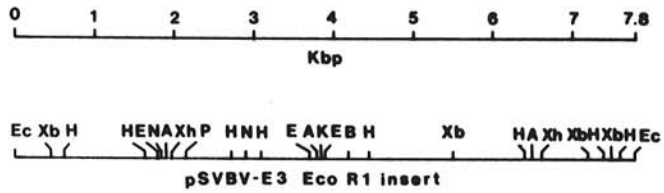

Fig. 4. Endonuclease restriction map of the 7.8-kbp *Eco*RI insert of pSVBV-E3. Abbreviations for endonuclease restriction enzymes are: Ec, *Eco*RI; Xb, *Xba*I; H, *Hin*dIII; E, *Eco*RV; N, *Nco*I; A, *Ava*I; Xh, *Xho*I; P, *Pst*I; K, *Kpn*I; and B, *Bam*HI.

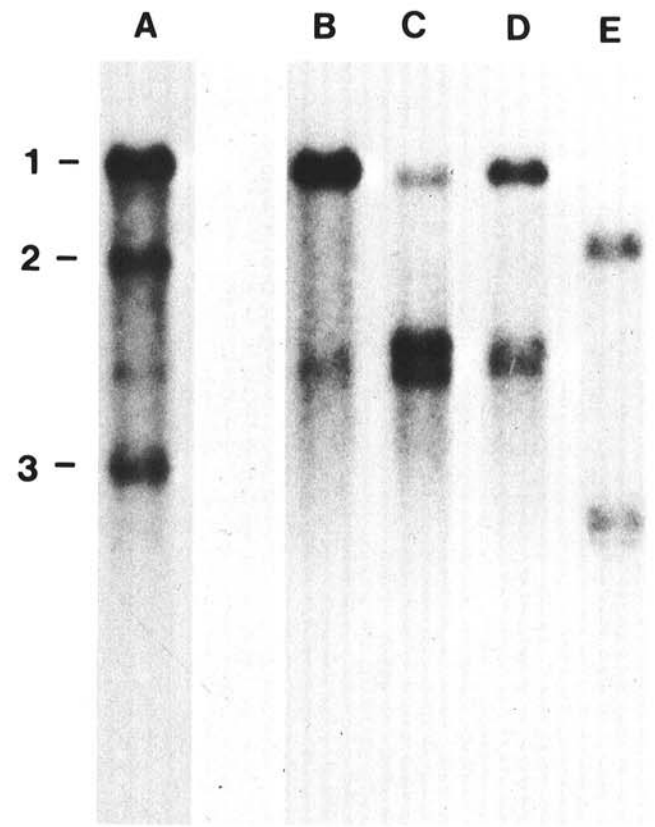

Fig. 5. Southern hybridization of alkali-denatured DNA of cauliflower mosaic virus (A), probed with pCaMV-1, and strawberry vein banding virus (B–E), probed with pSVBV-E3. Prior to alkali denaturation, strawberry vein banding virus DNA was digested with no enzyme (B), *Eco*RI (C), *Bam*HI (D), and *Pst*I (E). Numbers at left denote the mobility of the α (1), β (2), and γ (3) strands of cauliflower mosaic virus DNA.

single gap. In contrast, alkali-denatured CaMV DNA yielded three major single-stranded species, indicating this isolate of CaMV contained the usual complement of single-stranded discontinuities present in most CaMV isolates. That the major band observed for alkali-denatured SVBV DNA consisted of two single-stranded species of equal size was confirmed by restriction endonuclease digestion prior to alkali denaturation. When digested with *Eco*RI or *Bam*HI prior to alkali denaturation, SVBV DNA yielded three fragments of single-stranded DNA. For both enzymes, one of the fragments remained nearly the same size as in undigested alkali-denatured preparations, suggesting that one gap was situated near the *Eco*RI site, and the other gap was located close to the *Bam*HI site. The remaining two, smaller single-stranded fragments in both preparations migrated as closely spaced doublets. These fragments were probably derived from the respective complementary DNA strand, because the sum of their sizes, in each case, was close to the size estimated for full-length, undigested SVBV DNA. The location of the two single-stranded discontinuities was confirmed by *Pst*I digestion and alkali denaturation of SVBV DNA; only two single-stranded DNA bands, of 5.9 and 1.9 kb, were resolved by electrophoresis. Since the sum of the sizes of the two bands was only half of the expected total, each band was interpreted as consisting of two single-stranded species of equal size and mobility. This interpretation was consistent with the location of the *Pst*I site relative to the *Eco*RI and *Bam*HI sites. From these results it was possible to map the locations of the two single-stranded discontinuities relative to the positions of the *Eco*RI, *Pst*I, and *Bam*HI restriction sites on the circular molecule (Fig. 6).

Prior to gradient fractionation, many SVBV virion DNA preparations contained low amounts of a double-stranded, linear DNA of 3.9 kbp, which strongly hybridized to pSVBV-E3 in Southern hybridizations (data not shown). As full-length linear molecules likely arise from breakage of circular molecules at or near one of the gaps, breakage of SVBV DNA at both gaps should result in two equal-sized fragments of the same size as the observed 3.9-kbp band. In some SVBV DNA preparations, the 3.9-kbp band reappeared after gradient fractionation upon storage at 4 C. The minor band visible in the no-enzyme, alkali-denatured treatment of Figure 5 (migrating near the doublets produced in the adjacent *Eco*RI and *Bam*HI treatments) is the correct size expected for the single-stranded form of the 3.9-kbp band. The size, sequence-relatedness, and reappearance upon storage strongly suggest that the 3.9-kbp band is a specific breakdown product of the virion DNA and support the positioning of the gaps as depicted in Figure 6.

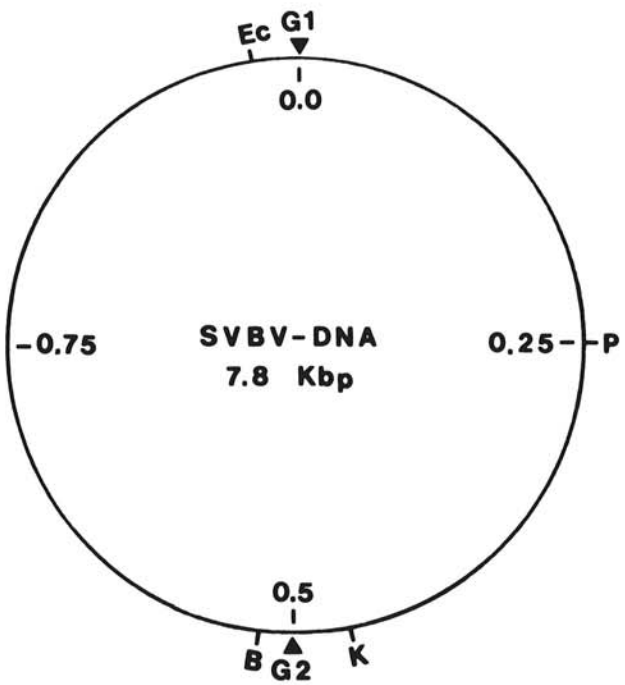

Fig. 6. Physical map of native circular virion DNA of strawberry vein banding virus. Numbers denote map units, single-stranded discontinuities are designated as G1 and G2, and sites for endonuclease restriction enzymes are abbreviated as in Figure 4.

Detection of SVBV DNA in tissue extracts. SVBV DNA was detected in tissue extracts from symptomatic leaves of *F. vesca* infected with SVBV, but not in extracts from healthy plants, by dot hybridization using pSVBV-E3 as probe (Fig. 7). Pretreatment of the samples with proteinase K (13) prior to alkali denaturation had no effect on hybridization. The distribution and titer of SVBV DNA in infected plants varied greatly between leaflets sampled from the same infected plant. When 24 leaflets were individually analyzed from a single plant, samples from leaflets with symptoms hybridized strongly, whereas symptomless leaflets contained little or no detectable SVBV DNA (Fig. 8). Uneven distribution of SVBV DNA was also observed in the symptomless commercial strawberry cultivar Tustin (data not shown).

Infectivity of cloned caulimovirus DNAs. *Pst*I-digested pCaMV-1 was infectious to turnip inoculated with a concentration of 10 $\mu g \cdot ml^{-1}$, and the symptoms produced were identical to those obtained with native CaMV virion DNA. No symptoms were observed in *F. vesca* or *F. virginiana* Duchesne after mechanical inoculation with *Eco*RI-digested pSVBV-E3 at concentrations up to 100 $\mu g \cdot ml^{-1}$. SVBV DNA was not detected in the inoculated plants by dot hybridization performed 30, 62, and 91 days after inoculation; nor was pSVBV-E3 infectious when the excised viral insert was circularized by ligation prior to inoculation. The infectivity of pCaMV-1 was not appreciably affected by

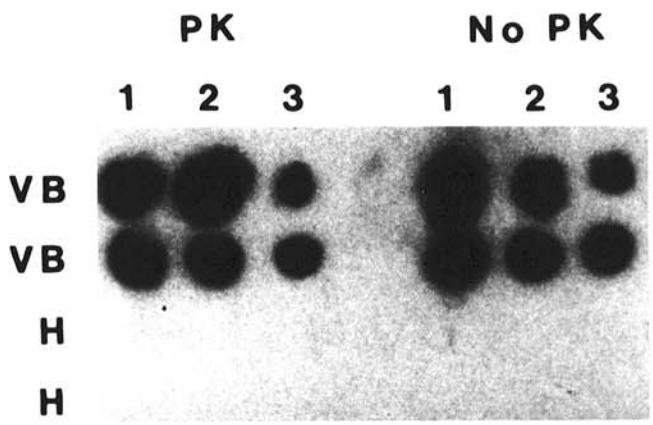

Fig. 7. Dot hybridization of extracts from healthy *Fragaria vesca* (H) and symptomatic plants infected with strawberry vein banding virus (VB), treated with proteinase K (PK) or untreated (No PK) and probed with nick-translated pSVBV-E3. Numbers denote the volume of each sample applied (1 = 25 μl, 2 = 5 μl, and 3 = 1 μl), where 1 μl is equivalent to 0.1 mg of plant tissue extracted.

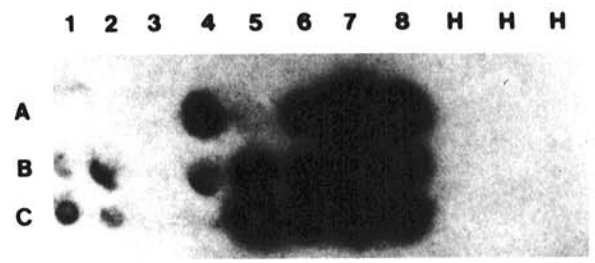

Fig. 8. Dot hybridization of extracts from individual leaflets (A–C) of eight leaves collected from a single *Fragaria vesca* plant chronically infected with strawberry vein banding virus (1–8) and from three leaves collected from a healthy plant (H). Leaflets from leaves 1, 2, and 3 were symptomless, as were leaflets 4C and 5A. The remaining leaflets from the infected plant were symptomatic, with varying amounts of chlorosis along the veins.

circularization of the excised viral insert. The infectivity of native SVBV virion DNA was not evaluated, because of the limited amount of DNA obtainable from infected plants.

DISCUSSION

The yield of recoverable SVBV DNA from infected *F. vesca* was too low to permit extensive physical analysis of DNA isolated directly from virions. This was due, in part, to the low titer and uneven distribution of the virus in the infected plants. Recovery of DNA of sufficient purity to permit electrophoretic analysis and restriction enzyme digestion required extensive purification to separate DNA from viscous host contaminants present in strawberry extracts. Host nucleic acids were removed by extensive nuclease digestion prior to virion disruption, although it was not always possible to completely eliminate host DNA at this stage of purification. Subsequent purification of DNA on DE 52 cellulose followed by rate-zonal centrifugation provided DNA of sufficient purity to permit manipulations necessary for cloning. Virion DNA preparations prepared in this manner were rarely pure, and it was often possible to visualize host DNA migrating as a smear between the circular and the linear viral DNA bands in stained gels. These limitations necessitated that the characterization of the viral genome be accomplished primarily from cloned sequences.

Southern hybridization experiments using cloned CaMV and SVBV DNAs as probes demonstrated that the two viral genomes shared no detectable sequence homology and that they are distinct caulimoviruses. Morris et al previously reported a distant serological relationship between the two viruses in enzyme-linked immunosorbent assay using CaMV antisera (16). The hybridization results are not inconsistent with this observation, in view of the limited sequence homology reported among caulimoviruses (2,17), which also share distant serological relationships (16,18).

Physical maps of caulimovirus DNA are by convention oriented with the gap in the α strand at the top (0.0 map units) with the 5′ end of the α strand on the left side of the α gap. For most other caulimoviruses, the α gap may easily be mapped, since the complementary strand contains two or three discontinuities. However, since SVBV DNA has only a single gap in each strand, we were unable to ascertain which strand is analogous to the α strand of other caulimoviruses. Therefore, the orientation of the SVBV map is arbitrary, with the gap located near the *Eco*RI site placed at the top and designated G1. Furthermore, the SVBV map was not oriented with regard to strand polarity. Thus, the SVBV map presented is only one of four possible orientations and may require corrections (rotation to place gap 2 at the top or conversion to the mirror image) to align it with maps of other caulimoviruses.

It has generally been possible to demonstrate the fidelity of cloned caulimovirus genomes by mechanical inoculation of susceptible host plants with excised full-length viral inserts (8). We were able to similarly demonstrate infectivity of *Pst*I-digested pCaMV-1 on turnip but could not infect *Fragaria* hosts with the *Eco*RI-digested pSVBV-E3 clone. Successful inoculation with the cloned DNA could not be achieved at high inoculum concentrations of either linear DNA or circular monomers of the cloned viral insert. The lack of infectivity of the SVBV clone might indicate that the insert did not contain the entire viral sequence, despite its apparent full length. A small fragment could be missing as a result of a second, undetected *Eco*RI site located close to the one used for cloning. To evaluate this possibility, *Sau*3A fragments spanning the *Eco*RI site were cloned from viral DNA and analyzed on 8% polyacrylamide gels after *Eco*RI digestion. No small *Eco*RI fragments were identified by this procedure. This suggests that the lack of infectivity of the clone may not result from incomplete cloning of the genome. Rosaceous hosts are, in general, particularly refractory to mechanical inoculation by plant viruses, and there have been few reports of success. We were unable, for example, to infect *F. vesca* with purified tobacco streak virus at concentrations (1 mg · ml^{-1}) that were highly infectious on other hosts (19). In view of the restricted host range of SVBV, it has not yet been possible to discriminate between host susceptibility (to mechanical inoculation) and clone integrity as the cause of the lack of infectivity of pSVBV-E3.

An important objective of this research was to develop an assay that would be useful for routine detection of SVBV in infected plants. The lack of overt symptoms produced by SVBV infection in most commercial strawberry cultivars and the inability to purify sufficient quantities of antigen for antisera production (16) led us to construct recombinant DNA clones that would be useful in dot hybridization tests. Nick-translated pSVBV-E3 readily detected viral DNA in infected plants and demonstrated that the distribution of SVBV DNA was uneven in infected plant tissues of the *Fragaria* indicator clone, UC 6. In this host, the intensity of the hybridization signal corresponded roughly to the severity of symptom expression on the different leaflets sampled from the same plant. Similar variation in signal intensity was also observed in assays of samples from an infected commercial strawberry cultivar in which no symptoms were expressed. These results indicate the dot hybridization assay is sufficiently sensitive and specific to be a useful diagnostic test for the routine detection of SVBV in strawberry if precautions are taken to ensure that a composite sample is evaluated from individual plants. The SVBV clone represents the second of two probes that are now available for the detection of strawberry virus diseases. We have demonstrated the utility of cloned DNA probes for the detection of both DNA viruses (in the present study) and RNA viruses (19) in strawberry. As additional DNA probes become available for the other viral diseases of strawberry, dot hybridization may supplement or replace traditional bioassay procedures for the indexing of strawberry cultivars in clean-stock programs.

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