Molecular Cloning and Physical Mapping of Potato Virus S Complementary DNA

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ABSTRACT

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Complementary DNA clones that represent almost the full length of the Andean strain of potato virus S (PVS-An) genomic RNA were produced. Complementary DNA of 7.5 kilobase pairs (kbp) was synthesized and was either digested with restriction enzymes to produce cohesive ends or attached to *Eco*RI linkers and cloned into *Escherichia coli* plasmids pUC19 and pTZ18R. The size of the overlapping clones ranged from 0.4 to 6.0 kbp. A physical map of the overlapping clones was produced by restriction endonuclease digestion and Southern blot hybridization analysis. The clones have been successfully used in northern and dot blot hybridization assays to detect PVS-An specific sequences in crude leaf extracts of potato and *Chenopodium quinoa* infected with this virus. Furthermore, the sequence of the 5′ end of PVS-An not represented in the cDNA (261 bases) was obtained.

Additional keyword: carlavirus.

Potato virus S (PVS) belongs to the carlavirus group. The virus particles of this group are rods about 610–700 nm in length, composed of a single coat protein subunit with an M_r of about 33K and one molecule of positive-sense-single-stranded RNA with an M_r between 2.3 and 2.6 $\times$ 10^6 (7–7.8 kb) (9). The Andean (An) strain of PVS produces systemic infection in *Chenopodium quinoa* Willd. and is aphid transmissible in a nonpersistent manner; aphids do not transmit the type strain (15). There is little information on the molecular biology of the genome of this virus. Our work showed that PVS-An has a plus-sense-single-stranded RNA with a M_r of 2.4 $\times$ 10^6 (7.5 kb), that is polyadenylated at its 3′ end (10). In vitro translational studies in rabbit reticulocyte lysates indicated that PVS-An genomic RNA directs the synthesis of at least four major protein products of M_r of 140K, 123K, 104K, and 34K (10). The 34K product was identified as the viral coat protein subunit and is the only polypeptide with a known function. To date it is not known which mechanism(s) is used by this virus in its genome expression. We found no evidence of proteolytic processing, but have not investigated the possibility of the production of readthrough proteins in the replication of PVS-An. To further understand the genomic strategy of this virus, we have produced and mapped complementary DNA clones that represent almost the full length of PVS-An genomic RNA. These cDNA clones were used in northern blot hybridization experiments to determine if PVS-An produces subgenomic RNAs during its replication cycle. Also, we used the cDNA clones as diagnostic tools for the detection of viral specific sequences in crude leaf extracts of leaves infected with PVS. The sequence of the 5′ end of PVS-An not represented in the cDNA clones is reported.

MATERIALS AND METHODS

Virus isolate. A strain of PVS isolated in Wisconsin was identified as the Andean strain (PVS-An) (15). It systemically infects *C. quinoa* and is aphid transmissible (15). The virus was purified as described by Monis and de Zoeten (10).

Isolation of RNA. RNA was isolated from purified virions by the guanidine thiocyanate-LiCl procedure described by Cathala et al (2). Total RNA was isolated from *C. quinoa* plants infected with PVS-An by a modification of the procedure described by Silflow et al (14). Infected leaves (1 g) were ground in liquid N_2 and homogenized in 50 mM Tris-HCl, pH 8.0, containing 4% *p*-aminosalicylic acid, 1% triisopropylnapthalenesulfonic acid, 10 mM dithiothreitol (DTT), and 10 mM sodium metabisulfite. The homogenate was first extracted with Tris-saturated phenol, and then extracted with chloroform-isoamyl alcohol (24:1). The RNA was precipitated in the presence of 3 M LiCl. The RNA pellet was washed with 2 M LiCl, suspended in H_2O, and ethanol precipitated.

Isolation of dsRNA. dsRNA was prepared as described by Morris et al (11). Briefly, leaves of *C. quinoa* (5 g) infected with PVS-An (9 days postinoculation) were powdered in the presence of liquid N_2. The powder was extracted with an equal volume (w/v) of STE (5 mM Tris-HCl, pH 7.0, 0.1 M NaCl, 1 mM ethylenediaminetetraacetic acid [EDTA]) and Tris-saturated phenol:chloroform:isoamyl alcohol (25:24:1). Ethanol was added to the supernatant to a final concentration of 15%. To recover the dsRNA the supernatant was passed through a column containing CF-11 cellulose (Whatman). The column was washed several times with STE containing 15% ethanol. The dsRNA was eluted with 4 to 5 volumes of STE. The dsRNA was ethanol precipitated and pellets were resuspended in STE.

Complementary DNA synthesis and cloning strategy. PVS cDNA was produced by a modification of the procedure described by Gubler and Hoffman (4). The first strand synthesis was primed with oligo $(dT)_{12\text{-}16}$ (Pharmacia-LKB Biotechnology Inc., Piscataway, NJ), and reverse transcribed with the avian myoblastosis (AMV) reverse transcriptase (Life Sciences Inc., St. Petersburg, FL). Unincorporated nucleotides were removed by Sepharose 2B-300 column chromatography (Sigma Chemical Co., St. Louis, MO) and PVS-An cDNA was concentrated by ethanol precipitation. The second strand was synthesized with 0.36 μg of PVS-An first-strand cDNA, *E. coli* DNA ligase, DNA Polymerase I, and RNase H. The reaction was incubated for 60 min at 12 C followed by 60 min at 25 C, stopped by the addition of EDTA, extracted with phenol:chloroform:isoamyl alcohol (25:24:1), and the cDNA was ethanol precipitated in the

presence of ammonium acetate, digested with *Hin*dIII and *Kpn*I (Boehringer-Mannheim Inc. and BRL, Inc.) producing cohesive ends, and ligated to appropriately digested and dephosphorylated pUC19 plasmids (Pharmacia-LKB Biotechnology Inc.) (pH and pK plasmids in Fig. 1). Competent *E. coli* DH5 ∝ cells were transformed with the recombinant plasmids as described by Hanahan (5). Transformants containing pH or pK plasmids were selected on LB plates containing ampicillin and X-Gal and incubated at 37 C overnight. In an alternative cloning strategy, the double stranded cDNA was blunt ended as described by Maniatis et al (8), treated with *Eco*RI methylase (Promega Biotec, Madison, WI) and attached to phosphorylated *Eco*RI linkers (New England Biolabs, Beverly, MA) (pR plasmids in Fig. 1). This was followed by digestion with *Eco*RI and chromatography through Sepharose 2B-300 to remove excess linkers. The cDNA was concentrated by ethanol precipitation and ligated to *Eco*RI digested and dephosphorylated pTZ18R. Competent DH5 ∝ cells were transformed with these pR plasmids and selected as above. All steps of the cDNA synthesis were analyzed by alkaline agarose gel electrophoresis to determine the size range of the cDNA under denaturing conditions (8).

Characterization of recombinant plasmids and determination of the physical map of PVS-An clones. Transformants that were β-galactosidase negative and resistant to ampicillin were grown on selective media and their plasmids were extracted by the alkaline lysis procedure (8). To determine the size of the inserts, the recombinant plasmids were digested with the appropriate restriction endonuclease and assayed by agarose gel electrophoresis. A preliminary physical map of the PVS-An cDNA clones was produced by single and double digests using the following enzymes: *Bam*HI, *Eco*RI, *Hin*cII, *Hin*dIII, *Kpn*I, *Pst*I, and *Sst*I. The presence of overlapping clones and physical map was confirmed by Southern blot hybridization (16). Briefly, DNA from selected PVS-An cDNA clones was digested with the appropriate restriction endonuclease, electrophoresed on a 0.7% agarose gel, denatured, and electroblotted onto a Genescreen plus membrane (Du Pont Co., Boston, MA), prehybridized and hybridized as described by the manufacturer. The blots were washed twice in 2× SSC (0.3 M NaCl, 30 mM sodium citrate, pH 7.0) for 5 min at room temperature, in 2× SSC containing 0.01% SDS for 30 min at 65 C, and 0.1× SSC for 30 min at room temperature, the blot was sealed in a plastic bag and exposed to X-ray film.

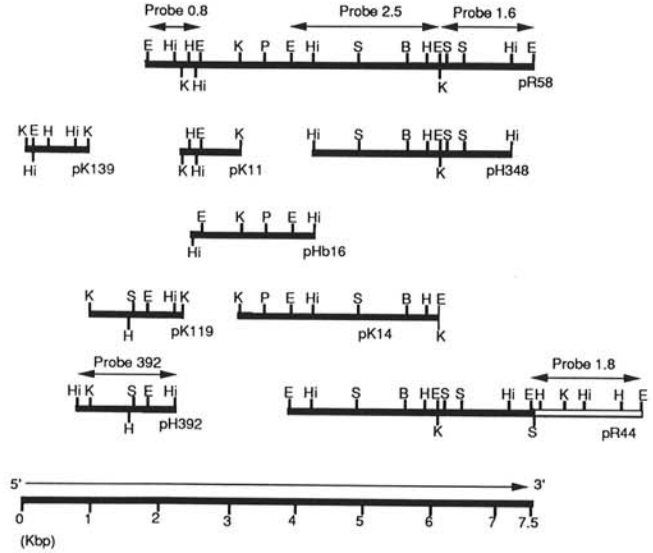

Fig. 1. Physical map of PVS-An cDNA clones produced by single and double digests with restriction endonucleases and Southern blot hybridization. The 0.8-kbp, 1.6-kbp, and 2.5-kbp *Eco*RI fragments from pR58 were subcloned into pTZ18R to produce labeled RNA probes. pH392 DNA was nick translated to produce probe H392. These probes were used to confirm the overlaps of the clones presented in this map. Restriction endonuclease sites are: *Bam*HI (B), *Eco*RI (E), *Hin*cII (H), *Hin*dIII (Hi), *Kpn*I (K), *Pst*I (P), and *Sst*I (S).

Hybridization probes. (^{32}P)-labeled cDNA probes were produced under the same conditions outlined for the first strand cDNA synthesis. To synthesize (^{32}P)-labeled RNA probes, RNA was transcribed from linearized pTZ18R plasmids containing the appropriate cDNA insert using T7 RNA polymerase (BRL Inc., Gaithersburg, MD) according to the manufacturer's directions. Nick translation of PVS-An specific cDNA clones for probes was performed as described by Maniatis et al (8).

Northern blot hybridization. To determine if PVS-An produces subgenomic mRNAs in virus infected tissue, total plant RNA and dsRNA from PVS-An-infected tissue and RNA isolated from virions were analyzed by the northern hybridization technique (17). The RNA was separated by 1% agarose gel electrophoresis, detected by ethidium bromide staining and electroblotted to a nylon membrane (Genescreen plus). The RNAs were hybridized to nick-translated probes representing the 3′ end (pR58) of PVS-An RNA. The blots were washed two times each in 2× SSC for 5 min at room temperature, 2× SSC containing 0.1% SDS for 30 min at 65 C, 0.1× SSC containing 0.1% SDS for 15 min at 65 C, and 0.1× SSC for 30 min at room temperature. X-ray film was exposed to the blots sealed in a plastic bag.

Dot blot hybridization. A dot blot hybridization system was used to determine if PVS-An cDNA clones would specifically detect only PVS-An specific sequences or would cross hybridize with the following viruses: potato virus A, potato leaf roll, potato virus M, potato virus S, potato virus X, and potato virus Y. A collection of tissue culture grown potato plants infected with these viruses was available (kindly provided by M. Peterson). Leaf samples (0.02 g) of potato grown in tissue culture infected with the aforementioned viruses and *C. quinoa* infected with PVS-

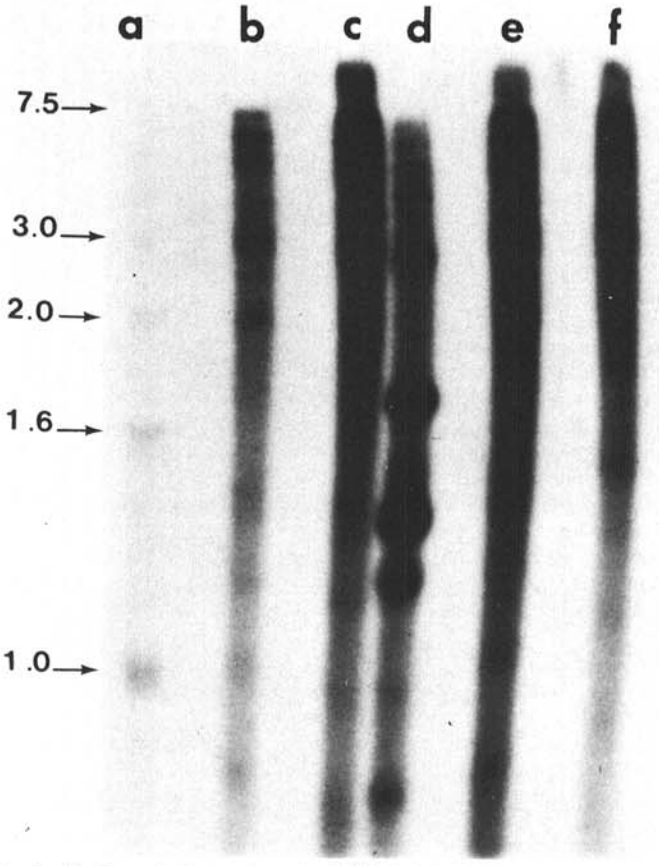

Fig. 2. Alkaline gel electrophoresis of (^{32}P)-labeled PVS-An cDNA. The labeled PVS-An cDNA was electrophoresed through a 1.4% alkaline agarose gel (30 mM NaOH), dried, and autoradiographed. The BRL 1 kbp DNA ladder labeled with (^{32}P) using the Klenow fragment of DNA Polymerase I (a). Samples consisted of first strand cDNA (b), second strand cDNA (c), second strand cDNA digested with *Eco*RI (d), second strand cDNA treated with *Eco*RI methylase and digested with *Eco*RI (e), and second strand cDNA not digested and incubated at 37 C (f).

An were ground in 10 mM sodium phosphate, pH 7.5, containing 0.01% SDS (13). The extracts were centrifuged and the supernatant (100 μl) was mixed with 20× SSC (120 μl) and formaldehyde (80 μl), then incubated at 65 C for 15 min to denature the RNA. A nylon membrane was prewetted in deionized water and mounted over filter paper in a suction manifold apparatus (BRL Inc.). Aliquots of the plant extracts (100 μl) were spotted on the membrane, and suction was applied. Purified PVS-An virions were treated in the same manner described for plant material. RNA of PVS-An, tobacco mosaic virus (TMV), and brome mosaic virus (BMV) was treated as described, except that only 50-μl aliquots were applied to the membranes. The membranes were baked at 80 C for 2 hr, prehybridized and hybridized to (^{32}P) nick-translated from cDNA clones (5×10^5 cpm/ml) as described above.

Five-prime-end sequence. Direct sequencing of PVS-An RNA was used to determine the sequence of the 5′ terminus. A synthetic primer (5′-TGTGACACTAGGTAGTTTT-3′) was deduced from the sequence of pK139. The primer was labeled with γ (^{32}P)-ATP (Amersham Corp.) (> 5,000 Ci/mmol) using T4 polynucleotide kinase (BRL Inc.). PVS-An RNA was heated at 95 C for 2 min and annealed to the primer (2:1 molar ratio) in the presence of 50 mM Tris-HCl, pH 8.3, 10 mM $MgCl_2$, and 40 mM KCl by a 2-min incubation at 65 C and cooled to room temperature over a period of 30 min. The annealed RNA-primer was used in dideoxy chain termination sequencing (12) reactions using a modification of the procedure described by Huisman et al (6). Briefly, after annealing of PVS-An RNA to the appropriate primer, DTT and AMV reverse transcriptase (Life Sciences, Inc.) were added at a final concentration of 10 mM and 2 units/μl, respectively. Aliquots of the synthesis reaction mixture were added to tubes containing the termination mixes for A, C, G, or T reactions (50 μM of all dNTPs and 25 μM ddATP, 18 μM ddCTP, 15 μM each of ddGTP and ddTTP). Samples were incubated at 42 C for 20 min and a 0.2 volume of 0.5 mM of all dNTPs was added and incubated for 15 additional minutes. The sequence data were analyzed using the University of Wisconsin Genetics Computer Group software (3).

RESULTS

cDNA synthesis and cloning. Alkaline agarose gel electrophoresis of the cDNA indicated that the size of the radioactive material ranged from 1.0 to 7.5 kilobases (kb) (Fig. 2). The larger product corresponds to the expected size for full-length cDNA of PVS-An genomic RNA. When PVS-An double-stranded cDNA was digested with *Eco*RI, *Hin*dIII, *Kpn*I, and *Pst*I, discrete fragments of cDNA were produced that corresponded to the size of fragments observed in the physically mapped PVS-An clones (Fig. 1 and data not shown). When PVS-An cDNA was treated with *Eco*RI methylase, the *Eco*RI sites of the cDNA were protected from *Eco*RI digestion (Fig. 2) as compared with non-methylated cDNA (Fig. 2). In preliminary experiments we found that smaller clones were preferentially selected during the cloning process. For this reason, fractionation of the methylated cDNA and the use of *Eco*RI linkers was necessary to obtain the larger clones presented in the map (pR plasmids in Fig. 1).

Characterization of recombinant plasmids and determination of the physical map of PVS-An clones. A total of 600 transformants were screened for insert size by restriction endonuclease digests of small-scale plasmid preparations (8). The sequence specificity of the inserted cDNA was confirmed by Southern blot hybridization using (^{32}P)-labeled PVS-An single-stranded cDNA as probe. The probe hybridized to the inserted cDNA but not to the plasmid DNA (data not shown). The size of the clones selected for further characterization range from 0.4 to 6.0 kilobase pairs (kbp). Identification of overlapping clones was determined by Southern blot hybridization using RNA probes transcribed from specific cDNA subclones or nick-translated cDNA probes (Fig. 1 and Table 1). The 0.8 kbp, 1.6 kbp, and 2.5 kbp *Eco*RI fragments from pR58 and the 1.8-kb fragment from pR44 were subcloned into the *Eco*RI site of pTZ18R from which (^{32}P)-labeled strand specific probes (probes 0.8, 2.5, 1.6, and 1.8, respectively, Fig. 1) were made. Probe H392 was produced by nick-translation of pH392. Southern blot hybridization experiments in which selected PVS-An cDNA clones were hybridized to the (^{32}P)-labeled probes are summarized in Table 1. The 5′-3′ polarity of the PVS-An cDNA clones was determined by sequencing through the poly (A) tract at the 3′-end of pR58 (data not shown). The remaining clones were oriented with respect to each other to give the complete map shown in Figure 1.

Northern blot hybridization analysis of PVS-An RNA. PVS RNA isolated from virions, infected and healthy *C. quinoa* supplemented with 90 μg of virions per gram of tissue (a concentration of virus comparable to that present in infected tissue) hybridized to a nick-translated PVS-An specific probe. A weak hybridization signal of two nucleic acid species of approximately 1.0×10^6 and 0.7×10^6 (3.0 and 2.0 kb, respectively) was regularly obtained from the total RNA isolated from PVS-An-infected plants (arrows shown in Fig. 3, b). The probe did not hybridize to similar-sized species in Northern blot hybridization of RNA extracted from virions (a) or from non-infected tissue supplemented with virions (c). These low molecular

TABLE 1. Size of Andean strain of potato virus S (PVS-An) DNA fragments (in kbp) that hybridized to specific probes

Probes	PVS-An clones								
	pK139	pH392	pK119	pR44	pR58	pK11	pHb16	pK14	pH348
5′ end									
pH392	1.3	1.6	1.1	1.8	0.8	nh[a]	nh	nh	nh
1.8 pR44	1.3	1.6	1.1	1.8	nh	nh	nh	nh	nh
0.8 pR58	nh	1.6	1.1	nh	0.8	1.1	2.0	nh	nh
3′ end									
2.5 pR58	na[b]	na	na	2.5	2.5	nh	2.0	3.4	3.5
1.6 pR58	na	na	na	1.6	1.6	nh	nh	nh	3.5

[a] No hybridization.
[b] Not analyzed in our experiments.

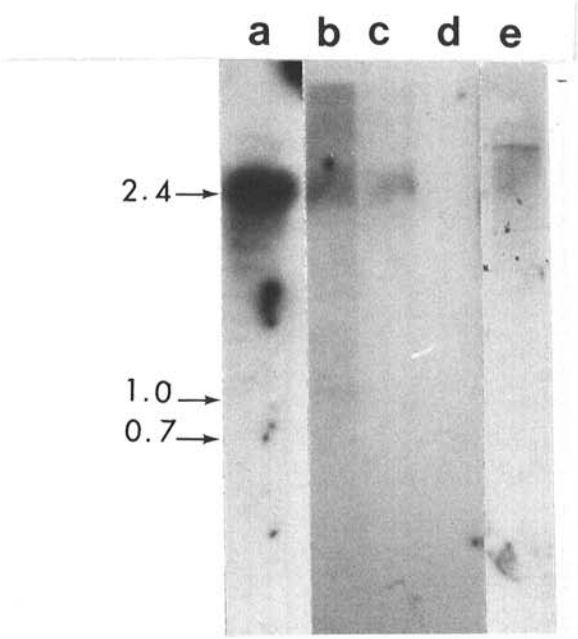

Fig. 3. Northern blot hybridization of PVS-An RNA. RNA was assayed by electrophoresis through a 1% agarose gel, electroblotted onto a nylon membrane, and hybridized to a probe produced by nick-translation (pR58). RNA analyzed was extracted from: a, PVS-An virions; b, *Chenopodium quinoa* infected with PVS-An; c, noninfected *C. quinoa* supplemented with PVS-An virions; d, noninfected *C. quinoa*; e, dsRNA from *C. quinoa* infected with PVS-An. The sizes of the RNA species are shown on the left ($\times 10^6$).

weight RNAs could represent putative subgenomic messenger RNAs that are only expressed in infected tissue. In dsRNA preparations extracted from PVS-An infected *C. quinoa* tissue (e) the probe did not detect any nucleic acid smaller than that expected for genome-length PVS-An dsRNA.

Dot blot hybridization. A PVS-An specific probe (pR58) hybridized to potato and leaf extracts of *C. quinoa* infected with an unidentified isolate of PVS, the Andean and type strains of PVS (Fig. 4, a, b, and c, respectively), as well as to disrupted PVS-An purified virions (Fig. 4, j). The probe did not hybridize to the RNA of other potato viruses tested (Fig. 4, d–h) or to healthy potato leaf extracts (Fig. 4, i). PVS-An RNA was detected in samples that contained as little as 0.8 μg of virions equivalent to 4 ng of PVS-An RNA (assuming 5% RNA per particle). Serial fivefold dilutions (Fig. 4, a–c, dilutions 4 and 5) of the infected leaf extracts had stronger hybridization signals than undiluted leaf extracts (Fig. 4, a–c, 1–5). At higher dilutions, the hybridization signal increased, indicating that components of plant sap interfered with the absorption or detection of viral RNA specific sequences. Probe 1.8 from pR44 hybridized to PVS-An RNA but did not hybridize to TMV or BMV RNA (Fig. 5).

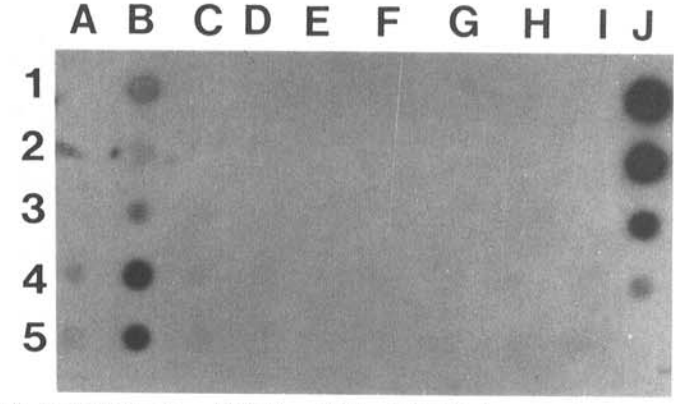

Fig. 4. Hybridization of PVS-An cDNA with PVS-An and other potato virus infected tissues by dot blot hybridization with nick-translated pR58 (5×10^5 cpm/ml). Leaf samples (0.02 g) were ground in 0.01 M sodium phosphate, pH 7.5, containing 0.01% SDS. The extracts were clarified by centrifugation, mixed with 1.2 volumes of 20× SSC and 0.8 volume of formaldehyde and incubated at 65 C for 15 min. Samples were serially diluted and applied to a nylon membrane. Numbers 1–5 represent the fivefold serial dilutions; 1 represents the undiluted plant extract. The following samples were applied: A, unidentified isolate of PVS; B, PVS-An; C, PVS-Ty; D, potato virus M; E, potato virus A; F, potato virus Y; G, potato leaf roll virus; H, potato virus X; I, uninfected potato; J, purified PVS-An virions (1 = 10 μg).

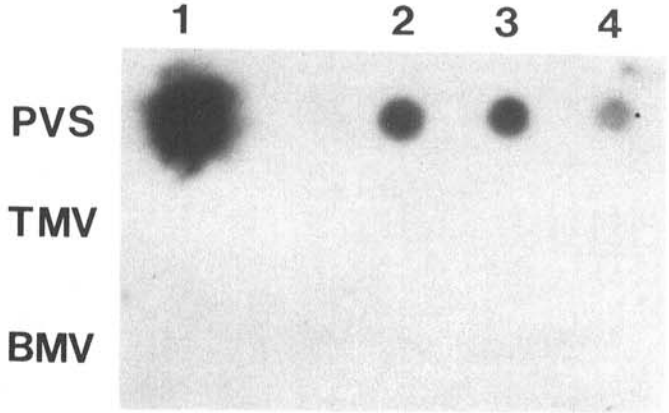

Fig. 5. Dot blot hybridization of PVS-An (PVS), tobacco mosaic virus (TMV), and brome mosaic virus (BMV) RNAs with the nick-translated 1.8-kbp fragment from pR44 (5×10^5 cpm/ml). The numbers represent the different concentrations of RNA spotted on the membrane: 1, 200 ng; 2, 60 ng; 3, 30 ng; 4, 3 ng.

Sequence of the 5′ end of PVS-An. The 5′ terminus of PVS-An RNA was sequenced by primer extension using reverse transcriptase and the dideoxy chain termination technique. It was not possible to resolve the complete nucleotide sequence (261 bases) in one gel reading. Therefore, two additional primers, designed from the sequence obtained (5′-TTGCTAAGATGTTCCTT-3′ and 5′-GGTGGCAACGTTGGAAA-3′), were used to determine the 5′-most nucleotide sequence. The sequence of the 5′ terminus of PVS-An RNA not represented in the cDNA clones is shown in Figure 6 (uppercase letters) and an autoradiogram showing the 5′-end terminus of PVS-An is shown in Figure 7.

DISCUSSION

Complementary DNA representing almost the full length of PVS-An genomic RNA has been cloned and mapped. Sequence specificity of the inserted cDNA was confirmed by dot hybridization of PVS-An RNA and Southern blot hybridization using PVS-An single-stranded cDNA as a probe. We were interested in obtaining clones that would represent most of the PVS-An RNA genome. We therefore used a cloning strategy that involved the use of methylation of restriction endonuclease sites and attachment of linkers. We were successful in obtaining large cDNA clones, but we also obtained recombinant clones probably because *Eco*RI methylase failed to methylate all sites present (Fig. 1, pR44). Complementary DNA clones were produced by digestion with *Hin*dIII and *Kpn*I and ligation to appropriately restricted plasmids (pH and pK plasmids in Fig. 1) and overlapping clones were found that confirmed the location of the different fragments in the large clones. The availability of well-characterized clones has facilitated the subcloning of specific fragments to be used in sequencing and mutagenesis experiments.

The sequence of the 5′ end of PVS-An RNA not represented in the cDNA clones is presented. We plan to construct full-length infectious clones that will be used to study different events in PVS replication (1).

When total RNA extracted from PVS-An-infected tissue of *C. quinoa* was assayed by northern blot hybridization two RNA species of approximately 1.0×10^6 (3.0 kb) and 0.7×10^6 (2.0 kb) hybridized to a probe specific to 75% of PVS-An genome (pR58). These RNAs could be considered putative subgenomic RNAs and if translated might code for proteins of 110K and 77K. One should be cautious, however, in interpreting these results: The small RNA species could have arisen from fragmented virions caused by the isolation procedure or they could represent less than full-length (−) viral RNA. The first hypothesis is unlikely, since the small M_r RNA species were not observed in RNA

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XTAAACACTCCCGAAAATAATTTGACTTAAACAACTCGACAGTTCAAGCAAAATTCT

TAAAATGGCACTTACTTACAGAAGTCCAATCGAGGAAGTGTTAACACTACTAGAGCC

CAATGCTCAATCCCTAATTTCCAACGTTGCCACCAGCAGCTTCCAAGAGAGTGAGAA

GGATAACTTCGCTTGGTTTTGTTATCATGTACCATGCTAATGCCAAGGAACATCTTA

GCAAAGCTGGGATTTACCTAAGCCCATACTCAGggtaccctcattctcacccggtgt

gtaaaacattgaaaactacctactgtcacaaaattcttcccaccactttaaataaca

ccttttttacttt
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Fig. 6. Nucleotide sequence of the 5′ terminus of PVS-An RNA. The RNA was sequenced directly by primer extension using AMV reverse transcriptase and the dideoxy chain termination technique. The sequence of the RNA not included in PVS-An cDNA clones is shown in uppercase letters. The sequence of pK139 from which the primer was designed is shown in lowercase letters. X = A, C, G, or T. Lines indicate the putative ATG initiation codons; * indicates the termination codon for the first putative ATG (frame b).

extracted from noninfected tissue supplemented with PVS virions (Fig. 3, c). It would be interesting to determine if these RNAs would produce any functional polypeptides when translated in vitro. The biological function of these putative RNAs needs to be confirmed using strand specific probes and translation studies. These small RNA species were not observed when RNA isolated from PVS-An virions and healthy tissue supplemented with virions were hybridized to the same probe. This suggests that these small RNA species are only produced during PVS infection.

The cDNA clones will be useful as diagnostic tools for the detection of this important potato virus in crude leaf extracts. The dot-hybridization experiments detected PVS-An specific sequences in infected potato grown in tissue culture and tissue of *C. quinoa*, but not in potato leaf extracts infected with various other potato viruses. A difference in the hybridization signal between an undetermined isolate of PVS, PVS-Ty, and PVS-An was found (Fig. 4, a–c). This difference may be due to the different degrees of homology of different PVS RNAs or alternatively to different titers of the virus present in the tissues used. Enzyme-linked immunoassay results showed that PVS-An was present in *C. quinoa* at a titer 3 times higher than the type strain in potato (data not shown).

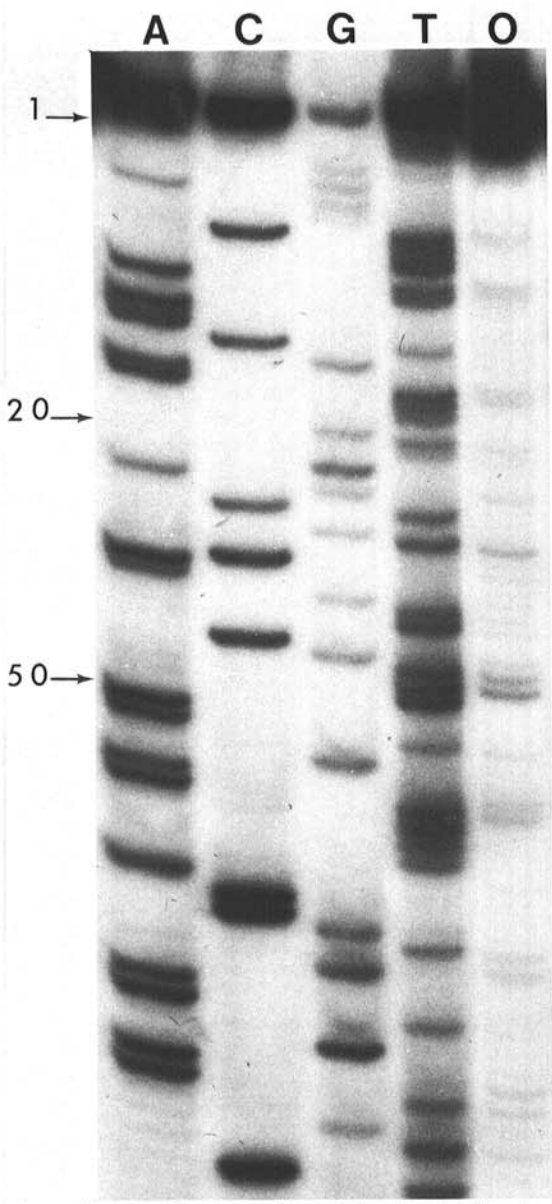

Fig. 7. Autoradiogram of a sequencing gel showing the 5′ end of PVS-An RNA. PVS-An RNA was sequenced by primer extension using AMV reverse transcriptase and the dideoxy chain termination technique. A, 25 μM ddATP; C, 18 μM ddCTP; G and T, 15 μM ddGTP and ddTTP, respectively; 0, no ddNTPs. Numbers on the left correspond to the position of the complementary base of PVS-An RNA.

The partial sequence determination of the Peruvian strain of PVS has been reported (7). Six open reading frames (ORFs) encoding potential polypeptides of 11K, 33K, 7K, 12K, 25K, and at least 41K were detected in the 3′-terminal 3553 nucleotides. The only ORF to which function was assigned was the coat protein which was the 33K polypeptide. There are a few differences between the restriction map presented for the Peruvian strain of PVS and that presented here. We did not detect any *Pst*I, *Sma*I, or *Xba*I sites in our restriction digest experiments at the 3′ end of the viral genome. However, only one *Pst*I site was found and it was located near the middle of the genome (see Fig. 1). It is possible that the missing sites are only present in the Peruvian strain of PVS or in the case of *Xba*I and *Pst*I, the sites could have been methylated and therefore not detected in our experiments. However, this is unlikely, since a preliminary experiment showed no difference between *Xba*I digested and undigested cDNA before cloning (data not shown). We cannot compare the 5′ end of PVS-An cDNA with the cDNA of the Peruvian strain because the latter clones do not represent this area of the genome. When the complete nucleotide sequence of PVS-An is obtained it will be possible to determine if there are differences in the nucleotide or amino acid sequence between these different strains of PVS.

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