Heterogeneity in Pepper Isolates of Cucumber Mosaic Virus

Gerardo Rodríguez-Alvarado, Gael Kurath, and **J. Allan Dodds,** Department of Plant Pathology, University of California, Riverside 92521

ABSTRACT

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Twenty-four cucumber mosaic cucumovirus (CMV) field isolates from pepper crops in California were characterized and compared by nucleic acid hybridization subgrouping, virion electrophoresis, and biological effects in several hosts. Isolates, belonging to subgroup I or subgroup II, were found that induced severe symptoms in mechanically inoculated bell peppers. Only two isolates, both from subgroup II, were mild. A group of 19 isolates collected from a single field were all in subgroup II and appeared identical by virion electrophoresis, but they exhibited varying degrees of symptom severity in peppers. As a more detailed indicator of heterogeneity, these 19 isolates were examined by RNase protection assays to detect sequence variation in the coat protein gene region of their genomes. The patterns of bands observed were complex and a high degree of genomic heterogeneity was detected between isolates, with no apparent correlation to symptomatology in bell pepper.

Cucumber mosaic cucumovirus (CMV) is the type member of the cucumovirus group of plant viruses. The genome of CMV consists of three plus-sense single-stranded (ss) RNA species called RNA 1, RNA 2, and RNA 3 in order of decreasing size. A fourth RNA (RNA 4) is found in the RNA extracted from virion particles, and is a subgenomic RNA generated from the 3′ half of RNA 3 (17). CMV has a worldwide distribution and infects a large variety of crops, but it is mainly significant in field-grown vegetables (22). Numerous strains have been reported (11), and the methodologies used to elucidate the taxonomy of CMV strains have been reviewed (17). Methodologies that have used RNA 3 characteristics for grouping of CMV strains include heterogeneity maps using a RNase protection assay (16), restriction mapping of the polymerase chain reaction–amplified coat protein gene (19), peptide mapping and enzyme-linked immunosorbent assay of the coat protein (8), and serological relationships of the coat protein using a double immunodiffusion technique (4). The majority of the CMV strains studied can be placed in one of two serogroups, DTL and ToRS (4), which are equivalent to subgroups I and II (16).

A high incidence of this virus in some Californian pepper growing areas has been reported (1). Recently, CMV isolates belonging to subgroups I and II were found infecting several hosts including peppers in California (3). In this study the genomic heterogeneity in the 3′ half of RNA 3 of subgroup II CMV isolates from a pepper field in southern California is described.

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Present address of first author: Plant Biology Division, The Samuel Roberts Noble Foundation, Inc. P. O. Box 2180, Ardmore, OK 73402. Address of second author: National Fisheries Research Center, Bldg. 204 Naval Station., Seattle, WA 98115-5007.

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MATERIALS AND METHODS

Virus isolates. In order to identify pepper fields with CMV infection, 324 pepper samples from six locations in California (Gilroy, Kern, Modesto, Orange, San Diego, and Ventura counties) were obtained during 1988, 1989, and 1990. The samples included leaves from young shoots, and in some cases fruits, from plants with or without distinct symptoms of viral infection. Each sample was divided into two parts. A portion was stored at –20 C. The remainder was ground in inoculation buffer (0.02 M potassium phosphate buffer, pH 7.0, 0.1% sodium thioglycollate, 0.3% sodium diethyldithiocarbamate, and 0.1% Celite) and rubbed on leaves of tobacco (*Nicotiana tabacum* L. 'Xanthi nc') seedlings. Symptomatic upper leaves of tobacco were used to inoculate leaves of *Chenopodium quinoa* Willd., and single local lesions were selected from this host. Each isolate was then propagated from a single local lesion tissue piece in tobacco. Double-stranded RNA (dsRNA) analysis was used to detect CMV or mixed virus infections in inoculated tobacco plants, or the presence of CMV satellite RNAs.

The CMV strains used as standards were CMV-S, originally isolated from squash (*Cucurbita pepo* L.) by M. H. V. van Regenmortel and obtained from J. M. Kaper, and CMV-P, isolated from purslane (*Portulaca oleracea* L.) by Dodds and Taylor (7). CMV strains were maintained in dried plant tissue at 4 C, and in tobacco plants. These two strains were used because they represent subgroup I (CMV-P) and subgroup II (CMV-S), and their virion mobilities have been characterized (5,6).

DsRNA analysis. DsRNA were isolated from 2.0 g of leaf tissue from infected tobacco plants by one cycle of chromatography on columns of CF-11 cellulose powder as previously described (6).

Virion analysis. Virions of CMV strains and isolates were extracted from 1.0 g of fresh tissue from young infected leaves (10–12 days postinoculation). Tissue was ground in 2 ml of cold 0.2 M sodium citrate (pH 6.5) buffer and 4 µl of 2-mercaptoethanol in a cold mortar on ice. The slurry was poured into a 15-ml centrifuge tube and placed on ice. One-half milliliter of chloroform was added to each tube, the sample was agitated for 30 s, centrifuged at 10,000 *g* for 10 min, and the supernatant was transferred to a clean centrifuge tube. An equal volume of 14% polyethylene glycol (8,000 molecular weight) dissolved in 0.2 M sodium citrate buffer, pH 6.5, was added. The mixture was incubated at 4 C overnight. The suspension was centrifuged at 5,000 *g* for 30 min and the pellets were resuspended in 1 ml of 5 mM EDTA, pH 7.0, 5% sucrose. A final centrifugation at 5,000 *g* for 30 min produced a supernatant with virions that was stored at 4 C. Ten to 30 µl per well of virion preparation was loaded into wells in horizontal 1.2% agarose gels, and electrophoresed at constant voltage (60 V) for 2 h in TAE (10 mM Tris, ph 7.8, 5 mM sodium acetate, and 0.5 mM EDTA) electrophoresis buffer. Gels were stained with ethidium bromide (50 ng/ml) for 20 min.

Dot blot hybridization. The method used to generate radioactively labeled hexamer-primed probes was previously described (9). As templates for the probes, recombinant CMV clones pFNY3 and pLS-87 (kindly supplied by J. Owen and P. Palukaitis) containing cDNA sequences representing RNA 3 sequences from CMV-Fny and CMV-L_2, respectively, were linearized with appropriate restriction enzymes. CMV strains Fny and L_2 belong to subgroups I and II, respectively (16). Viral RNA extraction was as previously described (13). For each set of samples, duplicate blotted sheets of nitrocellulose were baked, prehybridized, and hybridized at 42 C with one or the other of the two

different ^{32}P cDNA probes. The blots were washed six times in high stringency conditions; four times for 5 min (twice at room temperature and twice at 50 C) in 2× SSC (1× SSC is 0.15 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA, pH 7.7)/0.5% sodium dodecyl sulfate (SDS), and twice for 15 min in 0.1× SSC/0.5% SDS at 50 C. Autoradiograms were prepared as described previously (13).

Effect on selected hosts. Seedlings of *Lactuca saligna* L. (PI 261653) (kindly provided by R. Provvidenti), *Chenopodium quinoa*, and *Capsicum annuum* L. cvs. Keystone Resistant Giant No. 3 and Yolo Wonder B (Petoseed) were used for host response experiments. The seedlings were mechanically inoculated on the cotyledon leaves with sap extracts (1:5, in inoculation buffer) from tobacco plants infected for 6 days with each of the CMV field isolates. When it was necessary to confirm that systemic infection of the hosts had occurred, inoculations to *C. quinoa* (a local lesion host) were performed. *Lactuca saligna* (PI 261653) (18) and *C. quinoa* (2) have previously been used to correlate biological and serological subgroups of CMV strains. The bell pepper cultivars Keystone and Yolo Wonder B are commonly grown in southern California. They were used in this study to try to identify CMV pepper isolates that induce different symptoms in these commercial cultivars. Inoculated plants were grown in a greenhouse and observed during 2–4 mo. *Chenopodium quinoa* plants used to confirm systemic infection of other hosts were observed during 15 days. Average greenhouse temperatures were 34 C day and 20 C night during spring through summer, and 26.8 C day and 17.5 C night during fall through winter.

Construction of recombinant clones. Preparation of cDNA and construction of clones with sequences from RNA 3 of CMV-S are described elsewhere (20). A recombinant plasmid, pS51IIIX4, containing the coat protein gene together with 223 nucleotides (nt) upstream of the start codon and the complete untranslated 3′ end, was obtained by subcloning from clone pS51III. The cDNA insert of pS51IIIX4 was released from the multiple cloning site of pUC18 using the restriction enzymes *Pst*I and *Sst*I, and transferred to the corresponding sites in pBluescribe (pBS), to obtain pBS51IIIX4-6. The orientation of the cDNA insert ensured that the 3′ viral sequences were adjacent to the T7 promoter, with 27 nonviral bp between them. The 5′ end viral sequences were adjacent to the T3 promoter, with 33 nonviral bp between them.

Total RNA extraction, in vitro transcription, and RNase protection assays. Total RNA was extracted from infected plants according to Kurath et al (13). When tobacco plants were used as the source of total RNA, the final product was resuspended in 50 µl of 0.1 mM EDTA, pH 8.0. If pepper plants were the source of total RNA the final product was resuspended in 10 µl of 0.1 mM EDTA. The total RNA of the CMV isolates used in the RNase protection assays was extracted from experimentally infected tobacco plants, because the total RNA from field-collected pepper plants was low in quantity and the bands obtained were poorly resolved (data not shown).

The recombinant clone pBS51IIIX4-6 was linearized with *Sst*I to make plus-sense radioactively labeled transcripts, and with *Pst*I, *Hin*dIII, or *Sal*I to make minus-sense radioactively labeled transcripts (probes P1, P2, and P3, respectively; see Fig. 1). Probe P1 was transcribed from the T7 promoter to the *Pst*I site in the multiple cloning site of pBS to generate a probe of 1,237 nt with 8 nt of vector origin at the 5′ terminus, 1,202 nt of viral origin, and 27 nt of vector origin at the 3′ terminus. The P1 probe consisted of the entire coat protein open reading frame (ORF) together with 223 nt upstream of the start codon and the entire 3′ untranslated region. Probes P2 and P3 were transcribed from the T7 promoter to the internal restriction sites *Hin*dIII and *Sal*I, respectively, which produced probes P2 of 702 nt with 675 nt of viral origin, and P3 of 399 nt with 372

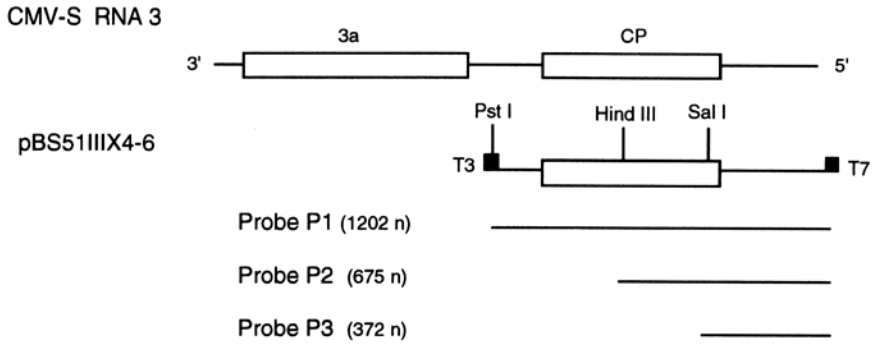

Fig. 1. Minus-sense probes generated from pBS51IIIX4-6, which contained cDNA from the 3' half of cucumber mosaic cucumovirus subgroup II (CMV-S) RNA 3. Three minus-sense transcripts of different lengths (P1, P2, and P3), represented as the lower three horizontal lines, were obtained using the restriction sites shown. In these transcripts the CMV 3' sequences are close to the T7 promoter of the pBS M13- vector.

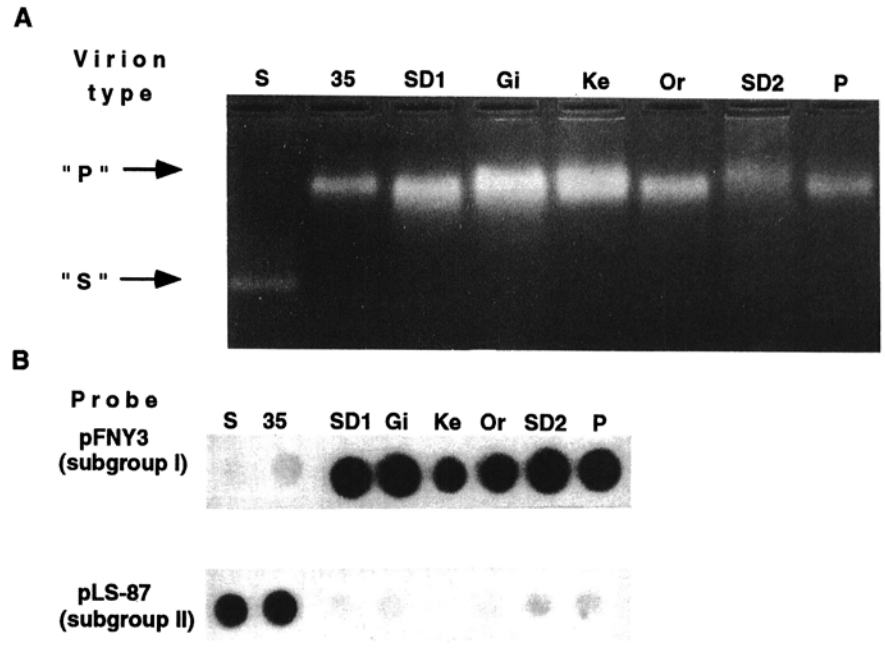

Fig. 2. Characterization of virions and subgrouping of cucumber mosaic cucumovirus (CMV) pepper isolates. **(A)** Comparison of virions of CMV pepper isolates obtained from tobacco plants. Analysis was made in 1% agarose gels electrophoresed for 1 h and stained with ethidium bromide. CMV-S (subgroup II, "fast") and CMV-P (subgroup I, "slow") were used as standards. Lanes labeled 35, SD1, Gi, Ke, Or, and SD2 contain virions from representative CMV isolates from each of the six collection sites. Isolate 35 is a representative of the 19 isolates found in one field in Ventura County. **(B)** Autoradiograph of a dot blot hybridization of RNA from CMV pepper isolates with two different subgroup-specific probes. Probes were generated from plasmids pFNY3 (subgroup I) and pLS-87 (subgroup II). CMV-P and CMV-S were used as standards representing subgroup I and II, respectively, and CMV isolates are labeled as in A.

nt of viral origin. The two smallest probes had 27 nt of vector origin at their 3′ terminus. The P2 probe consisted of the final 403 nt of the coat protein ORF, and the entire 3′ untranslated region. The P3 probe consisted of the final 50 nt of the coat protein ORF, and the entire 3′ untranslated region. The RNase protection assay protocol used was described by Winter et al (23) with some modifications by Kurath et al (13). The assay was performed with each CMV isolate three to four times.

RESULTS

CMV isolates. From six sites, a total of 324 pepper samples were collected and 118 of those samples caused systemic infections when sap from the plants was inoculated onto tobacco plants. Twenty-four isolates of CMV were identified using dsRNA analysis of the infected tobacco plants. DsRNAs of CMV satellite RNA were not detected. The CMV pepper isolates obtained were characterized by subgrouping, virion mobility in agarose gels, symptomatology in a host range, and genomic comparison of the 3′ half of RNA 3. From five locations, one CMV isolate was obtained per site. From the sixth site, a pepper field in Ventura County, 19 isolates were obtained. The following characterizations were made for all the isolates, but only one representative was selected from the Ventura County site for comparison and illustration in the figures.

Virion analysis. The electrophoretic mobility of the virions of the field isolates was compared in agarose gels with that of CMV-S and CMV-P. The virion of CMV-P has a slow electrophoretic mobility compared with CMV-S (5,15), and all the field isolates had type P virions by this assay (Fig. 2A).

Hybridization to specific probes. CMV isolates SD2, Or, Ke, Gi, and SD1, which were found in different locations, hybridized strongly to the pFNY3 probe (subgroup I) under high stringency conditions (Fig. 2B). The group of 19 isolates found in one pepper field in Ventura county during 1988 (represented by isolate 35 in Fig. 2) showed a much weaker signal with this probe. When the second probe, from pLS-87 (subgroup II), was utilized a strong hybridization signal to the 19 Ventura isolates was observed, and a weak signal was obtained for the other isolates (Fig. 1B). Viral RNAs of CMV-S hybridized to pLS-87 and viral RNAs of CMV-P hybridized to pFNY3, as expected (Table 1).

Symptomatology in a host range. The subgroup I field isolates (those that hybridized with a radioactive probe generated from pFNY3) produced two types of reaction in *C. quinoa*: local, or local and systemic (Table 1). The local reaction was abundant chlorotic lesions in inoculated leaves. The systemic symptoms induced by some isolates included chlorotic spots, small deformed leaves, and stunting. Those symptoms were clearest when plants were young (4–8 leaves) at inoculation time. All subgroup I field isolates tested also caused large chlorotic local lesions to develop in fully expanded leaves of *L. saligna*, with no systemic spread. The lack of systemic spread was confirmed by back inoculations to *C. quinoa* using sap from upper leaves. All 19 subgroup II field isolates (those that hybridized to a probe generated from pLS-87) were uniform in the symptoms they induced in *C. quinoa* (local lesions only), and all infected *L. saligna* systemically (Table 1), which was confirmed by back inoculations to *C. quinoa*. CMV-S induced the same response in those hosts. However, CMV-S, and the isolates 36 and 41, were asymptomatic in *L. saligna*, and in back inoculations to *C. quinoa* the local lesions obtained were few in number.

An experiment in two pepper cultivars, Keystone Resistant Giant #3 and Yolo Wonder B, was performed to test if the pepper isolates had different effects in pepper. Five plants were used for each cultivar and for each CMV strain or isolate. Degree of symptomatology was determined. Isolates inducing severe leaf deformation and strong mosaic were considered to be severe. Isolates inducing mild or no leaf deformation and mosaic were considered to be intermediate. Isolates inducing mild mosaic were considered to be mild. The majority of isolates, regardless of subgroup, induced severe mosaic and leaf deformation (Table 1). CMV-P (subgroup I type), which was used for comparison, was also severe in pepper. CMV-S (subgroup II type) induced milder symptom reactions.

In order to investigate variation in pathogenicity within the subgroup II isolates in pepper, two independent experiments done at different times were carried out. A total of 13 Keystone seedlings per isolate were mechanically inoculated and observed for 3 mo. Based on symptomatology two isolates (30 and 33) were identified as mild isolates, with the rest being severe or intermediate in severity.

Genomic comparison among subgroup II pepper isolates. Due to the variation in symptomatology within the 19 subgroup II isolates from a single field these isolates were examined for genomic heterogeneity by RNase protection assays. Probes P1, P2, and P3 (Fig. 1) were used in assays with total RNA from plants infected with each of the subgroup II isolates, and RNA fragments protected from RNase digestion were analyzed in denaturing polyacrylamide gels. The isolates analyzed in Figure 3 induced mild (30 and 33), severe (13, 35, 38, and 39), or intermediate (rest of the isolates) symptoms in pepper.

When probe P1 was annealed to total RNA of the CMV isolates from subgroup II, the patterns of protected fragments following RNase digestion were complex (Fig. 3A). The nineteen isolates could be placed in seven groups according to their pattern of bands (A–G, see Table 2). Some isolates (35, 18, 15, and 30) had a unique pattern, and were placed in groups A, C, D, and F, respectively. The rest of the isolates formed one small group, B, and two larger groups, E and G (Table 2). All of the isolates had two bands of the same size but with different intensity, one of about 100 nt and the other of about 200 nt. The 100-nt fragment was also readily detected in the CMV-S lane, but the other protected fragment was less abundant. Isolate 15 had the pattern most similar to that of CMV-S, except that the 200-nt fragment in this isolate was very prominent. When a probe with no RNA was digested with RNase a few fragments were observed as low intensity bands; their numbers were too low to interfere with the bands detected in the samples of the CMV isolates.

Using the two smaller minus-sense

Table 1. Summary of characteristics of six representative cucumber mosaic cucumovirus (CMV) pepper isolates and two CMV strains

CMV isolate	Source county	Virion	Member subgroup[a]	Host Reaction: *Chenopodium quinoa*	Host Reaction: *Lactuca saligna*	Host Reaction: *Capsicum annuum*[b]
35	Ventura[c]	P[d]	II	l[e]	s	Severe[f]
SD1	San Diego	P	I	l/s	nt	Severe
Gi	Gilroy	P	I	l	l	Severe
Ke	Kern	P	I	l/s	l	Severe
Or	Orange	P	I	l	l	Severe
SD2	San Diego	P	I	l/s	nt	Severe
CMV-S		S	II	l	s	Mild
CMV-P		P	I	l/s	l	Severe

[a] Subgrouping based on ability of viral RNA to hybridize to cDNA of either CMV-Fny RNA (subgroup I) or CMV-L2 RNA (subgroup II).
[b] Severe = severe mosaic, stunting, chlorosis, blistering, and leaf deformation; mild = mild mosaic.
[c] Eighteen other isolates from this site were all similar to 35 except for reaction on *C. annuum*.
[d] S = similar to CMV-S; P = similar to CMV-P.
[e] l = local reaction; s = systemic infection; nt = not tested.
[f] 19 isolates are represented here by isolate 35, of which 4 were severe, 13 were intermediate, and 2 were mild in *C. annuum*.

probes, P2 and P3 (Fig. 1), the 100-nt protected fragment observed before was still present, but the signal from the 200-nt fragment was very weak or absent. With probe P2 (Fig. 3B) the protected fragment patterns were still complex. The only change in grouping was for isolate 18, which now was identical to isolates in group G (Table 2). When probe P3 was utilized to protect total RNA from the CMV pepper isolates, only four types of fragment patterns were observed (Fig. 3C).

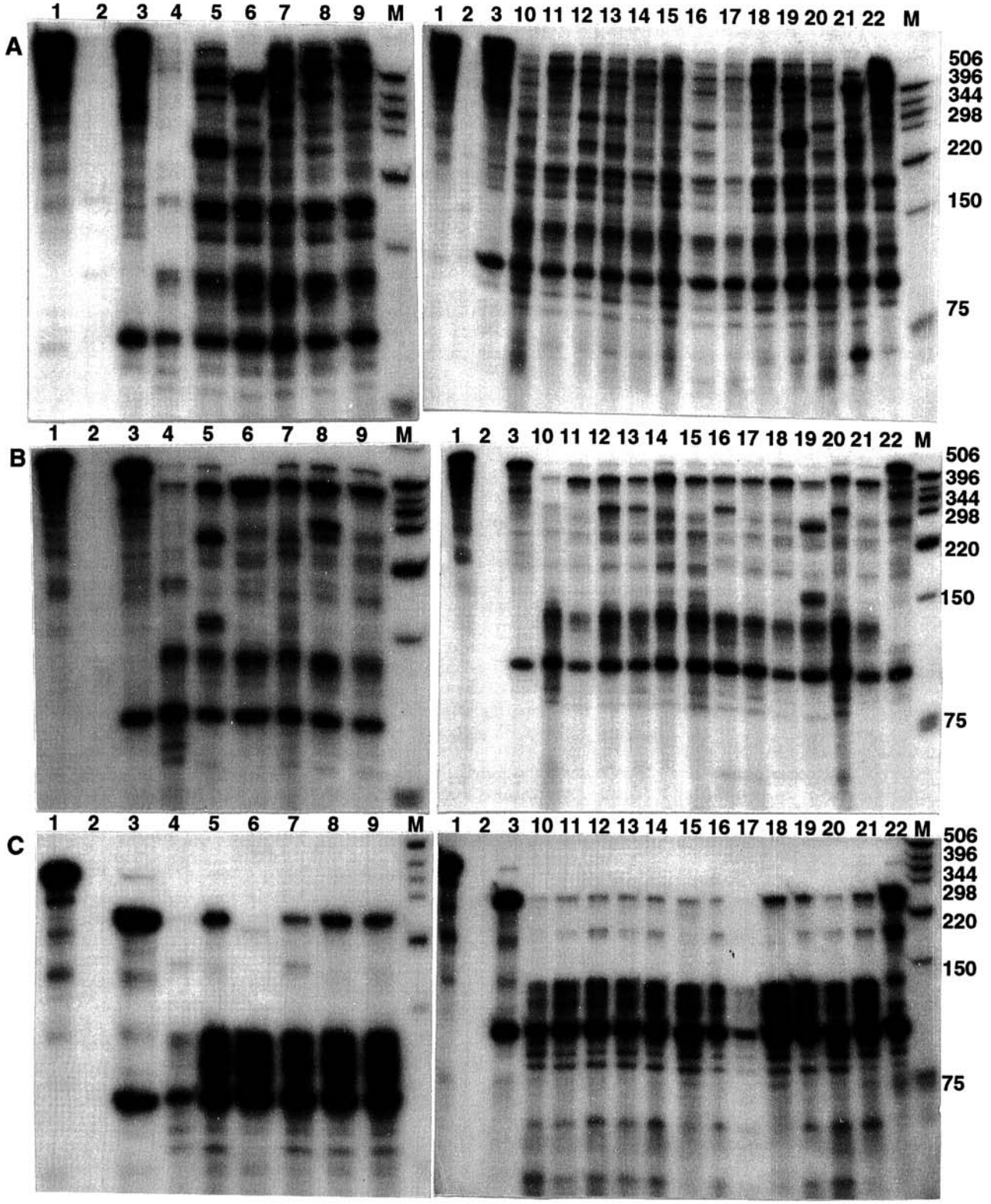

Fig. 3. RNase protection patterns of probes **(A),** P1; **(B),** P2; and **(C),** P3, with cucumber mosaic cucumovirus subgroup II (CMV-S) pepper isolates from a single field. Minus-sense probes were annealed to target RNAs indicated at the top of each lane: (1) Full length plus-sense transcript RNA of pBS51IIIX4-6 (no mismatch); (2) no RNA; (3) Total RNA from CMV-S infected tobacco; the next lanes are total RNA extracted from tobacco plants infected with the isolates: (4) 30, (5) 33, (6) 35, (7) 13, (8) 38, (9) 39, (10) 34, (11) 36, (12) 37, (13) 19, (14) 16, (15) 17, (16) 32, (17) 41, (18) 14, (19) 8, (20) 11, (21) 18, (22) 15. M indicates DNA size markers with sizes in nucleotides shown at the right margin. Isolates 30 and 33 are mild, isolates 13, 35, 38, and 39 are severe, and the rest of the isolates are intermediate in symptomatology in pepper.

Most of the isolates now formed a single group, but isolates 35, 15, and 30 appeared to still have unique band patterns. CMV-15 was very similar to CMV-S. The isolates in the main group were qualitatively similar to CMV-S but the relative intensity of the bands was different.

CMV-S RNA hybridized to the three minus-sense probes, P1, P2, and P3, was cleaved by the RNases to produce two major protected fragments in each case in addition to a full-length protected fragment, indicating either partial cleavage of the mismatch or a mixed population of RNA 3 genome molecules. A variable large fragment was obtained with each probe, and the small fragment of about 100 nt remained constant in size regardless of the probe used.

DISCUSSION

CMV pepper isolates found in different locations in southern California varied when analyzed for several characteristics. Isolates could be placed in either subgroup I or subgroup II (16) based on the signal obtained using group-specific hybridization probes, but comparison in agarose gels showed that virion mobility appeared to be a weak characteristic for distinguishing between CMV isolates. The twenty-four CMV pepper isolates found caused different degrees of symptom severity in cultivars of bell peppers but this did not correlate with subgrouping based on hybridization probes. The symptoms observed in *C. quinoa* inoculated with isolates used in this study are similar to those reported by Ahmad and Scott (2). They found Arkansas CMV isolates serologically related to the DTL serogroup (synonym for subgroup I) (17), which induced either a local reaction only or a local and systemic infection in *C. quinoa*. Although CMV isolates of subgroup I are prevalent in California (3, and this study), subgroup II isolates must be well established in some areas, as shown by the group of isolates from a Ventura County field. The conclusion, that severe diseases can be caused by strains of subgroup II as well as subgroup I (the most common source of CMV), should be noted in future pepper improvement programs.

Epidemiological studies with CMV that distinguish strains within the subgroup level are lacking because of the difficulty of differentiating strains (10) and subgroups within strains in field populations. An RNase protection method was used to characterize genetic diversity in field isolates of satellite tobacco mosaic virus (12). Since the 19 CMV isolates collected from a single pepper field for this study were similar in many characteristics, an RNase protection assay was used to determine if there were any detectable genomic differences between these isolates in the 3′ half of RNA 3 that contains the coat protein gene. Three 3′ (in mRNA sense) coterminal probes of different lengths of RNA 3, the largest including the entire sequence of the coat protein gene, were used for our studies to facilitate future comparisons of genomic variation among strains from subgroup I (16) and II. The patterns obtained were complex and seven different genome types were detected when the largest probe was used. This shows that there is detectable genetic diversity among similar isolates from a single field. Each CMV isolate was obtained from a single pepper plant. The ability to detect particular isolates in individual plants may have application in detecting reservoirs of viruses (10) and even in tracking the dispersal of such virus isolates in a field or area, without having to release artificially marked tester strains.

The difference in isolate 18 detected with the P1 and P2 probes indicates that this group of CMV isolates had a very high degree of sequence similarity in the region of the genome that started 223 nt upstream of the start codon of the coat protein, and ended after the first 304 nt of the same gene.

The smallest probe, P3, reduced the numbers of patterns of protected fragments to four. But three isolates, 35, 15, and 30, still had unique patterns, indicating that their genetic differences lie in the 3′ terminal 372 bases (Fig. 1). Most of the genetic variation between the isolates lies in the region 372 to 675; however, variability was detected along the entire length of the probe, suggesting that variation is not limited to a narrow region of the 3′ half of RNA 3. Analysis of the heterogeneity of subgroup I CMV strains showed that the coat protein ORF was more conserved than the 3a ORF. However, variation similar to that reported here was found inside the coat protein among three of eight strains (16). No correlation was observed between the grouping of isolates by RNase protections assays and symptom intensity in bell peppers. This result is not surprising because genetic mapping studies using pseudorecombinants have shown that symptom development can be controlled by individual RNAs or combinations of RNA 1, RNA 2, and RNA 3 (for a review see 17). Genetic mapping of the CMV domain(s) for symptom induction in pepper has not been reported.

The CMV isolates from subgroup II analyzed in this study that were found in a single pepper field may have originated from one or more plants of perennial hosts bordering or located near the sampled field. Diversity may be created here, since it has been shown that some hosts are able to induce or select mutants from the CMV population (14). Due to the possibility of multiple sources of infection, a certain degree of heterogeneity was expected among the CMV isolates found. On the other hand, the diversity observed in the field chosen for study may have developed following infection of the first pepper plant with a single isolate, which could have been the source for all subsequent infections each with the potential for slight variation. The generally low incidence of the type II CMV strains in pepper, and the absence of any type I strains in the field chosen for study, favor this possibility. The heterogeneity found for the CMV pepper isolates appears to be similar to that found for pepper mild mottle tobamovirus (PMMV) by Rodríguez-Cerezo et al (21). Twenty-six isolates of PMMV from greenhouse-grown peppers were classified into 10 different types according to their RNase T1 fingerprints. A main type was prevalent with closely related variants emerging from it.

The RNase protection assay, which has been confirmed as a useful tool for studying the genomic heterogeneity of different CMV strains (16), was used here to further characterize subgroup II CMV isolates. The present study is the first in which intrafield variability at the genomic level has been evaluated with field isolates of CMV. Evolution and comparison of nearly identical field isolates can be monitored

Table 2. Types of RNase protection assay patterns using three different probes and 19 different cucumber mosaic cucumovirus (CMV) isolates from a single field

	Type						
Probes	**A**	**B**	**C**	**D**	**E**	**F**	**G**
P1 (1202 nt)	35	33 8	18	15	37, 34 32, 11 38, 19	30	16, 36 39, 13 41, 12 14
P2 (675 nt)	35	33 8		15	37, 34 32, 11 38, 19	30	16, 36 39, 13 41, 12 14, 18
P3 (372 nt)	35			15		30	16, 36 39, 13 41, 12 14, 18 33, 8 37, 34 32, 11 38, 19

using this assay. Work of this kind may be useful in evaluating the viral genetic diversity available at a given site to challenge resistance obtained in transgenic lines.

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