Biological and Molecular Characterization of Lettuce Mosaic Potyvirus Isolates from the Salinas Valley of California

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ABSTRACT

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Recent lettuce mosaic outbreaks in the Salinas Valley of California have been attributed to the emergence or introduction of necrosis-inducing or severe strains of lettuce mosaic virus (LMV). A combination of molecular, serological, and biological methods were used to characterize LMV isolates associated with these outbreaks, including 5 putative necrosis-inducing isolates and a "type" LMV isolate. Comparisons of the deduced amino acid sequences of the coat protein (CP) N-terminal region (referred to here as the hypervariable [HV] region) did not indicate the presence of distinct strains. The amino acid identities of CP-HV ranged from 88 to 98%, and the sequences of the 5 putative necrosis-inducing isolates were no more identical to each other than they were to those of the other LMV isolates nor did they share common amino acid residues not present in the other isolates. All isolates were classified as pathotype II based on virulence on lettuce differential cultivars. Under the conditions of these inoculations, all isolates induced necrotic symptoms on susceptible cultivars. Induction of necrosis was attributed to host and environmental factors rather than to viral variability. Taken together, these results failed to indicate that new highly virulent LMV strains (pathotypes) are responsible for the recent lettuce mosaic outbreaks. This study illustrates the usefulness of polymerase chain reaction in the characterization of viral variability and establishes that individual isolates of a potyvirus strain may show as much as 12% divergence in the CP-HV region without changes in biological properties.

Lettuce mosaic potyvirus (LMV) is potentially the most destructive virus of lettuce (*Lactuca sativa* L.) worldwide (5,12). Infected plants usually show vein clearing, yellow mottling, and stunted growth. In iceberg lettuce, infection usually prevents head formation, rendering the plants unmarketable (13). The virus is seedborne in lettuce, transmitted by aphids in a noncirculative manner (31), and can rapidly reach epidemic levels in susceptible cultivars. Planting of LMV-infected seed combined with high levels of aphid activity can result in 100% yield losses (12,13).

LMV is a member of the family *Potyviridae*. Virions are long, flexuous rods measuring approximately 750 × 13 nm. The genome of all potyviruses studied to date is composed of one single-stranded, positive-sense RNA of approximately 10,000 nt with a viral-encoded protein (VPg) linked to its 5′-end. The 3′-end is polyadenylated (24). The LMV coat protein (CP) gene is located at the 3′-end of the RNA, and the CP has a predicted molecular mass of approximately 31 kDa (6).

In the Salinas Valley of California (Monterey County), where approximately 60% of the United States' iceberg lettuce is produced (38), lettuce mosaic is an endemic disease that almost destroyed the lettuce industry during the late 1940s and early 1950s (12). Through the combined efforts of the University of California and the lettuce industry, a series of management practices were developed and implemented that provided effective control of the disease (12). The most important practice was the implementation of a county-wide ordinance requiring that all lettuce seed be tested negative for the presence of LMV (0 infected in 30,000 tested) prior to planting. An LMV seed-indexing program, which is now based on enzyme-linked immunosorbent assay (ELISA) (9), currently is run by the University of California Cooperative Extension Service in Monterey County. Additional practices include the elimination of weed hosts and, more recently, the establishment of a lettuce-free period.

An increase in lettuce mosaic incidence beginning in 1987 raised concerns about the effectiveness of the LMV control program (23). Possible inoculum sources for these outbreaks were suggested, including the evolution or introduction of new, highly virulent LMV strains. The latter hypothesis was supported by the identification of necrosis-inducing LMV isolates, such as the so-called 'Firestone' strain (23), and the recent description of distinct LMV pathotypes from Europe and the Middle East (21,22). The emergence or introduction of necrosis-inducing strains of LMV in the Salinas Valley could cause serious yield reductions, as illustrated by the introduction of necrotic strains of bean common mosaic potyvirus (BCMV) into the United States (17,19). Therefore, we initiated a study to characterize LMV isolates associated with these outbreaks.

We used a polymerase chain reaction (PCR)-based approach to obtain sequence data, as well as biological and serological methods, to determine whether new LMV strains were responsible for the recent lettuce mosaic outbreaks. Our results indicate that new LMV strains or pathotypes are not responsible for the outbreaks but that

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up to 12% amino acid variability may be found in the CP N-terminal region of LMV isolates.

MATERIALS AND METHODS

Viral isolates and sap transmission. Lettuce plants having symptoms of LMV infection, including mosaic, leaf distortion, stunting, and/or necrotic spots on the leaves such as those attributed to the Firestone strain, were collected from fields throughout the Salinas Valley during 1991 and 1992. Additional LMV isolates were provided by B. W. Falk (University of California, Davis), H. Lot (INRA, Montfavet, France), P. Himmel (Asgrow Seed Co., San Juan Bautista, CA) and J. Watterson (Petoseed Co., Woodland, CA). Sap was prepared by grinding leaves in ice-cold 0.02 M potassium phosphate buffer, pH 7.2, containing 0.1% Na_2SO_3. *Chenopodium quinoa* and *Nicotiana benthamiana* plants at the four to six leaf stage, and lettuce plants, cv. Salinas, at the three to five leaf stage, were rub-inoculated with sap using Carborundum as an abrasive. Viral isolates were propagated in *N. benthamiana* and maintained in an insect-free greenhouse at 20 to 25°C.

To determine the virulence of LMV isolates on lettuce differential cultivars, plants at the three to five leaf stage were rub-inoculated as described above. Infection was assessed based on the appearance of symptoms 3 weeks after inoculation and by testing each plant by indirect ELISA (I-ELISA) with a polyclonal LMV antiserum (described below).

Preparation of polyclonal LMV antisera and ELISA. I-ELISA (3) was used to detect LMV in infected plants. An LMV polyclonal antiserum raised against an LMV strain from Florida (Florida AS) (9) was used to test field-collected samples. A new LMV polyclonal antiserum (Davis AS) was raised against a Salinas Valley isolate of LMV (LMV-R) using virions purified from infected *N. benthamiana* leaves according to the procedure of Lecoq and Pitrat (16). The Davis AS was used in some experiments.

Molecular biology procedures. A minipurification procedure was used to isolate virions from infected *N. benthamiana* leaves (15). Virions were resuspended in 1 ml of 0.01 M potassium phosphate buffer, pH 7.2. To verify the presence of virus in the preparations, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblot analyses were carried out with the Mini-Protean system (Bio-Rad Laboratories, Richmond, CA) according to the manufacturer's instructions.

To extract RNA from 200 µl of minipurified virions, 50 µl of buffer (0.2 M glycine, 0.2 M NaCl, 20 mM EDTA, pH 9.5) was added, and the solution was adjusted to 1.5% (wt/vol) SDS and 100 mg of Proteinase K (Bethesda Research Laboratories, Inc., Gaithersburg, MD) per ml. This solution was incubated at 37°C for 1 h. Viral RNA was extracted with phenol/chloroform and precipitated with ethanol. The RNA pellet was washed with 70% ethanol and resuspended in 20 µl of sterile water. cDNA was synthesized in a 20-µl reaction mixture containing 2 µl of RNA, 2 µl of 10× reaction buffer (0.5 M Tris-HCl, 0.7 M KCl, 0.1 M $MgCl_2$, pH 8.0), 10 mM β-mercaptoethanol, 200 pmol of oligo-dT primer, 0.1 mM each dNTP, and 10 units of avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI). The reaction was incubated at 42°C for 30 min, then boiled for 5 min. PCR amplification was performed in a 50-µl reaction mixture containing 5 µl of template cDNA, 5 µl of 10× reaction buffer (Perkin-Elmer, Foster City, CA), 2.5 mM $MgCl_2$, 0.2 mM each dNTP, 50 pmol of each primer, and 1 unit of *Taq* DNA polymerase (Perkin-Elmer). The PCR profile was 35 cycles with the following parameters: denaturing for 1 min at 94°C, annealing for 2 min at 42°C, and extension for 2 min at 72°C. PCR-generated fragments were cloned with the TA cloning kit (Invitrogen, San Diego, CA) and transformed into *Escherichia coli* according to the manufacturer's instructions. Recombinant plasmids were identified by restriction enzyme analysis and dideoxyribonucleotide sequencing (26) using Sequenase (U.S. Biomedical, Cleveland). Nucleotide (nt) and amino acid (aa) sequences were assembled and compared using the GCG software package (4).

RESULTS

LMV isolates and serological relationships. Lettuce plants showing symptoms of LMV infection were collected from various locations in the Salinas Valley during 1991 and 1992. All plants were collected in or around the areas where LMV outbreaks had occurred in previous years, and a number of plants showed necrotic spots or lesions, which are symptoms attributed to the necrosis-inducing LMV such as the Firestone strain. *C. quinoa* plants inoculated with sap prepared from these plants developed chlorotic local lesions on inoculated leaves, and systemic symptoms that included chlorotic lesions and leaf distortion (Table 1). These symptoms were identical to those induced by a "type" LMV isolate (LMV-P, provided by B. W. Falk). *N. benthamiana* plants inoculated with sap prepared from systemically infected *C. quinoa* leaves developed systemic mosaic symptoms, and lettuce plants inoculated with sap from infected *N. benthamiana* leaves developed mosaic symptoms that were similar to those observed in the field-collected plants. No differences were observed in the symptoms induced by each isolate in *C. quinoa*, *N. benthamiana*, or lettuce plants. Seven LMV isolates were selected for further characterization (Table 1), including three putative necrosis-inducing isolates (LMV-Fi, LMV-R, and LMV-Go) recovered from plants showing necrotic spots collected from fields near the Firestone factory in Salinas, where the occurrence of "severe" LMV isolates was first reported, hence the 'Firestone' strain (23). Two additional LMV isolates, also from the Salinas Valley, were provided by Asgrow (LMV-As) and Petoseed (LMV-Pe) companies. All LMV isolates collected from the Salinas Valley during 1991 and 1992 and the LMV-P isolate (originally recovered from a lettuce seed lot in France) reacted strongly in I-ELISA tests with the Florida AS, which has been used in the Monterey County seed testing program (Table 1).

Molecular characterization of the LMV isolates. To assess variability among the LMV isolates at the molecular level, partial nt and deduced aa sequences of the CP gene were determined. Initially, the 3′-end of the genomic RNA of the putative necrosis-inducing LMV-R isolate was amplified by PCR. An oligo-dT primer (5′-AGCTGGATCC$(T)_{14}$-3′, *Bam*HI site is underlined) was used to prime synthesis of cDNA from the viral RNA and then the oligo-dT primer and a 512-fold degenerate potyvirus primer (5′-GCGGGATCCGTNTGYGTNGAYGAYTTTYAAYAA-3′, *Bam*HI site is underlined; R. C. French, *personal communication*) were used to amplify the 3′-end of the NIb gene and the entire CP

TABLE 1. Symptoms induced in *Chenopodium quinoa* and *Nicotiana benthamiana* plants by lettuce mosaic virus (LMV) isolates from the Salinas Valley of California[a] and reaction against a polyclonal LMV antiserum (Florida AS) in indirect enzyme-linked immunosorbent assay

Isolate	Origin	Symptoms[b]		A_{405}[c]	
		C. quinoa	*N. benthamiana*	Lettuce[d]	*N. benthamiana*
As	Asgrow Seed Co.	ND	–/ld, mos	0.848	1.057
Pe	Petoseed Co.	ND	–/ld, mos	0.806	1.037
Fi	Firestone plant	cl/cl, ld	–/ld, mos	1.033	1.152
R	Firestone plant	cl/cl, ld	–/ld, mos	0.952	1.134
Go	Firestone plant	cl/cl, ld	–/ld, mos	0.830	0.846
Ha	Hartnell College	cl/cl, ld	–/ld, mos	1.091	1.001
Hi	Hitchcock Ln.	cl/cl, ld	–/ld, mos	0.952	1.200
Hu	Hunter Ln.	cl/cl, ld	–/ld, mos	1.074	1.182
Sp	Spreckles Rd.	cl/cl, ld	–/ld, mos	1.229	0.870
P	B. W. Falk	cl/cl, ld	–/ld, mos	0.946	1.085

[a] Isolates As, Pe, Fi, R, and Go represent a putative necrosis-inducing strain of LMV; isolate P represents the "type" strain of LMV and is originally from France.

[b] Symptoms in inoculated/noninoculated leaves: cl, chlorotic lesions; ld, leaf distortion; mos, mosaic; –, no symptoms; ND, not determined.

[c] The absorbance of healthy samples was 0.132 and 0.202 for lettuce and *N. benthamiana*, respectively; all results represent average readings of four wells.

[d] Cultivar Salinas.

gene. The predicted 2.0-kbp fragment was amplified (Fig. 1A and B), but attempts to clone this fragment with the TA cloning system were unsuccessful. Therefore, the 2.0-kbp fragment was digested with *Bam*HI, which resulted in two fragments of approximately 1.0 kbp (Fig. 1B). Because the primers had *Bam*HI sites engineered at their 5′-ends, the *Bam*HI-digested 1-kbp fragments were cloned into *Bam*HI-digested pKS+ (Stratagene, La Jolla, CA). The ends of these fragments were sequenced, and an LMV-specific primer was designed that annealed 250 nt upstream from the CP gene (5′-GGACCTGCAGAAGCGGAATTAGAAAAAT-3′, *Pst*I site is underlined). Using this primer and an oligo-dT primer containing a *Pst*I site (5′-AGCTCTGCAG $(T)_{14}$-3′, *Pst*I site is underlined), a 1.3-kbp fragment was amplified by PCR from viral cDNA of LMV-R (Fig. 1A and B). This fragment, which included the entire CP gene and the 3′-untranslated region, was cloned with the TA cloning system and sequenced on both strands using oligonucleotide primers to generate overlapping sequences (Fig. 1A; GenBank U24670).

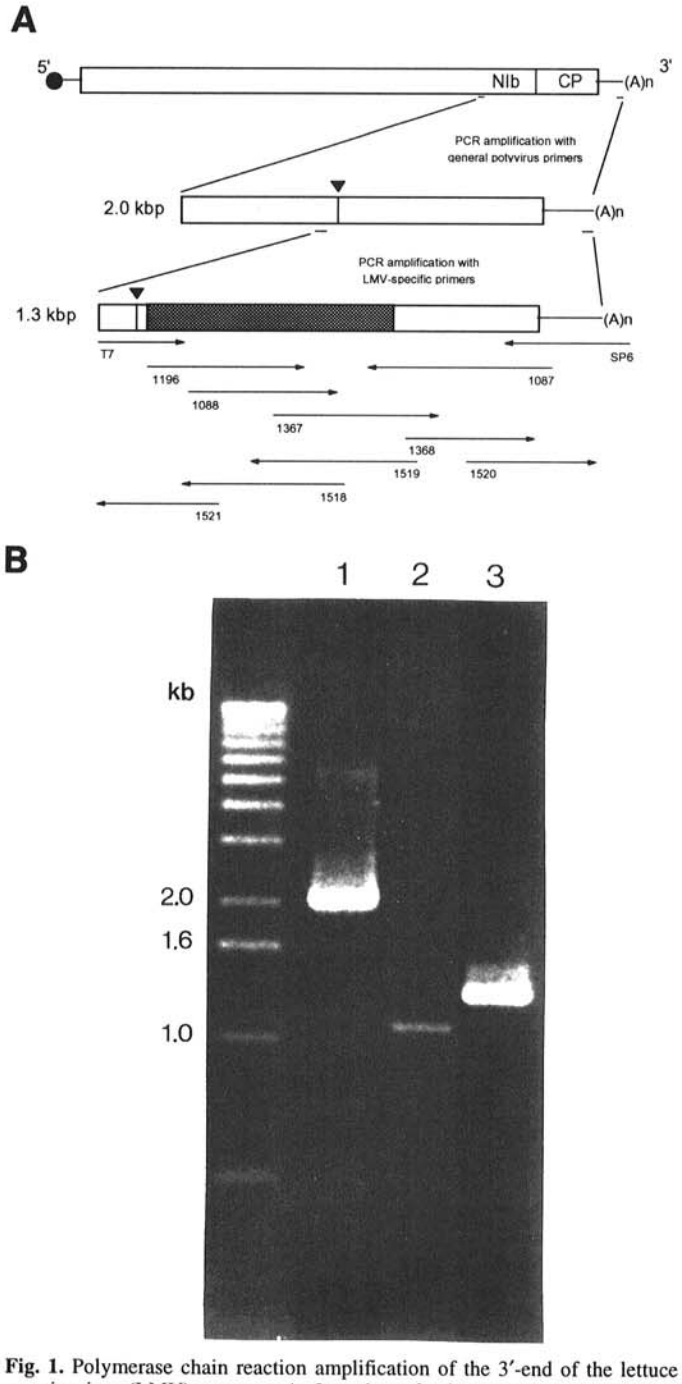

Fig. 1. Polymerase chain reaction amplification of the 3′-end of the lettuce mosaic virus (LMV) genome. **A,** Location of primers and amplified fragments with respect to the generic potyvirus genome and sequencing strategy for the LMV-R coat protein gene. Annealing sites for general and LMV-specific primers are indicated; numbers refer to individual primers. The 1.3-kbp fragment from LMV-R was sequenced on both strands. The hatched area in the 1.3-kbp fragment represents the 600-bp fragment sequenced for 9 other LMV isolates. **B,** Lane 1, amplification of a 2.0-kbp fragment with a degenerate potyvirus primer and an oligo-dT primer; lane 2, the 2.0-kbp fragment digested with *Bam*HI; lane 3, amplification of a 1.3-kbp fragment with LMV-specific primers.

The LMV-R CP gene encodes a predicted 278-aa protein with a molecular mass of approximately 31 kDa. The CP gene was followed by a 3′ untranslated region of 207 nt and a poly-A tail (Fig. 2). As expected for the CP of an aphid-transmissible potyvirus, the DAG triplet was present in the amino terminus (1,27). The deduced LMV-R CP aa sequence is 98% identical to that of the LMV-0 isolate from France (6), and the 3′-untranslated sequences of these isolates were 99% identical. A glutamine/glycine (Q/G) cleavage site was present between the NIb and the CP genes of LMV-R, which is consistent with the consensus potyvirus CP cleavage motif "V-x-x-Q/A,G,S" (7,8,27). Interestingly, the CP cleavage site of LMV-0 was Q/V (6), the first such cleavage site identified in a potyvirus.

A 600-bp CP gene fragment, which included most of the 5′-end of the coat protein gene (Fig. 1A), was amplified from the other 9 LMV isolates with LMV-specific primers designed from the LMV-R CP sequence (primer 1196, 5′-AAGGCAGTAAAACTGATG-3′, and primer 1519, 5′-CCTCGTAGCCGTCCGTAT-3′; Fig. 1A). RNA extraction, cDNA synthesis, and PCR conditions were as described above, except that the annealing temperature for PCR was 50°C. Reverse transcriptase (RT)-PCR products were cloned and sequenced as described (GenBank U24661 to U24671). Pairwise comparisons were made between sequences of all isolates, including LMV-0.

The derived amino acid sequences of the CP fragments of the 11 LMV isolates were 95 to 99% identical (Fig. 3; Table 2). Most of the differences were located in the N-terminal region (residues 10 to 80), which is the most variable region of the potyvirus CP (28,29) and will be referred to here as the hypervariable (HV) region. When only the HV regions were compared, the aa sequences were 88 to 98% identical (Table 2). The HV regions of the 5 putative necrosis-inducing isolates were 90 to 97% identical, whereas the HV region sequences of the other 5 isolates were 93 to 98% identical. The CP-HV region sequences of the 5 necrosis-inducing isolates were 88 to 98% identical to those of the other isolates. Alignment of the aa sequences of all 11 isolates failed to reveal highly conserved residues that would allow for grouping of the isolates based on the putative necrotic phenotype. Taken together, the CP aa sequence comparisons failed to provide evidence for distinct strains among these LMV isolates.

Virulence of LMV isolates on lettuce differential cultivars. To assess the possible biological significance of the genetic variability among these isolates, the 10 isolates in addition to a pathotype IV isolate (LMV-E, provided by H. Lot), were inoculated onto a series of lettuce differential cultivars that differ based on LMV resistance genes (21). Cvs. Salinas and Ithaca possess no resistance gene, Salinas 88 and Vanguard 75 possess the *mo* gene, and Calona and Malika possess the *g* gene. We determined the virulence of each isolate on each cultivar and also recorded the type of disease symptoms induced by these isolates to determine pathotype designations and identify necrosis-inducing isolates. Five plants of each cultivar were inoculated with each isolate, and the experiment was repeated three times. All 10 isolates induced symptoms only in the two cultivars without LMV resistance genes, whereas LMV-E infected all cultivars (Table 3). Viral antigen was detected by I-ELISA in some inoculated plants of cultivars possessing LMV resistance genes, particularly those with the *mo* gene, but these plants remained symptomless. These results indicate that all 10 LMV isolates are indistinguishable in virulence and that they are LMV pathotype II isolates (21).

Interestingly, all 10 isolates induced necrotic symptoms, particularly on cv. Ithaca. On cv. Salinas, necrotic symptoms also were induced by all isolates, but only under conditions of high temperature and long days. Thus, the ability to induce necrotic symptoms was a common characteristic of all isolates (including the type isolate, LMV-P) and appears to be influenced by environmental conditions and/or host genotype.

DISCUSSION

The reemergence of LMV in the Salinas Valley, along with the appearance of necrotic symptoms not usually associated with LMV infection, raised concerns that new LMV strains had evolved or had been introduced, such as those described for BCMV and potato virus Y (PVY) (19,33,34). Therefore, a combination of methods was used to define genetic variability among LMV isolates from the Salinas Valley and to establish whether new LMV strains were responsible for these outbreaks. Our results do not support the contention that new necrosis-inducing LMV strains (i.e., the Firestone strain) are responsible for these outbreaks.

Within the potyviruses, the sequence of the CP gene has been effectively used as a taxonomic tool and, in general, has supported previously differentiated species (2,10,14,25,32,35,36,39). It is now well established that distinct potyvirus species have CP aa identities of <70%, whereas strains of the same species have similarities of >90% (28,29,30,36). Unfortunately, there currently exists no clear sequence thresholds to support biological or serological evidence for whether a given isolate represents a distinct strain, and this has led to difficulty in classifying certain potyvirus isolates with respect to their strain designations. One approach to circumventing this problem has been to examine the N-terminal region of the CP gene, which is the location of the major virus-specific antigenic determinants and is referred to as the HV region. Some biologically distinct potyvirus strains (e.g., strains of plum pox [PPV] and sugarcane mosaic [SCMV]), which have overall CP aa identities of >90%, have far more divergent CP-HV regions (PPV strains, 74% identity [2]; SCMV strains 22% identity [11]). Similarly, the overall CP aa similarity among isolates of necrotic versus nonnecrotic BCMV strains ranges from 87 to 89% but is only 46 to 61% when CP-HV regions are compared (14). While these data suggest that divergence in the CP HV region may provide a reliable criterion to establish potyvirus strain relationships, a recent study of PVY isolates indicates that the CP-HV regions of biologically distinct strains are not necessarily highly divergent (33). The CP aa sequences of 21 PVY isolates representing necrotic and nonnecrotic strains were compared, and overall CP aa identities among isolates ranged from 93 to 99.6%. The CP-HV aa identities among isolates within each strain ranged from 94 to 99%, versus 86 to 92% between isolates of the two strains. However, based on conservation of CP cleavage sites and a number of amino acid differences, the isolates were placed into two groups that correlated with the ability of the isolates to induce necrotic symptoms. These results suggest that distinct potyvirus strains may be identified by a high degree of divergence in the CP-HV region and/or by a few highly conserved CP characteristics.

Therefore, we reasoned that if new necrosis-inducing strains were responsible for the LMV outbreaks in the Salinas Valley, then the CP-HV region of these isolates would be highly divergent or possess one or more conserved amino acid differences that would allow clear differentiation of these strains from the type LMV strain. Comparison of the CP-HV aa sequences of 9 LMV isolates associated with the recent outbreaks, including 5 putative necrosis-inducing or severe isolates (isolates considered to represent the Firestone strain of LMV) and 2 known LMV type isolates (LMV-P and LMV-0), did not reveal evidence of a distinct LMV strain in the Salinas Valley. The CP-HV sequences of the 5 putative necrosis-inducing or severe isolates were no more identical to each other than they were to those of the other LMV isolates (Table 3) nor did they share among them common amino acid residues not found in the other LMV isolates (Fig. 3). Therefore, we were unable to correlate CP sequence characteristics with the putative severe LMV isolates, and based on these results, these 11 isolates represent a single LMV strain. The 2 to 12% aa dif-

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                 ◆
 -3  caaggagacacaaagcttgatgcaggccaaggcagtaaaact
 -1   Q  G  D  T  K  L  D  A  G  Q  G  S  K  T

 40  gatgataggcagaagaactcagcggatccaaagaataatact
 14   D  D  R  Q  K  N  S  A  D  P  K  N  N  T

 82  atcacagagaaaggaagtggttctgtgcaggtgaagaaggac
 28   I  T  E  K  G  S  G  S  V  Q  V  K  K  D

124  gacgacatcaacgcagggctacatggtaaacacaccatacct
 42   D  D  I  N  A  G  L  H  G  K  H  T  I  P

166  cgtacaaaggcaatcacacagaaaatgaagttaccaatgatt
 56   R  T  K  A  I  T  Q  K  M  K  L  P  M  I

208  cgaggtaaagtggctttaaaccttgatcatttgctggagtac
 70   R  G  K  V  A  L  N  L  D  H  L  L  E  Y

250  gaaccaaaccagagagatatatcaaacacacgcgcaacccag
 84   E  P  N  Q  R  D  I  S  N  T  R  A  T  Q

292  aaacaatacgagtcatggtacgacggggttaagaatgactat
 98   K  Q  Y  E  S  W  Y  D  G  V  K  N  D  Y

334  gatgtggatgatagtggcatgcaattaattcttaatggattg
112   D  V  D  D  S  G  M  Q  L  I  L  N  G  L

376  atggtttggtgtatagaaaacgggacatccccgaatataaat
126   M  V  W  C  I  E  N  G  T  S  P  N  I  N

418  ggaacatgggtgatgatggatggcgaagaacaagtagaatat
140   G  T  W  V  M  M  D  G  E  E  Q  V  E  Y

460  gctctgaaacccatcatcgagcacgcaaaacccacgtttcgc
154   A  L  K  P  I  I  E  H  A  K  P  T  F  R

502  cagataatggcccacttcagtgacgcagccgaggcgtacatt
168   Q  I  M  A  H  F  S  D  A  A  E  A  Y  I

544  gaaatgagaaacaagaagaaaccgtatatgccacgatacgga
182   E  M  R  N  K  K  K  P  Y  M  P  R  Y  G

586  cggctacgaggcttgaacgatatggggttagctcgctatgct
196   R  L  R  G  L  N  D  M  G  L  A  R  Y  A

628  ttcgacttctacgaaacaacatcagcaaccccaaatcgggcg
210   F  D  F  Y  E  T  T  S  A  T  P  N  R  A

670  agagaggcgcacaatcaaatgaaggcagctgctctagtggga
224   R  E  A  H  N  Q  M  K  A  A  A  L  V  G

712  acacagaacagactgtttggaatggatggaggcggttcaacc
238   T  Q  N  R  L  F  G  M  D  G  G  G  S  T

754  caggaagagaacgcggagaggcacacagccgcagatgtgaat
252   Q  E  E  N  A  E  R  H  T  A  A  D  V  N

796  cagaatatgcatactctcttaggcgtgagagggttgcactaa
266   Q  N  M  H  T  L  L  G  V  R  G  L  H  *

832  aggcgtgtgttggcatttaaagactgtagtataaattataat

876  atagtgagtgttcactccttttatgtttatgtatgcacttat

918  ttagtattctctccctgtacttcgctcgtgagggcgtaggtt

970  cagctgagggcttttctagtttctgctgtgaggttttacctc

1012 gaaggttgctagtctggttttcagtcggagac(a)n
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Fig. 2. Nucleotide and derived amino acid sequence of the lettuce mosaic virus (LMV-R) coat protein (CP) gene. The cleavage site between the NIb and the CP gene (◆) and the DAG triplet required for aphid transmissibility are indicated.

ferences detected in the CP-HV aa sequences are randomly distributed, which probably represents typical variability among individual field isolates of a potyvirus, attributable to the lack of a proof-reading function of the viral RNA polymerase. A similar level of variability was found between the CP aa sequences of two biologically similar PPV isolates (37).

The biological properties of the LMV isolates were examined by inoculation onto a recently established series of lettuce differential cultivars, which can be used to differentiate four pathotypes of LMV (21,22). All 9 LMV isolates collected from the

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Asgrow      ---------- ---T-E---- G--------- Q-----P--- ---I------
Peto        ---------- ---A-A---- --------R- ---------- --L-V-----
Firestone   ------R--D ---------K ---------- ---------- ----------
Romaine     ---------- ---------- ---------- ---------- ----------
Gould       ---------N --D------- ---------- ---------- ----------
Hartnell    ---------- ---------- ---------- ---------- ---------V
Hitchcock   -------R-- ---------- ---------- ---------- ----M-----
Hunter      -------R-N ---------- ---------- ---------- ----M-----
Spreckles   ---------- -----A---- DR-------- ---------- ----------
LMV-P       ---------- --D------- --------I- ---------- ----------
LMV-0       ---------N --D------- ---------- ---------- ----------
CONSENSUS   GSKTDDkqKs SAhpKdNIIt ekGSGSGQmK kDDDINaGLH GKhtiPRTKa
            10                                                  59

Asgrow      ---------- ---------- V--------E -------E-- ----------
Peto        ---------- ------I--- ---------- --------S- ----------
Firestone   ---------- ---------- ---------- ---------- ----------
Romaine     ---------- ---------- ---------- ---------- ----------
Gould       ---------- ---------- ---------- ---------- ----------
Hartnell    ---------- ---------- ---------- ---------- --------E-
Hitchcock   ---------- ---------- ---------- ---------- ----------
Hunter      ---------- ---------- ---------- ---------- ----------
Spreckles   ---------- ---------- ---------- ---------- ----------
LMV-P       ---------- A--------- ---------- ---------- ----------
LMV-0       ---------- A--------- ---------- ---------- ----------
CONSENSUS   ITQKMKLPMI rGKVALnLDH lLEYEPNQRd ISNTRATqkQ YESWYDGVkN
            60                                                  109

Asgrow      ---------- ---------- ---------- ---------- ----------
Peto        ---------- ---------- ---------- ---------- ----------
Firestone   ---------- ---------- ---------- ---------- ----------
Romaine     ---------- ---------- ---------- ---------- ----------
Gould       ---------- ---------- ---------- ---------- ----------
Hartnell    --------S- ---------- ---------- ---------- ----------
Hitchcock   ---------- ---------- ---------- ---------- ----------
Hunter      ---------- ---------- ---------- ---------- ----------
Spreckles   ---------- ---------- ---------- ---------- ----------
LMV-P       ---------- ---------- ---------- ---------- ----------
LMV-0       ---------- ---------- ---------- ---------- ----------
CONSENSUS   DYDVDDSGmQ LILNGLMVWC IENGTSPNIN GTWVMMDGEE QVEYALKPII
            110                                                 159

Asgrow      --------H- ------V--- ---------- ---------
Peto        ------L--- ---------- ---------- ---------
Firestone   ---------- ---------- ---------- ---------
Romaine     ---------- ---------- ---------- ---------
Gould       ---------- ---------- ---------- ---------
Hartnell    ---------- ---------- ---------- ---------
Hitchcock   ---------- ---------- ---------- ---------
Hunter      ---------- ---------- ---------- ---------
Spreckles   ---------- ---------- ---------- ---------
LMV-P       ---------- ---------- ---------- ---------
LMV-0       ---------- ---------- ---------- ---------
CONSENSUS   EHAKPTfRqI MAHFSDaAEA YIEMRNKKKP YMPRYGRLR
            160                                     198
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Fig. 3. Alignment of the deduced coat protein (CP) amino acid sequences of lettuce mosaic virus (LMV) isolates from the Salinas Valley of California and LMV-P. The computer-generated consensus sequence is shown at the bottom. Amino acids that differ from the consensus sequence in each isolate are indicated. Isolates As, Pe, Fi, R, and Go represent a putative necrosis-inducing strain of LMV.

Salinas Valley, including those recovered from plants with necrotic symptoms, had identical virulence properties and were classified as pathotype II, the most common pathotype. These results provide additional evidence that isolates associated with the Salinas Valley outbreaks do not represent a new LMV strain or pathotype. The severe LMV isolates may be more aggressive pathotype II isolates, as suggested by Pink et al. (21) for a Firestone isolate of LMV from the Salinas Valley.

An explanation for the necrotic symptoms observed in LMV-infected plants is provided by the results of the infectivity experiments. All LMV isolates, including the type isolate, induced necrosis on susceptible cultivars, indicating that this property may be intrinsic to LMV, with expression of necrotic symptoms dependent on host or environmental factors. The occurrence of necrotic symptoms associated with LMV infection in the Salinas Valley has been reported previously (18,41; A. O. Paulus, *personal communication*), which provides additional evidence that necrosis-inducing LMV isolates have not recently appeared. We have observed similar necrotic symptoms in lettuce plants regenerated in vitro, suggesting that necrosis may be a more generalized host response to physiological stress, such as that caused by a viral infection.

The low level of genetic and biological variability detected among LMV isolates in the Salinas Valley, an area where lettuce has been intensively cultivated for at least 50 years, may be surprising, particularly when compared to LMV in Europe, where distinct pathotypes have been identified (21,22). One possible explanation for this difference is the method by which lettuce mosaic is managed. In the Salinas Valley, LMV management is based on the use of virus-indexed seed, weed management, and a lettuce-free period. Although resistant lettuce cultivars are available, they have not been extensively used, probably due to the success of the seed-indexing program. In Europe, LMV control is based on the use of resistant cultivars (5), which may place greater selection pressure on the virus, resulting in the emergence of new resistance-breaking pathotypes. Because this kind of selection pressure has not been placed on LMV in the Salinas Valley, new virulent strains may be less likely to evolve.

We are currently investigating the role of alternate hosts, such as weeds and/or ornamental plants, as the inoculum source for the LMV outbreaks. Although alternate hosts have been regarded as relatively unimportant in the epidemiology of lettuce mosaic in the Salinas Valley (13), they can be responsible for localized outbreaks (41). LMV-infected ornamental plants have been recently identified in the Salinas Valley, including the freeway daisy (*Osteospermum fruticosum*) (20) and the widely planted *Gazania* spp. (40). These plants are vegetatively propagated perennials that can be an inoculum source of LMV during the lettuce-growing season and can harbor the virus over the lettuce-free period. We believe that these plants may be the primary inoculum source for the recent lettuce mosaic outbreaks.

In conclusion, molecular (CP sequence comparisons) and biological (infectivity in differential cultivars) characterization of LMV isolates associated with recent lettuce mosaic outbreaks in the Salinas Valley failed to provide evidence that new LMV strains are responsible for these outbreaks. Individual LMV isolates had as much as 12% aa variability in the CP-HV region, but this did not affect their infectivity in lettuce. It will be of interest to determine the CP sequences for LMV pathotype I, III, and IV isolates, compare these to the sequences of the Salinas Valley isolates (pathotype II), and establish whether CP sequence properties can be used to differentiate LMV pathotypes.

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TABLE 2. Percent coat protein (CP) amino acid identities between lettuce mosaic virus (LMV) isolates from the Salinas Valley of California[a]

	Asgrow (As)	Peto (Pe)	Firestone (Fi)	Romaine (R)	Gould (Go)	Hartnell (Ha)	Hitchcock (Hi)	Hunter (Hu)	Spreckles (Sp)	LMV-P (P)	LMV-0 (0)
Asgrow (As)	—	90	93	96	92	94	94	93	94	93	90
Peto (Pe)	95	—	91	94	91	93	93	91	96	91	88
Firestone (Fi)	97	96	—	97	97	96	96	97	96	94	95
Romaine (R)	98	97	99	—	97	98	98	97	98	97	95
Gould (Go)	97	96	99	99	—	96	96	97	96	97	98
Hartnell (Ha)	97	96	98	99	98	—	97	96	97	96	93
Hitchcock (Hi)	97	96	98	99	98	98	—	98	97	96	95
Hunter (Hu)	97	96	99	99	99	98	99	—	96	94	97
Spreckles (Sp)	97	97	98	99	98	98	99	98	—	96	93
LMV-P (P)	97	96	98	99	97	98	98	98	98	—	98
LMV-0 (0)	96	95	98	98	99	97	98	98	98	99	—

[a] Identities are presented for a CP fragment, including residues 10 to 198 (below the diagonal), and for the hypervariable region, including residues 10 to 80 (above the diagonal). Isolates As, Pe, Fi, R, and Go represent a putative necrosis-inducing strain of LMV; isolates P and 0 represent the "type" strain of LMV and are originally from France.

TABLE 3. Infectivity and symptoms in lettuce differential cultivars inoculated with lettuce mosaic virus (LMV) isolates from the Salinas Valley of California[a]

Cultivar	Asgrow (As)	Firestone (Fi)	Gould (Go)	Hartnell (Ha)	Hitchcock (Hi)	Hunter (Hu)	LMV-P (P)	Peto (Pe)	Romaine (R)	Spreckles (Sp)	LMV-E
Ithaca	mo/n[b] (5/5)[c]	mo/n (8/15)	mo/n (6/10)	mo/n (12/15)	mo/n (8/15)	mo/n (10/15)	mo/n (9/15)	mo/n (11/15)	mo/n (13/15)	mo/n (10/15)	mo/n (5/5)
Salinas	mo/n (5/5)	mo/n (11/15)	mo/n (8/10)	mo/n (8/15)	mo/n (9/15)	mo/n (13/15)	mo/n (11/15)	mo/n (12/15)	mo/n (14/15)	mo/n (12/15)	mo/n (5/5)
Calona	– (1/5)	– (1/15)	– (0/10)	– (2/15)	– (2/15)	– (0/17)	– (0/15)	– (1/15)	– (1/15)	– (2/15)	mo/n (4/5)
Malika	– (0/0)	– (0/10)	– (0/10)	– (0/10)	– (0/10)	– (0/8)	– (0/10)	– (0/10)	– (0/10)	– (0/10)	mo/n (4/5)
Salinas 88	– (1/5)	– (0/15)	– (0/10)	– (2/15)	– (2/15)	– (1/15)	– (0/15)	– (2/15)	– (0/15)	– (1/15)	mo/n (5/5)
Vanguard 75	– (0/5)	– (2/15)	– (1/10)	– (0/15)	– (0/15)	– (1/15)	– (1/15)	– (1/15)	– (0/15)	– (1/15)	mo/n (3/5)

[a] Isolates As, Pe, Fi, R, and Go represent a putative necrosis-inducing strain of LMV; isolate P represents the "type" strain of LMV and is originally from France; isolate E represents LMV pathotype IV and is originally from Spain.

[b] Symptoms: mo, mosaic; n, necrosis; –, no symptoms.

[c] Number of plants positive in indirect enzyme-linked immunosorbent assay/number of plants inoculated. Plants were tested 4 wk after inoculation.

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