Role of *Vigna* species in the Appearance of Pathogenic Variants of Cucumber Mosaic Virus

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ABSTRACT

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To determine the role of host plants in the appearance of new strains of cucumber mosaic virus (CMV), two cowpea cultivars, namely, *Vigna unguiculata* ssp. *unguiculata* 'Blackeye' and *V. unguiculata* ssp. *cylindrica* 'Catjang,' were investigated as model systems. Four strains of CMV, selected by several single-lesion transfers through *Chenopodium quinoa*, caused numerous necrotic lesions with diameters of 0.1–0.2 cm and ≤0.1 cm on the inoculated primary leaves of Blackeye and Catjang, respectively. Additionally, large necrotic lesions (0.4–0.6 cm in diameter) appeared at a rate of 0.11–5.26% (0.53% average) of the total lesions on Catjang and only 0.02% of those on Blackeye. Inocula from large lesions derived from Catjang caused similar lesions on Blackeye. Unlike their parent strains, some large-lesion isolates became systemic to Blackeye. Large-lesion isolates were stable under continual passages through Catjang but reverted to small-lesion types after four serial passages by mass inoculation through *C. quinoa*, cucumber, squash, or tobacco. Cross protection and serological tests indicated that the large-lesion isolates were CMV. They were also indistinguishable from respective parent strains as determined by coat protein peptide mapping, RNA profile, and symptomatology on differential hosts. This suggested that the large-lesion isolates were mutants and not contaminants already present in the various isolates. Presence or absence of satellite RNA did not affect the appearance and/or size of large lesions on Catjang. Their initital expression on Catjang provides an example of a specific host-selection mechanism which might add to the high variability of CMV.

Additional key words: CARNA 5, host passage effect, peptide mapping.

Numerous strains of cucumber mosaic virus (CMV) are known to occur throughout the world (14). This high variability could be a problem for growing cultivars resistant to this virus. For example, soon after the discovery of CMV resistance in wild lettuce (*Lactuca saligna* L.) and its incorporation into lettuce breeding lines, a CMV strain was identified that could infect resistant lines (25). Because of the importance of many diseases caused by CMV strains, the nature and cause of variability in CMV needs to be determined. While mutation is presumably the primary cause of variation, the association of small satellite RNAs with many CMV strains (29) and host adaptation phenomena (31,32) have been implicated in the pathogenic variability of CMV strains.

In a preliminary investigation of the reaction of various cowpea species to CMV strains, it was noted that inoculated primary leaves of cowpea, *Vigna unguiculata* ssp. *cylindrica* 'Catjang' occasionally developed some large necrotic lesions (0.4–0.6 cm in diameter) in addition to numerous typical small necrotic lesions ≤0.1 cm in diameter. No such large lesions were observed when these CMV strains were also inoculated to Blackeye. Thus, it appeared that Catjang induced or specifically selected large-lesion mutants from the CMV population. The biological and biochemical properties of these isolates that induce large local lesions are presented in this report. The importance of such mutations and host-selection mechanisms in the variability of CMV is discussed.

MATERIAL AND METHODS

Maintenance of virus culture and host plants. Inoculum of CMV-C (24), CMV-WL (9), and CMV-L-2 (6) were maintained in zucchini squash (*Cucurbita pepo* L. 'President'). The CMV-N (7,27) was maintained in periwinkle (*Vinca rosea* L.). Test plants were grown in 10-cm-diameter plastic pots containing synthetic potting mix amended with fertilizers. Greenhouses were maintained at 25–30 C and supplemented with fluorescent light to ensure at least 16 hr of light per day. The two subspecies of cowpea, *V. unguiculata* ssp. *unguiculata* 'Blackeye' and *V. unguiculata* ssp. *cylindrica* 'Catjang' used in this study will be henceforth referred to by their cultivar names. Corundum-dusted young primary leaves of Blackeye and Catjang were inoculated with CMV-infected squash or periwinkle tissue ground in 0.01 M phosphate buffer, pH 7.0 (1:10, w/v). Seedlings were placed in the dark for 24 hr before inoculation and rinsed with tap water after inoculation.

Biological purification of virus isolates by single-lesion isolation. The CMV strains and large-lesion isolates were selected by serial passages of isolates from single lesions through *Chenopodium quinoa* L. and/or Catjang by using inoculum diluted to obtain about one lesion per leaf in each passage.

Serology. CMV-C rabbit antiserum was used for immunodiffusion tests in a medium containing 0.75% Ionagar and 0.1% NaN_3. Extracted tissue was prepared (1:1, w/v) in 0.01 M phosphate buffer, pH 7.0. For intra-gel cross absorption tests (28), the peripheral wells were filled with 0, 10, 15, 20, and 25 μg of purified CMV-C large-lesion isolate #7 and incubated for 18 hr at room temperature. The peripheral wells were then refilled with 30 μl each of CMV-C antiserum and the central well with leaf extract of CMV-C-infected squash. The whole plate was incubated for another 48 hr at room temperature in a humid chamber before observation.

Cross protection test. Young plants of tobacco (*Nicotiana tabacum* L. 'H-423') were initially inoculated with CMV strains and the large-lesion isolates. Control plants were inoculated with sterile buffer only. Three plants were used per test. Ten to 12 days later, both previously inoculated and systemically invaded leaves were challenge inoculated with the necrotic-lesion-producing CMV-N strain. Observations were made for the appearance of local lesions 6–8 days after the challenge inoculation.

Virus and RNA purification. All CMV isolates except CMV-N were purified from zucchini squash 12–15 days after inoculation by

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using the method of Lot et al (17) except that the final virus pellet was resuspended in PEN buffer (0.01 M NaH_2PO_4, 0.001 M EDTA, 0.01 M NaN_3, pH 7.0) (9). The CMV-N was purified from cucumber (*Cucumis sativus* L. 'Marketer'). RNA was extracted from purified virus by the method of Brakke and Van Pelt (1) with modifications (10). The final RNA pellet was resuspended in sterile PEN buffer. Electrophoresis of the total CMV RNA was carried out in 2.4% polyacrylamide-*bis* acrylamide (20:1) gels in Loening buffer (16) at pH 7.8. Plastic tubes (13.5 × 0.6 cm) were filled with acrylamide solution (2.4% acrylamide, 0.12% bisacrylamide) 9 cm from the bottom and allowed to set for 4 hr at room temperature. The gels were prerun at 3 milliamperes (mA) per tube for 30 min. Viral RNA (40 μg) was applied to each tube and electrophoresed for 3 hr at 3 mA per gel at room temperature. Following electrophoresis, gels were soaked in 0.2 M NaCl for 1 hr at 4 C and scanned at 260 nm with a Beckman Model 25 spectrophotometer.

Peptide mapping. Peptide mapping of CMV coat proteins was performed by using the technique of Cleveland et al (2) with some modifications (5). Coat proteins of CMV-C, CMV-N, CMV-WL, and their large-lesion isolates were purified from virus preparations by polyacrylamide gel electrophoresis (11,15). Chymotrypsin, V8 Staphylococcus aureus protease, and papain were used for limited proteolysis (2). Protein digests were separated by electrophoresis in SDS-15% polyacrylamide-*bis* acrylamide (30:0.8) gels in a minislab gel apparatus (Idea Scientific Co., Corvallis, OR). The peptide patterns were visualized by staining with Coomassie blue R-250.

RESULTS

Appearance of cucumber mosaic virus large-lesion isolates on Catjang and Blackeye cowpeas. Four to five days after the inoculation of young primary leaves of Blackeye and Catjang with CMV-C, CMV-N, CMV-WL, and CMV-L-2, numerous small necrotic lesions were observed. The frequency of infection in Blackeye was always much higher than in Catjang. In the latter, some lesions were found enlarged to a diameter of 0.4–0.6 cm within 5–7 days after inoculation (Fig. 1A). The frequency of appearance of these large lesions varied from experiment to experiment (0.23–5.26%) with an average of 0.76% of total lesions in Catjang (Table 1). However, only one large lesion was observed on leaves of Blackeye inoculated with any of the four CMV strains (Table 1).

The role of interference (21) in the inconsistent and relatively lower frequency of large lesions on Blackeye was assessed. The CMV-C inoculum was diluted with phosphate buffer at 1:10, 1:50, 1:100, and 1:200 (w/v) and inoculated to Blackeye. If interference was limiting the formation of large lesions, it would be expected that the frequency of large lesion appearance would increase with progressive dilution of inoculum. However, no marked increase in frequency of their appearance was noticed with higher dilutions of inoculum (Table 2). Thus, the lower frequency of large lesions could not be correlated with increased interference.

Rate of appearance of large lesion phenotype. Biologically pure inocula were used to estimate the mutation rate to the large-lesion

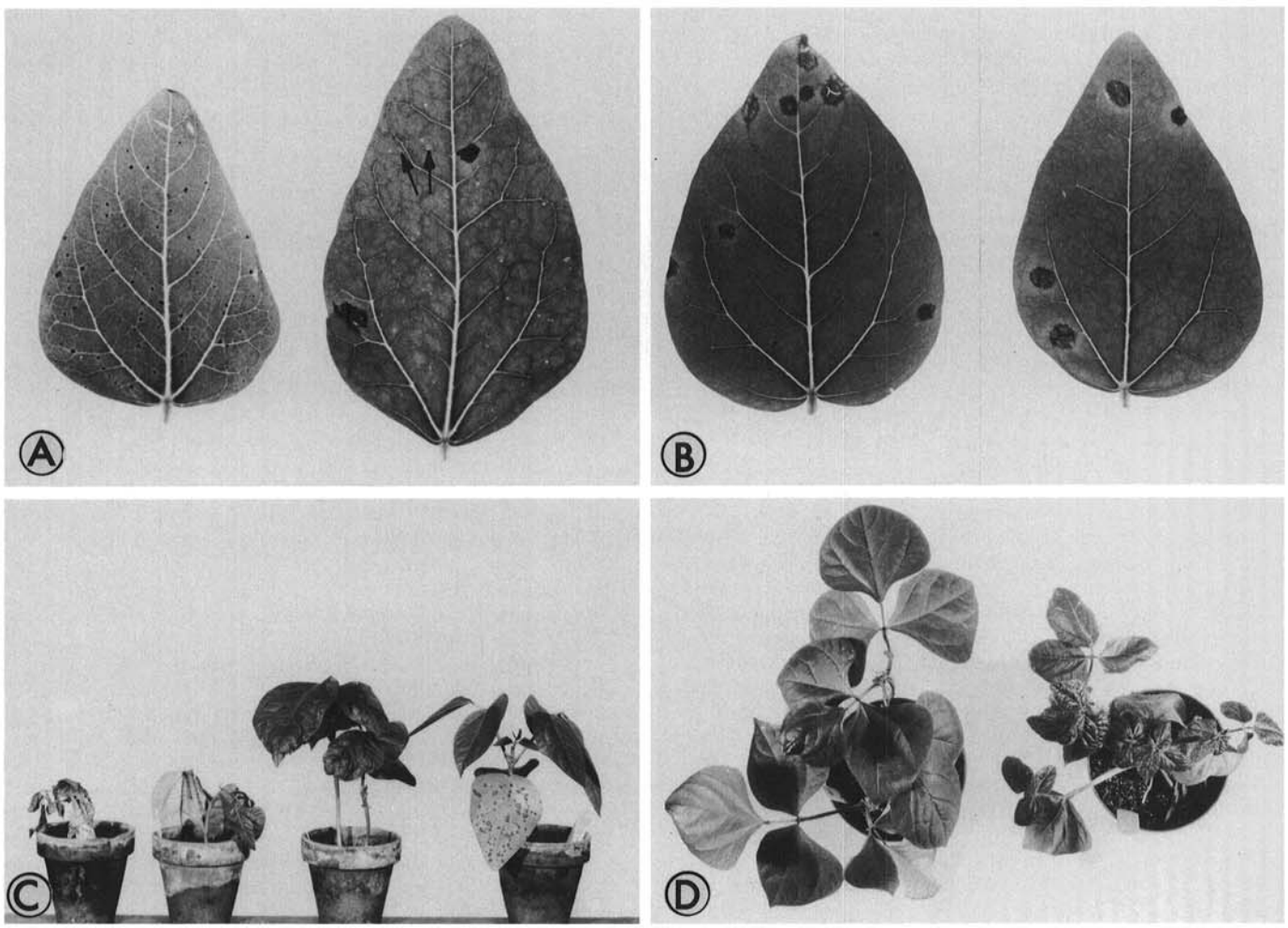

Fig. 1. Symptoms induced by cucumber mosaic virus (CMV) strains and large-lesion isolates on *Vigna unguiculata* ssp. *unguiculata* 'Blackeye' and *V. unguiculata* ssp. *cylindrica* 'Catjang'. **A,** Small necrotic lesions on Blackeye (left) and small plus large necrotic lesions on Catjang (right) following inoculation with CMV-C (small lesions on Catjang are indicated with arrows; white spots were not due to infection). **B,** Symptoms of large-lesion isolate CMV-CL #5 upon subinoculation to Blackeye (left) and Catjang (right). **C,** Progression of systemic necrosis and death of Blackeye seedlings following inoculation with large-lesion isolate CMV-NL #1 (from right to left). *D,* Mosaic with mild necrosis on Blackeye (right) following inoculation with large lesion isolate CMV-CL #7 chl; healthy plant (left).

phenotype. After a minimum of six single-lesion transfers of the original CMV isolates in *C. quinoa*, the virus isolates were reinoculated to squash, the host used to maintain stock cultures. Inoculation of these pure cultures to both *Vigna* species resulted in the appearance of large lesions in most cases (Table 3). The average frequency of these large lesions was 0.53% in Catjang and 0.02% in Blackeye.

In another test, the mutation rate was determined with small-lesion isolates selected from an inoculated Catjang leaf. It was assumed that these isolates would likely be free from admixtures with large-lesion types because Catjang is a good host for expression of large-lesion phenotypes (Tables 1 and 3). From such inoculations, an average of 2.22 and 0.20% large lesions appeared on Catjang and Blackeye, respectively (Table 4).

The average mutation rates derived from data presented in Tables 1, 3, and 4 were 0.67 and 0.02% for Catjang and Blackeye, respectively.

Stability of large-lesion isolates. Initial observations indicated that large-lesion inoculum taken from Catjang and subinoculated to Catjang produced only large lesions, whereas on similarly inoculated Blackeye a mixture of large, medium, and small lesions appeared (Fig. 1B). In the latter, some plants also produced only small lesions, while others produced only large lesions. Subsequently, in nine experiments, large-lesion isolates were selected by four to five single-lesion passages through *C. quinoa* or Catjang, inoculated once to *C. quinoa* leaves to build up inoculum, and then inoculated to Catjang and Blackeye. Only large lesions appeared on Catjang and a mixture of large, medium, and small lesions appeared on Blackeye (Table 5). To study the nature of these small-lesion progenies, two small lesions each from Blackeye separately inoculated with five different large-lesion isolates were isolated, enhanced by one passage in *C. quinoa*, and reinoculated to both *Vigna* species. Only large lesions appeared on Catjang and a mixture of large, medium, and small lesions appeared on Blackeye. These results indicated that the heterologous reactions on Blackeye were not due to contamination in the large-lesion inocula.

In other experiments, purified single-lesion isolates were serially passed by mass inoculation through various hosts, followed by inoculation to Catjang and Blackeye. In an initial test, the large-lesion isolate CMV-NL #1, mass-transferred serially five times through *C. quinoa*, produced only large lesions when inoculated to Catjang. In a similar test, four large-lesion isolates from CMV-C were passed through *C. quinoa*, zucchini squash, tobacco, and cucumber four times. At the end of the last passage, the isolates were back-inoculated to Catjang and Blackeye. In four cases a mixture of large, medium, and small lesions appeared on Catjang, while in four cases only small lesions occurred (Table 6). Only small lesions appeared on Blackeye in all such cases (Table 6).

Change of local to systemic infection by large-lesion isolates in cowpeas. Of the 50 large-lesion isolates investigated, most remained localized in Catjang and Blackeye. However, 12 isolates spread along leaf veins and produced necrosis of petioles and stems in both hosts (Fig. 1C). In addition, a total of seven large-lesion isolates from all four CMV strains produced chlorotic lesions in addition to large, medium, and small necrotic lesions on Blackeye. In such cases, severe systemic mosaic developed on uninoculated leaves with or without mild necrosis (Fig. 1D). Inoculum from the leaves with mosaic produced only large necrotic lesions on Catjang and again a mixture of large, medium, and small lesions plus chlorotic lesions on Blackeye. Systemic mosaic reappeared in the latter host. This property is unlike that of CMV-B (6,23) which causes large chlorotic lesions only on the inoculated primary leaves and mosaic on the trifoliolate leaves of both *Vigna* species.

Cross protection. To determine the relationship of large-lesion isolates with CMV strains, a cross protection test was carried out. After challenge inoculation with CMV-N, no local lesions appeared on those tobacco plants previously inoculated with CMV-C, CMV-WL, and their large-lesion isolates CMV-CL #5, CMV-CL #6, and CMV-WLL #1. On the control sets, an average of 61 lesions appeared per inoculated leaf.

Host reactions. To determine if the large-lesion isolates might have arisen either as a common contaminant CMV strain in association with other CMV strains or from mutation of various CMV strains, investigations were made on the pathogenic marker properties and the coat protein peptide maps of CMV strains and their large-lesion isolates. The CMV-C, CMV-N, CMV-WL, and CMV-L-2 can be distinguished on the basis of pathogenicity to cucumber, *L. saligna*, squash, tobacco, and tomato (*Lycopericum esculentum*, line 80-18-1) (Table 7). Symptoms induced by a particular CMV strain and its large-lesion isolate were identical, but distinctly different from the large-lesion isolate of another CMV strain (Table 7). The large-lesion isolate #1 from CMV-N strain, however, failed to produce typical necrotic lesions on tobacco. Instead, it produced only minute chlorotic-to-necrotic spots on the inoculated lower leaves. Its ability to cause necrotic local lesions on inoculated squash cotyledons and its inability to move systemically in tobacco in most cases confirmed its CMV-N origin.

Serology. In the immunodiffusion test with CMV-C antiserum, all large-lesion isolates showed reactions of identity with their parent CMV strains (Fig. 2). After the cross absorption of CMV-C antiserum with large-lesion isolates, no further reaction could be detected with CMV-C.

RNA profiles. The RNA profiles of CMV-C, CMV-WL, CMV-L-2 (previously designated as CMV-LsS), and CMV-N were reported previously (6,9,30). When the total RNA of CMV strains and their large-lesion isolates were electrophoresed in 2.4% polyacrylamide gels, no differences were noticed between the RNA

TABLE 1. Appearance of large-lesion variants of four cucumber mosaic virus (CMV)[z] strains upon mechanical inoculation to two cowpea cultivars, *Vigna unguiculata* ssp. *cylindrica* 'Catjang' and *V. unguiculata* ssp. *unguiculata* 'Blackeye'

	Catjang			Blackeye		
CMV strain	Plants (no.)	Large/small lesions	Large lesion (%)	Plants (no.)	Large/small lesions	Large lesion (%)
C	101	4/867	0.46	25	0/125	0.00
	52	9/2,342	0.38	42	1/6,257	0.01
	22	4/119	3.25	7	0/109	0.00
N	25	4/905	0.44	25	0/3,728	0.00
WL	33	26/1,992	1.27	36	0/6,466	0.00
	33	4/76	5.26	32	0/2,159	0.00
L-2	23	2/840	0.23	5	0/879	0.00
	100	12/1,288	0.92	100	0/5,910	0.00
Totals	388	65/8,429		272	1/25,633	
Average			0.76			0.004

[z]Stock cultures of CMV strains C, WL, and L-2 used to inoculate *Vigna* spp. were maintained in cultivar President zucchini squash; N strain was maintained in periwinkle.

TABLE 2. Appearance of large lesions upon inoculation of cucumber mosaic virus (CMV)-C at various dilutions to *Vigna unguiculata* ssp. *unguiculata* 'Blackeye'

Experiment	Plants (no.)	Inoculum dilution	Total lesions		Large lesion (%)
			Small	Large	
A	57	1:10[z]	16,648	7	0.04
	54	1:50	10,767	1	0.01
	60	1:100	10,049	0	0.00
	61	1:200	4,841	0	0.00
B	61	1:10	6,596	3	0.04
	60	1:50	4,331	3	0.07
	45	1:100	2,965	1	0.03
	44	1:200	1,846	1	0.05

[z]For 1:10 (w/v) inoculum, 5 gm of CMV-C-infected squash leaves were homogenized with 45 ml of buffer.

TABLE 3. Appearance of large lesions after inoculation of single-lesion isolates of cucumber mosaic virus (CMV) on *Vigna unguiculata* ssp. *cylindrica* 'Catjang' and *V. unguiculata* ssp. *unguiculata* 'Blackeye'

CMV strain	Isolate	SLT[x]	Catjang			Blackeye		
			Plants (no.)	Large/ small lesions	Large lesion (%)	Plants (no.)	Large/ small lesions	Lesion (%)
C	A	6	40	1/898	0.11	46	1/6,577	0.015
	B	6	51	1/299	0.33	44	0/2,310	0.000
	C	11	43	0/78	0.00	21	7/4,081	0.170
	D	11	43	9/2,342	0.38	42	1/6,257	0.016
	E	6	40	0/951	0.00	...[y]	...	...
	F	6	49	0/1,090	0.00	...	...	...
	G	6	57	2/1,099	0.18	...	...	...
WL	#1[z]	8	33	4/76	5.26	32	0/5,159	0.000
	#2	6	33	9/877	1.02	28	0/6,096	0.000
	#3	6	33	26/2,035	0.34	36	0/6,480	0.000
Total			422	52/9,745		249	9/36,960	
Average					0.53			0.020

[x]SLT = number of single-lesion transfers serially through *Chenopodium quinoa*. At the end of single-lesion passage, the isolates were inoculated to squash which was then used as a further source of inocula.
[y]Not tested.
[z]The results of WL isolate #1 were also included in Table 1.

TABLE 4. Expression of cucumber mosaic virus (CMV)-C[z] small-lesion isolates selected from *Vigna unguiculata* ssp. *cylindrica* 'Catjang' and subsequently inoculated to *V. unguiculata* ssp. *cylindrica* and *V. unguiculata* ssp. *unguiculata* 'Blackeye'

CMV isolate	Catjang			Blackeye		
	Plants (no.)	Large/ small lesions	Large lesion (%)	Plants (no.)	Large/ small lesions	Large lesion (%)
#1	21	4/233	1.68	7	1/380	0.26
#2	22	4/119	3.25	7	0/109	0.00
Total	43	8/360		14	1/489	
Average			2.22			0.20

[z]Initial inoculum was prepared by mixing RNA (1 + 2) and RNA 3 of CMV-C total RNA separated by two cycles of sucrose density gradient centrifugation and then inoculated to Catjang. RNA from two small lesions were isolated and enhanced by two successive passages through *Chenopodium quinoa* before being inoculated to Catjang and Blackeye.

TABLE 5. Expression of large-lesion isolates of cucumber mosaic virus (CMV) on *Vigna unguiculata* ssp. *unguiculata* 'Blackeye' and *V. unguiculata* ssp. *cylindrica* 'Catjang'

CMV strain	Large lesion isolate[w]	Lesion types[x] on inoculated primary leaves of:	
		Catjang	Blackeye
C	#5 (5SLT-C[y]	L	M, S
C	#6 (6SLT-C)	L	L, M, S
C	#7 (4SLT-V)[z]	L	L, M, S
C	#5 (4SLT-V)	L	L, M, S
C	#6 (4SLT-V)	L	L, M, S
C	#9 (4STL-V)	L	L, M, S
L-2	#1 (4SLT-C)	L, Vs	L, M, S
WL	#3 (4SLT-C)	L, Gi	Lcn, S

[w]Source of inoculum was *Chenopodium quinoa* for all the single-lesion isolates.
[x]Gi = Green island in the center of lesions, L = large necrotic lesions, Lcn = large chlorotic lesions with interspersed necrosis, M = medium necrotic lesions, S = small necrotic lesions, Vs = lesions spreading through leaf veins. An average of 6–15 and 10–30 lesions per leaf were observed on Catjang and Blackeye, respectively.
[y]SLT-C = Single-lesion transfer of inoculum serially through *C. quinoa*; 5 = transferred five times, etc.
[z]SLT-V = Single-lesion transfers of inoculum serially through *V. unguiculata* ssp. *cylindrica*; 4 = transferred four times, etc.

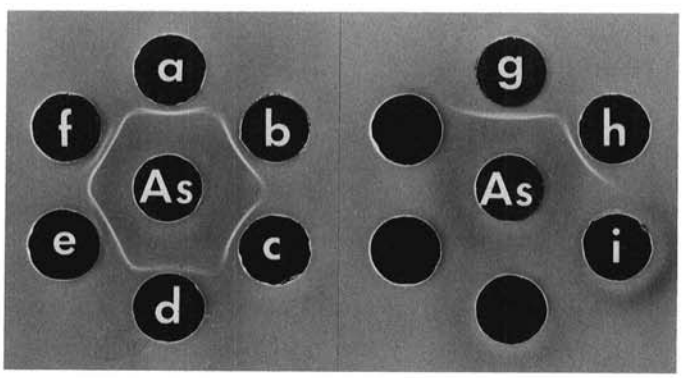

Fig. 2. Serological relationships among various strains of cucumber mosaic virus (CMV) and their large-lesion isolates. Center wells of the two immunodiffusion agar plates contained antiserum to CMV-C. Outer wells contained crude sap from infected *Cucumis sativus* 'Marketer' tissue: well a, CMV-C; well b, CMV-CL #5; well c, CMV-N; well d, CMV-NL #1; well e, CMV-WL; well f, CMV-WLL #1; well g, CMV-L-2; well h, CMV-L-2L #1; and well i, healthy sap.

bands of a CMV strain and its large-lesion isolate. Except for CMV-N and its large-lesion isolate, all other strains and large-lesion isolates carried RNA 5 (Fig. 3). However, RNA 5 in the large-lesion isolate of CMV-C (CMV-CL #5) was barely detectable.

Peptide mapping. Limited proteolytic digests of coat proteins from CMV-C, CMV-N, CMV-WL, and their large-lesion isolates using chymotrypsin, V8 protease, and papain were compared (Fig. 4). The peptide profiles of strains C and N and the respective large-lesion isolates CL #5 and NL #1 were indistinguishable from each other but strikingly different from strain WL and its large lesion isolate WLL #1. However, the peptide profiles of WL and WLL #1 were indistinguishable. These corresponded with the two subgroups as reported by Edwards and Gonsalves (5).

Role of RNA 5. The role of CMV-associated RNA 5 (CARNA 5) on the initial appearance of large lesions and their subsequent expression was assessed. In one experiment, inoculum was made from the purified RNA 1 + 2 and RNA 3 of CMV-C after two cycles of sucrose density gradient centrifugation and inoculated to both *Vigna* species. Large lesions appeared on Catjang after this inoculation (Table 4). In another experiment, RNA 1 + 2 + 3 of large lesion isolate #5 of CMV-C (CMV-CL #5) was purified through two cycles of gel electrophoresis to remove RNA 5 and

inoculated to *C. quinoa*. Ten local lesions were then recovered from inoculated leaves of *C. quinoa* and further inoculated to Catjang. All ten isolates produced only large lesions. Although CMV-N did not have RNA 5 (Fig. 3C; [30]), it still produced large lesions on Catjang. Finally, obvious differences between the relative amounts of RNA 5 of the parent CMV strains and of their large-lesion isolates were not observed (Fig. 3).

DISCUSSION

With the use of serology and cross protection tests, we have shown that the large-lesion isolates obtainable after inoculation of CMV to Catjang are isolates of CMV and not other contaminating virus(es). Furthermore, various facts indicate that the large-lesion isolates are mutants of several CMV strains and not any contaminating strains of CMV. First, large lesions appear on Catjang upon inoculation with various CMV strains and isolates selected through four or more single-lesion transfers in *C. quinoa*. Local lesions frequently have been regarded as a source of biologically pure strains of virus, with certain exceptions (20). Second, the pathogenic marker properties as well as the coat protein peptide maps of CMV strains and their large-lesion isolates were similar. Host reactions have similarly been used by MacNeill and Boxall (18) and De Jager and Wesseling (3) as markers to identify spontaneous mutations in plant viruses. Third, the observed mutation rate to large lesions (0.53%) is within the range of mutation rates of RNA viruses of plants, animals, and bacteria (12). RNA viruses in general show high mutation frequency, which is considered to be significant in their rapid evolution (12).

The fact that such isolates derived from various strains of CMV indicates the general occurrence of this phenomenon. Such changes in the pathogenic expression of virus after one or more passages through specific hosts have been reported for several plant viruses and termed "host passage" effects (31,32). Initial experiments showed that large-lesion isolates could be selected in Catjang and occasionally in Blackeye. However, the frequency of appearance of large lesions on the latter host was very low and inconsistent (Tables 1, 3, and 4) as compared to Catjang. Interference apparently is not involved. However, it was observed that not all of the large-lesion isolates selected from Catjang could produce uniformly large lesions on Blackeye. Due to such highly heterogeneous reactions of Blackeye with large-lesion isolates, the latter might go undetected on this host. This could explain the less frequent appearance of large lesions on Blackeye.

Localization of CMV in cowpea is determined by the single dominant host-gene C (4). It was observed that some large-lesion isolates of all four CMV strains would spread through leaf veins

TABLE 6. Effect of four serial passages of mass inocula through various hosts on the stability of some large-lesion isolates of cucumber mosaic virus strain C

Large-lesion isolate	Passage host	Lesion types (average lesions/leaf)[y]					
		Catjang			Blackeye		
		Large	Medium	Small	Large	Medium	Small
CL#5[z]	*Chenopodium quinoa*	30.9	8.0	0.5	0	0	52
	Cucurbita pepo 'President'	4.3	0.0	1.3	0	0	39
	Cucumis sativus 'Marketer'	0.03	0.0	1.9	0	0	47
	Nicotiana tabacum H-423	0.0	0.0	18.5	0	0	47
CL#7[z]	*C. quinoa*	0.5	0.4	2.7	0	0	40
	C. pepo 'President'	0.0	0.0	5.0	0	0	35
	C. sativus 'Marketer'	0.0	0.0	3.9	0	0	37
	N. tabacum 'H-423'	0.0	0.0	4.5	0	0	44

[y] Number based on average of 10 inoculated primary leaves: Large = 0.4–0.6 cm, medium = 0.2–0.3 cm, and small = <0.2 cm in diameter.
[z] Purified through five single-lesion passages on *C. quinoa*.

TABLE 7. Symptoms induced by cucumber mosaic virus (CMV) strains and large-lesion isolates on some diagnostic hosts

CMV strains and isolates	Host reactions[v]				
	Cucurbita pepo 'President'	*Lycopersicum esculentum* line 80-18-1	*Lactuca saligna*	*Nicotiana tabacum* 'H-423'	*Cucumis sativus* 'Marketer'
C	M	M, Fl	Cp, Ns	M	M
CL#5[w]	M	M, Fl	Cp, Ns	M	M
WL	M	Lc	M	M	M
WLL#1	M	Lc	M	M	M
N	Nls (cotyledon)	...[y]	Cp	Nls	Nls
	Ns[x]		Ns	Ns[z]	M, Ssn
NL#1	Nls (cotyledon)	...	Cp	Cnl	Nls
	Ns[x]		Ns	Ns[z]	M, Ssn
NL#4	Nls (cotyledon)	...	Cp	Nls	Nls
	Ns[x]		Ns	Ns[z]	M, Ssn
L-2	M	Lc	M	M	M
L-2L#1	M	Lc	M	M	M

[v] Cnl = chlorotic and necrotic lesions, Cp = chlorotic spots, Fl = fern leaf, Lc = leaf curl, M = mosaic, Nls = necrotic local lesions, Ns = nonsystemic, Ssn = severe stem necrosis.
[w] Use of L after the strain designation means large-lesion isolate; thus, CL#5 is the large lesion isolate of CMV-C.
[x] Occasionally, mild mosaic and necrosis appeared on uninoculated leaves.
[y] Not tested.
[z] Rarely, systemic yellow spots appeared on uninoculated leaves.

and petioles of both *Vigna* species, causing severe stem necrosis and death of seedlings. Some large-lesion isolates produced typical large necrotic lesions on Catjang, whereas chlorotic and necrotic lesions were produced on the inoculated leaves and severe mosaic with mild necrosis on the trifoliolate leaves of Blackeye. These results suggest that there are separate mechanisms for necrotization and localization of CMV in cowpea, which may act either in parallel or independently. For example, if resistance of cowpea was due solely to necrotic reaction, we would not have expected systemic necrosis on uninoculated leaves. Appearance of CMV variants overcoming hypersensitive cowpea has also been reported by previous workers (19,31). However, to our knowledge, no thorough attempt was made to determine whether such host-adapted forms are preexisting contaminants or mutants selected by the hosts. From experiments with four different CMV strains, we suggest that this particular host-adapted variation was due to mutation rather than contamination in CMV.

The large-lesion isolates are stable during single-lesion transfer through *C. quinoa*. However, with one exception, mass transfer of such isolates through *C. quinoa* resulted in reappearance of small lesions upon inoculation to Catjang. Although contamination with the parent strain could not be completely excluded, the most likely explanation is that mass transfer (high multiplicity) of large-lesion isolates allows gradual accumulation of revertants; whereas, serial single-lesion passage by high inoculum dilution (low multiplicity) does not. The mutation rate is high in both modes of passage, but the rate of accumulation of genome mutations is quite different (12). Mass transfer of large-lesion inocula through cucumber,

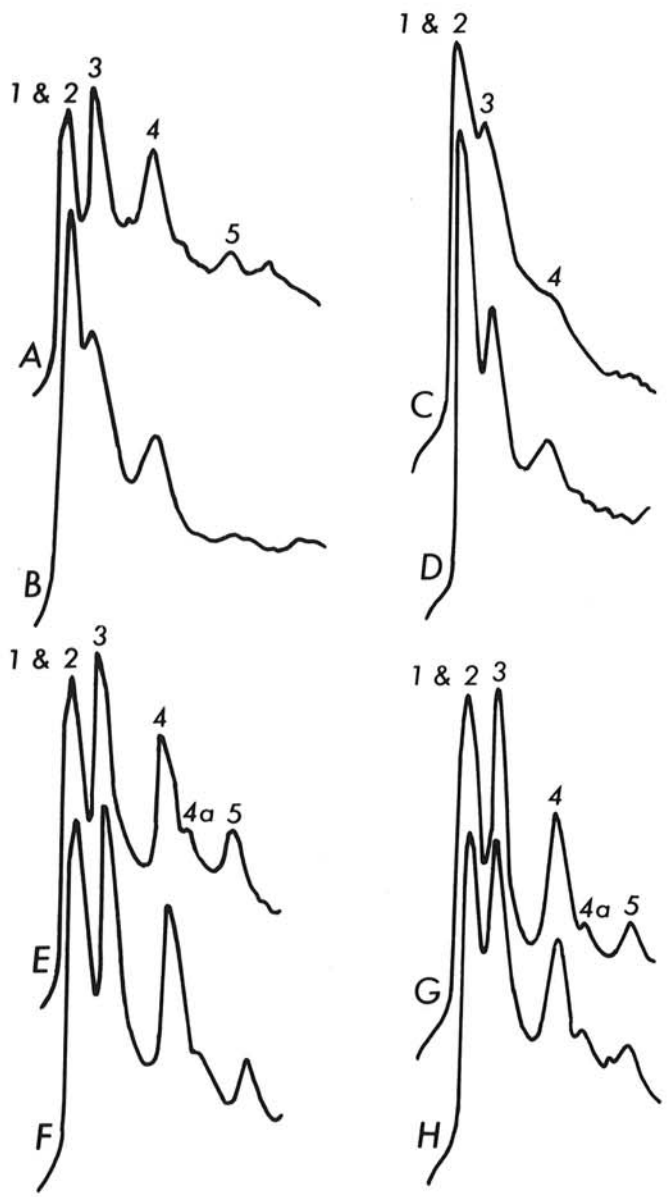

Fig. 3. Ultraviolet absorbance (260 nm) of RNA preparations from cucumber mosaic virus (CMV) strains (A, C, E, and G) and their respective large-lesion isolates (B, D, F, and H) electrophoresed in 2.4% polyacrylamide gel. Curve A, CMV-C; curve B, CMV-CL #5; curve C, CMV-N; curve D, CMV-NL #1; curve E, CMV-WL; curve F, CMV-WLL #3; curve G, CMV-L-2; and curve H, CMV-L-2L #7.

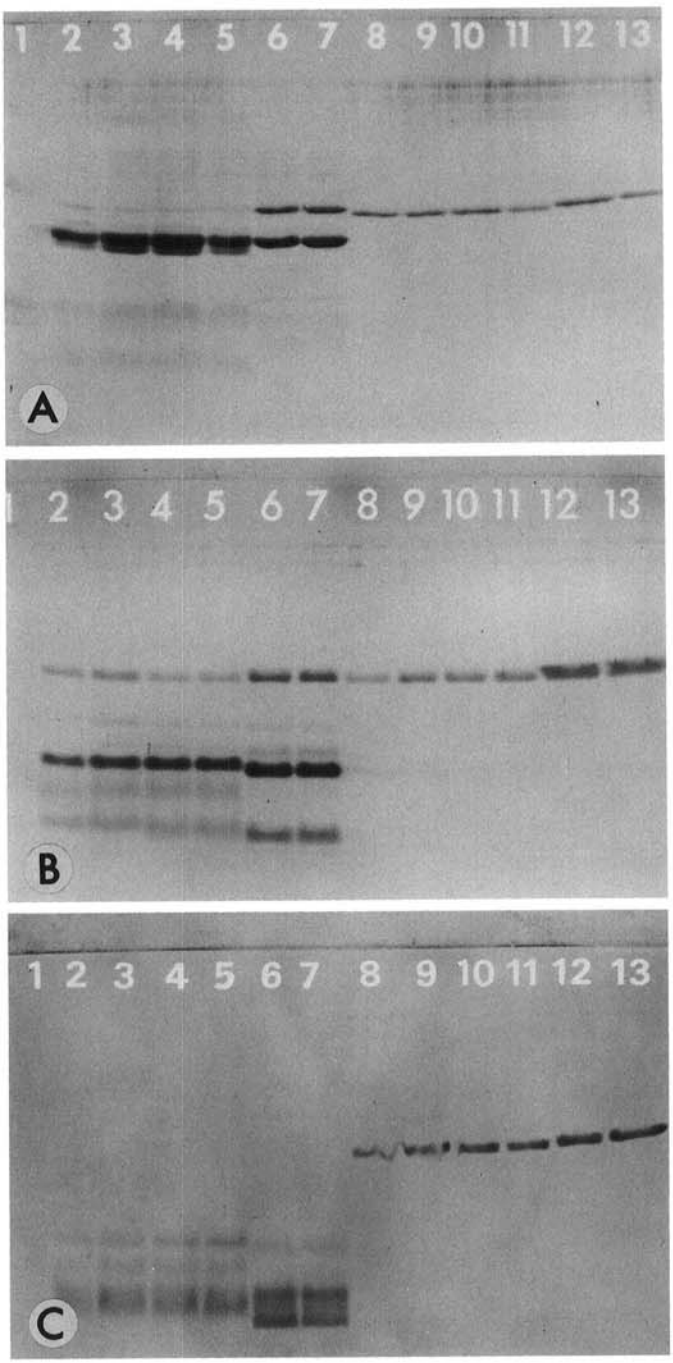

Fig. 4. Comparison of coat proteins among various cucumber mosaic virus (CMV) strains and their large-lesion isolates following partial proteolysis with several proteases and polyacrylamide gel electrophoresis: **A,** V8 protease digest; **B,** chymotrypsin digest; and **C,** papain digest. Well 1 in each figure was loaded with 0.1 μg of protease. Wells 2–7 were loaded with 0.5 μg each of protease-digested coat protein from CMV-C, CMV-CL #5, CMV-N, CMV-NL #1, CMV-WL, and CMV-WLL #1, respectively. Wells 8–13 were loaded with 0.1 μg each of undigested coat protein from CMV-C, CMV-CL #5, CMV-N, CMV-NL #1, CMV-WL, and CMV-WLL #1, respectively.

squash, and tobacco produced similar results. Instability of CMV variants through host passages has also been reported by previous workers (19,31).

The satellite RNAs, or RNA 5s, of CMV have been found either to induce new disease symptoms (9,13,26) or to suppress the severity of CMV-induced symptoms (13,29) in various hosts. Experiments conducted in our investigations have indicated that the presence or absence of RNA 5 seemed to have no effect either on the appearance of large-lesion isolates or on the size of the large lesions. This suggests that the large-lesion phenotype is not affected by RNA 5 and is due to mutation at some genomic site(s) of the virus. Since the genome of CMV is divided into three RNAs (22), genetic studies conducted by using pseudorecombination analysis would be useful in assigning the mutation to a particular RNA.

The selection of pathogenic mutants of CMV by specific hosts (e.g., Catjang) only partly explains the highly variable nature of CMV. Other than the presence of satellite RNAs, the high mutation rate and specific host selection, the potential mechanism which might add to CMV variability is genetic reassortment. However, to date, there is no report of in vivo genetic reassortment in CMV. For this to happen, virus strains should coinfect the same plant and overcome the barriers of cross protection and incompatibility (8).

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