Genetic Analysis of Cucumber Mosaic, Peanut Stunt and Chrysanthemum Mild Mottle Viruses

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花田 薫*・栃原比呂志*：キュウリモザイクウイルス，Peanut stunt virus およびキク微斑ウイルスの遺伝的解析

Abstract

Four strains of cucumber mosaic virus (CMV-Y, CMV-L, CMV-E and CMV-P), Peanut stunt virus (PSV) and chrysanthemum mild mottle virus (CMMV) had a tripartite genome. Molecular weights of RNA's of CMV, PSV, and CMMV in aqueous gels were determined by polyacrylamide gel electrophoresis under non-denaturing conditions. Analysis utilizing pseudo-recombinants constructed by RNA3 exchange between CMV strains or between CMV-L and CMMV revealed that serotypes were determined by RNA3, while symptoms on cucumber and cowpea plants depended on RNA1+2. Furthermore when RNA 1 and RNA 2 were exchanged between CMV strains, symptoms on cowpea depended on RNA2.

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Introduction

Viruses belonging to cucumovirus group are small spherical plant viruses with ribonucleic acid (RNA) as genetic material¹⁾. Members of this group so far reported are cucumber mosaic virus (CMV), tomato aspermy virus (TAV), peanut stunt virus (PSV) and chrysanthemum mild mottle virus (CMMV). CMV is the type member of the cucumovirus group and is very common in Japan. Many different strains of CMV have been described on the basis of host range or pathogenicity on plant^{8,17)}, whereas only two serotypes of CMV were reported¹⁵⁾. PSV has been isolated from bean¹⁸⁾, and CMMV was isolated from chrysanthemum¹⁴⁾. RNA isolated from CMV has been separated by polyacrylamide gel electrophoresis into four or five major RNA species (RNA's)¹¹⁾. RNA's are named RNA1-4 or 1-5 from the largest to the smallest. Since the three largest RNA's are necessary for the infection to occur^{11,12)}, CMV has been considered to have a tripartite genome. It is possible for viruses with a multipartite genome to construct new viruses by exchanging RNA's between two related viruses. These newly constructed viruses are called pseudo-recombinants (PRs)¹⁾. By comparing genetic markers of PRs with two parent viruses, it is possible to decide the location of genetic determinants. Many studies have been carried out to determine the location of genetic markers on RNA's with the aid of PRs constructed by exchange of RNA's between viruses with a tripartite genome¹⁹⁾.

We report here the results of analysis of viral RNA's utilizing polyacrylamide gel electrophoresis for several viruses of cucumovirus group isolated in Japan. We will also describe determinants of serotypes and symptoms on RNA3, RNA1+2 and RNA2.

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Materials and Methods

Viruses and purification. The following viruses were used: Cucumber mosaic virus (CMV); (1) CMV-Y, an isolate from tobacco supplied by Dr. K. Kiriya¹⁶⁾; (2) CMV-E, a culture from pea sent by Dr. T. Inouye⁷⁾; (3) CMV-L, another culture from pea supplied by Dr. M. Iwaki (unpublished); (4) CMV-P, an isolate from Japanese butterbur¹⁵⁾. Peanut stunt virus (PSV), an isolate from bean supplied by Dr. T. Tsuchizaki¹⁸⁾. Chrysanthemum mild mottle virus (CMMV), a culture obtained from chrysanthemum¹⁴⁾. All CMV strains, PSV and CMMV were purified as previously described¹⁴⁾. Prior to use, single lesion isolation was done for each virus using *Chenopodium quinoa* Willd. as a local lesion host. Purified virus preparations were used immediately or stored at -70°C until use.

Serological tests. Antiserum to PSV was supplied by Dr. T. Tsuchizaki¹⁸⁾. Antisera to CMV-Y and CMMV were prepared beforehand¹⁴⁾. Antiserum to CMV-P was prepared according to methods similar to those for CMV-Y antiserum. The titer of CMV-P antiserum was 1024 by microprecipitin tests. Gel diffusion tests were performed as described previously¹⁴⁾, using 0.7 % or 1 % agarose instead of 1 % agar. Purified virus or partially purified virus (without sucrose density gradient centrifugation) was used as antigen.

Polyacrylamide gel electrophoresis. 2.5 % polyacrylamide gel electrophoresis was done essentially as described by Murant *et al.*¹⁰⁾ The electrophoretic patterns of RNA's of CMV-Y separated from disrupted virus by sodium dodecyl sulfate (SDS) were indistinguishable from patterns of phenol extracted RNA's. Therefore, disrupted virus by addition of 1 % SDS to purified virus solution was directly loaded on the top of gels after incubation at 40°C for one minute to ensure the disruption. All electrophoreses were done at $5-10^{\circ}\text{C}$. After electrophoresis the gels were scanned with a Gilford spectrophotometer (2400-S) equipped with a linear transporter or Shimadzu double-beam chromatoscanner (CS910) at 260 nm. Gels were scanned at 280 nm also to check the ratio of absorption at 260 and 280 nm. RNA's were recovered by phenol extraction of excised bands located by staining with Toluidine Blue O¹¹⁾. When RNA 1 and RNA 2 were excised together, the product was called RNA1+2. Sterile glassware and solution were used for all RNA preparations.

Molecular weight determination. The molecular weights of RNA's obtained from SDS disrupted viruses were determined by coelectrophoresis with marker *Escherichia coli* ribosomal RNA (23 S and 16 S) in aqueous gels¹¹⁾.

Infectivity assay and single lesion isolation. Infectivity assay and single lesion isolation were done according to methods described by Harrison *et al.*⁵⁾ using *C. quinoa* plants. The buffer used for dilution of RNA's contained 0.1M NaCl, 20mM Tris (hydroxymethyl) aminomethan-HCl (pH 8.5) and 5 mM Ethylenediaminetetra-acetic acid¹¹⁾. Inoculated plants were placed in a glasshouse maintained at $20-32^{\circ}\text{C}$ (usually $22-26^{\circ}\text{C}$) and local lesions were counted 3-5 days after inoculation. For isolation of pseudo-recombinants, local lesions induced by inocula containing RNA's from different viruses (or strains) were excised and ground separately, then each extract was inoculated to a separate tobacco plant (Ky 57 or Xanthi nc). Each single lesion isolate was subcultured on a new tobacco plant, and the serotype of each single lesion isolate was determined by gel diffusion tests. Pseudo-recombinants and parent viruses were inoculated to cowpea (cv. Kurodane 16) and cucumber (cv. Ochiai) plants, and maintained in the glasshouse until symptoms developed.

Results

Serological relationship among CMV strains, PSV and CMMV

Gel diffusion tests in agarose gels clearly demonstrated a serological relationship among

Fig. 1. a-d Immunodiffusion tests with CMV strains, PSV and CMMV. Each outer well contains 0.1mg purified viruses of (L) CMV-L, (Y) CMV-Y, (P) CMV-P, (E) CMV-E, (S) PSV, and (M) CMMV. Central wells contain antisera to (Y) CMV-Y, (P) CMV-P, (S) PSV, and (M) CMMV.

e-h Immunodiffusion tests with pseudo-recombinants (PRs) constructed by RNA3 exchange between CMV-Y and CMV-P (e and f) or between CMV-L and CMMV (g and h). Outer wells contain purified viruses of (Y) parental CMV-Y, (P) CMV-P, (L) CMV-L, and (M) CMMV; the others were PRs and controls, the first letter represents the parent contributing RNA1+2 and the second letter the parent supplying RNA3. Central wells contain antisera to (Y) CMV-Y, (P) CMV-P, and (M) CMMV.

CMVs, PSV and CMMV (Fig. 1, a-d). All of four CMV strains reacted with antisera to CMV-Y and to CMV-P, while none of those strains reacted with anti-PSV and anti-CMMV sera. Purified PSV and CMMV reacted with anti-PSV and anti-CMMV sera, although PSV and CMMV did not reveal clear reactions with antisera to CMV-Y and to CMV-P. CMV-P and other three CMV strains (CMV-Y, CMV-E and CMV-L) were distinguished by spur formation. PSV and CMMV formed a spur also. Therefore, we designated the serotype of CMV-Y (also CMV-E and CMV-L) as Y type, CMV-P as P type, PSV as S type and CMMV as M type.

Electrophoretic patterns of CMVs, PSV and CMMV

Electrophoretic patterns of four CMV strains, PSV and CMMV are shown in Fig. 2. All of these viruses contained four major RNA's. RNA1 and RNA2 of CMV-P and PSV were well separated, while RNA1 and RNA2 of CMMV were poorly separated. Separation of RNA1 and RNA2 of the other CMVs seemed intermediate. The ratio of RNA's of each virus differed from preparation to preparation. Molecular weights of RNA 1-4

Fig. 2. Polyacrylamide gel electrophoretic patterns of RNA's of CMV strains, PSV and CMMV. Purified viruses (30 $\mu\text{g/gel}$) were subjected to electrophoresis on 2.5% acrylamide gels containing 0.1% SDS. Migration is from left to right. Molecular weights of viral RNA's were estimated by relative mobility in gels to *E. coli* rRNA, and results were shown in Table 1. (A) CMV-Y, (B) CMV-P, (C) CMV-L, (D) and (H) *E. coli* rRNA, (E) CMV-E, (F) PSV, and (G) CMMV.

Fig. 3. Detection of RNA5 of CMV strains, PSV and CMMV by polyacrylamide gel electrophoresis. Purified viruses (70 $\mu\text{g/gel}$) were subjected to electrophoresis on 2.5% acrylamide gels containing 0.1% SDS. Migration is from left to right. (A) CMV-Y, (B) CMV-P, (C) CMV-E, (D) CMV-L, (E) PSV, and (F) CMMV.

Table 1. Molecular weights of RNA's of CMVs, PSV and CMMV

Virus	Molecular weights ^{a)} ($\times 10^{-6}$ daltons)			
	RNA1	RNA2	RNA3	RNA4
CMV-Y	1.20 $\pm$ 0.02	1.09 $\pm$ 0.02	0.76 $\pm$ 0.02	0.34 $\pm$ 0.01
CMV-L	1.17 $\pm$ 0.01	1.06 $\pm$ 0.02	0.75 $\pm$ 0.02	0.35 $\pm$ 0.02
CMV-P	1.22 $\pm$ 0.01	1.06 $\pm$ 0.02	0.72 $\pm$ 0.02	0.33 $\pm$ 0.01
PSV	1.19 $\pm$ 0.02	1.02 $\pm$ 0.02	0.73 $\pm$ 0.01	0.35 $\pm$ 0.01
CMMV	1.16 $\pm$ 0.02	1.08 $\pm$ 0.02	0.79 $\pm$ 0.01	0.37 $\pm$ 0.01

a) The values are means of 6-10 determinations.

Table 2. Infectivity of RNA's of CMVs, PSV and CMMV

Experiment Number	Virus	Infectivity of RNA's ^{a)}			
		1+2	3	1+2+3	1+2+3+4
1	CMV-Y	30	1	310	NT ^{b)}
	CMV-P	42	0	527	NT
2	CMV-P	14	0	311	312
	CMV-L	13	0	244	166
3	CMV-P	1	2	131	NT
	PSV	20	10	515	NT
4	PSV	31	0 ^{c)}	373	337
5	CMV-E	5	0 ^{d)}	143	NT
6	CMMV	500	0	725	NT

a) Total numbers of local lesions in four leaves (Expt. 1, 2 and 6), six leaves (Expt. 3) or eight half-leaves (Expt. 4 and 5) of *C. quinoa*.

b) Not tested.

c) Inoculum contained RNA4 also.

d) Inoculum contained RNA2 also.

Table 3. Infectivity of RNA's of CMV-L and CMMV in homologous or in heterogeneous combinations

Donor of RNA's		Infectivity ^{a)}	
RNA1+2	RNA3	Expt. 1	Expt. 2
CMV-L	—	103	50
—	CMV-L	1	1
CMV-L	CMV-L	583	425
CMV-L	CMMV	596	500
CMMV	—	415	643
—	CMMV	0	0
CMMV	CMMV	410	596
CMMV	CMV-L	500	576

a) Total numbers of local lesions in four leaves of *C. quinoa*.

of three CMV strains, PSV and CMMV were determined by coelectrophoresis of viral RNA's with *E. coli* rRNA (as shown in Fig. 2). Molecular weights of each RNA's did not differ strikingly among these viruses (Table 1). When electrophoresis were done for a shorter time, all tested CMV-Y purified preparations revealed RNA5 (Fig. 3). CMV-L, PSV and CMMV did not contain detectable amount of RNA5. Some purified preparations of CMV-E and CMV-P revealed RNA5, while the others did not. RNA5 and viral protein were easily distinguished by comparison of absorbance of at 260 and 280 nm. Occasionally RNA3 of CMV-P, CMV-L and PSV were further separated into two peaks.

Tripartite genome of CMVs, PSV and CMMV

The role played by RNA's in infectivity was tested by inoculating RNA's singly or in combinations to *C. quinoa* plants (Table 2 and Table 3). Inoculum containing RNA1+2 alone obtained from all viruses except CMMV was infectious to some extent, while RNA3 alone was almost non-infectious in all trials. Inoculum containing RNA1+2 and RNA3 showed the highest infectivity in all instances. Low infectivity of RNA1+2 alone was considered to be due to contamination of RNA3, in the cases of CMVs and PSV. Further addition of RNA4 to inoculum containing RNA1+2 and RNA3 did not affect the infectivity. For CMMV, inoculum containing RNA1+2 alone was highly infectious and adding RNA3 of CMMV or of CMV-L did not enhance the infectivity of

RNA1+2 of CMMV remarkably. However, addition of RNA3 of CMV increased the infectivity of RNA1+2 of CMV-L very much. Occasionally RNA3 of PSV and of CMV-P showed low infectivity, although the reason for this is not known.

Exchange of RNA3 between CMV strains

RNA1+2 preparations from CMV-Y or CMV-P were mixed together with RNA3 from CMV-P or CMV-Y, respectively, and they were subsequently inoculated to *C. quinoa* plants. For the control experiments, homologous mixtures of RNA1+2 and RNA3 from either strain were inoculated separately. After single lesion isolation, the serotype of each single lesion isolate (SLI) was determined by gel diffusion tests using partially purified virus preparation from each SLI as antigen. As shown in Table 4, almost all SLIs had the serotype of the strain supplying RNA3 to the original inoculum. A few exceptional SLIs with the serotype of RNA1+2 donor probably originated from contamination of RNA3 in the original RNA1+2 preparations. Similar results were obtained by RNA3 exchange trials between CMV-L and CMV-P (Table 4). Hence, serotypes of CMVs were determined by RNA3. SLIs with the serotype of RNA3 donor were considered as pseudo-recombinants (PRs). Gel diffusion tests using purified virus preparations of PRs constructed by RNA3 exchange between CMV-Y and CMV-P are shown in Fig. 1 (e and f). Symptoms on cucumber and cowpea induced by PRs were compared with those by parent

Table 4. Serotypes of single lesion isolates produced by RNA3 exchange between CMV strains or between CMV and CMMV

Donor of RNA's RNA1+2 RNA3		Numbers of isolates with the serotype of	
Between CMV-Y(Y) and CMV-P(P)		Y type	P type
Y	P	1	5
P	Y	6	2
Y	Y	7	0
P	P	0	6
Between CMV-(L) and CMV-P(P)		Y type	P type
L	P	1	6
P	L	4	0
L	L	4	0
P	P	0	3
Between CMV-L(L) and CMMV(M)		Y type	M type
L	M	4	4
M	L	3	3
L	L	3	0
M	M	0	4

Table 5. Symptoms produced by pseudo-recombinants constructed by RNA3 exchange between CMV strains or between CMV and CMMV

RNA's in inoculum RNA1+2 RNA3		Symptoms ^{a)} on Cucumber Cowpea	
Between CMV-Y(Y) and CMV-P(P)			
Y	P	ChS, ^{b)} YM	NL, —
P	Y	—, —	NL, —
Y	Y	ChS, YM	NL, —
P	P	—, —	NL, —
Between CMV-L(L) and CMV-P(P)			
L	P	—, YM	ChS, M
P	L	—, —	NL, —
L	L	—, YM	ChS, M
P	P	—, —	NL, —
Between CMV-L(L) and CMMV(M)			
L	M	—, YM	ChS, M
M	L	—, —	NL, —
L	L	—, YM	ChS, M
M	M	—, —	NL, —

a) Symptoms produced on plants: ChS = chlorotic spots, YM = yellow mottling, M = mosaic, NL = necrotic lesion, and — = no symptoms appeared.

b) Symptoms on inoculated leaves.

c) Systemic symptoms.

Table 6. Regeneration of parent stains by RNA3 exchange between two types of pseudo-recombinants

Donor of RNA's		Number of isolates with the serotype of		Symptoms ^{a)} on cucumber
RNA1+2	RNA3	Y type	P type	
YP ^{b)}	PY	3	0	ChS, YM
PY	YP	0	2	—, —
YP	YP	0	2	ChS, YM
PY	PY	2	0	—, —

a) See Table 5.

b) One of pseudo-recombinants constructed by RNA3 exchange between CMV-Y and CMV-P. The first and the second letter represents the origin of RNA1+2 and RNA3, respectively.

Table 7. Symptoms on cowpea caused by single leion isolates produced by exchanging RNA1 and RNA2 between CMV strains

Donor of RNA's			Total numbers of isolates tested	Number of isolates which caused symptoms of
RNA1	RNA2	RNA3		
Between CMV-Y(Y) and CMV-L(L)				
L	Y	L	12	All CMV-Y
Y	L	Y	12	All CMV-L
Y	L	L	8	All CMV-L
L	Y	Y	6	All CMV-Y
L	L	L	8	All CMV-L
Y	Y	Y	8	All CMV-Y
Between CMV-Y(Y) and CMV-E(E)				
E	Y	E	9	All CMV-Y
Y	E	Y	5	4 CMV-E, 1 CMV-Y
Y	E	E	7	All CMV-E
E	Y	Y	7	All CMV-Y
E	E	E	6	All CMV-E
Y	Y	Y	6	All CMV-Y

and gel diffusion tests were shown in Fig. 1 (g and h). When symptoms were compared, it was found that symptoms on cucumber and cowpea were determined by RNA 1+2 (Table 5), which is in good agreement with results obtained above.

Exchange of RNA1 and RNA2 between CMV strains

To test which one of RNA1 and RNA2 of CMV determines symptom, RNA1 and RNA2 were exchanged between CMV-Y and either CMV-E or CMV-L. These three strains were serologically indistinguishable from each other (Fig. 1, a-d) and caused similar

viruses, and it was found that symptoms on cucumber and cowpea were determined by RNA1+2 (Table 5). Back cross experiments were carried out by re-exchanging RNA3 between PRs (which had been constructed by RNA3 exchange between CMV-Y and CMV-P) in order to confirm PRs were made simply by re-assorting RNA's of parent viruses. As shown in Table 6, parent strains were regenerated in all instances as judged from serotype as a marker of RNA3 and the symptom on cucumber as a marker of RNA1+2, confirming that PRs were made by re-assorting RNA's of parents.

Exchange of RNA3 between CMV and CMMV

To see whether RNA1+2 determines symptom and RNA3 decides serotype also in the case of CMMV, preparations of RNA1+2 and RNA3 of CMV-L and CMMV were mixed in four combinations and were inoculated. The serotype of each SLI was tested by gel diffusion tests and it was found that half of the tested SLIs derived from heterogenous combinations of RNA's had the serotype of RNA3 donor (Table 4). SLIs with the serotype of the virus contributing RNA1+2 induced symptoms of RNA1+2 donor, therefore SLIs with the serotype of RNA3 donor were considered as PRs

symptoms on cucumber. On cowpea CMV-Y induced necrotic local lesions and did not infect systemically, while CMV-E and CMV-L caused chlorotic spots in inoculated leaves and infect systemically. The purity of RNA1, RNA2 and RNA3 preparations were checked prior to use (data not shown). RNA preparations used here were only slightly contaminated by each other except that RNA1 of CMV-L was contaminated by RNA2 to some extent. Symptoms on cowpea of each SLI obtained by exchange RNA1 or RNA2 were compared (Table 7). Almost all tested SLI caused symptoms of the strain contributing RNA2.

Exchange of RNA2 between CMV strains with different serotype was done similarly, using CMV-L and CMV-P. RNA1 of these two strains could be identified by coelectrophoresis in sister gels (Fig. 4). Although symptoms on cowpea of each SLI obtained by exchanging RNA2 were checked, in this case it was not clear whether RNA2 determined symptoms or not. Then, RNA1 of SLIs obtained was identified by electrophoresis, whereas RNA3 was identified by serological methods. Two SLIs causing different symptoms had the same RNA1 which comigrate with RNA1 of CMV-L (Fig. 4), and the serotype of both SLIs was found to be Y type. Hence, only RNA2 could differ between two SLIs which caused different symptoms on cowpea. Furthermore, RNA1 and RNA3 of two more SLIs obtained by inoculum containing RNA1 of CMV-L, RNA2 of CMV-P and RNA3 of CMV-L were identified similarly, then RNA1 and RNA3 of these two more SLIs were found to originate from CMV-L. These two more SLIs caused similar symptoms of CMV-P. Hence, in this case also it was concluded that RNA2 determined symptoms on cowpea, although we failed to obtain another type of PRs containing RNA1 of CMV-P, RNA2 of CMV-L and RNA3 of CMV-P so far.

Discussion

CMV-P with a serotype different from CMV-Y was first isolated in Japan from Japanese butterbur plant¹⁵⁾. It was reported that PSV found in Japan was serologically distantly related to CMMV¹⁸⁾. Gel diffusion tests shown in Fig. 2 demonstrate the serological

Fig. 4. Electrophoretic identification of RNA1 of single lesion isolates produced by RNA2 exchange between CMV-L and CMV-P. Purified viruses (30 μ g/gel) were subjected to electrophoresis. Migration is from left to right. (A) CMV-L; (B) CMV-P; (C) and (D) isolates produced by inoculum containing RNA1 of CMV-L+RNA2 of CMV-P+RNA3 of CMV-L. Isolate C induced symptoms of CMV-L on cowpea, while isolate D caused symptoms of CMV-P.

relationship among four CMV strains, PSV and CMMV. Viruses of cucumovirus isolated in Japan could be grouped serologically into two groups. CMVs (serotype of Y and P types) belong to one group, while PSV and CMMV (serotype of S and M types) belong to another group. Between these two groups no clear reactions could be detected by gel diffusion tests. Between viruses of the same group spur formation could be observed.

It was demonstrated that CMV has a tripartite genome¹¹⁾. CMV-Y and CMV common strain contained five or four major RNA's respectively, and the mixture of RNA1-3 had the highest infectivity¹²⁾. Although RNA of PSV was separated into four major RNA's effects on infectivity of each RNA have not been determined yet⁹⁾. Data shown in Fig. 2 and Fig. 3 demonstrate that four CMV strains, PSV and CMMV contained four or five major RNA's. Molecular weights (MWs) of RNA's of CMV-Y were reported to be 1.23, 1.13, 0.83 and 0.33 ($\times 10^6$ daltons) for RNA1-4, respectively, under denaturing conditions¹³⁾. MWs of RNA1-3 of CMV-Y in aqueous gels were slightly smaller than MWs in 8 M Urea, but MW of RNA4 was almost the same value (Table 1). MWs of RNA1-4 were slightly different among CMV strains, PSV and CMMV. No biological characters of the viruses (e. g. serotype or symptom) could be correlated with MWs of viral RNA's. The mixture of RNA1-3 showed the highest infectivity in the cases of CMVs and PSV (Table 2 and Table 3). In the case of CMMV, RNA1+2 was highly infectious itself and adding RNA3 did not enhance the infectivity of RNA1+2 too much. The finding that RNA3 of CMMV activated RNA1+2 of CMV-L indicated heavy contamination of RNA1+2 preparations of CMMV with its RNA3 under RNA's separation condition used. Hence, it was concluded that CMMV had a tripartite genome also.

It was shown that serological specificity of CMV and TAV was determined by RNA 3 and symptoms on cucumber and several *Nicotiana* spp. were decided by RNA1+2, comparing CMV, TAV and one of PRs constructed by RNA3 exchange between these two viruses⁹⁾. Our results confirmed these determinants on RNA's using four CMV strains and CMMV (Table 4 and Table 5). RNA3 determined serotypes and RNA1+2 decided symptoms on cucumber and cowpea, when two types of PRs were compared with parent strains. Both types of PRs were obtained between two different viruses (CMV and CMMV) by exchange of RNA3, and both types showed dependency of serotypes on RNA3 and symptoms on RNA1+2. When RNA1 and RNA2 or only RNA2 was exchanged between CMV strains, RNA2 determined symptoms on cowpea (Table 7 and Fig. 4). Our preliminary attempts to construct PRs between PSV and one of the other viruses have failed so far.

CMV-Y contained a considerable amount of RNA5 and this strain caused necrosis on tomato (cv. Fukuju Number 2 and Sekaiichi) at 28 C but not at 22 C (unpublished results). This RNA5 would be similar to CMV associated RNA5 reported in USA⁶⁾, but might not be identical. CMV-E, CMV-L, CMV-P, PSV and CMMV did not cause necrosis on tomato, although some of them contained RNA5. An isolate of CMV of Australian origin contained a large amount of RNA5, although it did not induce necrosis on tomato²⁾. Analysis of RNA5 of cucumovirus isolated in Japan is in progress.

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和 文 摘 要

キュウリモザイクウイルス, Peanut stunt virus およびキク微斑
ウイルスの遺伝的解析

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キュウリモザイクウイルス (CMV) の4系統, Peanut stunt virus (PSV) およびキク微斑ウイルスの核酸 (RNA) は, ポリアクリルアミドゲル電気泳動により4つまたは5つの成分 (分子量の大きい方から順に RNA1, RNA2, RNA3, RNA4, RNA5 とよぶ) に分けられ, 大きい方から3つの成分が感染に必須であった。CMVの3系統, PSVおよびCMMVのRNA1-4の分子量を決定した。CMVの系統間およびCMMVとの間でRNA3を交換してえられた Pseudo-recombinant の性質を検討したところ, 血清型はRNA3が, ササゲおよびキュウリの病徴はRNA1+2が支配していた。さらにCMVの系統間でRNA1とRNA2の交換を行い, ササゲの病徴はRNA2が決定していることを明らかにした。