Comparison of Cucumber Mosaic Virus Isolates by Double-Stranded RNA Analysis*

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Cucumber mosaic virus (CMV), the type member of the cucumovirus group, contains four positive sense single-stranded(ss) RNA species designated RNA1~4. CMV has a very broad host range and is one of the most widespread and economically important viruses. Numerous isolates (strains) of CMV have been reported on the basis of host range and symptoms¹⁾, but it is difficult to classify and identify genuine differences among them. Replicative form double-stranded RNAs (RF-dsRNAs), that are twice the size of viral genomic ssRNA have been isolated from plants infected with RNA viruses²⁾. Analytical results of the dsRNA species have been used for diagnosis as well as for virus classification³⁻⁶⁾. This report deals with the comparison of the CMV isolates by dsRNA analysis, demonstrating the effectiveness of the method in such identification, and suggests that the individual isolates may be distinguished by this means.

Six CMV isolates were isolated from naturally infected plants collected in Tochigi, Ibaraki, and Shizuoka prefectures and designated PU, TU, KU, IU, EU and SU, respectively, for this study (Table 1). All isolates were propagated in *Nicotiana tabacum* cv. Xanthi.

DsRNAs were extracted from plants infected by the six isolates as described by Dodds and Bar-Joseph (1983)³⁾. Frozen leaf tissue (5 g) which had been stored at -80 C was ground with a mortar and pestle to a fine powder and homogenized with 10 ml of 2×STE buffer (1×STE=0.1 M NaCl, 0.05 M Tris-HCl, 1 mM EDTA, pH 7.0) containing 1% SDS, 0.1 ml of 2-mercaptoethanol, 10 ml of 80% phenol containing 0.1% 8-hydroxyquinoline, 10 ml of chloroform and 0.4 ml of pentanol. After the mixture was centrifuged at 8,000×g for 10 min, ethanol was added to a final concentration of 15%. Following a second centrifugation, 1.2 g of CF-11 cellulose powder (Whatman) was added to the supernatant and the mixture was stirred for 15 min at 4 C. The powder was placed in a glass column and washed with 100 ml of 15% ethanol/85% STE buffer. The dsRNA was eluted with 20 ml of ethanol-free STE buffer. The eluate was then mixed with 1 g of CF-11 cellulose powder and 3.5 ml of ethanol and subjected to a second cycle of chromatography. The second column was washed with 100 ml of 15% ethanol/85% STE. Finally the dsRNA was eluted with 16 ml of STE buffer, made to 30 mM MgCl₂, incubated for 20 min at 30 C with 20 µg of DNase I and concentrated by ethanol precipitation. The dsRNA

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pellet was dried under vacuum and then suspended in 80 μ l of sterilized distilled water containing 10% sucrose and 0.001% bromophenol blue.

The dsRNAs were analyzed by electrophoresis in 5% polyacrylamide slab gels (135 $\times$ 120 $\times$ 1 mm) prepared with an acrylamide-bisacrylamide ratio of 30 : 1.5 (w/w) and Tris-Borate-EDTA (TBE) buffer. Each dsRNA sample of 1 to 5 μ l was electrophoresed. After electrophoresis at 10 mA for 10~12 hr, the dsRNA bands were visualized using a Daiichi silver staining kit (Daiichi-kagaku Co., Tokyo, Japan).

The CMV antiserum was produced against the cucumber isolate which was serologically identical to serotype Y⁷⁾ of CMV (unpublished data). When six CMV isolates were compared in agar gel diffusion test with the antiserum, a single continuous line produced by four isolates (TU, KU, IU, and SU) spurred over the line between EU and PU isolates (Fig. 1). Serological comparisons with CMV-Y and CMV-P showed that TU, KU, IU, and SU isolates were identified as serotype Y⁷⁾ and the others were serotype P⁷⁾ (data not shown; Table 2).

The effect of host species or cultivars on electrophoretic patterns of dsRNAs was examined. No variation was observed among profiles of dsRNAs isolated from CMV-KU infected plants of *N. tabacum* cv. Xanthi, cv. Xanthi nc, *N. glutinosa*, *Lycopersicon esculentum* cv. Fukuji and *Cucumis sativus* cv. Suyo (Fig. 2).

DsRNAs prepared from tobacco tissue infected with CMV isolates were co-electrophoresed. Differences in the electrophoretic mobility of dsRNA1~3 associated with each CMV isolate were observed (Figs. 3 and 4) and dsRNAs of CMV-IU and CMV-SU were found to be the same (Fig. 3K). No difference was detected in dsRNA4 mobility among all isolates. In the case of dsRNA3, the mobility was indistinct among isolates belonging to the serotype Y but it was apparently distinguishable from isolates of serotype P. The dsRNA3 of CMV-EU and CMV-

Table 1. Isolates of CMV used in this study

Isolate	Host	Symptoms	Location and date of isolation
PU	Tomato	stem and leaf necrosis	Kanuma, Tochigi Pref., Dec. 1982
TU	Tomato	stem necrosis	Mooka, Tochigi Pref., July 1983
KU	Squash	mosaic, fruit deformation	Oyama, Tochigi Pref., Aug. 1984
IU	Tomato	mosaic, fanleaf	Ishioka, Ibaraki Pref., Oct. 1984
EU	Eggplant	mild mosaic	Edosaki, Ibaraki Pref., July 1985
SU	Tomato	mosaic, fanleaf	Kikugawa, Shizuoka Pref., Oct. 1985

Fig. 1. Gel immunodiffusion test: central well (As) contained antiserum to CMV-Y, the rest contained CMV-TU (T), KU (K), IU (I), SU (S), EU (E) and PU (P).

Fig. 2. Effect of host on electrophoretic pattern of dsRNAs from plants infected with CMV-KU. *Nicotiana tabacum* cv. Xanthi (a), cv. Xanthi nc (b), *N. glutinosa* (c), *Lycopersicon esculentum* cv. Fukuju (d) and *Cucumis sativus* cv. Suyo (e).

7) Hanada, K. and Tochiara, H. (1980). Ann. Phytopath. Soc. Japan 46: 159-168.

Table 2. Serotype of CMV isolates and molecular weight estimates of double-stranded RNAs associated with the isolates

Isolate	Serotype	Molecular weight ($\times 10^6$)*		
		dsRNA1	dsRNA2	dsRNA3
TU	Y	2.40	2.25	1.56
KU	Y	2.55	2.25	1.56
IU	Y	2.40	2.27	1.56
SU	Y	2.40	2.27	1.56
EU	P	2.35	2.29	1.62
PU	P	2.35	2.31	1.62

* Molecular weight estimates determined from the mobilities of CMV isolate dsRNAs relative to rice dwarf virus genomic dsRNA⁸⁾.

Fig. 3. Electrophoresis of dsRNAs from *N. tabacum* cv. Xanthi infected with CMV isolates belonging to serotype Y. Rice dwarf virus genomic dsRNA as markers (a), CMV-TU (b), KU (c), IU (d), SU (e), TU+KU (f), TU+IU (g), TU+SU (h), TU+KU (i), KU+SU (j) and IU+SU (k).

Fig. 4. Electrophoresis of dsRNAs associated with CMV-EU, PU or TU. RDV dsRNA (a), CMV-TU (b), PU (l), EU (m), TU+PU (n), TU+EU (o) and PU+EU (p).

PU migrated more slowly than that of other isolates. Differences in dsRNA1 and dsRNA2 were detected, which were sufficient to distinguish the CMV isolates used in this study. The estimated molecular weights for dsRNAs were determined using rice dwarf virus dsRNA⁸⁾ as markers and were approximately double the value for CMV genomic ssRNAs^{7,9)} (Table 2). Additional minor dsRNA were also observed (Figs. 3 and 4). It will be necessary to determine whether the minor dsRNAs are virus specific or related to the host⁹⁾.

The observation that the mobility of dsRNA3 on electrophoresis was correlated with the serotype can be explained by the coat protein gene being on CMV genomic RNA3⁹⁾. The

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detection of three kinds of dsRNA1 and four kinds of dsRNA2 from six isolates suggests that a large number of CMV variations are present in nature.

Our results indicate that five CMV isolates can be distinguished on the basis of electrophoretic migration of RF-dsRNAs on polyacrylamide gels. The lack of difference between CMV-IU and SU in dsRNAs mobility, in symptoms of original host and serological characteristics suggests that both isolates are the same. Thus, dsRNA analysis has the potential to provide reliable evidence by which to categorize CMV isolates (strains) that have been confused until the present.

和 文 摘 要

王 蔚芹・夏秋知英・奥田誠一・寺中理明：複製型二本鎖 RNA による数種キュウリモザイクウイルス分離株の比較

キュウリモザイクウイルス (CMV) の分離株間における核酸ゲノムの差異を知るために、CMV 感染葉から複製型二本鎖 RNA を CF-11 セルローズにより抽出し、銀染色を用いたポリアクリルアミドゲル電気泳動による比較を行った。抽出に用いる植物の種類によって複製型二本鎖 RNA の電気泳動パターンは変化しなかった。用いた CMV の 6 分離株からは、電気泳動度の異なる 3 種の RNA1, 4 種の RNA2, 2 種の RNA3 が検出された。RNA3 の泳動度の差は血清型の差に一致していた。複製型二本鎖 RNA の電気泳動により CMV の系統判別が迅速容易に行えると考えられた。

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