# CMVサテライトRNAのCMV感染植物への伝染


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# Cucumber Mosaic Virus Satellite RNA Transmissible to Plants Infected with a Different Isolate of CMV

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**Key words:** cucumber mosaic virus, satellite RNA, transmission, cross-protection.

Cucumber mosaic virus (CMV)-associated RNA5 is a satellite RNA (s-RNA) which often causes effects on the symptoms induced by CMV[1]. It has been shown that s-RNA can be introduced into CMV isolates by mix inoculation with an isolate containing no s-RNA[2] or with genomic RNAs 1～3[3]. S-RNA might be transferred from one isolate to another and affect CMV symptoms in the field, though no experimental evidence has been obtained so far. In this paper, we report the transmission of s-RNA in a CMV isolate to plants which had been infected with another isolate containing no s-RNA. The possibility of protecting plants from s-RNA infection by applying another s-RNA was also examined.

***Virus isolates, plants, and vector.*** Three isolates of CMV, CMV-pepo[4], CMV-HT86TM, and CMV-ab were used. CMV-pepo expresses mosaic in tomato as well as in tobacco and contains no s-RNAs. CMV-HT86TM, isolated from tomato in 1986, causes severe necrosis in tomato and mild mosaic in tobacco. CMV-ab, isolated from *Abutilon pictum* in 1984, develops striking yellow mosaic in tobacco and mild mosaic in tomato. HT86TM and ab both contain one s-RNA species. All the isolates were propagated in tobacco and purified by Takanami's procedure[2].

Tomato (*Lycopersicon esculentum* cv. Ogata-fukuju), tobacco (*Nicotiana tabacum* cv. Xanthi), and *N. glutinosa* were used as host plants. First inoculation was done at the 5～6 leaf stage for tobacco or *N. glutinosa* and at the 4～5 leaf stage for tomato seedlings. Insect transmission was performed using *Myzus persicae* propagated on healthy tobacco seedlings. Experiments were conducted in a greenhouse at 20～25 C.

***Double-stranded (ds) RNAs analysis.*** DsRNA species from 5 g leaf tissue were extracted by SDS-phenol, purified through cellulose column[5], and visualized on 6% polyacrylamide slab gels.

***Transmission of s-RNA to CMV-infected plants.*** To investigate whether s-RNA in CMV-HT86TM or in CMV-ab is transmissible to plants already infected with CMV-pepo, the following experiments were conducted. The plants were mechanically inoculated on the two largest leaves with purified CMV-pepo at 100 μg/ml. Five days later, the plants were again treated with CMV-HT86TM, -ab, or 0.1 M phosphate buffer pH 7.0 on the same leaves by the following methods; 1) sap inoculation: mechanically inoculated with purified virus at 100 μg/ml, 2) aphid inoculation: inoculated with the virus from infected plants by means of aphids at five per plant in a nonpersistent manner (starved for 2 hr, acquired for 5 min, inoculated for 20 min), 3) contact inoculation with a razor blade: edge of the inoculated leaves was cut three times for the length of 1 cm with a razor blade dipped in leaf sap from the infected plants, and 4) contact

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inoculation by rubbing leaf surface: surface of the inoculated leaves was gently rubbed three times for the length of 5 cm with a detached leaf from the infected plants.

The results are presented in Table 1. Tomato plants inoculated with CMV-pepo developed a systemic mild mosaic. However, most of the tomato seedlings inoculated with CMV-pepo and then applied with CMV-HT86TM developed mosaic and severe necrosis in the shoot. Gel electrophoresis showed that the necrotic leaves as well as CMV-HT86TM-inoculated tomato plants contained dsRNAs 1～5, whereas plants infected with CMV-pepo contained only dsRNAs 1～4. Likewise, tobacco plants inoculated with CMV-pepo showed ordinary mosaic, however, tobacco seedlings inoculated with CMV-pepo followed by treatment with CMV-ab mostly developed mosaic and striking bright yellow mosaic. DsRNA 5 was detected from the yellow mosaic leaves and from tobacco inoculated with CMV-ab. Table 1 shows that s-RNAs were highly transmissible not only by sap and by aphid, but also by contact. Thus, it is possible that these pathogenicity-related s-RNAs are transferred from one CMV isolate to another in field conditions.

***Cross-protection of s-RNA by s-RNA.*** The possibility of protecting plants from s-RNA infection by applying another s-RNA was examined. Plants were mechanically inoculated on the two largest leaves with either of CMV-HT86TM or CMV-ab at 100 μg/ml and then inoculated on the same leaves with the other virus at 100 μg/ml five days after the first inoculation. Tomato plants inoculated with CMV-ab and treated with CMV-HT86TM did not show necrosis.

Table 1. Symptom development in plants infected with non-s-RNA-containing CMV and subjected to inoculation with s-RNA-containing CMV

| Host | Inoculum | | Method of inoculation | No. of plants tested | Symptoms appeared | | |
|---|---|---|---|---|---|---|---|
| | 1st | 2nd | | | N[a] | Y[a] | M[a] |
| Tomato | pepo[b] | HT86TM[b] | Sap | 6 | 4 | 0 | 2 |
| | pepo | HT86TM | Aphid | 6 | 5 | 0 | 1 |
| | pepo | HT86TM | Razor cont.[c] | 6 | 4 | 0 | 2 |
| | pepo | HT86TM | Razor cont. | 6 | 5 | 0 | 1 |
| | pepo | HT86TM | Leaf cont.[c] | 6 | 6 | 0 | 0 |
| | pepo | HT86TM | Leaf cont. | 6 | 6 | 0 | 0 |
| | pepo | Buffer[d] | Sap | 6 | 0 | 0 | 6 |
| | pepo | Buffer | Sap | 6 | 0 | 0 | 6 |
| Tobacco | pepo | ab[b] | Sap | 6 | 0 | 6 | 0 |
| | pepo | ab | Aphid | 6 | 0 | 4 | 2 |
| | pepo | ab | Razor cont. | 6 | 0 | 4 | 2 |
| | pepo | ab | Leaf cont. | 6 | 0 | 6 | 0 |
| | pepo | Buffer | Sap | 6 | 0 | 0 | 6 |
| *N.g.*[e] | pepo | ab | Sap | 6 | 0 | 6 | 0 |
| | pepo | ab | Aphid | 6 | 0 | 5 | 1 |
| | pepo | ab | Razor cont. | 6 | 0 | 3 | 3 |
| | pepo | ab | Leaf cont. | 6 | 0 | 4 | 2 |
| | pepo | Buffer | Sap | 6 | 0 | 0 | 6 |

a) N: necrosis, Y: yellow mosaic, M: mosaic only.
b) CMV isolates. Pepo contains no s-RNAs, HT86TM and ab contain s-RNA.
c) Razor cont.: Edge of the leaves were cut three times for a length of 1 cm by a razor blade dipped in leaf sap containing challenge virus.
Leaf cont.: Surface of the inoculated leaves was gently rubbed three times for a length of 5 cm by a detached leaf infected with challenge virus.
d) 0.1 M phosphate buffer pH 7.0.
e) *Nicotiana glutinosa.*

Table 2. Symptom development in plants applied with two CMV isolates containing s-RNA

| Host | Inoculum | | No. of plants tested | Symptoms appeared | | |
|---|---|---|---|---|---|---|
| | 1st | 2nd | | N [a)] | Y [a)] | M [a)] |
| Tomato | ab [b)] | HT86TM [b)] | 6 | 0 | 0 | 6 |
| | ab | HT86TM | 6 | 0 | 0 | 6 |
| | HT86TM | ab | 6 | 6 | 0 | 0 |
| | pepo | HT86TM | 6 | 5 | 0 | 1 |
| | Buffer [d)] | HT86TM | 6 | 6 | 0 | 0 |
| | ab | Buffer | 6 | 0 | 0 | 6 |
| Tobacco | HT86TM | ab | 6 | 0 | 0 | 6 |
| | HT86TM | ab | 6 | 0 | 0 | 6 |
| | ab | HT86TM | 6 | 0 | 6 | 0 |
| | pepo | ab | 6 | 0 | 4 | 2 |
| | Buffer | ab | 6 | 0 | 6 | 0 |
| | HT86TM | Buffer | 6 | 0 | 0 | 6 |

a, b, d) See legend in Table 1.

Similarly, tobacco plants inoculated with CMV-HT86TM and applied with CMV-ab did not develop yellow mosaic (Table 2). These facts suggest that the plants were protected from severer disease by applying the first s-RNA. DsRNA 5 was detected from the tissues in all the treatments, however, the two s-RNAs could not be distinguished by the mobility on the slab gels.

From these results, it is concluded that a pathogenicity-related s-RNA could be transmitted to plants that had been infected with different isolates containing no s-RNA. Since CMV is widely distributed in the field[6], these s-RNAs may rapidly spread and increase disease severity in crops. Careful consideration must be given to control such s-RNAs, because they are easily transmitted by contact and they are physically very stable[7]. Our results indicate the possibility to use attenuated s-RNA to control these pathogenicity-related s-RNAs.

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## 和 文 摘 要

大木 理・田中尚智・井上忠男：CMV サテライト RNA の CMV 感染植物への伝染

サテライト RNA がサテライト RNA をもたない CMV に感染している宿主に移行して病徴を変化させうるか否かについて，宿主に明瞭な病徴をひき起こすサテライト RNA を用いて実験したところ，汁液接種，アブラムシ接種，刃物による接触，葉面による接触の 4 とおりの方法で容易に移行が起こることが知られた。また，用いた二つのサテライト RNA の間では干渉が認められ，サテライト RNA に起因する被害の回避に弱毒化したサテライト RNA が利用できる可能性が示された。

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