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# A Strain of Cucumber Mosaic Virus Causing Systemic Necrosis on Tomato*

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## Summary

Tomato plants (*Lycopersicon esculentum* cv. Fukujunigo) in the experimental field of Tohoku University were often struck with a lethal necrotic disease. As the causal agent of the disease, a strain of cucumber mosaic virus (CMV) was isolated. RNA of the CMV consists of five fragments, as well as that of the yellow strain of CMV (CMV-Y). MW of the virus coat protein was $26 \times 10^3$ dalton, the same as that of CMV-Y. The same strain of the present virus was also isolated from tomato plants, showing top necrosis in the fields in Miyagi prefecture.

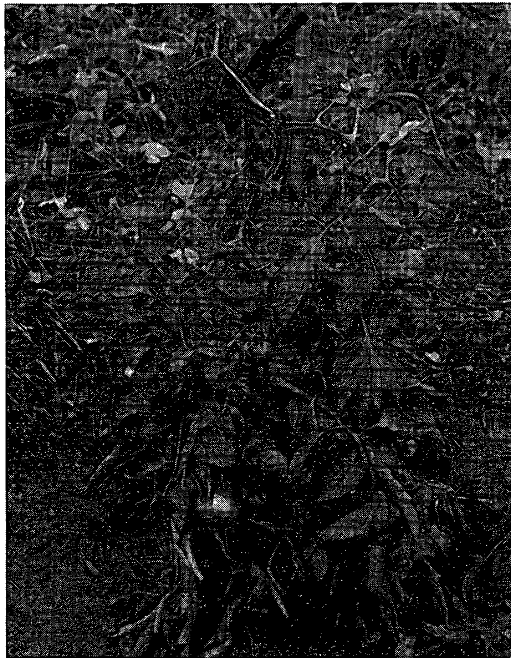
Since 1979, tomato plants in the experimental field of Tohoku University were often affected with a severe necrotic disorder. The necrosis in the diseased tomato first appeared in parts of the veins, the petiols and the stem, and kept spreading in these tissues. Thereafter, the necrosis developed to the top leaves and young green fruits, and later the whole plant collapsed. From the diseased tomato plants, we separated a strain of cucumber mosaic virus. In this paper, we will describe some properties of the virus.

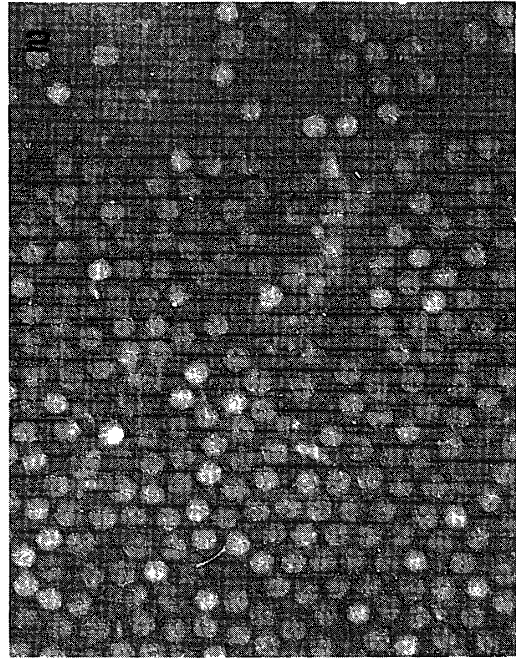
## Materials and Methods

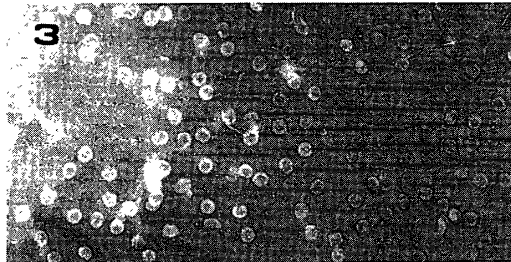
*Plants and virus isolation.* From tomato (*Lycopersicon esculentum* cv. Fukujunigo) plants showing top necrosis in the field of Tohoku University, before collapse, leaves were harvested and stored at −20°C for 2 days. Virus fraction was separated from the samples by the virus purification methods of Scott(12), and Honkura et al(7). Inoculated tomato and test plants were placed in a green house.

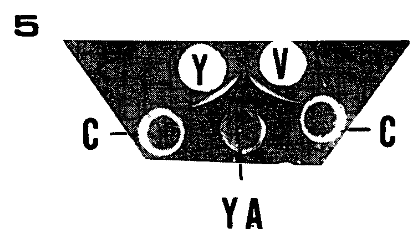
As a standard, cucumber mosaic virus strain Y (CMV-Y)(13) was used. The virus contains five RNA fragments including RNA 5 termed satellite RNA.

* Part of this work was reported at the Meeting of the Tohoku Division of the Phytopathological Society of Japan held at Hirosaki University in 1980.








5
Y
V
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4

TABLE 1. *Symptoms on Test Plants Inoculated with the Virus Fraction Extracted from Tomato Leaves Showing Top Necrosis*

| Test Plants | Symptom | |
|---|---|---|
| | Inoculated leaves | Top leaves |
| *Cucumis sativus* (cv. Yamato sanjaku) | — | M |
| *Nicotiana tabacum* (cv. ky57) | — | M* |
| *Nicotiana glutinosa* | — | M |
| *Chenopodium amaranticolor* | N | — |
| *Chenopodium quinoa* | N | — |
| *Vigna sinenis* (cv. Kurodane sanjaku) | N | — |
| *Vicia faba* (cv. Wasesoramame) | N | — |
| *Phaseolus vulgaris* (cv. Top crop) | (N) | — |

M : Mosaic symptom, N : Necrotic lesion, — : No symptom
( ) : Sometimes appear. * faint

*Electronmicroscopic observations.* The virus fraction was mixed with an equal volume of 2% phosphotungstic acid (PTA), pH 6.0 and placed onto a carbon-coated collodion film supported by a copper mesh grid, and excess sample solution was removed with filter paper. The grid was examined with a JEOL model 100 B electron microscope.

*Immunological reactions.* Rabbits were immunized by an intramuscular infection of purified cucumber mosaic virus (CMV-Y) emulsified with Freund's complete adjuvant (Difco) (1 : 1) and followed by two intravenous injections at weekly intervals. Serum was collected by standard methods 7 days after the last injection and stored at −20°C with 0.1% sodium azide. For the serological studies, Ouchterlony double-diffusion tests were performed in agar plate containing 1% agarose (Sigma, Type II : medium EEO), in 0.15 M sodium chloride, 10 mM sodium phosphate buffer, pH 7.0, 1 mM sodium ethylendiaminetetraacetate and 0.1% sodium azide. The plates were incubated at room temperature for 2 days.

*SDS-polyacrylamide gel electrophoresis.* Purified virus was dissociated in 2% SDS, 1% 2-mercaptoethanol, 50 mM Tris-HCl pH 9.0, at 100°C for 5 min and

FIG. 1. A tomato plant showing top necrosis (arrow).

FIG. 2. Particles in the virus fraction stained with 2% PTA, pH 6. (×120,000)

FIG. 3. Virus particles detected by direct dip method using 2% PTA, pH 6. (×58,000)

FIG. 4. A tomato plant inoculated with the present virus caused systemic necrosis (right), and that inoculated with CMV-Y did not cause such systemic necrosis though the growth was inhibited (middle). Left is a healthy tomato plant inoculated with 0.01 M phosphate buffer (pH 7.0) as a control.

FIG. 5. Double diffusion test of purified CMV-Y (Y) and the present virus (V) against CMV-Y antiserum (YA). C : Phosphate buffer

electrophoresed in a 10% polyacrylamide gel as described by Laemmli[9]. Phosphorylase a (molecular weight (MW) $92.5\times10^3$), bovine serum albumin (MW $66\times10^3$), aldolase (MW $89\times10^3$), carbonic anhydrase (MW $28.8\times10^3$), TMV coat protein (MW $17.5\times10^3$) and cytochrome C (MW $12.3\times10^3$) were used as the MW standards.

*Extraction of viral nucleic acid.* Virus fraction was dissociated in 2% sodium dodecyl sulfate (SDS), 30 mM sodium phosphate, pH 7.2 containing 100 $\mu$g/ml of bentonite at 60°C for 5 min, with or without preincubation in 200 $\mu$g/ml of proteinase K at 37°C for 1 hr. Protein was extracted three times with phenol saturated with 30 mM sodium phosphate, pH 7.2, containing 0.1% 8-hydroxyquinoline. Nucleic acid in the aqueous phase was precipitated in 70% ethanol at −80°C overnight and pelleted at 10,000 rpm for 5 min. The pellet was washed with ethanol, dried and suspended in water.

*Agarose gel electrophoresis.* Extracted viral nucleic acid was denatured by formaldehyde and electrophoresed in 1.7% agarose gels in tube (9 cm long with 7 mm diameter) at 7 mA/tube for 1 hr. As markers, E. coli 23 s ribosomal RNA (MW $1.07\times10^6$) and 16 s ribosomal RNA (MW $0.53\times10^6$) were used. After electrophoresis, the gels were stained in 0.005% Stains-all in formamide and destained in water under dim light.[1]

## Results

A tomato plant showing typical symptoms, top necrosis, is shown in Fig. 1. When the virus fraction extracted from leaves of the diseased plants was examined with an electron microscope, only spherical particles with 30 nm in diameter were detected (Fig. 2). Similar particles were also detected in negatively stained dip-preparations from the leaves (Fig. 3). Neither rod shaped particles such as tobacco mosaic virus nor flexuous particles such as potato virus x were detected in the virus fraction.

When the virus fraction was inoculated to young tomato plants (10-12 cm in height) of the same cultivar, they showed the same symptoms as naturally diseased tomato plants; the necrosis first appeared in the veins, the petioles and the stems, and developed to the top leaves 20 to 30 days after inoculation (Fig. 4).

The virus fraction prepared as described above was inoculated to several indicator plants (Table 1). Inoculated leaves of *Cucumis sativus*, *Nicotiana tabacum* (cv. ky57) and *N. glutinosa* were symptomless; however, their top leaves showed mosaic symptom. On the other hand, the inoculated leaves of *Chenopodium amaranticolor*, *C. quinoa*, *Vicia faba* and *Vigna sinensis* (cv. Kurodane sanjaku) caused necrotic local lesions and no symptoms on their developing top leaves. The inoculated leaves of *Phaseolus vulgaris* sometimes showed a small number of necrotic local lesions, but the top leaves were without symptom.

The virus fraction reacted with CMV-Y antiserum, and a precipitation band

was formed and was continuous with that of CMV-Y (Fig. 5).

From the results of particle morphology, host reactions and serological tests mentioned above, the causal agent of the tomato disease was identified as a strain of CMV.

Next, the compositions of the present virus were compared with those of CMV-Y. Thus, MW of coat proteins of both virures was determined to be $26\times 10^3$ dalton. The nucleic acids of CMV-Y had MWs of 1.20, 1.09, 0.75, 0.33 and 0.15 ($\times 10^6$ dalton), and those of the present virus were not different from those of CMV-Y.

From tomato (*Lycopersicon esculentum* cv. Eiko) plants showing top necrosis collected from a tomato field in Yamoto-cho, Miyagi prefecture in 1985, the same strain as the present CMV was isolated.

Incidentally, in the present experiments, CMV-Y did not cause lethal necrosis in any tomato plants inoculated with it.

## Discussion

A detailed study regarding occurrence of systemic necrosis on tomato caused by a strain of CMV was first reported by Kaper and Waterworth.[8] RNAs of the virus consisted of five fragments, and they pointed out that the smallest RNA fragment (RNA5) of the CMV is the causal agent for the necrosis. In Japan, also, similar tomato disease caused by a strain of CMV has been reported by several researchers.[2,3,4,10,14,15] For instance, Yoshida et al.[15] isolated a strain belonging to paper strain of CMV from tomato plants caused a lethal necrotic disease and detected a considerable amount of RNA5 in addition to RNA 1-4 from the virus. It is not clear that whether or not the present strain of CMV in the same strain as reported by them. Yoshida et al.[16] reported that RNA5 of CMV-PF (fl) which shows fern-leaf symptoms on tomato plants causes an attenuation of the symptoms when it was simultaneously inoculated with RNA5-free CMV. Thus, the role of RNA5 of CMV is not necessarily the same for symptom expressions. Recently nucleotide sequence of RNA5 in several strains of CMV has been analyzed[5,6,11] and compared with each other in relation to characteristics of symptoms.

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