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The Identification and the Classification of Tulip Breaking Virus and Cucumber Mosaic Virus found Infecting Tulip and Lily Plants

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Introduction

Lily mottle viruses, lily rosette virus, lily symptomless virus and cucumber mosaic virus (CMV), including tulip virus, were described as lily virus by BRIERLEY *et al.*(3). In Japan, CMV was isolated from *Lilium Longiflorum* (7), and other species of *Lilium* (6). But there are no reports on lily virus apart from those on CMV. On the other hand, tulip breaking virus (2, 9, 10, 14, 15, 16, 17), tobacco necrosis virus (5), and tulip white streak virus (12) are described collectively as tulip virus. CMV was isolated from tulip by AINTHWORTH (1), KASSANIS (5) and TAKAHASHI *et al.* (13).

The relationship between three lily mottle viruses; virulent coarse mottle virus (VCM), coarse mottle virus (CM), and latent virus (LT) and tulip breaking virus were reported by BRIERLEY *et al.* (3, 4) and MCWHORTER (9, 10). Tulip breaking viruses were divided into two types; tulip virus 1 (TV1) showing color removing and tulip virus 2 (TV2) showing color adding in the petals of tulip (10). BRIERLEY *et al.* (4) demonstrated that lily mottle viruses (VCM, CM and LT) showed color breaking in tulip petals, and they suggested that these five viruses should be considered as strains or subspecies of tulip breaking virus.

In 1963, two types of virus were isolated from tulip plants showing mosaic symptoms, and three types of virus were isolated from virus-infected lily plants. This paper identifies theses viruses and demonstrates the relationship of the viruses isolated from tulip and lily.

Materials and Methods

The naturally infected tulip plants (T1–T8) which showed color adding or color removing breakings on their petals and mosaic on their leaves were collected in May, 1963 in Osaka. The naturally infected lily plants (L1–L5) showed breakings on petals and mottle on leaves.

The infected leaves or bulbs were homogenated with phosphate buffer (0.1 M, pH 7.0) and then centrifuged at low speed. Inoculation was made by the commonly-used method of dusting leaves with using cotton pads. The plants used were grown in a greenhouse. The tulip breaking virus isolated from tulips, produced symptoms on *L. formosanum*, plants of which were grown from seed. CMV produced symptoms on the following indicator plants; *Nicotiana tabacum* (var. Xanthi), *N. glutinosa*, cowpea, cucumber, pinto bean and tomato. Also, many species of lily and test plants of CMV were used in inoculation experiments with lily mottle viruses and other virus. The symptoms were observed over a period of 40 days.

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Results

1. Tulip breaking virus

Tulip breaking virus was isolated from the infected tulip plants (T1–T6). The virus failed to show symptoms on plants other than *L. formosanum* and tulip (Table 1). Although the virus failed to produce symptoms on *L. longiflorum*, when reinoculated onto tulip or *L. formosanum*, symptoms were obtained. Symptoms of mottle appeared on the developed leaves of lily about two weeks after inoculation, and distortion and malformation of leaves followed.

Table 1. Host range of the viruses of T1–T6

Test Plants	Plant affected*					
	T1	T2	T3	T4	T5	T6
N. tabacum	—	—	—	—	—	—
N. glutinosa	—	—	—	—	—	—
Solanum lycopersicum	—	—	—	—	—	—
Cucumis sativus	—	—	—	—	—	—
Phaseolus vulgaris	—	—	—	—	—	—
Vigna sinensis	—	—	—	—	—	—
Pisum sativum	—	—	—	—	—	—
Beta vulgaris var. Rapa	—	—	—	—	—	—
Spinacia oleracea	—	—	—	—	—	—
Raphanus sativum	—	—	—	—	—	—
Brassica campestris	—	—	—	—	—	—
Lilium formosanum	S	S	S	S	S	S
Tulipa Gesneriana William pitt	S	S	S	S	S	S

S : Systemic symptoms
—: No symptoms

L. formosanum was used as an indicator of virus activity. The thermal inactivation point of the virus was between 65° and 75°C after 10 min. exposure. Aging of leaf sap of virus-infected *L. formosanum* occurred after storage at 25°C for 6 days. Infectivity was lost at a dilution of 1:50,000 (Table 4).

The virus particles were observed under the electron microscope by the dipping method, and were found to be rod-shaped and about 750 mμ in length.

2. Tulip-cucumber mosaic virus

The viruses of T7 and T8 have a wide host range different from those of T1–T6. T7 and T8 showed symptoms on *Nicotiana tabacum, N. glutonosa,* tomato, cucumber, and produced local lesions on inoculated leaves of pea, cowpea and sesame (Table 2). These viruses can be considered as cucumber mosaic virus (CMV). Tobacco necrosis virus was not found in T7 and T8. Symptoms induced by the viruses of T7 and T8 were as follows; 1) *N. glutinosa*: systemic infection showed as a slight clearing of the veins about 4–7 days after inoculation, followed by general mosaic. Narrowing of the leaves sometimes occurred. 2) *N. tabacum*: yellowing appeared in youg leaves about 4–7 days after inoculation, and then yellowing became severe mosaic and there were dark green blisters on the leaves. Whole plants became stunted. 3) cucumber: the first symptom appeared on the young leaves which developed small greenish-yellow areas. The characteristic symptom, a yellow mosaic,

Table 2. Host range and symptoms of the viruses of T8 & T7.

Test Plants	Plant affected*		Symptoms
	T7	T8	
N. tabacum	21/21	15/15	S
N. glutinosa	21/21	15/15	S
Solanum melongena	43/44	31/37	S
S. lycopersicum	20/38	15/28	S
Capsium annum	69/70	56/59	S
Cucumis sativus	28/28	35/35	S
C. melo var. common Makino	12/14	27/35	S
C. melo	14/18	12/13	S
Citrullus vulgaris	13/18	13/18	L
Phaseolus vulgaris	0/19	0/23	—
Vigna sinensis	56/56	19/19	L
Pisum sativum	13/14	11/12	L
Vicia faba	16/17	15/17	L
Zinnia elegans	22/22	21/21	S
Spinacia oleracea	28/46	20/35	L, S
Beta vulgaris	34/35	32/38	L
B. vulgaris var. Rapa	37/48	30/34	L
Gomphrena globosa	31/36	8/16	S
Raphanus sativus	0/52	0/28	—
Brassica campestris	0/43	0/36	—
Lilium formosanum	8/21	3/10	S

* : Number of plants inoculated over number of plants infected.
L: Local lesion
S : Systemic symptoms

appeared on all leaves developed after 7 days. 4) cowpea: It produced local lesions on inoculated leaves about 16–48 hours after inoculation. 5) Broad bean: It produced about ten local lesions on each inoculated leaf. The viruses of T7 and T8 showed the same symptoms with CMV on the indicator plants.

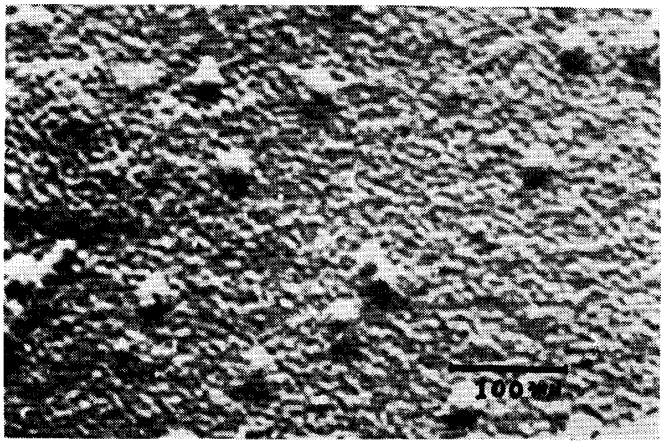

Fig. 1. Electronmicrograph of CMV.

Table 3. Host range and symptoms of the viruses of L1–L5

Test plants	Viruses of				
	L 1	L 2	L 3	L 4	L 5
Lilium formosanum	Mottle	Mottle	Severe mottle	Severe mottle flower distortion	Mottle flower distortion
L. longiflorum	Mottle soon masked	Mottle	Severe mottle	Mottle soon masked	Mottle
L. elegans	Mottle flower distortion	Mottle flower distortion	Mottle Color removing	Mottle Color adding	Mottle flower distortion
Tulipa Gesneriana					
William pitt	Color removing	Color removing	Color removing	Color adding	Color removing
Roland	Color adding	Color adding	Color removing	Color adding	Color adding
Allium fistulosum	—	—	Mottle	—	—
A. cepa	—	—	Mottle	—	—
Vigna sinensis	—	—	—	—	—
N. tabacum	—	—	—	—	—
N. glutinosa	—	—	—	—	—
Cucumis sativus	—	—	—	—	—
Solanium lycopersicum	—	—	—	—	—

Color adding: Color adding breaking on petals
Color removing: Color removing breaking on petals
—: No symptoms

In this experiment, cowpea was used as a test plant. The thermal inactivation point was between 65° and 75°C for 10 min. exposure, resistance to aging was about 3 days at 20°C and approximately 40 days at 0°C. Dilution end point was found to be 1:10,000 (Table 4).

The virus was purified by the method described by SCOTT (11). Examination of purified CMV in the electron microscope revealed polyhedral particles 30 mμ in diameter (Fig. 1).

3. Lily mottle virus

The host range of five isolates distinguished by the principal test reactions are shown in table 3. None of the isolates showed symptoms on tobacco, pintobean, cowpea, tomato, cucumber and radish. The viruses of L1 and L2 produced color removing breaks in tulip (var. William pitt) and green mottling in *L. formosanum* and *L. longiflorum* about two weeks after inoculation. Buds of inoculated *L. formosanum* undergrew and did not flower. The virus of L1 could be considered as coarse mottle virus (CM) because that it produced symptoms on *L. longiflorum* and color removing break on tulip.

Onion and *Allium* infected with the virus of L3 produced fine yellow mottled and curled leaves. The virus also produced mosaic symptom on leaves and caused non development of buds.

The virus of L4 produced color adding breaks on tulip and *L. elegans*. The viruses of L3 and L4 corresponded closely with VCM and TV2 respectively described by BRIERLEY *et al.* (4). The virus of L5 produced color removing breaks and no flowering on tulip and *L. elegans*. It also produced coarse mottling, twisted, curled leaves and distorted flowers. The virus of L5 is identical with that of L3 and it can considered as VCM.

The properties of the three viruses were determined in *L. formosanum*. The thermal inactivation point of CM was found to be between 70° and 75°C, those of VCM, between 65° and 70°C; those of TV2, between 60° and 65°C. CM, VCM and TV2 were active after dilution to 1:5,000. At 24°C, all three viruses were active after aging 3 days (Table 5).

Table 4. Inactivation points of the viruses of T1–T8

Viruses	Thermal inactivation point	Dilution end point	Tolerance of aging
T1–T6	65–75°C	1: 50,000	5–6 days
T7 & T8	65–75°C	1: 10,000	2–3 days

Table 5. Inactivation points of the viruses of L1–L5

Viruses	Thermal inactivation point	Dilution end point	Tolerance of aging
L1	70–75°C	1: 1,000–5,000	3–4 days
L2	65–70°C	1: 1,000–5,000	2–3
L3	65–70°C	1: 1,000–5,000	3–4
L4	60–70°C	1: 1,000–5,000	1–3
L5	65–70°C	1: 1,000–1,500	3–4

Five viruses were observed under the electron microscope by using dipping method. All virus particles were rod shaped, and they measured as follows; L1: 725–750 mμ, L2: approximately 775 mμ, L3: 750–775 mμ, L4: 750 mμ, L5: 750–775 mμ (Fig. 2). Accordingly, it could be considered that they are the same strain regarding particle size.

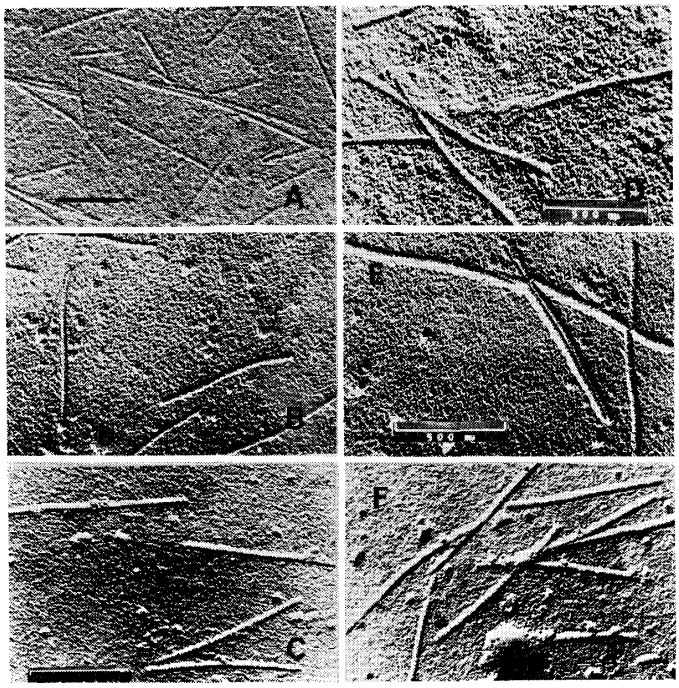

Fig. 2. A : Electronmicrograph of L1.
B : ,, L2.
C : ,, L3.
D : ,, L4.
E : ,, L5.
F : ,, TV.

Discussion

Tulip-CMV has not been reported in Japan so far. YAMAMOTO *et al.* (19) reported that CMV was infective against tulip plants, whereas CMV was isolated from the petals of breaking on tulip (1). BRIERLEY and DOOLITTLE (2) demonstrated that CMV produced the breaking of the petals on tulip. We found that CMV caused the breaking of the petals on tulip. CMV isolated from the tulip was not infective against the *Cruciferae* and it did not show any systemic symptoms in the *Leguminosae*. From these observations together with further findings on host range and symptoms, this virus appears to be similar to the ordinary strain of CMV. But this virus, as reported by KOMURO (8), is considered as strains of lily CMV because of its infectivity against the *Liliaceae*.

Three kinds of viruses which have known no host apart from the *Liliaceae* were isolated from the infected lilies. These viruses seem to be VCM, CM and tulip virus 2 according to the classification described by BRIERLEY *et al.* (4). We were not able to obtain CMV from lilies.

Tulip viruses were classified TV 1 and TV 2 by MCWHORTER (9) on the basis of color adding and removal respectively. BRIERLEY *et al.* (4) suggested that lily mottle viruses and tulip viruses were considered as strain or subspecies of tulip breaking virus from the point of view of host range, symptoms and thermal inactivation points. Also MCWHORTER (10) described lily mottle viruses as tulip breaking virus. The symptoms produced by TV 2 isolated from *L. elegans* Thunb. corresponds with those produced by the tulip virus reported by MCWHORTER (9). In Japan, tulip breaking virus was isolated from the infected tulip, and the viruses which produced breaking on the tulip were isolated from the *Liliaceae*, and particles of these viruses resemble those of tulip breaking virus (14, 15, 16, 17, 18).

In this experiment the type of color breaking of the petals on tulip caused by lily mottle virus (VCM, CM) was different from that caused by tulip viruses (TV 1, TV 2). Both host range and thermal inactivation points were, however, similar to each other. No difference was found in size and shape among these virus particles and the size was about 750 mμ in length which has already been reported (17, 18). Thus lily mottle viruses and tulip viruses can be considered as substrains or subspecies of tulip breaking virus.

Summary

It is possible that there is more than one or virus strain associated with the disease known as breaking on tulip. It was attempted to obtain further information on the identification of the viruses. Two types of viruses viz, tulip virus (TV) and cucumber mosaic virus (CMV) were isolated in our experiments from tulip plants, naturally showing symptoms of breaking. TV showed symptoms only on tulip and lily (*Lilium formosanum*) by mechanical inoculation of leaf sap from tulip, and no symptoms were observed on other test plants. Mottle symptoms appeared on the developed leaves of lily about two weeks after inoculation followed by distortion and malformation of the leaves.

The thermal inactivation point was between 65° and 75°C for 10 min. exposure, and aging occurred after about 6 day-storage at 25°C for leaf sap of infected *L. formosanum*. In fectvity was lost at a dilution of 1 : 50,000. The particles of TV were about 750 mμ in length and were rod-shaped.

The virus identified as CMV showed systemic symptoms on test plants such as *Nicotiana tabacum, N. glutinosa, Cucumis sativus* and *Solanum melogena*. The thermal inactivation point was 65°–75°C, resistance to aging was 3–4 days at room temperature and about 40 days at −5°C. The dilution end point was 1 : 10,000. No tobacco necrosis virus was found in naturally virus-infected tulip in this experiment.

On the other hand, three kinds of viruses were isolated from naturally infected lily plants, viz, coarse mottle virus (CM), virulent coarse mottle virus (VCM) and tulip virus (TV). The host range of CM, VCM and TV were found to be limited to the *Liliaceae* including tulip and onion, and no symptoms were observed on test plants of CMV or other families. Three viruses produced coarse or fine mottling of several species of lily leaves, and when strong symptom expression occurs at the bud stage, the flowers were varioulsy deformed with curled and narrowed perianth segments. They produced color adding or color removing in petals of tulip. The thermal inactivation point of CM was found to be between 70° and 75°C; those of VCM, between 65° and 75°C; those of TV, between 60° and 65°C. CM, VCM and TV were active after dilution to 1 : 5,000 and all these viruses were active after 3 days at 24°C. The particles of CM, VCM and TV were all about 750 mμ in length.

It is strongly suggested that TV, CM and VCM would be considered as a strain of tulip breaking virus.

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