Plant Pathol. J. 15(2) : 119-123 (1999)

The Plant Pathology Journal

Identification of Tomato Aspermy Virus (TAV) and Chrysanthemum Virus B (CVB) from *Dendranthema indicum* in Korea

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(Received March 7, 1999)

Chrysanthemums showing leaf mottling were collected from three southern locations in Korea in 1998. Two kinds of viruses were isolated from the leaves and were identified as tomato aspermy virus (ch-TAV) and chrysanthemum virus B (ch-CVB), according to their host range, morphology, intracellular location, agar gel double diffusion test, and double-stranded RNA (dsRNA) analysis. The purified ch-TAV was spherical particles of approximately 29 nm in diameter and ch-CVB was filamentous particles of 685 nm long. Inclusion bodies were not observed in ch-TAV and/or ch-CVB infected chrysanthemum. ch-TAV showed positive serological reaction with TAV antiserum (ATCC-127) but not with CMV-pepper antiserum. In dsRNA analysis, four kinds of viral dsRNA were observed on ch-TAV and one viral dsRNA was shown on ch-CVB. Rate of co-infection with TAV and CVB in commercial chrysanthemums was 20.9%. On the other hand, infection with CVB alone was 97.2%. However, chrysanthemums naturally infected with TAV alone were not found.

Keywords **:** chrysanthemum, CVB, TAV, virus.

Chrysanthemum is an important cut flower in Korea. It accounts for 29 percent in terms of the cut flower cultivation area. Six kinds of viruses and two viroids were reported as infectious pathogenic agents on chrysanthemum worldwide (Bouwen and Annemarie, 1995). These are tomato aspermy virus (David et al., 1991; Hill et al., 1996; Hollings and Stone, 1971), chrysanthemum virus B (Brunt, 1995; Hakkaart and Maat, 1974; Hill et al., 1996; Hollings and Stone, 1972), tomato spotted wilt virus (Bouwen and Annemarie, 1995; Hill et al., 1996; Verhoeven et al., 1996), cucumber mosaic virus (Bouwen and Annemarie, 1995), impatiens necrotic spot virus (Bouwen and Annemarie, 1995), garland chrysanthemum albinic mosaic virus (Chen et al., 1996), chrysanthemum stunt viroid (Dusi et al., 1990), and chrysanthemum chlorotic mottle viroid (Bouwen and Annemarie, 1995; Li et al., 1987; McGovern et al., 1988). Of these viruses, the most important two viruses are chrysanthemum virus B (CVB) and tomato aspermy virus (TAV). In the case of viroid, the chrysanthemum stunt viroid causes significant damage on chrysanthemum (Bouwen and Annemarie, 1995; Dusi et al., 1990). CVB is a virus with rather straight rod-shaped particles about 685 nm long and belongs to the carlavirus group (Bouwen and Annemarie, 1995). It occurs throughout the world in many cultivated chrysanthemum varieties (Hollings and Stone, 1972). On the other hand, TAV belongs to the cucumovirus group which has isometric in shape and 29 nm in diameter (Bouwen and Annemarie, 1995).

At present, no report has been made on the occurrence of viruses affecting chrysanthemums in Korea. Here we report the identification of two viruses from chrysanthemums showing leaf mottling. The rate of infection with viruses and the viral disease symptoms were investigated on some chrysanthemum cultivars in three provinces of Korea.

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Materials and Methods

Source of virus. Chrysanthemums showing leaf mottling were collected from three southern locations namely: Masan, Changwon and Pusan. In order to isolate TAV and CVB, saps of leaf samples were inoculated on indicator plants like *Chenopodium quinoa* and *Petunia hybrida* 'Carpet red'. Single local lesion of *C. quinoa* was used as an inoculum on *C. quinoa* three times successively and the virus was propagated and maintained in *Nicotiana tabacum* 'Samsun'. Chlorotic local lesion of *P. hybrida* 'Carpet red' was also used as an inoculum on *N. clevalandii* and propagated in it. Systemically infected *N. tabacum* 'Samsun' with spherical virus isolate and *N. clevalandii* with filamentous virus isolate were used as the sources of identification of TAV and CVB, respectively.

Virus purification. Systemically infected leaves of *N. tabacum* 'Samsun' with spherical virus isolate were harvested 10 days after inoculation (Hollings and Stone, 1971). It was purified using the modified methods according to Scott (Hollings and Stone, 1971). *N. clevalandii* leaves systemically infected with filamentous virus isolate were harvested 3 weeks after inoculation and purified using the Oertel procedure (Hollings and Stone, 1972).

Host range test. Seventeen indicator plants (Table 1) were inoculated with spherical or filamentous virus isolates in 0.02 M

Table 1. Symptomatological responses[a] of indicator plants to ch-TAV and ch-CVB infection

Indicator plants	ch-CVB	ch-TAV	cu-CMV
Chenopodium quinoa	C/-	sNS/-	sNS/-
C. amaranticolor	-/-	sNS/-	sNS/-
Nicotiana tabacum 'Samsun'	-/-	N/M	-/M
N. rustica	-/-	C/M	C/M
N. occidentalis	-/M	N/M	N/M
N. glutinosa	-/-	-/M	-/M
N. bentamiana	-/M	N/M	-/M
N. clevalandii	-/M	N/M	N/M
Vigna unguiculata	-/-	N/-	N/-
Cucumis sativus	-/-	-/-	-/M
Datura stramonium	-/-	-/M	-/-
Capsicum annuum	-/-	-/M	-/M
Petunia hybrida 'Carpet red'	C/-	-,GI/M	-/M
Gomphrena globosa	-/-	N/M	N/M
Physalis floridana	-/-	-/M	-/M
Tetragonia expansa	-/-	N/-	N/-
Lycopersicon eseulentum 'Rutgers'	-/-	N/M,ST	N/M

C: chlorotic lesion, M: mosaic or mottle, GI: green island, N: necrotic lesion, sNS: small necrotic spot, ST: stunt, - : latent or no infection
[a]Test was conducted from April to May 1998.

phosphate buffer, pH 7.2. Test was conducted from April to May in green house. Reaction was observed starting from 5 days up to 15 days after inoculation. In order to compare the host range of spherical virus isolate with CMV, cu-CMV isolates from cucumber (not published) was used as a control.

Electron microscopy. The virus particles in leaf samples in the test plants were negatively stained and examined in a LEO 906 transmission electron microscope. Chrysanthemum leaves showing mottling symptoms were prefixed in 1% Karnovsky's fixative solution. After postfixation in 1% Osmium tetroxide, dehydration was carried out in an ethanol series. Embedding was conducted in Spurr resin.

Serological analysis. Spherical virus isolates were tested using the 1% agar gel double diffusion technique against antiserum to TAV (ATCC-127) and to CMV pepper isolate (CMV-pepper antiserum) previously prepared in our laboratory (Choi, et al., 1998).

DsRNA analysis. Viral dsRNA extraction from systemically infected *N. tabacum* 'Samsun' and *N. clevalandii* was conducted as described by Morris and Dodds (15) using CF-11 cellulose column chromatography (Whatman). The viral dsRNA of CMV, CGMMV and ZYMV were used as molecular size markers. These were extracted from systemically infected cucumber, watermelon, and zucchini, respectively. RNA preparations were separated in 6% polyacrylamide gel in 44.5 mM Tris-borate buffer containing 1.0 mM EDTA (pH 8.0). The dsRNA bands were visualized by silver staining methods.

Viral infection rate. Around 20 commercial chrysanthemum farms were surveyed to investigate the rate of virus infection. 35 to 60 plants were randomly collected per cultivar. The leaf samples of chrysanthemum were analysed for virus infection using the leaf dip method for CVB and indicator plants, *C. quinoa* and *Cucumis sativus* were used for TAV.

Results

Host range test. Among the seventeen test plants inoculated with ch-TAV, *Chenopodium* spp., *Vigna unguiculata* and *Tetragonia expansa* showed local necrotic spots on inoculated leaves. *Nicotiana* spp., *Capsicum annuum, Datura stramonium, Petunia hybrida, Gomphrena globosa, Physalis floridana* and *Lycopersicon esculentum* 'Rutgers' showed systemic infection. *Cucumis sativus* was not infected with ch-TAV, but it was systemically infected with cu-CMV and vice versa in *D. stramonium.* Among the seventeen test plants inoculated with ch-CVB, only *N. clevalandii, N. occidentalis,* and *N. bentamiana* were systemically infected. *C. quinoa* and *P. hybrida* showed local chlorotic lesion on ch-CVB inoculated leaves (Table 1).

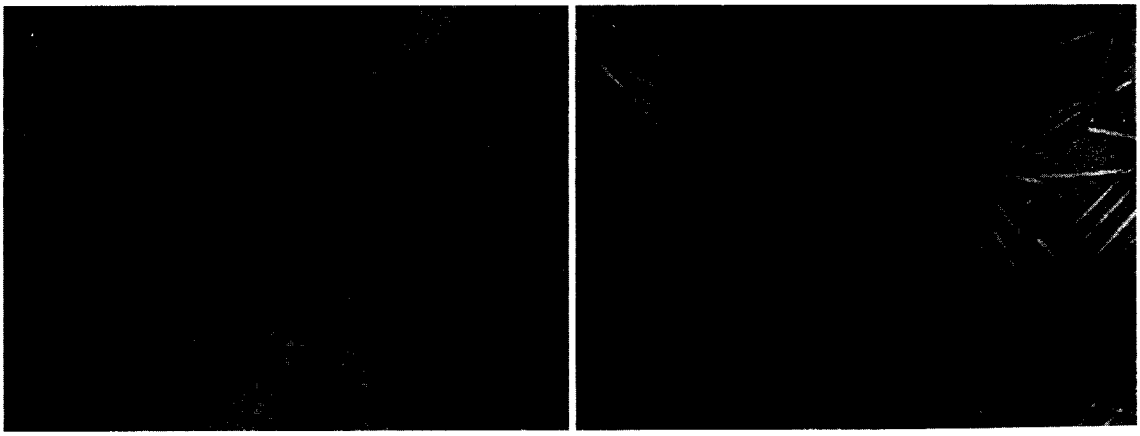

Fig. 1. Electron micrograph of purified ch-TAV (A) and ch-CVB (B) stained in phosphotungstic acid. Scale bar represents 50 nm (A) and 500 nm (B).

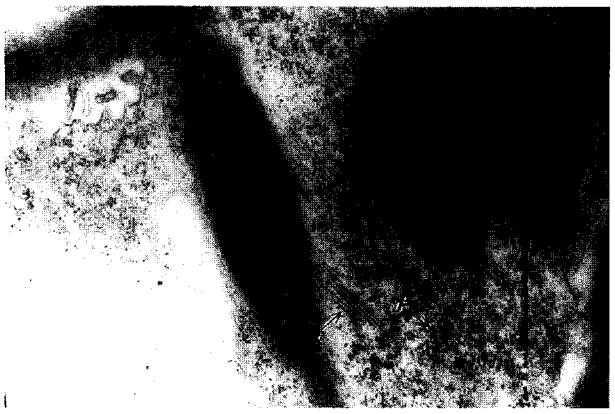

Fig. 2. Location of the viruses in tissue sections of chrysanthemums infected with TAV and CVB. Numerous spherical (A) and filamentous (B) virus particles in the cytoplasm.

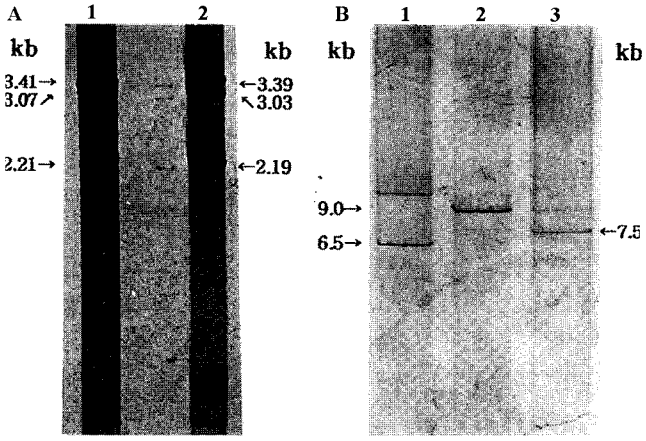

Fig. 4. DsRNA analysis of ch-TAV (A) and ch-CVB (B) extracted from infected *N. tabacum* 'Samsun' and *N. clevalandii*, respectively. Electrophoresis was carried out in 6% acrylamide gel and the gels were visualized by silver staining methods. In A, lane 1, ch-TAV; lane 2, cu-CMV (size marker). In B, lane 1, CGMMV(size maker), lane 2, ZYMV (size marker); lane 3, ch-CVB.

Virus purification. Purified ch-TAV were spherical particles of approximately 29 nm in diameter (Fig. 1A) and ch-CVB was filamentous particle of 685 nm long (Fig. 1B). The UV absorbance of purified ch-TAV virions showed maximum at 260 nm and minimum at 240 nm (data not shown).

Electron microscopy. Inclusion bodies were not observed in ch-CVB and ch-TAV infected chrysanthemums. Both virus particles were commonly scattered within the cytoplasm (Fig. 2).

DsRNA analysis. Four kinds of RNAs were observed in dsRNA analysis of ch-TAV (Fig. 4A). In comparison of dsRNA band pattern of ch-TAV with cu-CMV in polyacrylamide gel, migration of the second largest RNA of the former virus was faster than that of the latter one (Fig. 4A). In the case of ch-CVB, a band of 7.5 kbp was observed in polyacrylamide gel (Fig. 4B).

Serological analysis. In agar gel double diffusion test, *N. tabacum* 'Samsun' infected with ch-TAV positively reacted with antiserum to TAV (ATCC-127) but not with CMV-pepper antiserum (Fig. 3A, B).

Table 2. Infection rate of TAV and/or CVB in commercially cultivated chrysanthemums in Masan, Changwon and Pusan, Korea

Cultivars	No. of plants tested	Infection rates (%)[b]		
		TAV+CVB[a]	CVB	TAV
Puma	40	0	97.5	0
Backwang	55	0	98.2	0
Sulpung	50	9.3	98.0	0
Chunkwang	50	0	98.0	0
Subangryuck	50	3.3	98.0	0
Owallchung	60	45.0	96.7	0
Goldencasandra	35	88.7	94.3	0
Average	-	20.9	97.2	0

[a]TAV and CVB were tested using indicator plants and EM, respectively.
[b]Viral infection rate was investigated from March to July 1998.

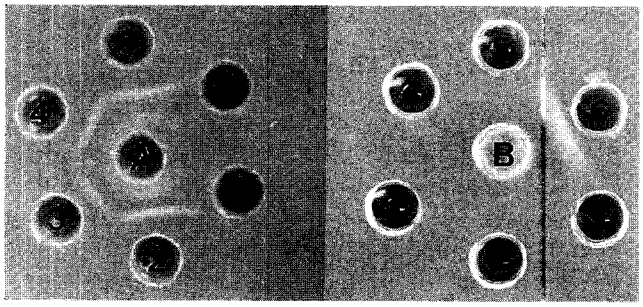

Fig. 3. Serological reaction of TAV antiserum (ATCC-127) (A), and CMV-pepper antiserum (B) to the healthy sap of *N. tabacum* 'Samsun' (1), sap from infected with ch-TAV (2~5), and sap from inected with cu-CMV (6).

Viral infection rates. Rate of co-infection with TAV and CVB in commercially available chrysanthemums was 20.9 % and infection with CVB alone was 97.2% (Table 2). Virus infected chrysanthemums usually showed leaf mottling (Fig. 5E). In the case of co-infected chrysanthemum with TAV and CVB, mosaics in 'Chunkwang' (Fig. 5A), necrotic lesions around veins in 'Subangryuck' (Fig. 5B), necrotic green islands in 'Sulpung' (Fig. 5C), and necrotic staining around leaf edge in 'Namjung' (Fig. 5D) were observed. CVB infected 'Subangryuck' (Fig. 5E) and 'Namjung' (Fig. 5F) showed mottling and yellow spots on their leaves, respectively.

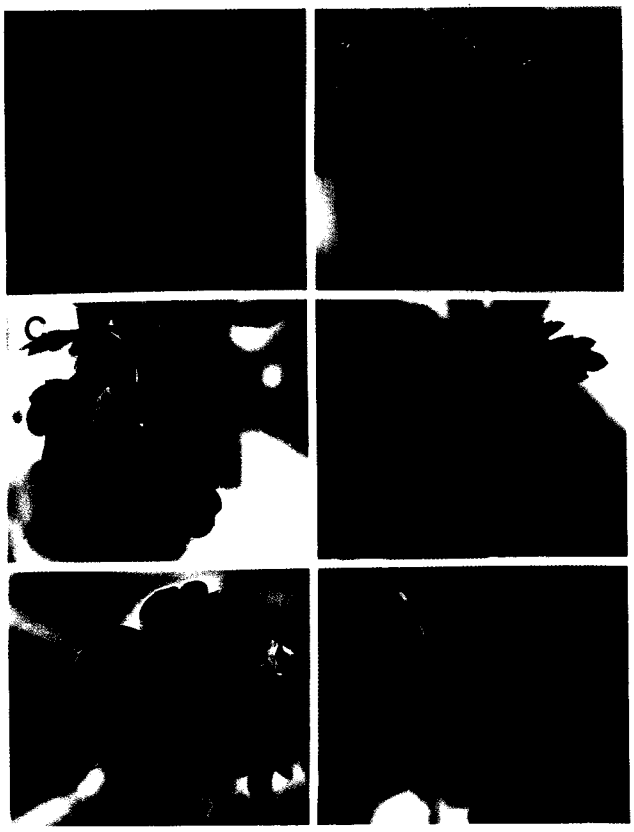

Fig. 5. Disease symptoms of chrysanthemums infected with TAV (A~D) or CVB (E~F). A, 'Chunkwang'; B and E, 'Subangryuck'; C, 'Sulpung'; D and F, 'Namjung'.

Discussion

From this study, it was identified that mottle symptom on chrysanthemum leaves was caused by TAV and/or CVB infection. The analysis of host range, morphology, serology, and dsRNA of ch-TAV or ch-CVB share the typical characteristics of cucumovirus and carlavirus, respectively (Brunt, 1995; Hollings and Stone, 1972; Morris and Dodds, 1979). *Chenopodium quinoa* was reported not to be diagnostically available to ch-CVB but we observed that ch-CVB infected *C. quinoa* showed local cholorotic spots on inoculated leaves under the condition of 25~30°C. This result was similar to the previous report by Brunt (1995). *N. bentamiana* or *N. occidentalis* could not be used as a host to ch-CVB but under the condition of 25~30°C and long day photoperiods (12~15 hrs) these plants turned out systemic symptoms. So, these plants could be used as new indicator plants for CVB in the limited culture conditions described above.

TAV and CMV share many common properties but there have been some controversies on their serological relationship (Daniels and Campbell, 1992; Srivastava, 1992). Rao et al. (1982) described that serological relationship between TAV and CMV was different in various virus strains. ch-TAV isolated in this study was not serologically related to the CMV-pepper antiserum.

This study showed that CVB infection rate of chrysanthemums in Korea is higher than that of Australia. Rate of co-infection with TAV and CVB in commercially available chrysanthemums was 20.9% and infection with CVB alone was 97.2%. Jane and Jenny (1985) reported that viral infection rates of chrysanthemums with CVB and TAV were 81% and 23%, respectively, in Australia.

Viral disease symptoms and infection rate were different among cultivars. But some cultivars were so severely affected by the virus infection that in-depth studies on the interactions between virus and chrysanthemums could be conducted. Further research achieves may be directed towards the study of obtaining virus-free chrysanthemum.

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