Clover Yellow Vein Virus and a Carlavirus Isolated from *Impatiens sultani* in Japan

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Impatiens sultani is a widely grown ornamental plant, however, no virus has so far been described for it in Japan. In 1989, we detected two types of filamentous viruses in leaf-dip preparations from the plant collected in Osaka. This paper reports the characterization of a potyvirus and a carlavirus isolated from *I. sultani* which appeared necrotic ring symptom.

Clover yellow vein virus. A potyvirus, tentatively named as I89-1, was isolated from the diseased leaves showing necrotic ring symptom. It was mechanically transmitted to *Phaseolus vulgaris* cv. Topcrop and subjected to three successive single lesion transfers on *Chenopodium quinoa*.

Mechanical inoculation experiment revealed that I89-1 had a wide host range and appeared necrotic symptoms in most species. I89-1 was infectious to 16 species in 5 families. It systemically infected *Chenopodium amaranticolor*, *C. quinoa*, *Glycine max*, *Nicotiana clevelandii*, *N. debneyi*, *Phaseolus vulgaris* cv. Topcrop, *Pisum sativum* cv. Sanjunichi-kinusaya, *Sesamum indicum*, *Trifolium repens*, *Vicia faba*, *Vigna unguiculata* subsp. *sesquipedalis* cv. Kurodane-sanjaku. Species locally infected were *Beta vulgaris*, *N. tabacum*, *Petunia hybrida*, *Spinacia oleracea*, *Tetragonia expansa*, *Vigna unguiculata* cv. Daruma. The virus did not infect 16 species including *Cucumis sativus*, *Gomphrena globosa* and *Pisum sativum* cv. Wisconsin Perfection. The virus was also transmitted by *Myzus persicae* in non-persistent manner. The infected *I. sultani* appeared necrotic ring symptom (Plate I-1).

Filamentous virus particles c. 750 nm long and 12 nm wide were detected by electron microscopy in crude sap of the infected *I. sultani*. Fragments of pinwheel inclusions and rhombic, crystalline inclusions (dense body)¹⁾ were also observed in the leaf dip preparations negatively stained with 2% PTA, pH 6.5. In thin sections of the infected leaves of *I. sultani* or *N. clevelandii*, pinwheel- and laminated aggregate-type inclusions and crystalline inclusions as well as filamentous virus particles were observed in the cytoplasm (Plate I-2, 3).

I89-1 in crude sap of *I. sultani* or *C. quinoa* strongly reacted with the antisera to Cal. 35²⁾ and No. 30³⁾ isolates of clover yellow vein virus (CYVV) and to the N strain (P180)⁴⁾ of bean yellow mosaic virus (BYMV-N) in the double diffusion test (DDT) on 0.15% Gelrite gels⁵⁾ (Plate I-4). The virus particles positively decorated with the antibodies were observed in the immuno-

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sorbent electron microscopy (ISEM). On the other hand, I89-1 did not react with antiserum to B strain (V124)⁴⁾, P strain (P242)⁴⁾ or O strain (BH-8)⁴⁾ of bean yellow mosaic virus by ISEM as well as DDT.

From these results, I89-1 was identified as CYVV⁶⁾. CYVV has been isolated from many plant species, however, this may be the first report for *I. sultani* as the natural host of CYVV. The name "necrotic ring" is proposed for the disease of *I. sultani* by CYVV.

Host range study and other experiments showed that the isolate used in this study was identical to BYMV-N⁴⁾. The precipitin lines of the antigen to CYVV antisera and to BYMV-N antiserum were completely fused (Plate I-4). Therefore, it is concluded that BYMV-N is a synonym for CYVV^{2,3,6)}. Since the name "BYMV-N" has been used only in Japan, it is recommendable to use "CYVV" for the virus.

A carlavirus. Another type of filamentous virus, tentatively named as I89-2, was detected in addition to I89-1 in the original *I. sultani* and also in *C. quinoa* which has been mechanically inoculated with the sap of *I. sultani*. Isolation of I89-2 was successfully conducted through single lesion transfers on *C. quinoa*.

In mechanical inoculation experiment using 38 species out of 11 families, only *C. amaranticolor* and *C. quinoa* were susceptible to I89-2 besides *I. sultani*. The virus produced local chlorotic spots on *C. amaranticolor* or *C. quinoa* 8–10 days after inoculation, however, no symptoms were observed on the infected *I. sultani*. Trials to transmit I89-2 from *I. sultani* to *I. sultani* or from *C. quinoa* to *C. quinoa* by *Myzus persicae* in the non-persistent condition were failed.

Slightly curved virus particles c. 640 nm long and 12 nm wide were detected by electron microscopy in the crude sap preparations negatively stained with 2% PTA, pH 6.5. The filamentous particles were also observed in cytoplasm of the infected *I. sultani* or *C. quinoa*.

I89-2 was purified from the inoculated leaves of *C. quinoa* (Plate I-6). The extract was clarified by chloroform and carbon tetrachloride. Then, the virus was precipitated by PEG 6,000 and purified by differential and sucrose density gradient centrifugation. Purified I89-2 had an absorption spectrum typical of nucleoprotein with a minimum absorbance at 242 nm and a maximum at 258 nm with a A_{260}/A_{280} ratio of 1.22. Single band with a buoyant density of 1.32 g/cm³ was obtained for the virus in the equilibrium CsCl density gradient.

The orcinol, the diphenylamine and the formaldehyde treatments indicated that the extracted nucleic acid preparation was single-stranded RNA. Polyacrylamide gel electrophoresis under denaturing conditions revealed that the RNA consisted of single component with a molecular weight of c. 1.9×10^6 daltons. Protein of the virus electrophoresed in SDS-polyacrylamide gel migrated as one band with a molecular weight of c. 34,000 daltons.

Antiserum prepared against I89-2 in a rabbit had a titer of 1/1,024 with the purified virus preparation in microprecipitin test. I89-2 did not react with antisera to any of the following viruses in gel diffusion tests: carnation latent virus⁷⁾, garlic latent virus⁸⁾, helenium virus S (HVS)⁹⁾, shallot latent virus¹⁰⁾ and strawberry pseudo mild yellow edge virus (SPMYEV)¹¹⁾. However, I89-2 reacted with antiserum to SPMYEV by the immunosorbent electron microscopy to a dilution of 1/200 (Plate I-7).

The results obtained in this study strongly indicated that I89-2 is a carlavirus. Although it related to SPMYEV and has a common natural host with HVS, it is considered that the virus is an undescribed virus because of its very specific host reactions. The name *impatiens* latent virus is proposed for the virus. Since *I. sultani* is an annual plant in Japan, the virus might be transmitted to it from another natural host by an unknown means.

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和 文 摘 要

向 本春・大木 理・尾崎武司・井上忠男：日本の *Impatiens sultani* から分離された clover yellow vein virus ならびに carlavirus について

えそ輪紋病徴を示すインパチエンスから 2 種のひも状ウイルスが分離されたので同定を試みた。一つは長さ 750 nm の potyvirus で、インパチエンスにはえそ輪紋病徴を表し、諸性質から clover yellow vein virus と同定された。ウイルス和名をクローバ葉脈黄化ウイルス、インパチエンス病名をえそ輪紋病 (necrotic ring) としたい。第 2 のウイルスは長さ約 640 nm の carlavirus で、インパチエンスに無病徴感染したほか *Chenopodium amaranticolor* と *C. quinoa* に局部感染した。精製ウイルスの理化学的性質は既報の carlavirus にほぼ一致し、5 種の carlavirus のうち、strawberry pseudo mild yellow edge virus とのみ免疫電顕法で遠い血清関連が認められた。未記載ウイルスと考えられるのでウイルス名としてインパチエンス潜在ウイルス (impatiens latent virus) を提案したい。

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Explanation of plate

Plate I

1. Necrotic rings appeared on systemic leaves of *Impatiens sultani* infected with I89-1 (clover yellow vein virus, potyvirus), 18 days after inoculation.
2. Pinwheel- and laminated aggregate-type inclusions observed in cytoplasm of a leaf parenchyma cell of *I. sultani* infected with I89-1.
3. Crystalline inclusions and laminated aggregates (arrows) appeared in cytoplasm of a leaf parenchyma cell of *I. sultani* infected with I89-1.
4. Double diffusion test performed in 0.15% Gelrite gel. The central well contains crude sap of *I. sultani* infected with I89-1 (AG). Outer wells are the antisera of bean yellow mosaic virus (BYMV) and clover yellow vein virus (CYVV): B, BYMV-B (V124); P, BYMV-P (P242); O, BYMV-O (BH-8); N, BYMV-N (P180); C1, CYVV-Cal.35; C2, CYVV-No. 30. Rings around the outer wells are formed by non-specific reaction. Precipitation between O/P and AG are due to antibodies to the healthy components.
5. Chlorotic spots appeared on a inoculated leaf of *Chenopodium amaranticolor* infected with I89-2 (impatiens latent virus, carlavirus), 10 days after inoculation.
6. Purified virus particles of I89-2.
7. Virus particles of I89-2 decorated with the antiserum to strawberry pseudo mild yellow edge virus at a dilution of 1/100.

Bar represents 200 nm in Figs. 2, 3, 6 and 7.

Plate I