

観賞用Alliumから分離されたleek yellow stripe virus

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Leek Yellow Stripe Virus Isolated from an Ornamental *Allium* Plant in Japan*

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Abstract

A potyvirus isolated from an ornamental *Allium ampeloprasum* cv. 'Murasame' showing mosaic and white necrosis on the leaves, collected in Kurashiki, was identified as an isolate of leek yellow stripe virus (LYSV). LYSV was transmitted by aphids, *Myzus persicae* and *Aphis gossypii*, in a non-persistent manner and by sap-inoculation to 6 of 49 species from 4 of 11 families. *A. ampeloprasum* cv. 'Miyako' and *A. giganteum* were infected systemically and produced mosaic symptoms on leaves. Local lesions were produced on *Chenopodium amaranticolor* and *C. quinoa*, whereas systemic latent infection was produced on *Spinacia oleracea* and latent local infection was on *Gomphrena globosa*. Sap from the diseased *C. quinoa* was infective after 10-min heating at 50 C but not 55 C, after dilution to 10^{-3} but not 10^{-4} and after 4 days but not 8 days at 20 C. The virus particles were filamentous, about 820 nm long. The virus was purified from locally infected *C. quinoa* leaves by clarifying sap extracted in 0.5 M phosphate buffer with chloroform/carbon tetrachloride mixture followed by PEG precipitation, differential centrifugations through sucrose cushion and sucrose density gradient centrifugation. LYSV contained a single protein species of mol. wt. 36,500 daltons. In ultrathin sections of the virus-infected *C. quinoa* leaves, cytoplasmic inclusions containing pinwheels, scrolls and short curved laminated aggregates were observed in the cytoplasm. LYSV reacted with LYSV antiserum donated from Ir. D. Z. Maat (the Netherlands) and the antiserum to LYSV produced by immunizing a rabbit (titer: 1/512), but not with antisera to bean yellow mosaic virus, clover yellow vein virus, freesia mosaic virus, iris mild mosaic virus, iris severe mosaic virus, narcissus yellow streak virus, onion yellow dwarf virus, turnip mosaic virus and potato virus Y.

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Key words: leek yellow stripe virus, ornamental *Allium* plant.

INTRODUCTION

Some of ornamental *Allium* plants have been grown for cut flowers in recent years. In the spring of 1986, a potyvirus was isolated from ornamental *Allium* plants collected from a commercial greenhouse in Kurashiki that showed severe mosaic and white necrosis on the leaves. The virus differed in host range and serological nature from onion yellow dwarf virus (OYDV) and any other potyviruses from *Allium* plants. It was identified as an isolate of leek yellow stripe virus (LYSV), which had not been reported in Japan. This paper mainly deals the characterization of the virus with host range, symptomatology, vector transmission, physical properties, purification, viral protein, serological tests, electron microscopy and ultrastructures of the infected plants.

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MATERIALS AND METHODS

Virus isolate. An isolate of LYSV in this study was isolated by mechanical inoculation from a diseased leaf of an ornamental plant, *Allium ampeloprasum* cv. "Murasame", collected at Tamashima in Kurashiki in April, 1986, showing severe mosaic and white necrosis on the leaves (Plate I-1). In all experiments, the virus isolate was used after three successive single lesion transfers on *Chenopodium quinoa*.

Host range. Plants were inoculated mechanically with sap from leaves of *C. quinoa* showing chlorotic lesions. The inoculated plants were maintained in a greenhouse and symptoms were observed up to 3 wk after inoculation. Back inoculations were made on *C. quinoa*.

Aphid transmission. Aphid transmission tests were done in a greenhouse using *Myzus persicae* and *Aphis gossypii* reared on *Brassica rapa* and *Solanum melongena*, respectively. Apterous aphids were starved for 1~2 hr and then allowed an acquisition feeding period of 5 min on leaves of infected *C. quinoa* and an inoculation access period of 24 hr on leaves of healthy *C. quinoa*. Following test periods, the aphids were killed by spraying an insecticide (DDVP).

Virus purification. Inoculated leaves (100 g) of *C. quinoa* were harvested at about 2 wk after inoculation and were homogenized with a mortar and pestle in 300 ml of 0.5 M potassium phosphate buffer (PB), pH 7.5, containing 0.2% thioglycolic acid. The homogenate was expressed through 2 layers of cheesecloth and 10% (v/v) carbon tetrachloride was added to the extract, and then stirred for 15 min. The mixture was centrifuged for 15 min at $4,000 \times g$ and the aqueous phase was collected. After an additional low-speed centrifugation ($7,000 \times g$ for 15 min), 1% (v/v) Triton X-100, 4% (w/v) polyethylene glycol (mol. wt. 6,000) and 0.1 M NaCl were added to the supernatant and the mixture was stirred for 1 hr. The suspension was centrifuged at $7,000 \times g$ for 15 min. The pellet was resuspended in 100 ml of 0.5 M PB, containing 0.5 M urea (pH 7.5), and stirred gently for 30 min. After centrifugation ($7,000 \times g$ for 15 min), the supernatant was placed on a 2 ml cushion of 20% sucrose in 0.5 M PB containing 0.5 M urea (pH 7.5) and centrifuged at 23,000 rpm for 90 min in a Beckman SW 27 rotor. The pellet was resuspended in 3 ml of 0.5 M PB containing 0.5 M urea (pH 7.5), then layered on 10~40% sucrose density gradients and centrifuged at 23,000 rpm for 120 min (Beckman SW 27). The virus zone was collected and concentrated by high speed centrifugation.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE in 2 mm thick polyacrylamide slab gel (4% stacking gel and 12% separation gel) was done by the method of Laemmli¹²⁾. For molecular weight estimation, SDS-degraded virus protein was co-electrophoresed with the Pharmacia marker proteins (phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and α -lactalbumin) and bean yellow mosaic virus coat protein at 60 V for 6 hr.

Serology. Antiserum to LYSV was prepared by injecting a rabbit intravenously with partially purified virus and followed by five intramuscular injections of the virus emulsified with Freund's incomplete adjuvant, at 7~10-day intervals. Per injection the virus obtained from 100~150 g of plant materials was administered. The rabbit was bled 3 wk after the final injection. The antiserum was absorbed with concentrated extracts from non-inoculated *C. quinoa* leaves before use. Antiserum titer was determined by the microprecipitin test. Other antisera were kindly donated by the following: bean yellow mosaic virus (BYMV) and clover yellow vein virus (CYVV), A. A. Brunt, U.K.; freesia mosaic virus (FMV), LYSV and OYDV, D. Z. Maat, the Netherlands; iris mild mosaic virus (IMMV) and iris severe mosaic virus (ISMV), A. F. L. M. Derks, the Netherlands; narcissus yellow stripe virus (NYSV), M. Kameya-Iwaki, Japan; turnip mosaic virus (TuMV), H. Tochiwara, Japan, and potato virus Y (PVY), D. E. Purcifull, U.S.A. Double diffusion tests were made in a medium consisting of 0.8% agar (Difco, Agar Noble), 0.5% sodium dodecyl sulfate (SDS), and 1% sodium azide (w/v) in deionized water.

Some serological reactions were checked by immunoelectron microscopical decoration test.

Electron microscopy. Electron microscopy was applied to the preparations using a Hitachi HU-12. Preparations of crude sap and purified virus were observed after negative staining with 1% aqueous phosphotungstic acid (PTA), pH 7.0. For thin sectioning, tissue samples of LYSV-infected *C. quinoa* leaves, showing chlorotic local lesions, were fixed in 6% glutaraldehyde in 0.1 M PB for 2 hr, followed by post-fixation in 1% osmium tetroxide for 2 hr at 4 C. After dehydration, the tissues were embedded in epoxy resin. Thin sections were cut with glass-knives and post-stained with uranyl acetate and lead citrate.

RESULTS

Host range and symptoms

The virus from *Allium ampeloprasum* cv. "Murasame" was transmitted by inoculation with sap to 6 of 49 species from 4 of 11 families (Table 1). Severe mosaic symptoms were produced systemically on *A. ampeloprasum* cv. "Miyako" and light mosaic symptoms were produced systemically on *A. giganteum* (Plate I-2). Local lesions were produced on *Chenopodium quinoa* and *C. amaranticolor*, showing chlorotic spots. The small vague local chlorotic spots were produced about 7 days after inoculation, but soon thereafter became more clear without further enlarging (Plate I-3). On *C. amaranticolor*, the local lesions were often appeared as pinpoint dry lesions. Latent systemic infection was produced on *Spinacia oleracea* and latent local infection was on *Gomphrena globosa*.

No infection was obtained on *Allium cepa*, *A. chinense* and *A. fistulosum* and the same held for all other 40 plant species or cultivars tested, viz. *Tetragonia expansa*, *Beta vulgaris* var. *cicla*, *Chenopodium murale*, *Callistephus chinensis*, *Chrysanthemum coronarium*, *Zinnia elegans*, *Raphanus sativus*, *Brassica campestris*, *B. rapa* var. *komatsuna*, *Cucumis melo* var. *conomon*, *C. sativus*, *Cucurbita moschata*, *C. maxima*, *Citrullus vulgaris*, *Zea mays*, *Sesamum indicum*, *Lilium longiflorum*, *Tulipa gesneriana*, *Astragalus sinicus*, *Glycine max*, *Pisum sativum* (Kinuzaya, France), *Vicia faba*, *Phaseolus angularis*, *P. vulgaris* (Kurosando, Top Crop, Satsukimidori), *Vigna sinensis* (Kurodane-Sanjaku, Akadane-Sanjaku), *V. radiata*, *Nicotiana tabacum* (Samsun, White Burley), *N. clevelandii*, *N. glutinosa*, *N. rustica*, *Petunia hybrida*, *Datura stramonium*, *Lycopersicon esculentum*, *Solanum melongena*, and *Hyoscyamus niger*.

Aphid transmission

The virus was transmitted by *Myzus persicae* and *Aphis gossypii* in a non-persistent manner after acquiring period for 5 min, using local lesion assay on the leaves of *C. quinoa*.

Properties in crude sap

Sap from *C. quinoa* showing local lesions was infective to *C. quinoa* after dilution to 10^{-3} but not 10^{-4} , after 10-min heating at 50 C but not 55 C, and after 4 days at 20 C but not 8 days.

Table 1. Susceptible hosts and symptoms of LYSV

Host species	Symptoms ^{a)}	
	Inoculated leaves	Uninoculated leaves
<i>Allium ampeloprasum</i> cv. "Miyako"	Mo	Mo
<i>A. giganteum</i>	Mo	Mo
<i>Gomphrena globosa</i>	l	—
<i>Spinacia oleracea</i>	l	l
<i>Chenopodium amaranticolor</i>	CS-NS	—
<i>C. quinoa</i>	CS	—

a) Symptoms represent: CS, chlorotic spots; NS, necrotic spots; l, latent infection; Mo, mosaic; —, not infected.

Electron microscopy

Leaf-dip preparations from the infected plants contained elongated flexuous filamentous particles. Measurements of 290 flexuous rods revealed that about 70% of them were 650~850 nm long, with a principal frequency peak at 820 nm. Plate I-4 shows an electron micrograph of purified preparations of LYSV. Fragments of cylindrical inclusions were observed in the leaf-dip preparations from diseased plants (Plate I-5). The plates of the inclusions showed a pattern of fine linear striations with a periodicity of approximately 5 nm, as observed on inclusions induced by other potyviruses.

Serology

In a microprecipitin test, LYSV antiserum donated from Ir. D. Z. Maat (titer: 1/1,024) reacted with LYSV up to dilution of 1/512. The antiserum to LYSV in this study, after absorption with clarified sap of healthy *C. quinoa*, reacted up to dilution of 1/512 with the homologous antigen. The antiserum reacted with a clear precipitin line in the agar gel double-diffusion test with sap from infected plants but not from uninfected ones (Plate I-6). Immunoelectron microscopical test showed LYSV reacted strongly with the homologous antiserum (Plate I-7) and LYSV antiserum donated from Ir. D. Z. Maat but not with antisera to BYMV, CYVV, FMV, IMMV, ISMV, NYSV, OYDV, TuMV and PVY.

Molecular weight of coat protein

SDS-PAGE of virus protein showed that LYSV consisted of one species of polypeptide which was estimated to have mol. wt. of 36,500 daltons (Plate II-1). Co-electrophoresed BYMV protein (34,500 daltons) was separated clearly from LYSV protein in polyacrylamide gel.

Ultrastructure of infected plants

Plate II-2, 3, 4 and 5 show the ultrastructures of mesophyll cells of diseased *C. quinoa*. In cytoplasm of infected plant cells, cytoplasmic inclusions were observed. The inclusions appeared as pinwheels, scrolls and laminated aggregates. Virus particles were dispersed or clustered in the cytoplasm.

DISCUSSION

Garlic mosaic virus (GMV)¹³, garlic yellow streak virus (GYSV)¹⁵, leek yellow stripe virus (LYSV)^{2-4,7,16,17} and onion yellow dwarf virus (OYDV)^{1,4,14} are known as potyviruses occurring in vegetable *Allium* plants. In Japan, only GMV and OYDV were found until now. A potyvirus isolated from an ornamental *Allium ampeloprasum* cv. "Murasame" showing mosaic and white necrosis on the leaves was identified as LYSV. LYSV is distinguished from OYDV by its host range. LYSV produces local lesions on *C. amaranticolor* and *C. quinoa*, local latent infection on *G. globosa* and is poorly infectious to onion (*A. cepa*) and welsh onion (*A. fistulosum*)^{2-4,7}, whereas OYDV does not infect *C. amaranticolor* and *C. quinoa* and easily infects onion and welsh onion^{1,4,14}. LYSV is also different in host range from GMV, which produces latent local infection on *C. amaranticolor* and *C. quinoa*¹³. Recently Mohamed and Young¹⁵ reported a potyvirus, GYSV, from garlic (*A. sativum*) in New Zealand. GYSV differed from LYSV in host range as it does not infect *C. amaranticolor* and *C. quinoa* and infects *Tetragonia expansa* and *C. murale*. The virus reported here is very similar to LYSV² in host range since both produce local lesions on *C. amaranticolor* and *C. quinoa* and local latent infection on *G. globosa*. However, unlike LYSV reported previously, the isolate was found to infect *Spinacia oleracea* systemically without symptoms. The modal length of the virus particles was observed to be 820 nm, which is consistent with that of LYSV² though it is slightly longer than those of many potyviruses including OYDV (755 nm)¹³, GMV (750 nm)¹³ and GYSV (755 nm)¹⁵.

In immunoelectron microscopical tests, LYSV reacted with LYSV antiserum donated from D. Z. Maat and homologous antiserum, but it was not serologically related with BYMV, CYVV, FMV, IMMV, ISMV, NYSV, OYDV, PVY and TuMV tested.

Huttinga¹⁰ purified LYSV from infected leek (*A. ampeloprasum*) leaves using molecular

sieving on Sephadex G-200 column to eliminate the contamination of mucilage of leek leaves. We purified the virus from *C. quinoa* leaves by adopting a common method using PEG-precipitation for concentrating virus. Molecular weight (36,500 daltons) of the coat protein subunit was slightly higher than 34,000 daltons of LYSV described by Huttinga¹⁰⁾ and 34,500 daltons of BYMV¹¹⁾.

LYSV induces the cylindrical inclusions^{8,9)} in mesophyll cells. The structure of the cylindrical inclusions of this virus showed pinwheels, scrolls and short curved laminated aggregates in the cytoplasm. According to the classification of Edwardson^{5,6)}, the ultrastructure of the inclusion was assigned to subdivision IV of potyvirus.

LYSV occurs on leek in a wide part in Europe and causes a serious damage specially in autumn and winter, but it has not been found in Japan on neither leek nor any other plants.

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和 文 摘 要

野田千代一・井上成信：観賞用 *Allium* から分離された leek yellow stripe virus

1986年に岡山県倉敷市玉島で、葉に明瞭なモザイク斑と灰白色のえそ斑とを示す観賞用のアリウム属の *Allium ampeloprasum* から potyvirus が分離された。本ウイルスはモモアカアブラムシおよびワタアブラムシで非永続的に伝搬された。汁液接種が容易で、供試した13科46種の植物のうち *A. giganteum* に全身感染して軽いモザイク症状を生じ、*Chenopodium quinoa* と *C. amaranticolor* とに局部感染、またホウレンソウに無病徴全身感染、センニチコウに無病徴局部感染したが、ネギとタマネギには感染しなかった。不活化温度は50~55℃(10分)、希釈限界は 10^{-3} ~ 10^{-4} 、保存期限は4~8日であった。DN法観察によると、本ウイルスは多くの potyvirus よりやや長い820nmの屈曲したひも状であった。0.5Mリン酸緩衝液で磨砕後、クロロホルム-四塩化炭素で清澄化、PEGでの濃縮、クッション遠心分離およびショ糖密度勾配遠心分離を採用することで純化を行った。SDS-ポリアクリルアミドゲル電気泳動による外被タンパク質の分析では分子量36,500ダルトンの1本のバンドが認められた。感染植物葉の超薄切片像には、細胞質内に散在または集塊するウイルス粒子と pinwheel, scroll, short curved laminated aggregate などの細胞質内封入体が多数認められた。免疫電顕法によると、本ウイルスは bean yellow mosaic virus, clover yellow vein virus, freesia mosaic virus, iris mild mosaic virus, iris severe mosaic virus, potato virus Y, narcissus yellow streak virus, onion yellow dwarf virus, turnip mosaic virus の各抗血清とは反応せず、オランダの D. Z. Maat 氏より分譲された leek yellow stripe virus 抗血清とのみ反応した。以上の結果から、本ウイルスは leek yellow stripe virus (LYSV) と同定された。

Explanation of plates

Plate I.

1. Symptoms in *Allium ampeloprasum* naturally infected with LYSV, showing mosaic and white necrosis.
2. *A. giganteum* inoculated with LYSV, showing mild mosaic symptom on an uninoculated upper leaf.
3. *Chenopodium quinoa* inoculated with LYSV, showing chlorotic spots on an inoculated leaf.
4. Electron micrograph of purified preparations of LYSV negatively stained in PTA. Bar represents 200 nm.
5. Electron micrograph of cytoplasmic inclusions in leaf dip preparations, showing a striation pattern. Bar represents 200 nm.
6. Double-diffusion test of LYSV antiserum (AS) with crude sap extracts from healthy (H) and LYSV infected (V) *C. quinoa*.
7. Electron micrograph of leaf dip preparations of LYSV incubated with a 1/60 dilution of antiserum to LYSV for 30 min before negative staining. Bar represents 200 nm.

Plate II.

1. SDS-polyacrylamide gel electrophoresis of coat protein subunit of LYSV. Lane a: marker proteins of molecular weight containing (from top) phosphorylase b (94,000 daltons), bovine serum albumin (67,000 daltons), ovalbumin (43,000 daltons), carbonic anhydrase (30,000 daltons), soybean trypsin inhibitor (20,100 daltons) and α -lactalbumin (14,400 daltons). Lane b: BYMV coat protein (B) and LYSV coat protein (L). Lane c: LYSV coat protein (L) only.
- 2~5. Thin sections of leaf cells of *C. quinoa* infected with LYSV, showing tubular inclusions, pin-wheels, scrolls, short curved laminated aggregates (2~3) and aggregates of virus particles (4). Some inclusions can be seen as bundles attaching to cell wall (5). Bars represent 50 nm (2, 3, 5) and 500 nm (4).

Plate I

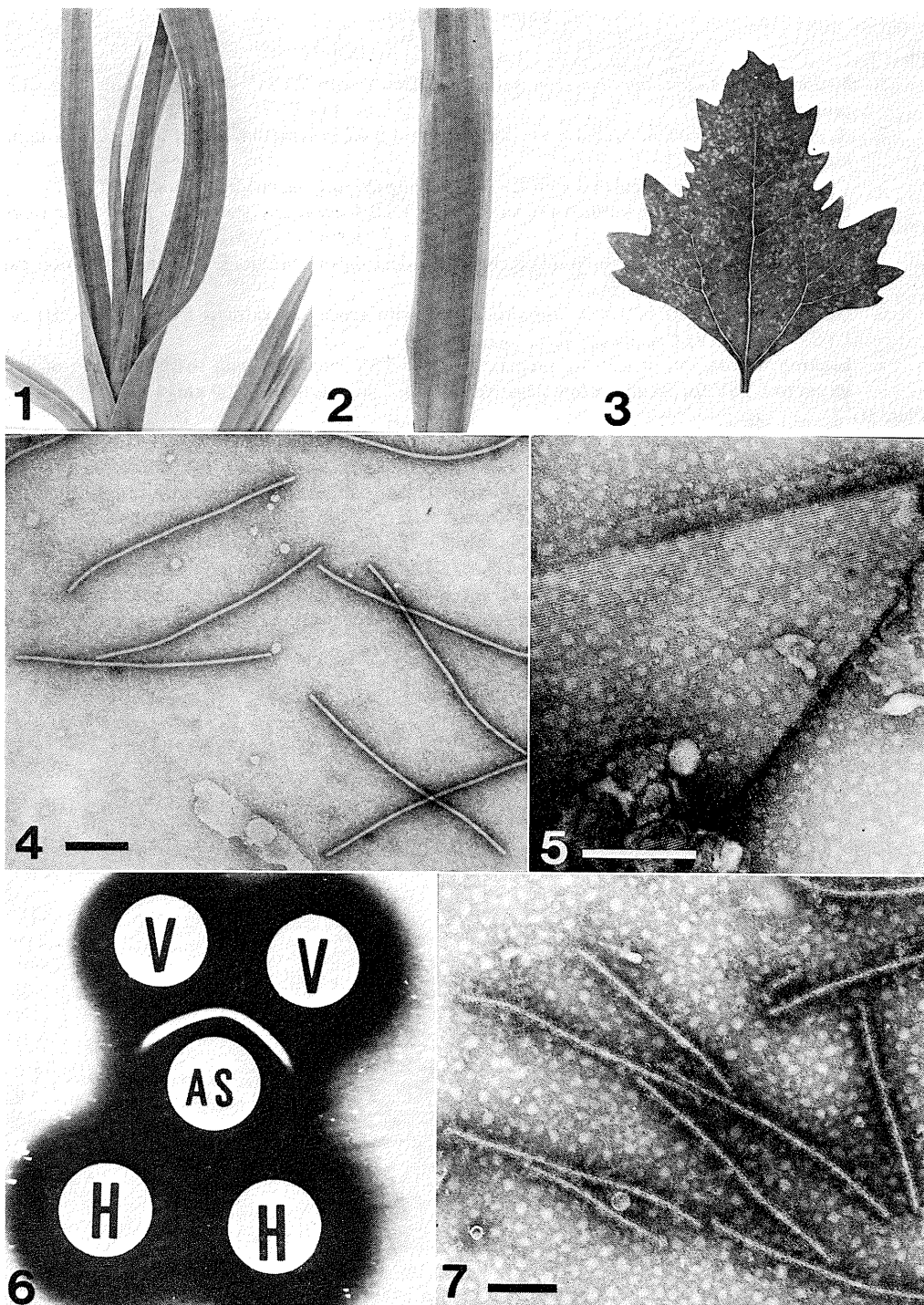


Plate II

