Host Range and Some Properties of Asparagus Virus 1 Isolated from *Asparagus officinalis* in Japan

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分離された asparagus virus 1 について

Abstract

A virus was isolated from symptomless asparagus plants (*Asparagus officinalis*) in Hokkaido. The virus was sap transmissible to 7 species belonging to 4 families, and caused local lesions on *Chenopodium* spp., *Tetragonia expansa*, *Spinacia oleracea* and *Gomphrena globosa*. The virus was transmitted by *Myzus persicae* in a non-persistent manner. Sap from infected *C. quinoa* was infective after heating for 10 min at 50 C but not 55 C, after dilution to 10^{-3} but not 2×10^{-4} , and after 8 days but not 11 days at 20 C. The virus particles were elongated flexuous rods, with a modal length of 746 nm $\times$ 13 nm. On the basis of the symptomatology, host range, transmission mode, physical properties, and particle morphology, the virus was identified as asparagus virus 1 described by Hein (1960). Ultraviolet absorption spectrum of purified virus preparation was characteristic of that of nucleoprotein with an A 260/280 ratio of 1.24. The titer of the antiserum against the virus was 1/512 in ring interface precipitin test. This antiserum reacted positively with the purified virus, but not with some other potyviruses in SDS-agar gel double diffusion test. However, in ring interface precipitin test, the antiserum reacted weakly with turnip mosaic virus, bean yellow mosaic virus and lettuce mosaic virus. Ultrathin sections of infected asparagus leaves showed cytoplasmic inclusions of pinwheel, bundle and laminated aggregates.

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Key Words : asparagus virus 1, asparagus.

Introduction

Several viruses have been isolated from asparagus (*Asparagus officinalis*) in Europe and North America. Hein^{8,9,10} reported that asparagus virus 1 (AV1) and asparagus virus 2 (AV2) were widespread in Germany, occurring separately or together, although neither virus produced any symptoms on asparagus. It has been demonstrated that AV1 has a limited host range, consists of flexuous filamentous particles 754 nm in length, and is transmitted by aphids¹⁰. Recently, a virus similar to AV1 has been described in the U. S. A.¹¹ There are no reports of viruses occurring on asparagus in Japan. A virus was isolated from asparagus, and identified as a strain of AV1,

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which have first been described in Japan. This reports mainly presents the host range, physical properties, purification method, serological characteristics and electron microscopic observation of the virus isolated from asparagus.

Materials and Methods

Virus source and maintenance. During field surveys of virus diseases in Hokkaido, a virus isolated from symptomless asparagus plants was tentatively designated as asparagus virus 1-J (AV1-J). The virus was maintained with asparagus plants and *Chenopodium quinoa* in a greenhouse. Mechanical inoculation was carried out using a glass spatula dipped in sap of infected leaves on carborundum dusted fully expanded cotyledons or leaves of test plants. The sap was prepared by grinding infected leaves in a mortar with 0.1 M phosphate buffer, pH 7.0, containing 0.1% thioglycolic acid (approximately 1:5 w/v). Inoculated plants were kept under observation for 20 days and checked for the presence of the virus by back-inoculation to *C. amaranticolor*. Five other viruses belonging to the potyvirus group, *i.e.* bean yellow mosaic virus (BYMV)³⁾, beet mosaic virus (BMV)⁴⁾, lettuce mosaic virus (LMV)⁶⁾, potato virus Y (PVY)¹²⁾ and turnip mosaic virus (TuMV)⁶⁾, were used for serological comparison with AV1-J.

Purification. Infected asparagus leaves were homogenized in 0.5 M borate buffer, pH 8.0, containing 0.05 M ethylenediaminetetraacetic acid (EDTA) and 0.1% thioglycolic acid, and chloroform at the rate of 1:1:1 (w/v/v). After low-speed centrifugation (5,000 rpm for 10 min in a Hitachi RPR 12-2 rotor), n-butanol was added to the aqueous phase up to a final concentration of 8%, and stirred for 1 hr. The mixture was centrifuged at 8,000 rpm for 10 min, and then the supernatant was centrifuged at 40,000 rpm for 90 min in an International 410 rotor. The resulting pellets were each dissolved in 0.05 M borate buffer, pH 8.0, containing 0.002 M EDTA (resuspending buffer), and the pooled suspension was centrifuged at 40,000 rpm for 90 min. The resulting pellets were dissolved in the resuspending buffer. After low-speed centrifugation (8,000 rpm for 10 min), these suspensions were layered onto 10-40% sucrose density-gradients in suspending buffer, and the gradients were centrifuged at 24,000 rpm for 120 min in an International SB110 rotor. The resulting opalescent band was collected by using an ISCO density-gradient fractionator. Infectivity of the band was evaluated by inoculation to *C. amaranticolor*.

Serology. Antiserum against the virus was obtained by subjecting a rabbit to three intramuscular injections of 1 ml of purified AV1-J (0.5 mg/ml in saline) emulsified with an equal volume of Freund's complete adjuvant at 3-week intervals. Blood was collected from the rabbit 3 weeks after the final injection. For comparison with this virus, each of the antisera against TuMV, BYMV, LMV, BMV and PVY was prepared using the same procedure as mentioned above. SDS-immunodiffusion test was conducted according to the method of Gooding and Bing⁷⁾.

Electron microscopy. The purified virus and crude leaf extract were negatively stained with 2% phosphotungstic acid, pH 7.0, and examined under a Hitachi HU12 electron microscope. Small pieces of systemically infected leaves of asparagus and locally infected leaves of *C. amaranticolor* were fixed with 2.5% glutaraldehyde in

Millonig's buffer for 3 hr at 4 C, and washed with the same buffer. The specimens were postfixed with 2 % osmium tetroxide in Millonig's buffer for 3 hr at 4 C. After washing and dehydration, the specimens were embedded in spurr resin. Thin sections stained with uranyl acetate and lead citrate were examined under an electron microscope.

Results

Occurrence and symptoms in asparagus

Asparagus plants growing in Hokkaido seldom showed definite symptoms, although some showed faint, light-green mosaic symptoms on the leaves. Asparagus leaf samples were collected from various localities in Hokkaido during the period 1979 to 1981, and examined by inoculation to indicator plants and by electron microscopy. Three out of the 89 samples collected caused symptoms on indicator plants, which resembled those induced by AV1-J, and one of the samples proved to be doubly infected with AV1-J and AV2³).

AV1-J failed to infect asparagus seedlings after mechanical inoculation with the extract of infected asparagus leaves or locally infected leaves of *C. quinoa*. On the other hand, asparagus plants were readily infected when the spears were inoculated with partially purified AV1-J. The infected asparagus plants, however, failed to demonstrate any distinct leaf symptoms or plant stunting throughout the close observations in a greenhouse for 2 years.

Host range and symptomatology

Fifty-six herbaceous species belonging to 13 families were inoculated, and only 7 species from 4 families became infected (Table 1). The virus induced characteristic local lesions without subsequent systemic infection on *Tetragonia expansa*, *Spinacia oleracea*, *Gomphrena globosa*, and three species of *Chenopodium*. Circular necrotic lesions 2-3 mm in diameter were produced on *S. oleracea* and *C. amaranticolor* about 7-10 days after inoculation (Plate I. 1 and 2). *T. expansa* developed faint chlorotic lesions 5-7 mm in diameter 15 days after inoculation (Plate I. 3).

Aphid transmission

Myzus persicae and *Aphis gossypii* were used for tests of aphid transmission. Aphids were starved for 2 hr and then fed on infected asparagus for 15 min, and 15 aphids were transferred to each healthy asparagus seedling. The aphids were killed after remaining on the plant overnight. AV1-J was transmitted to seven of ten plants by *M. persicae*, but not by *A. gossypii*.

Properties in vitro

In crude sap from locally infected leaves of *C. quinoa*, the virus had a dilution end point ranging between 10^{-3} and 2×10^{-4} , when diluted with 0.1 M phosphate buffer (pH 7.0), and retained its infectivity for 8 to 11 days at 20 C. It was infective after heating for 10 min at 50 C but not at 55 C.

Table 1. Host range of asparagus virus 1-J isolated from asparagus

Plants susceptible	
Liliaceae	<i>Asparagus officinalis</i>
Chenopodiaceae	<i>Spinacia oleracea</i> , <i>Chenopodium amaranticolor</i> , <i>C. quinoa</i> , <i>C. capitatum</i>
Amaranthaceae	<i>Gomphrena globosa</i>
Aizoaceae	<i>Tetragonia expansa</i>
Plants insusceptible	
Liliaceae	<i>Lilium elegans</i> , <i>Allium fistulosum</i> , <i>A. cepa</i> , <i>A. tuberosum</i>
Chenopodiaceae	<i>Beta vulgaris</i> , <i>B. vulgaris</i> var. <i>cicla</i>
Solanaceae	<i>Nicotiana tabacum</i> (Samsun, Bright Yellow, Xanthi nc), <i>N. glutinosa</i> , <i>N. sylvestris</i> , <i>N. clelandii</i> , <i>N. benthamiana</i> , <i>Petunia hybrida</i> , <i>Datura stramonium</i> , <i>Lycopersicon esculentum</i> , <i>Capsicum frutescens</i> , <i>Solanum tuberosum</i>
Cucurbitaceae	<i>Cucurbita pepo</i> , <i>Cucumis sativus</i>
Cruciferae	<i>Brassica rapa</i> var. <i>Komatsuna</i> , <i>B. rapa</i>
Amaranthaceae	<i>Celosia cristata</i> , <i>Amaranthus retroflexus</i>
Pedaliaceae	<i>Sesamum indicum</i>
Leguminosae	<i>Phaseolus vulgaris</i> , <i>P. angularis</i> , <i>Pisum sativum</i> , <i>Vicia faba</i> , <i>Vigna sesquipedalis</i> , <i>Glycine max</i>
Compositae	<i>Chrysanthemum coronarium</i> , <i>Lactuca sativa</i> , <i>Zinnia elegans</i>
Umbelliferae	<i>Daucus carota</i> , <i>Apium graveolens</i> , <i>Cryptotaenia canadensis</i>
Gramineae	<i>Zea mays</i>
Caryophyllaceae	<i>Dianthus caryophyllus</i>

Purification

Infectivity was restricted to the resulting opalescent band purified by the procedure mentioned above. In electron micrographs of the purified virus preparations, numerous flexuous rods about 750 nm in length were observed (Plate I. 5). The ultraviolet absorption spectrum of purified virus that showed maximum values at 260 nm and minimum values at 247 nm was typical of that of nucleoprotein. A 260/280 ratio was 1.24.

Serology

The homologous titers of the antisera used in the present study were as follows: AV1-J 1/512, TuMV 1/1024, LMV 1/1024, BYMV 1/1024, PVY 1/1024 and BMV 1/512. SDS-immunodiffusion test and ring interface precipitin test were carried out to determine the serological relationships between AV1-J and five other potyviruses. In SDS-agar gel plate, AV1-J antiserum reacted positively with partially purified AV1-J and crude sap of infected leaves, but did not react with TuMV, BYMV, LMV, BMV, and PVY. However, in ring interface precipitin test, TuMV, BYMV and LMV reacted with diluted AV1-J antiserum at the titers of 1/16, 1/8 and 1/16, respectively. PVY or BMV failed to react with undiluted AV1-J antiserum. In the ring interface precipitin test, AV1-J reacted with each of the diluted antisera against TuMV, LMV and BYMV at the titers of 1/64, 1/64 and 1/8, respectively, but not with the antisera against PVY or BMV.

Electron microscopy

Filamentous particles of AV1-J were readily observed in leaf dip-preparations from asparagus. One hundred sixty-two particles were measured, using tobacco mosaic virus as standard. The distribution of particle length is shown in Fig. 1. In forty-five percent of the particles the length ranged between 700 and 750 nm, with a modal length of 746 nm and the width of the particle was 13 nm (Plate I. 4). In purified preparations, particles with the modal length and also fragmented particles were contained (Plate I. 5). In thin sections of asparagus and *C. amaranticolor* infected with AV1-J, cytoplasmic inclusions were found (Plate I. 6, 7 and Plate II. 3), besides the presence of characteristic

Fig. 1. Length distribution of asparagus virus 1-J particles in negatively stained leaf dip-preparation from infected asparagus

vesicular structures (Plate I. 6). Viruslike particles were found to be scattered in the periphery of the vesicular structures in the cytoplasm (Plate II. 1 and 2) or regularly distributed along the arms of the pinwheels (Plate II. 3). No viruslike particles or cytoplasmic inclusions were observed in healthy asparagus tissues.

Discussion

So far, several viruses have been isolated from asparagus plants such as asparagus virus 2^{9,11,13}), tobacco streak virus^{1,11}), cucumber mosaic virus¹⁵), tobacco mosaic virus²), arabis mosaic virus¹⁴), strawberry latent ring spot virus¹⁴), and tomato black ring virus¹⁴). In 1960, filamentous virus particles were isolated from asparagus in Germany for the first time, and designated as asparagus virus 1 (AV1)⁸). On the basis of the morphology of the virus particles, types of inclusions induced by the virus, and mode of aphid-transmission, it was considered that AV1-J belonged to the potyvirus group. AV1-J had a limited host range, and induced characteristic local lesions without subsequent systemic infection on hosts except for asparagus plants. Although the infectivity to *S. oleracea* and *B. vulgaris*, and physical properties were slightly different from those of AV1, AV1-J reported here closely resembled AV1 described by Hein¹⁰).

In SDS-agar gel double diffusion tests, no reaction was observed between AV1-J antiserum and TuMV, BYMV, LMV, BMV and PVY, respectively. In the ring interface precipitin tests, however, AV1-J antiserum reacted weakly with TuMV, LMV and BYMV. Furthermore, AV1-J reacted weakly with the antisera against TuMV, LMV and BYMV. From these results, it is considered that AV1-J might be serologically related to TuMV, LMV and BYMV, but not to PVY and BMV.

Although infection with AV1 was reported to cause yield reduction of asparagus¹⁰), infection with AV1-J did not appear to any visible symptoms on plants either in greenhouse or field. Since asparagus plant is a long-life crop, we are currently

investigating the possible effect of AV1-J infection on asparagus growth.

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Literature cited

1. Brunt, A. A. and Paludan, N. (1970). *Phytopathol. Z.* 69: 277-282.
2. Faccioli, G. (1965). *Phytopath. Mediterranea* 4: 163-167. (Rev. Appl. Mycol. 46: 158. 1967).
3. Fujisawa, I. and Tsuchizaki, T. (1979). *Ann. Phytopath. Soc. Japan* 45: 566. (Abstr. in Japanese).
4. Fujisawa, I. and Tsuchizaki, T. (1980). *Ibid.* 46: 422 (Abstr. in Japanese).
5. Fujisawa, I., Goto, T. and Tsuchizaki, T. (1980). *Ibid.* 46: 100 (Abstr. in Japanese).
6. Fujisawa, I. and Iizuka, N. (1982). *Ibid.* 48: 121 (Abstr. in Japanese).
7. Gooding, G. V., Jr. and Bing, W. W. (1970). *Phytopathology* 60: 1293 (Abstr.).
8. Hein, A. (1960). *Z. Pflanzenkrankh.* 67: 217-219.
9. Hein, A. (1963). *Mitt. Biol. Bundesanst. Land-Forstwirtschaft. Berl.-Dahlem.* 108: 70-74 (Rev. Appl. Mycol. 43: 455. 1964).
10. Hein, A. (1969). *Z. Pflanzenkrankh.* 76: 395-406.
11. Mink, G. I. and Uyeda, I. (1977). *Plant Dis. Repr.* 61: 398-401.
12. Oshima, N. (1951). *Ann. Phytopath. Soc. Japan* 15: 121-126 (in Japanese).
13. Paludan, N. (1964). *Maanedsovers. Plantesygd.* 407: 11-16 (Rev. Appl. Mycol. 43: 559. 1964).
14. Posnette, A. F. (1969). *J. hort. Sci.* 44: 403-406.
15. Schade, C. (1969). *NachrBl. dt. Pflschtzdienst. Berl.*, 23: 38-40 (Rev. Plant Pathol. 49: 56. 1970).

和文摘要

アスパラガスから分離された asparagus virus 1 について

藤沢一郎・後藤忠則・土崎常男・飯塚典男

北海道内の外観健全なアスパラガスから検出されたひも状ウイルスの諸性質を調べた。本ウイルスは、汁液接種した13科56種の植物のうち、アスパラガスに全身感染し、*C. amaranticolor*, *C. quinoa*, *C. capitatum*, ツルナ、センニチコウ、ハウレンソウに局部感染した。また本ウイルスはモモアカアブラムシにより非永続的に伝搬され、粗汁液中での不活化温度は50~55℃(10分)、希釈限界は $2 \times 10^{-4} \sim 10^{-3}$ 、保存期限は8~11日(20℃)であった。ウイルス粒子は 746×13 nmで、感染植物細胞の超薄切片像で細胞質中に散在するウイルス粒子が認められ、また pinwheel, bundle, laminated aggregates などの細胞質封入体と vesicle からなる膜状封入体が観察された。純化ウイルスを用いて作製した抗血清の力価は1/512(重層法による)であった。本抗血清は、SDSを含む寒天ゲル内で、純化ウイルスおよび感染葉粗汁液と明瞭な反応を示し、重層法ではカブモザイクウイルス、インゲン黄斑モザイクウイルスおよびレタスモザイクウイルスと違い関係だが反応が認められた。以上の諸結果から本ウイルスは既報の asparagus virus 1 の1系統と同定された。

Explanations of Plates**Plate I.**

1. Necrotic local lesions induced by asparagus virus 1-J on *Spinacia oleracea*.
2. Necrotic local lesions induced by asparagus virus 1-J on *Chenopodium amaranticolor*.
3. Faint chlorotic local lesions induced by asparagus virus 1-J on *Tetragonia expansa*.
4. Asparagus virus 1-J particles in leaf dip preparation mixed with tobacco mosaic virus. Bar represents 500 nm.
5. Purified asparagus virus 1-J particles. Bar represents 500 nm.
- 6-7. Ultrastructure of asparagus virus 1-J infected leaf cell of asparagus. Pinwheel and bundle type inclusions within characteristic vesicular structure in the cytoplasm. Bar represents 500 nm.

Plate II.

- 1-2. Ultrastructure of asparagus virus 1-J infected leaf cell of asparagus. Viruslike particles in the cytoplasm. Bar represents 500 nm.
3. Ultrastructure of leaf cell of *C. amaranticolor* locally infected with asparagus virus 1-J. Pinwheel, laminated aggregates type inclusions. Bar represents 500 nm.

Plate I

Plate II