

アスパラガスから分離された Potexvirus : asparagus virus III

誌名	日本植物病理學會報 = Annals of the Phytopathological Society of Japan
ISSN	00319473
著者名	藤澤, 一郎
発行元	日本植物病理學會
巻/号	52巻2号
巻号補足	
掲載ページ	p. 193-200
発行年月	1986年4月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター

Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council
Secretariat



Asparagus Virus III : a New Member of Potexvirus from Asparagus

Ichiro FUJISAWA

Abstract

A potexvirus which had not been described previously, was isolated from *Asparagus officinalis* in Japan and the name asparagus virus III (AVIII) is proposed. AVIII infected 26 species from 8 families by sap inoculation, out of 37 species from 11 families tested. Artificially infected asparagus plants did not show any distinct leaf symptoms in the greenhouse for 2 years. The virus was not transmitted by aphids and through seeds of infected asparagus. Sap from infected *Nicotiana megalosiphon* was infective after heating for 10 min at 55 C but not at 60 C, after dilution to 2×10^{-4} but not 10^{-4} , and after 21 days but not 23 days at 20 C. The virus particles were elongated flexuous rods, with a modal length of 580 nm $\times$ 13 nm. Ultraviolet absorption spectrum of purified virus preparation was characteristic of that of nucleoprotein with an A 260/280 ratio of 1.18-1.19. The titer of the antiserum against the virus was 1/1024 in ring interface precipitin test. This antiserum reacted positively with AVIII but not with potato virus X (PVX) in SDS-agar gel immunodiffusion test. In tests with immune electron microscopy using the antibody decorating technique, the virus reacted with antisera against cactus virus X and narcissus mosaic virus but not with antisera against white clover mosaic virus, PVX, clover yellow mosaic virus and tulip virus X. All of the properties described are consistent with AVIII being a distinct member of potexvirus group.

(Received September 17, 1985)

Key words : asparagus, asparagus virus III, potexvirus.

Introduction

Several viruses are known to infect asparagus (*Asparagus officinalis*). It was already reported that asparagus virus I (AVI) and asparagus virus II (AVII) had been isolated from asparagus plants in Hokkaido in Japan, occurring separately or together, although neither virus produced any distinct symptoms on asparagus^{3,4}. In the course of further surveys of asparagus fields in Hokkaido, filamentous particles with a length ranging from 500 to 600 nm which were isolated from *A. officinalis* exhibiting faint yellowing symptoms on young leaves were found to correspond to a potexvirus which had not been described previously and for which the name asparagus virus III (AVIII) is proposed. This paper describes the host range of the virus, some of its properties and its relationship with other members of the potexvirus group.

Materials and Methods

Virus isolate. The isolate of AVIII used throughout this work was obtained from Vegetable and Ornamental Crops Research Station, Ano, Mie, Japan 農林水産省野菜試験場

asparagus plants showing faint yellowing symptoms which were collected at Date, Hokkaido, Japan, in August 1982. The virus was maintained on *Nicotiana megalosiphon* throughout the present work.

Host range and physical properties. In host range studies, leaves of test plants were dusted with 400-mesh Carborundum and inoculated manually with infective *N. megalosiphon* sap. Inoculated plants were grown at 20–25 C and tested for infection by back-inoculation to *Chenopodium amaranticolor*. Assays to determine the *in vitro* properties of AVIII were made with *N. megalosiphon* and *C. amaranticolor* as described above.

Electron microscopy. For the determination of particle length, crude sap preparations from AVIII-infected *N. megalosiphon* and tobacco mosaic virus (TMV)-infected tobacco cultivar Samsun sap were mixed with 2% neutral sodium phosphotungstate. The TMV particles were assumed to be 300 nm long and served as an internal length standard. The method of Milne and Luisoni (1975) was applied for tests with immune electron microscopy (IEM) using the antibody decoration technique. Crude plant extracts were adsorbed to carbon-coated grids at room temperature. The grids that had floated on droplets of antiserum at a dilution of 1:100 or 1:50 were washed by distilled water, and then negatively stained with 2% sodium phosphotungstate pH 7.0. Small pieces of systemically infected leaves of *N. megalosiphon* were examined by thin-sectioning as described previously³⁾.

Virus purification. The virus was purified by homogenizing 100 g of infected *N. debneyi* leaves in 150 ml of 0.1 M K_2HPO_4 buffer, pH 9.4, containing 0.1% 2-mercaptoethanol, 1% Triton X-100 and 0.005 M ethylenediaminetetraacetic acid (EDTA). Equal volume of chloroform was added to the homogenate and blended for 5 min. The emulsion was centrifuged at 5,500 rpm for 15 min in a Hitachi RPR12-2 rotor, and polyethylene glycol-6,000 was added to the aqueous phase up to a final concentration of 4%, and stirred for 2 hr. The mixture was centrifuged at 9,000 rpm for 15 min, and the pellets were resuspended over a period of 2–4 hr in the extraction buffer without mercaptoethanol, and then the suspension was centrifuged at 8,000 rpm for 10 min. 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 Tris (Tris (hydroxymethyl) aminomethane), pH 7.5, containing 0.001 M EDTA. After low-speed centrifugation (9,000 rpm for 10 min), these suspensions were layered onto 10–40% sucrose density-gradients in suspending buffer, and the gradients were centrifuged at 25,000 rpm for 4 hr in an Hitachi SW27 rotor. The resulting opalescent band was collected by using an ISCO density-gradient fractionator. Infectivity of the band was evaluated by inoculation to tobacco plants (*N. tabacum* cv. Samsun).

Serology. Antiserum against the virus was obtained by subjecting a rabbit to three intramuscular injections of 1 ml of purified AVIII emulsified with an equal volume of Freund's complete adjuvant at 3-weeks intervals. Blood was removed from the rabbit 3 weeks after the last injection. Antisera to tulip virus X (TVX) and narcissus mosaic virus (NMV) were supplied by Dr. W. P. Mowat; antisera to clover yellow mosaic virus (CYMV) and cactus virus X (CaVX) by Dr. R. Koenig; antiserum to white clover mosaic virus (WCMV) by Dr. N. Iizuka; and potato virus X (PVX) and antiserum to

PVX by Dr. T. Goto.

Results

Symptoms in asparagus

When two small fields of asparagus plants in the locality of Date in Hokkaido were examined in 1982, some of the plants showed faint yellowing symptoms on young leaves. Three out of the 7 samples collected from both fields caused symptoms on indicator plants such as *N. tabacum* cv. Samsun, *N. benthamiana* and *C. amaranticolor*, which resembled those induced by AVIII, and the rest of the samples was confirmed to be doubly infected with AVII⁴⁾ and AVIII. Asparagus plants were readily infected when the spears were inoculated with partially purified AVIII. However, the infected asparagus plants failed to exhibit any distinct leaf symptoms in the greenhouse for two years.

Host range and symptomatology

Thirty-seven herbaceous species belonging to 11 families were inoculated, and 26 species from 8 families became infected (Table 1). Plants such as *C. amaranticolor*, tobacco, *N. megalosiphon* and *N. benthamiana* developed characteristic symptoms, which made them suitable as assay plants for the detection of the virus. On *C. amaranticolor*, a few chlorotic spots were formed on the inoculated leaves 7 days after inoculation, followed by clear vein yellow symptoms on the newly developed leaves (Plate I-1). In tobacco, the virus induced characteristic chlorotic lesions on the inoculated leaves 7 days after inoculation without subsequent systemic infection. *N. megalosiphon* developed chlorotic spots on inoculated leaves 5-7 days after inoculation, followed by clear systemic mosaic symptoms (Plate I-3 right). The virus induced distinct systemic vein necrosis in young leaves of *N. benthamiana* 5 days after inoculation, resulting occasionally in the death of the plants. The following species failed to develop symptoms when inoculated with AVIII and no viruses were detected in them by back-inoculation to indicator hosts; Liliaceae: *Allium cepa*, *A. fistulosum*; Solanaceae: *Lycopersicon esculentum*, *Petunia hybrida*; Leguminosae: *Phaseolus vulgaris*; Cruciferae: *Raphanus sativus*, *Brassica rapa*; Gramineae: *Zea mays*, *Triticum aestivum*; Cucurbitaceae: *Cucurbita pepo*, *Cucumis sativus*;

Properties in vitro

In the crude sap from systemically infected leaves of *N. megalosiphon*, the virus had a dilution end point ranging between 2×10^{-4} and 10^{-4} , when diluted with 0.1 M phosphate buffer (pH 7.0), and retained its infectivity for 21 to 23 days at 20 C. It was infective after heating for 10 min at 55 C but not at 60 C.

Electron microscopy

Electron micrographs of sap from infected plants, and of purified preparations showed filamentous particles (Plate I-4). Ninety-two particles were measured in leaf dip-preparations from *N. debneyi*. The distribution of the particle length is shown in Fig. 1. In fifty-four percent of the particles the length ranged between 570-590 nm, with a modal length of 580 nm and the width of the particle was 13 nm. In ultrathin sections of infected *N. megalosiphon* leaf material, masses of filamentous virus-like particles were observed within the cytoplasm (Plate I-6 and 7). Although scattered par-

Table 1. Host range and symptomatology of asparagus virus III

Plant species	Symptoms
Liliaceae	
<i>Asparagus officinalis</i>	SSI
Chenopodiaceae	
<i>Chenopodium amaranticolor</i>	LNS, SVC
<i>C. quinoa</i>	LNS, M
<i>C. murale</i>	LNS, SVC
<i>Spinacia oleracea</i>	LCS, SSI
<i>Beta vulgaris</i>	LCS, NS
Solanaceae	
<i>Nicotiana tabacum</i>	
cv. Blight Yellow	LNS, NS
cv. Ambalema	LNS, NS
cv. Samsun	LCS, NS
<i>N. clevelandii</i>	LNS, M
<i>N. debneyi</i>	LCS, MM
<i>N. rustica</i>	LCS, NS
<i>N. megalosiphon</i>	LCS, M
<i>N. glutinosa</i>	SLI, NS
<i>N. benthamiana</i>	LCS, SVN, D
<i>Solanum tuberosum</i>	SLI, NS
<i>Datura stramonium</i>	LCS, NS
Leguminosae	
<i>Vicia faba</i>	LNS, NS
<i>Pisum sativum</i>	LNS, NS
<i>Glycine max</i>	SLI, MM
<i>Vigna angularis</i>	SLI, SSI
Amaranthaceae	
<i>Gomphrena globosa</i>	LNS, NS
<i>Celosia cristata</i>	SLI, M
Compositae	
<i>Zinnia elegans</i>	LCS, NS
<i>Chrysanthemum coronarium</i>	LCS, NS
Aizoaceae	
<i>Tetragonia expansa</i>	LCS, SCR
Pedaliaceae	
<i>Sesamum indicum</i>	LNS, NS

Coded symptom descriptions: LCS=local chlorotic spots; LNS=local necrotic spots; SLI=symptomless local infections; NS=no systemic infection; SVC=systemic vein clearing; SVN=systemic vein necrosis; SCR=systemic chlorotic rings; M=mosaic; MM=mild mosaic; SSI=symptomless systemic infections; D=death of plant.

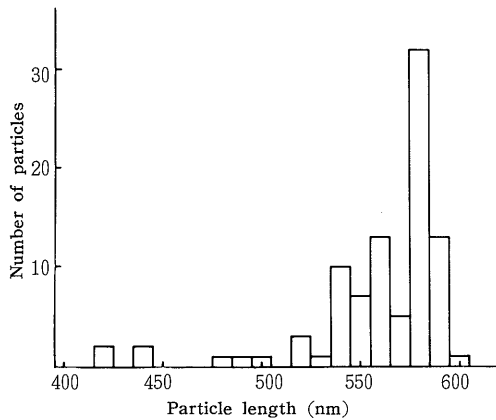


Fig. 1. Length distribution of asparagus virus III particles in negatively stained leaf dip-preparation from infected *Nicotiana debneyi*.

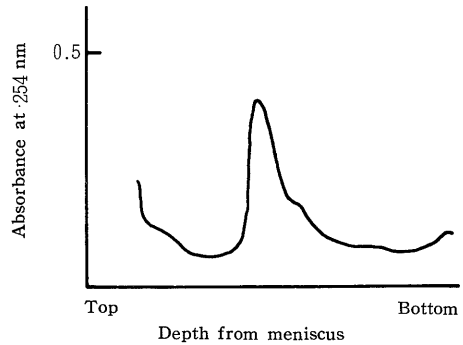


Fig. 2. Scanning pattern after sucrose density gradient centrifugation of partially purified asparagus virus III. Centrifugation was at 25,000 rpm for 4 hr in a Hitachi SW 27 rotor at 4 C in a linear gradient containing 100-400 mg sucrose per ml.

ticles were rarely found, large aggregates in the cytoplasm were common (Plate I-7). However, no structural alterations of the infected cells nor cytoplasmic pinwheel or bundle type inclusions were observed.

Purification

Partially purified AVIII preparations centrifuged through sucrose density gradients formed a single light-scattering band about half way down the tube with a sharp upper boundary and a diffuse lower one (Fig. 2). Infectivity on tobacco was associated with this light-scattering zone. The ultraviolet absorption spectrum of purified virus showed the maximum value at 260 nm and the minimum one at 247 nm. The A 260/280 ratio was 1.18-1.19.

Transmission

The virus was not transmitted from infected to healthy *N. megalosiphon* or *N. benthamiana* by aphids, *Myzus persicae* and *Aphis gossypii*, apparently regardless of the length of acquisition feeding periods. Furthermore, tests of virus transmission through seeds collected from infected asparagus, *Glycine max* or *Vigna angularis* gave negative results.

Serological relationships

Antiserum prepared against AVIII had a homologous titer of 1/1024 in ring interface precipitin test, and formed a single precipitation line with the virus, but not with PVX in double diffusion agar gels containing sodium lauryl sulfate. The antisera against CaVX, CYMV, NMV, PVX, TVX and WCMV were used for IEM antibody decoration test with AVIII. AVIII reacted strongly with its homologous antiserum and even coating of antibodies accumulated along the virus particles (Plate I-5a). Heterologous reactions occurred between AVIII and antisera to CaVX (Plate I-5b) and NMV (Plate I-5c). Particles of AVIII coated with CaVX antibodies resembled those coated with homologous antiserum. However, reactions with NMV antibodies were inconsistent, often with a markedly sparser coating of antibodies. The antisera to CYMV, PVX, TVX and

WCMV did not react with AVIII in IEM antibody decoration tests.

Discussion

AVIII resembles the potexviruses in its particle size and shape, properties in sap, mechanical transmissibility, and failure of transmission by aphids⁷⁾. Moreover, it is serologically related to CaVX and NMV both of which have been established members of the group⁷⁾. AVIII is more closely related serologically to CaVX than to NMV. And the intracellular appearance of the AVIII particles resembles those of potexviruses such as NMV¹¹⁾ and plantain virus X⁵⁾. Three viruses, AVIII, CaVX and NMV have a very different host range: that of AVIII seems to be relatively wide, whereas CaVX has a narrow host range and infects species in the Chenopodiaceae, Amaranthaceae and Caryophyllaceae families^{1,10)}. Also, AVIII infects many species of Solanaceae systemically, whereas only *N. clevelandii* in this family is known to be infected systemically by NMV^{2,9)}. In addition, there are differences among these three viruses in their particle size. All of the particles are flexuous filaments, but in those of AVIII the length is 580×13 nm, while 520×13 nm in CaVX¹⁾ and 550×13 nm in NMV⁹⁾. Because of these differences, AVIII should be considered as a distinct virus rather than as a strain of CaVX or NMV.

Although the field-grown asparagus plants that contained AVIII, exhibited faint yellowing symptoms on young leaves, the artificially infected asparagus failed to exhibit any distinct leaf symptoms in the greenhouse for two years. Since asparagus plant is a long-life crop, it is considered that the symptoms such as yellowing or mottling could appear on those inoculated plants few years later. And then, we are currently investigating the possible effect on asparagus growth.

The author would like to thank Dr. N. Iizuka and Dr. T. Goto, Hokkaido National Agricultural Experiment Station, for their useful suggestions.

Literature cited

1. Berck, R. (1971). C.M.I./A.A.B. Descriptions of plant viruses. No. 58.
2. Brunt, A. A. (1966). Ann. appl. Biol. 58 : 13-23.
3. Fujisawa, I., Goto, T., Tsuchizaki, T. and Iizuka, N. (1983). Ann. Phytopath. Soc. Japan 49 : 299-307.
4. Fujisawa, I., Goto, T., Tsuchizaki, T. and Iizuka, N. (1983). Ibid. 49 : 683-688.
5. Hammond, J. and Hull, R. (1981). J. gen. Virol. 54 : 75-90.
6. Jones, R. A. C., Koenig, R. and Lesemann, D. E. (1980). Ann. appl. Biol. 94 : 61-68.
7. Koenig, R. and Lesemann, D. E. (1978). C.M.I./A.A.B. Descriptions of plant viruses. No. 200.
8. Milne, R. G. and Luisoni, E. (1975). Virology 68 : 270-274.
9. Mowat, W. P. (1971). C.M.I./A.A.B. Descriptions of plant viruses. No. 45.
10. Plese, N. and Milicic, D. (1966). Phytopath. Z. 55 : 197-210.
11. Turner, R. H. (1971). J. gen. Virol. 13 : 177-179.

和 文 摘 要

藤澤一郎：アスパラガスから分離された Potexvirus : asparagus virus III

北海道内のアスパラガスから分離された長さ 500-600 nm のひも状ウイルスの寄主範囲と諸性質について調べた。本ウイルスは汁液接種した11科37種の植物のうち8科26種に感染が認められ、ナス科、アカザ科の多くの植物に感染し、アスパラガスには無病徴感染した。本ウイルスの粗汁液中での不活化限界は、耐熱性 (10分) 55~60 C, 耐希釈性 5,000-10,000倍, 耐保存性 (20 C) 21~23日であり, dip 法で電顕観察したウイルス粒子の多くは、長さ 580 nm, 巾 13 nm の形態を示した。感染葉の超薄切片像ではウイルス様粒子の集団は細胞質中のみ観察され、感染による細胞器官の変化は認められなかった。純化ウイルスを用いて作製した抗血清の力価は 1/1024 (重層法による) で、SDS を含む寒天ゲル内で本ウイルスと明瞭に反応した。また、本ウイルスと Potexvirus 群の数種ウイルスとの血清学的関係を免疫電顕法で調べた結果、本ウイルスは cactus virus X および narcissus mosaic virus の各抗血清とは陽性の反応を示した。以上の諸結果から、本ウイルスは Potexvirus 群に属する未記載のウイルスと考えられ asparagus virus III と命名した。

Explanation of plate

Plate I

- 1-3. Symptoms induced by asparagus virus III on various hosts.
1. *Chenopodium amaranticolor*, chlorotic local lesions on inoculated leaves and vein chlorosis on upper leaves.
2. *C. quinoa*, systemic mosaic.
3. *Nicotiana debneyi* (left), systemic mild mosaic. *N. megalosiphon* (right), systemic clear mosaic.
4. Electron micrograph of purified asparagus virus III particles after sucrose density gradient centrifugation. Bar represents 100 nm.
- 5a-5c. Particles of asparagus virus III incubated with asparagus virus III antiserum at 1:100 dilution (5a), cactus virus X antiserum at 1:50 dilution (5b) and narcissus mosaic virus antiserum at 1:50 dilution (5c). Each was negatively stained with 2 % sodium phosphotungstate pH 7.0. Bar represents 100 nm.
- 6-7. Ultrastructure of asparagus virus III-infected leaf cells of *Nicotiana megalosiphon*. Virus like particles (V) in the cytoplasm. N: Nucleus, C: Chloroplast. Bar represents 500 nm.

Plate I

