

ピーマンに発生した葉脈黄化病とその病原ウイルス,ピーマン葉脈黄化ウイルス(新称)について

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Pepper Vein Yellows Virus, a Novel Luteovirus from Bell Pepper Plants in Japan

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Abstract

Bell pepper plants showing vein yellowing and leaf roll symptoms were observed in plastic houses at Kitanakagusuku, Okinawa Prefecture in 1981. The causal agent did not infect plants by mechanical inoculation or through seed but was transmitted by grafting and two species of aphids, *Aphis gossypii* Glover, collected from pepper and *Myzus persicae* Sulz. in a persistent manner. The causal agent has limited host range, only infect *Capsicum* species. In serological tests this agent did not react with antisera to potato leaf roll virus, tobacco necrotic dwarf virus, barley yellow dwarf virus and beet western yellows virus. Partially purified preparations contained spherical particles 25 nm in diameter. In ultrathin sections virus-like particles were observed only in the phloem cells of infected pepper plants. Based on these results the causal agent was classified as a new member of the luteovirus group and named pepper vein yellows virus (PeVYV).

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Key words: pepper vein yellows virus, aphid transmission, serology, electron microscopy, bell pepper.

INTRODUCTION

In 1981, bell pepper plants (*Capsicum annuum* L. var. *grossum* Sendt.) showing vein yellowing and leaf roll were observed in plastic houses at Kitanakagusuku, Okinawa Prefecture. These syndromes were previously thought to be due to magnesium deficiency, since similar syndromes had previously been found in other areas of Okinawa, Ishigaki and Miyako Islands. We considered that this syndrome may have been induced by a virus infection. Based on symptomatology the causal agent was clearly different from tobacco mosaic virus, cucumber mosaic virus, broad bean wilt virus, chrysanthemum mild mottle virus, potato virus Y and tomato spotted wilt virus which had been isolated previously from pepper plants in Japan¹⁴⁾. We report here on the host range, aphid transmission, morphology, serology, and electron microscopy of the agent. In this paper, we refer to this disease as pepper vein yellows and to the causal agent as pepper vein yellows virus (PeVYV).

MATERIALS AND METHODS

Virus isolation. Bell pepper plants (cv. Ace)

showing vein yellowing were collected in plastic houses at Kitanakagusuku, Okinawa Prefecture (Plate I-1). The virus isolate was maintained by grafting and serial transmission to bell pepper plants (cv. Ace) with green peach aphids (*Myzus persicae* Sulz.) and melon aphids (*Aphis gossypii* Glover.).

Sap inoculation. All test plants were grown in an insect-free glasshouse. Bell pepper (cv. Ace) was used as an indicator plant. Inoculum was prepared by grinding infected pepper leaves in 0.1 M phosphate buffer pH 7.2 with a mortar and a pestle and squeezing through double layers of cheesecloth. The crude sap was inoculated to test plants dusted with Carborundum. Inoculated plants were observed after about 1 month and then symptomless plants were back-inoculated to indicator plants by sap inoculation.

Grafting. Scions from infected pepper were grafted into the axils of test plants. The grafted plants showing no symptoms were back grafted to the indicator plants to confirm whether the test plants were infected or not.

Transmission through seed. Seed transmission tests were performed with mature seeds obtained from infected plants of two *Capsicum* varieties (*C. annuum* L. var. *grossum* Sendt. cv. Wase high-green, and *C. an-*

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num L. var. *longum* Sendt.). Seeds were sown in nursery boxes in an insect-free glasshouse and observed for symptoms until the third true leaf stage when disease incidence was measured.

Aphid transmission. Transmission experiments were conducted with the three cultures of aphids, *M. persicae*, *A. gossypii* (AG-P) collected from pepper and *A. gossypii* (AG-C) collected from cucumber.

Experiments done to determine the mode of transmission included fasting nonviruliferous aphids for 1 to 2 hr, feeding them on virus source plants for 5 to 15 min, and then transferring them to indicator plants for 12 hr.

Unless otherwise stated, the following procedure was used in all other aphid transmission tests. Nonviruliferous aphids were allowed to feed on infected leaves in a petri dish for various acquisition access periods (AAP). The aphids were then transferred to young healthy indicator plants to feed for various inoculation access periods (IAP).

Inoculation experiments were performed on several plant species using aphids given AAP and IAP for 72 hr, respectively. Symptomless plants were assayed by back inoculation using aphids and grafting.

The persistence of this virus was determined by serial transmission of AG-P aphids through sequential 24-hr transfer for 7 days to individual indicator plants. All transmission tests were performed at 25°C, and after inoculation feeding, all infested plants were sprayed with an insecticide and maintained in the glasshouse for

about one month.

Serology. Virus was partially purified as described by Takanami and Kubo¹¹. The preparation was used to produce antiserum against this virus by injecting a rabbit with a series of intravenous and intramuscular injections. Agar gel double diffusion tests (100 ml, 0.1 M PB, pH 7.2, 0.8% Difco noble agarose, and 0.02% sodium azide) were done using the partially purified virus preparation and homologous antiserum and heterologous antisera to tobacco necrotic dwarf virus (TNDV)⁸ and potato leaf roll virus (PLRV)⁷. Enzyme-linked immunosorbent assays (ELISA) were also tried using antisera to barley yellow dwarf virus (BYDV)¹⁰, beet western yellows virus (BWYV), TNDV, and PLRV.

Electron microscopy. Crude sap and partially purified preparations were examined with an electron microscope after negative staining in 2% phosphotungstic acid (PTA), pH 7.0. Ultrathin sections of phloem leaf tissue were cut after fixing with glutaraldehyde and osmium tetroxide, dehydration with ethanol and embedding in epoxy resin¹⁵.

Specimens were stained with uranyl acetate and lead citrate and examined with an electron microscope (JEM-100B, 80 kV).

RESULTS

Mechanical transmission

No test plants developed symptoms after sap inoculation. Back inoculation revealed that none of the inoculated plants were infected by sap inoculation (Table 1). After grafting, bell pepper and long pepper (*C. annuum* L. var. *longum* Sendt.) plants initially showed clear vein yellowing symptoms on the upper leaves about 10 days later (Plate I-2) and subsequently all leaves developed leaf roll and yellowing symptoms. The fruit formed on infected plants was malformed. Eight other species challenged by grafting did not become infected.

Transmission through seed

Virus infection through seed did not occur in either *Capsicum* varieties tested.

Table 1. Reactions of some plants inoculated with pepper vein yellows virus

Plants inoculated	Methods of inoculation	
	Sap	Graft
<i>Capsicum annuum</i> L. var. <i>grossum</i> Sendt.		
cv. Ace	—	+
<i>C. annuum</i> L. var. <i>longum</i> Sendt.	NT	+
<i>C. frutescens</i> L.	NT	—
<i>Nicotiana tabacum</i> L.		
cv. White burley	—	—
cv. Samsun NN	NT	—
<i>N. rustica</i> L.	NT	—
<i>N. glutinosa</i> L.	—	—
<i>Lycopersicon esculentum</i> Mill.		
cv. Hukujyu	—	—
<i>Solanum melongena</i> L.		
cv. Nagasakicho	—	—
<i>Datura stramonium</i> L.	—	—
<i>Physalis floridana</i> Rydb.	—	NT
<i>Petunia hybrida</i> Vilm.	—	NT
<i>Zinnia elegans</i> Jacq.	—	NT
<i>Vigna unguiculata</i> L.	—	NT
<i>Vicia fava</i> L.	—	NT
<i>Tetragonia expansa</i> Murr.	—	NT
<i>Chenopodium quinoa</i> Willd.	—	NT
<i>C. amaranticolor</i> Coste & Reyn.	—	NT

+: infected, —: not infected, NT: not tested.

Table 2. Transmission of pepper vein yellows virus with *Myzus persicae*

Experiments	AAP (hr) ^{a)}	IAP (hr) ^{b)}	Transmission ^{c)}
1	24	72	0/10
	48	48	1/10
	72	72	0/10
2	24	24	0/6
	48	48	3/6
	72	72	3/6
3	24	48	0/6
	48	72	3/5

a) Acquisition access period.

b) Inoculation access period.

c) Number of infected plants/Number of infested pepper plants.

Table 3. Effect of duration of AAP^{a)} or IAP^{b)} on transmissibility of pepper vein yellows virus by *A. gossypii*^{c)}

Duration of AAP ^{d)}		Duration of IAP ^{e)}	
Time	Transmission	Time	Transmission
5 (min)	1/5 ^{f)}	5 (min)	0/5
10	0/5	10	0/5
30	1/5	30	2/5
1 (hr)	3/5	1 (hr)	1/5
2	4/5	2	3/5
4	5/5	4	5/5
6	5/5	6	5/5
24	5/5	16	5/5
144	5/5	96	5/5
		120	5/5
		148	5/5

a) Acquisition access period.

b) Inoculation access period.

c) *A. gossypii* collected from pepper. 5 aphids/plant were used.

d) Gave IAP for 24 hr.

e) Gave AAP for 24 hr.

f) Number of infected plants/number of inoculated plants.

Aphid transmission by *M. persicae*

Green peach aphids (*M. persicae*) did not transmit this virus with short feeding times. As the AAP and IAP increased, the transmission efficiency increased (Table 2) but the transmission efficiency was still only about 50% when the AAP and IAP were 48 to 72 hr.

Aphid transmission by *A. gossypii*

In 1991, we found melon aphids (*A. gossypii*) on pepper plants showing vein yellowing symptoms. Some of these aphids were collected from the pepper plants and transferred to healthy ones. Most of the inoculated plants were infected and we therefore conducted further aphid transmission experiments using *A. gossypii* (AG-P) from a virus-free culture.

The virus was transmitted by following AAP as short as 5 min, but the number of infected plants increased as the AAP increased from 30 min to 4 hr (Table 3).

No transmission occurred when the IAP was less than 30 min. The transmission rate reached 100% when the IAP was 4 hr or more (Table 3).

Host range of PeVYV using *A. gossypii*

Inoculation experiments using aphids were also carried out to determine the host range of pepper vein yellows virus (PeVYV). Although the viruliferous aphids were transferred to 5 species such as *Nicotiana glutinosa* L., *N. benthamiana* L., *Solanum melongena* L. cv. Nagasakicho., *Vicia faba* L., *Vigna unguiculata* L., and *Cucumis melo* L. cv. Rohran., aphids were died on the infested plants. Although the viruliferous aphids were able to feed on the following species such as *Physalis floridana* Rydb., *Hordeum vulgare* L. cv. Hokutohadaka., *Triticum aestivum* L. cv. Mukakomugi, and cv. Asahikaze., *Zea mays* L. cv. American sweet No. 1, and

Table 4. Serial transmission of pepper vein yellows virus with *A. gossypii* (AG-P)*)

Number	1	2	3	4	5	6	7 (day)
1	●	●	●	●	●	●	○
2	●	●	●	●	●	○	○
3	●	●	●	●	○	○	○
4	●	●	●	●	●	●	●
5	●	○	●	●	●	○	●
6	●	●	○	●	○	●	○
7	●	●	●	●	○	●	○
8	●	●	●	●	●	●	○
9	○	●	—	—	—	—	—
10	○	●	●	●	○	○	○

●: Infected, ○: Not infected, —: Death of aphids.

*): *A. gossypii* collected from pepper.Table 5. Comparison of transmissibility of pepper vein yellows virus by two *A. gossypii* populations

Aphids	AAP (hr)	IAP (hr)	Transmission
AG-P ^{a)}	24	24	13/13 ^{c)}
AG-P	24	24	3/10
AG-C ^{b)}	24	24	0/11
AG-C	24	24	0/10

a) *A. gossypii* collected from pepper.b) *A. gossypii* collected from cucumber.

c) Number of infected plants/Numbers of infested indicator plants.

Gossypium barbadense L., any plant species excepting bell pepper did not develop symptoms. Back inoculations using aphids indicated that PeVYV did not infect these plants.

Virus persistence in aphid vector

The virus was transmitted much more efficiently by AG-P than *M. persicae*. Viruliferous AG-P continued to transmit the virus at a high level for 4 days and the rate then gradually decreased (Table 4). Serial transmission tests showed that the vector could retain the virus for at least 7 days (Table 4).

Transmission efficiency of *A. gossypii*

Transmission efficiency of this virus was compared between both vectors AG-P and AG-C. In contrast to the high efficiency of AG-P, AG-C did not transmit this virus at all (Table 5).

Serology

In agar gel double diffusion tests, partially purified preparations reacted only with the homologous antiserum having a titer of 1/124 and did not react with heterologous antisera to PLRV and TNDV. In ELISA tests, this virus did not react with antibodies to PLRV, BYDV and BWYV.

Electron microscopy

No virus-like particles (VLPs) were observed in the specimens of crude sap from infected plants. Partially purified preparations contained some spherical particles with a diameter of approximately 25 nm (Fig. 1). In

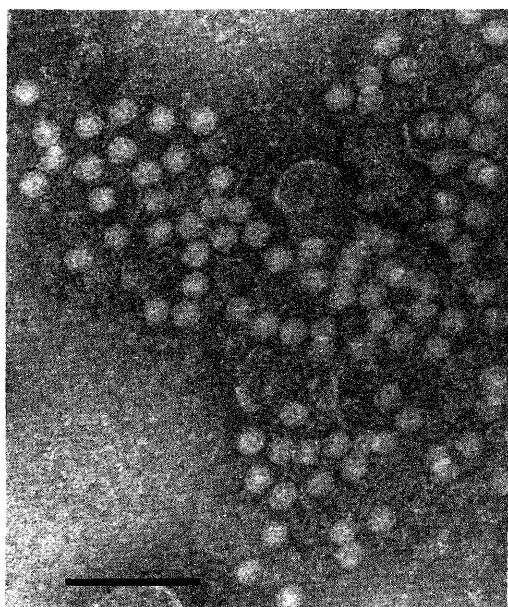


Fig. 1. Partially purified preparation of PeVYV stained with 2% PTA pH 7.0. Bar represents 100 nm.

ultrathin sections of the infected plants, VLPs were found restricted to the phloem cells and these showed organelle degeneration (Plate II-1). VLPs were mainly confined to cytoplasm of phloem cells but were occasionally found in the nucleus (Plate II-2).

DISCUSSION

The work described here indicated that this virus is: 1) not transmitted by sap inoculation or through seed, 2) persistently transmitted by *A. gossypii*, 3) limited to a narrow host range, 4) associated with spherical VLP's 25 nm in diameter that are found restricted to the phloem cells of infected plants. These characteristics are similar to those of luteoviruses¹³⁾.

Of three cultures of aphids studied, *A. gossypii* collected from pepper transmitted more efficiently than *M. persicae* while no transmission occurred with *A. gossypii* collected from cucumber. Similar results had been obtained in transmission trials using the two strains of soybean dwarf virus and the three populations of *Aulacorthum solani* collected from different countries²⁾. On the basis of life cycle and host range, Japanese populations of *A. gossypii* were classified into 4 biotypes by Inaizumi⁶⁾ but we did not establish which biotypes the two cultures of *A. gossypii* used in these experiments corresponded to.

One possible member of the luteovirus group, cotton anthocyanosis virus (CoAV) was transmitted only by *A. gossypii* and induced anthocyanosis symptoms similar to those of magnesium deficiency¹⁾. Host range study could be distinguished CoAV from PeVYV. Serological relationships between CoAV and other luteoviruses has not been reported.

Fletcher *et al.*⁴⁾ investigated the causal agent of pep-

per yellow vein (PYVA) which induced yellow vein symptom in pepper in England. It was not mechanically transmitted or by aphids, but was transmitted by grafting and fungus *Olpidium*. PYVA could be different from PeVYV on transmission vector.

Two luteoviruses, BWYV¹²⁾ and Capsicum yellow virus (CYV)⁵⁾ have been isolated from pepper plants in America and Australia, respectively. BWYV has a wide range of host plants³⁾ and distant serological relationships to PLRV⁹⁾. CYV is able to infect some *Solanaceous* hosts and has close relationships to PLRV⁵⁾. The results of serological assay showed that PeVYV could be differed from BWYV and CYV.

Prior to this study, no luteoviruses had been isolated from pepper plants in Japan. PeVYV was found to be clearly distinct from other luteoviruses and luteo-like viruses infecting pepper plants and from other viruses which had been isolated from pepper plants in Japan. Based on these facts, PeVYV is presumably a new member of the luteovirus group.

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和 文 摘 要

与那覇哲義・豊里哲也・河野伸二・尾崎武司：ピーマンに発

生した葉脈黄化病とその病原ウイルス、ピーマン葉脈黄化ウイルス（新称）について

1981年3月、沖縄県北中城村のハウス栽培のピーマンに葉脈黄化病が発生した。その病徴より本病はウイルスに起因するものと考えられた。その病原は汁液接種では伝染しなかったが、接ぎ木伝染し、また2種のアブラムシ、モモアカアブラムシ (*Myzus persicae* Sulz.), ワタアブラムシ (*Aphis gossypii* Glover.) により永続的に伝搬された。この病原の宿主範囲は狭く、トウガラシ属植物 (ピーマン, トウガラシ) にのみ感染し、感染した植物は葉脈黄化, 葉巻症状を示し, また奇形果も観察された。感染ピーマンを用いた部分純化標品中に直径約 25 nm の球状粒子が観察された。同様に感染ピーマン葉を用いた超薄切片では宿主の篩部細胞にのみウイルス様粒子が観察された。寒天ゲル内拡散法, ELISA を用いて血清学的関係を調査したところ, オオムギ黄萎ウイルス, ジャガイモ葉巻ウイルス, タバコえそ萎縮ウイルス, ビート西部萎黄ウイルスの各 Luteovirus 抗血清とは反応しなかった。以上の実験結果から, 本ウイルスは Luteovirus group に属する新しいウイルスと考えられ, 本病をピーマン葉脈黄化病, 本ウイルスをピーマン葉脈黄化ウイルス (Pepper vein yellows virus) と命名したい。

Explanation of plates

Plate I

1. A naturally infected pepper plant showing vein yellowing and leaf roll symptoms.
2. A pepper plant (cv. Ace) infected with PeVYV using *A. gossypii*. Note vein yellow symptom in upper leaves.

Plate II

- 1, 2. An ultrathin section of phloem cells of a pepper plant infected with PeVYV. (×45,000)
Note virus-like particles (V) scattered through cytoplasm and nucleus (II-2).
CW: cell wall, N: nucleus.

Plate I



Plate II

