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An Unusual Potyvirus from Pepper in Taiwan

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대만에서 고추에 발생한 미보고 Potyvirus에 관한 연구

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ABSTRACT

A virus which induced yellowing, vein banding and ruffling on pepper in the field was investigated. The virus reacted strongly with PVY-antiserum in ELISA, but not with antisera of cucumber mosaic virus, tobacco mosaic virus, tomato black ring virus, alfalfa mosaic virus, tomato spotted wilt virus, tobacco etch virus, pepper mottle virus, and tobacco ringspot virus. Electron micrographs revealed that the virus was a flexuous rod of 750-760nm in length. The virus was transmitted mechanically and by *Myzus persicae* in a nonpersistent manner. The host range was similar to that of PVY, except that *Chenopodium amaranticolor* and *C. quinoa* were infected systemically.

Key words : potyvirus, pepper.

要 約

圃場에서 고추에 黃化, 葉脈綠帶 그리고 葉脈주름을 일으키는 바이러스가 分離되었다. 이 바이러스는 PVY抗血淸과 ELISA 檢定에서 反應하였으나 CMV, TMV, TBRV, AMV, TSWV, TEV, PMV, TRSV와는 反應하지 않았다. 바이러스 粒子의 電子顯微鏡 檢鏡 結果 길이가 750-760nm의 絲狀形 粒子였다. 이 바이러스는 진딧물에 의해 쉽게 傳染되었으며 奇主範囲는 PVY와 비슷하였으나 *Chenopodium amaranticolor* 와 *C. quinoa*에 全身 感染을 일으켰다.

INTRODUCTION

More than 40 different viruses are known to infect peppers. Of these, ten are aphid transmitted potyviruses (unpublished). Virus infection of peppers in Taiwan ranges from approx. 30 to 70%. The most frequently encountered viruses are cucumber mosaic virus, potato virus Y and tobacco mosaic virus(1). Other viruses are probably also present, tomato black ring virus, alfalfa mosaic virus, tomato spotted wilt virus, tobacco etch virus, pepper mottle virus, and tobacco ringspot virus. This paper reports investigations on one of the

potyviruses isolated from peppers in Taiwan and its properties.

MATERIALS AND METHODS

Virus source. Field samples were inoculated to *Nicotiana tabacum* 'Xanthi'. Leaves of *N. tabacum* 'Xanthi' showing mosaic were inoculated to *Chenopodium amaranticolor*. The upper leaves of *C. amaranticolor* showing systemic chlorotic spots and veinal spreading lesions were inoculated to healthy *C. amaranticolor*. After 3 successive lesions transfers, *N. tabacum* 'Xanthi' was inoculated, where the virus was maintained.

Mechanical inoculation. Inoculum consisted of infected leaves, homogenized 1 : 4 fold in 0.01 M sodium phosphate buffer, pH 7.0.

Physical properties. Crude sap of infected leaves of *N. tabacum* 'Xanthi' was used to determine the physical properties of the virus. For thermal inactivation point determination, sap was heated from 30°C to 70°C. For dilution end point determination, dilutions of crude sap from 10^{-1} to 10^{-6} were made with 0.01 M sodium phosphate buffer, pH 7.0. For determination of longevity *in vitro*. crude sap was left at room temperature from 1 day to 8 days. The samples were inoculated to *C. amaranticolor* and local lesions were counted in one square centimeter with 3 replications.

Insect transmission. The green peach aphid (*Myzus persicae* Sulz) was used in insect transmission studies, using *N. tabacum* 'Xanthi' as the virus source plant. Non-viruliferous aphids were fasted for 1.5 hr. and then allowed an acquisition feeding for 30 seconds on the infected plant. Groups of 5 and 10 aphids were transferred immediately to healthy *N. tabacum* 'Xanthi', where they were left for 12 hr. before they were killed by insecticide.

ELISA test. Infected *N. tabacum* 'Xanthi' and *C. amaranticolor* were tested by ELISA(5), with antisera to cucumber mosaic virus(CMV), potato virus Y(PVY), tobacco mosaic virus(TMV), tomato black ring virus(TBRV), alfalfa mosaic virus(AMV), tomato spotted wilt virus(TSWA), tobacco etch virus(TEV), pepper mottle virus (PMV), and tobacco ringspot virus(TRSV). The coating immunogloblins were used at 1 : 500 dilutions except that of PVY, which was used at 1 : 1000 dilution. The concentration of the enzyme conjugates was 1 : 1000 for CMV, PVY and TSWV, and 1 : 500 for TMV, AMV, TEV, PMV, TRSV, and TBRV. Leaves were homogenized in phosphate buffer, pH 7.4, containing 0.05% Tween 20 and 2% polyvinylpyrolidone with a leaf press(Meku Pollaehne W. Germany). Two hundred microliters of the homogenate were added to wells of the microplate after coating with gamma globlin. The plates were incubated overnight in the refrigerator. After washing, 20 μl of enzyme labeled gamma globlin was added. P-nitrophenyl phosphate was used as the substrate. Color intensity was measured at A 405nm with a Titertek Multiskam MC. colorimeter.

Purification. Infected leaves of *N. tabacum* 'Xanthi' were homogenized in 0.1 M sodium citrate buffer, pH 7.4, containing 0.01 M Na-DIECA. The homogenate was clarified using 8% of a 1 : 1 mixture of n-butanol and chloroform, and centrifuged at 8,000g for 10 min. Polyethyleneglycol, MW 6,000, 4%, was added to the acqueous phase and the mixture stirred gently for one hour. After centrifugation at 9,000g for 20 min, pellets were resuspended in 0.1M phosphate buffer, pH 7.4, containing 0.1M $MgCl_2$. The resuspended pellets were centrifuged at 8,000g for 20 min. The supernatant was layered on a 30% sucrose cushion prepared in 0.1 M phosphate buffer, pH 7.4, and centrifuged at 70,000g for 120 min. Following lowspeed centrifugation(8000g/20 min) the virus solution was mixed with CsCl(0.47g/ml = 1.35 density) and then centrifuged at 100,000g for 17 hours. The virus containing fractions were collected with a ISCO UA-5 fraction collector.

Electron microscopy. Leaf dips were made from systemically infected *N. tabacum* 'Xanthi', *N. tabacum* 'V-20' and *C. amaranticolor*, and negatively stained with 2% phosphotungstic acid. Observation of virus particles was done with a

Table 1. Symptoms of peppers infected with the unusual virus in the field

Isolate	Collected Area	Symptoms	Virus[a]
4	AVRDC	yellowing, veinbanding, ruffling	PVY
22	AVRDC	veinbanding, ruffling	PVY
117	Hualien	stem necrosis, wilting	PVY, CMV, TMV
121	Hualien	yellowing, veinclearing	PVY
122	Hualien	veinbanding	PVY
132	Hualien	yellowing, mosaic	PVY
133	Hualien	veinbanding, stem necrosis	PVY

[a]Viruses detected from field samples by ELISA.

electronmicroscope JOEL 200EX.

RESULTS

Surveys of the symptoms of pepper in field. Out of the total of 47 samples, seven were founded to be infected with the unusual potyvirus. Plants infected with the unusual potyvirus showed yellowing, veinbanding and ruffling on leaves. All isolates were infected only with PVY, but isolate 117 was infected also with CMV and TMV (Table 1).

Host range and symptoms. Fourteen of twenty-three host plant species, inoculated by mechanical inoculation showed symptoms (Table 2). Mosaic

Table 2. Reactions of host plants inoculated with the virus isolated from pepper by mechanical inoculation

Plant species	Symptoms[a] of	
	inoculated leaves	uninoculated leaves
Chenopodium amaranticolor	CS	CS, VS
C. quinoa	CS	CS, VS
Nicotiana tabacum 'Xanthi'	–	M
N. tabacum 'Xanthi NC'	–	–
N. tabacum 'Samsun'	–	M
N. tabacum 'Samsun NN'	–	M
N. tabacum 'White Burley'	–	M
N. tabacum 'V-20'	–	M
N. benthamiana	–	M
N. debneyii	–	M
N. glutinosa	–	M
Datura stramonium	–	–
Physalis floridana	NS	Y, D
Petunia hybrida	–	M, VB
Capsicum annuum 'Early Calwonder'	–	SN, Y, D
C. annuum 'Yolo Wonder'	–	M
C. annuum 'Florida VR-2'	–	–
C. annuum 'Perennial'	–	–
Vigna unguiculata	–	–
Phaseolus vulgaris	–	–
P. angularis	–	–
Glycine max cv. 'Shih-Shih'	–	–
Gomphrena globosa	–	–
Cucumis sativus	–	–

[a]CS : chlorotic spots VS : veinal spreading lesions
M : mosaic NS : necrotic spots
D : dead SN : stem necrosis
Y : yellowing – : no reaction, no virus detected by ELISA.
VB : vein banding

symptoms were produced on *N. tabacum* 'Xanthi' (Plate 1-4, 5), *N. tabacum* 'Samsum', *N. tabacum* 'Samsun NN', *N. tabacum* 'White Burley'(Plate 2-2), *N. tabacum* 'V-20'(Plate 2-1), *N. benthamiana*, *N. debneyii*, *N. glutinosa*, *Petunia hybrida* (Plate 2-3), and *Capsicum annuum* 'Yolo Wonder'. *Physalis floridana* reacted with necrotic spots on the inoculated leaves, followed by wilt and death of the whole plant(Plate 2-4, 5). On *C. annuum* 'Early Calwonder', necrosis and yellowing of the leaves was produced(Plate 1-3). *Chenopodium amaranticolor* and *C. quinoa* reacted with local lesions on the inoculated leaves followed by systemic chlorotic spots and veinal spreading lesions on the upper leaves(Plate 1-1, 2). The virus could not infect *N. tabacum* 'Xanthi NC', *Datura stramonium*, *C. annuum* 'Perennial', *C. annuum* 'Florida VR-2', *Vigna unguiculata*, *Phaseolus vulgaris*, *P. angularis*, *Glycine max* 'Shih-Shih', *Gomphrena globosa* and *Cucumis sativus*.

Table 3. Thermal inactivation point

Temperature (°C)	No. of local lesions	Upper leaves
30	31	+[a]
40	27	+
50	17	+
60	1	+
70	0	-
80	0	-

[a] +: systemic infection.

Table 4. Dilution end point

Dilution	No. of local lesions	Upper leaves
Crude sap	21	+[a]
10^{-1}	8	+
10^{-2}	7	+
10^{-3}	8	+
10^{-4}	3	+
10^{-5}	0	-
10^{-6}	0	-

[a] +: systemic infection.

Properties in crude sap. The physical properties of the unusual virus were shown in Table 3, 4, and 5.

Insect transmission. The virus was transmitted by *Myzus persicae* in a nonpersistant manner. All tested plants became infected(Table 6).

ELISA test. All 7 isolates reacted with PVY-antiserum but not with the other 6 antisera(Table 7). The reaction of isolates, No. 4 and No. 22, was very strong and that of isolate 117 was weak.

Purification. A virus band was obtained after centrifugation with CsCl(Plate 3-2). The buoyant density in CsCl of the unusual potyvirus isolate is approx. 1.5(Fig. 1).

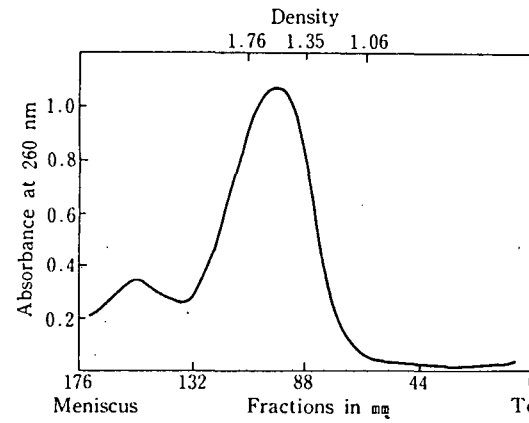

Fig. 1. Fractions collect of the virus band and absorbance curve(Fraction collector; ISCO UA-5, UV-spectrometer; Perkin-Elmer)

Table 5. Longevity in vitro[a]

Day	No. of local lesions	Upper leaves
1	28	+[b]
2	13	+
3	4	+
4	4	+
5	2	+
6	5	+
7	4	+
8	4	+

[a]: storage at room temperature
[b] +: systemic infection.

Table 6. Insect transmission of the virus from pepper by *Myzus persicae*

Plant tested	No. of aphids per plant	No. of plants		% of infection
		tested	infected	
N. tabacum 'Xanthi'	5	6	6	100
N. tabacum 'Xanthi'	10	6	6	100

Table. 7. ELISA test for the virus from pepper with PVY-antiserum

Isolate[a]		Numerical Value
4	A	> 2.0
	B	0.4
22	A	> 2.0
	B	0.1
117	A	0.006
	B	0.711
121	A	0.634
	B	0.584
122	A	1.965
	B	0.269
132	A	1.967
	B	0.306
133	A	1.297
	B	0.296
Healthy		0.003
PVY infected leaf		1.754

[a]A : *N. tabacum* 'Xanthi'
B : Systemically infected leaves of *C. amaranticolor*.

Electronmicroscopy. Flexuous rod shape particles typical of potyvirus were observed in the crude sap of infected *N. tabacum* 'Xanthi', *N. tabacum* 'V-20' and *C. amaranticolor* (Plate 3-1). About 60% of the particles were 750-760 nm in length with normal length of 750 nm (Fig. 2).

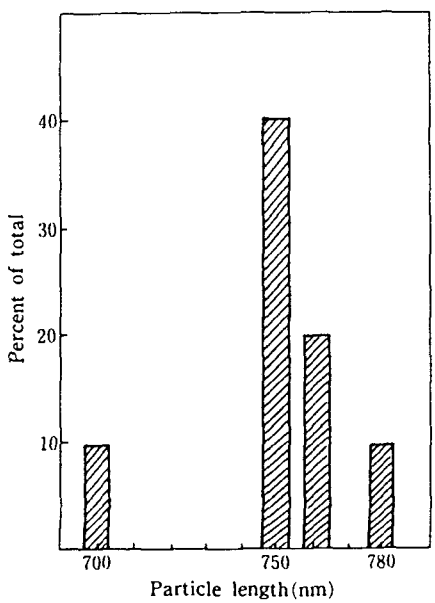

Fig. 2. Particle length distribution negatively stained preparation of the unusual potyvirus from pepper

DISCUSSION

The flexuous rod shape and the lengh of the particles suggest that the unusual virus isolated from pepper in Taiwan is a member of potyvirus group.

Ten potyviruses reported from pepper, Arizona pepper virus (17), chilli veinal virus(20), pepper mild mosaic virus(12), pepper mottle virus(24), pepper severe mosaic virus(7), pepper veinal mottle virus(2), unnamed virus(14), potato virus Y(15), tobacco etch virus(13), and Peru tomato virus(8). However none are able to systemically infect on *Chenopodium amaranticolor*, and *C. quinoa*.

The unusual virus is similar to PVY in host plants reactions, but this virus kill *Capsicum annuum* 'Early Calwonder' and *Physalis floridana*. PVY typically cause mild mottle in Capsicum(2, 9). The necrotic strain of PVY cause necrosis on leaves of *C. annuum* (2, 9) and the common strain cause systemic necrosis on *P. floridana* but do not kill(2, 9).

Based on host plants reactions and serological close relationship with PVY, the unusual virus isolated from pepper in Taiwan is distinguishable from the other ten potyviruses which infect peppers. Further serological studies will be necessary to if the unusual potyvirus is an previously undescribed strain of PVY or a new virus in pepper.

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PLATE 1

PLATE 1

Systemic chlorotic spots on *C. amaraniticolor*
Veinal spreading lesions and chlorotic spots on upper leaves of *C. amaranticolor*, 15–17 days after inoculation
Stem necrosis and leaf yellowing of *C. annuum* 'Early Calwonder'.
Vein clearing on the leaves of *N. tabacum* 'Xanthi', 8 days after inoculation.
Mosaic of *N. tabacum* 'Xanthi', 13 days after inoculation.

PLATE 2

PLATE 2

1 : Veinclearing (very mild) on the leaves of *N. tabacum* 'V-20'(upside ; healthy plants).
2 : Mosaic symptoms of *N. tabacum* 'White Burley'.
3 : Mosaic symptoms of *P. hybrida*.
4 : Necrotic spots and veinal necrosis on the leaves of *P. floridana*, 12 days after inoculation.
5 : Leaf death of *P. floridana*, 15 days after inoculation.

PLATE 3

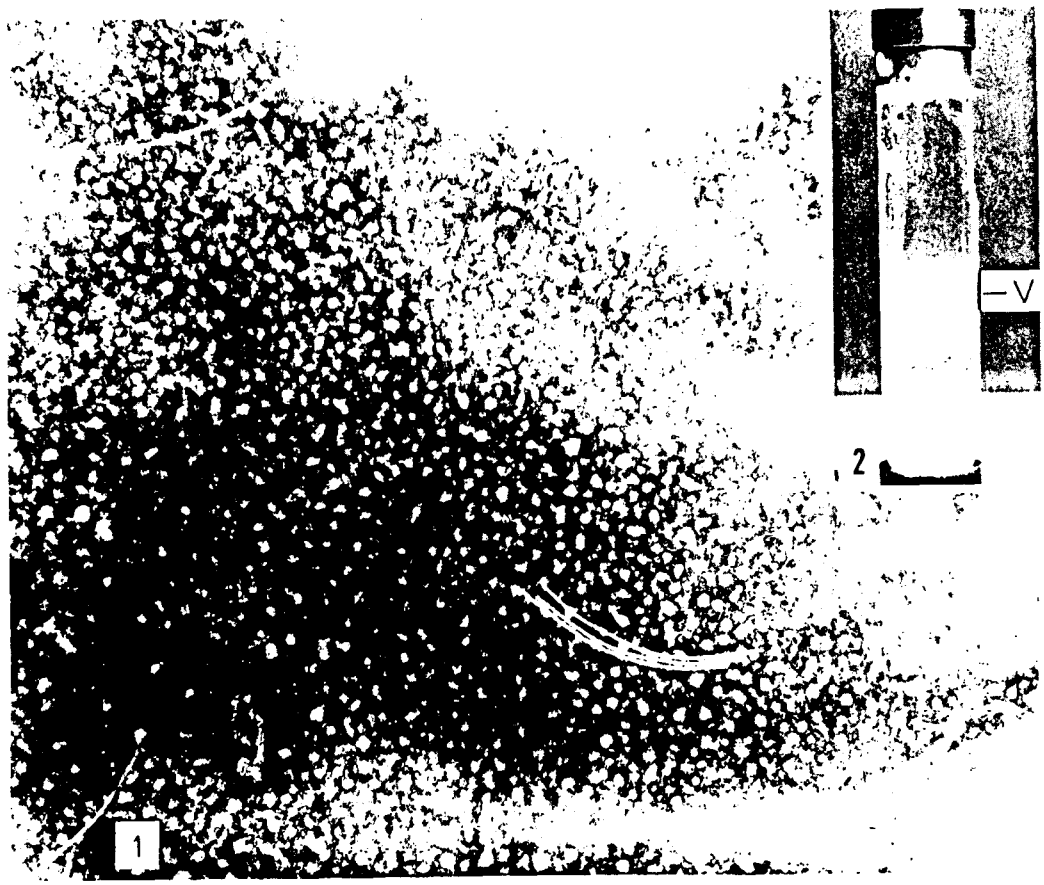

PLATE 3

1 : Electronmicrograph of the unusual virus stained negatively. Bar represents 200 nm.
2 : The virus band after centrifugation with CsCl for 17 hours.

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