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Broad Bean Wilt Fabaviruses and Their Specific Ultrastructures

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ABSTRACT

Pathogenicities of the five BBWV isolates were differentiated by the reactions on the 29 host plants including *Chenopodium amaranticolor*. Three specific ultrastructures were observed in cells infected with BBWV. The first ultrastructure was the tube made of 1~2 layers of virus particles. The second one was the comb structure consists of round and angled structures. The last one was the membrane proliferation in the cytosol.

Key words : Broad bean wilt fabavirus, Comb, Membrane proliferation, Tube, Ultrastructure

INTRODUCTION

Comoviridae has three genera; *Comovirus*, *Nepovirus*, and *Fabavirus* classified by the transmission vector of beetles, nematodes and aphids, orderly. The genome of *Comoviridae* is bipartite composed of positive sense single stranded RNA. Comoviruses have their specific ultrastructures. In case of broad bean wilt fabavirus (BBWV), the specific ultrastructures were cylindrical tubes of 80 nm in diameter with nine viral particles in cross sectioned face, and hexagonally packed viral sheets, where the virus particles embedded in multi-layered sheet (Tayler & Stubbs, 1972). Other reported ultrastructures are the membrane proliferation in cytosol (Francki et al., 1985) and the quadrangular tube that virus particles make multi-rectangular shapes with two layers (Vega et al., 1980). In Korea, the ultrastructural

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studies for the cells infected with BBWV from spinach (Lee et al., 1979) and lily (Chang & Chung, 1987) were reported. However, they did not show the virus crystals and vacuolation in lily. BBWV out of comoviruses infects *Chenopodiaceae*, *Lycopersicon esculentum* and *Cucurbits* systemically. Because BBWV was found in various crops in Korea and the virus isolates had a diverse virulence, the biological and ultrastructural characteristics were compared among the isolates from several crops.

MATERIALS AND METHODS

Virus source : Leaves of spinach, perilla, pea and red pepper showing symptoms of virus infection were collected from the fields and inoculated mechanically to the indicator plants. Virus in spinach was purified biologically from the lesions on *Chenopodium quinoa*, *Vigna sesquipedalis*, *Nicotiana tabacum* 'Ky-57', *Pisum sativum*, *Vicia faba* and *Datura stramonium*. Virus in perilla was purified from the lesions on *C. quinoa, C. amaranticolor, Nicotiana benthamiana*, *Vicia faba* and *N. glutinosa*. In the pea-infecting virus, the lesions of *Pisum sativum, Vigna sesquipedalis, N. benthamiana, N. rustica* and *C. quinoa* were used as virus source. In red pepper, the lesions of *C. quinoa, Physalis floridana* and *Vicia faba* were used as virus sources. These isolates were compared each other by the symptomatology. The virus sources were purified biologically through three transfers of single local using *C. amaranticolor* before testing of host range.

Mechanical inoculation : Leaves of diseased plants were homogenized in 0.01 M sodium phosphate buffer, pH 7.0, with a mortar and pestle. The homogenized samples were inoculated with wooden towel to the 33 kinds of healthy plants after scattering carborundum 600 mesh. The inoculated plants were washed with tap water immediately after mechanical inoculation.

Electron microscopy : Infected tissues at 2 weeks after inoculation were fixed in 2.5% glutaraldehyde with Millonig's phosphate buffer, pH 7.4, for 90 minutes at 4 °C. After clearly rinsed with Millonig's phosphate buffer, post fixation was done by 2% osmium tetroxide for 90 minutes at 4°C. The tissues were allowed to soak in 1% uranyl acetate for overnight in the refrigerator following rinsing with distilled water. Dehydration was done with an ascending series of ethyl alcohol. The dehydrated tissues were embedded in Spurr resin and polymerized at 60°C for 24 hours. Ultrathin sections of 80 nm thickness were obtained with an ultramicrotome using a diamond knife. The sections were double stained with 2% uranyl acetate and 0.5% lead citrate for 20 minutes and 10 minutes, respectively.

RESULTS

Biological tests for the new isolates : Two out of five BBWV isolates were found newly from *Perilla frutescens* and *Pisum sativum* in Korea. The biological reactions for the isolates from *Perilla frutescens* (PE-ASA3) and from *Pisum sativum* (P-K2) are shown in Table 1. Perilla produced the severe mosaic in the field (Plate 1-1). The PE-ASA3 could systemically infect 10 indicator plants including *Chenopodium amaranticolor* out of 29 tested plants. Five plants including *Nicotiana tabacum* 'Bright yellow' were infected locally. The isolate of BBWV could systemically infect and produce mosaic on red pepper, Hanbyul cultivar (Plate 1-2), and produced the systemic chlorotic spots and severe mosaic on *C. amaranticolor* (Plate 1-3). *C. quinoa* was killed at one week after mechanical inoculation (Plate 1-4). Mosaic disease was produced on *Pisum sativum* and *Physalis floridana* (Plate 1-5). Large necrotic spots were produced on the inoculated leaves of *Nicotiana glutinosa* (Plate 1-7) and then severe mosaic was produced on the upper leaves. The typical large necrotic double ring spots were produced on the inoculated leaves of *Datura stramonium* (Plate 1-8) and *N. tabacum*

Table 1. Symptomatology on the indicator plants inoculated mechanically with BBWV isolates

Indicator plant	Reactions for the isolate of		Indicator plant	Reactions for the isolate of	
	PE-ASA3[a]	P-K2[b]		PE-ASA3[a]	P-K2[b]
Chenopodium amaranticolor	CL/SM,MAL,TD[c]	CS/CS,SM	*Phaseolus anguralis*	-/VC	-/-
C. quinoa	CL/SM,MAL,TD	CS/CS,SM	*Vigna sesquipedalis*	NL/-	-/-
Nicotiana benthamiana	NL/SM	CS/CS,SM	*Sesamum indicum*	-/-	-/-
N. glutinosa	NL/SM,MAL	CRS/CRS	*Perilla frutescens*	VC/VC,VN	-/-
N. rustica	-/-	NS/M	*Impatiens balsamina*	-/-	-/-
N. tabacum cv. Bright yellow	NL/-	-/-	*Lycopersicon esculentum*	-/-	-/-
N. tabacum cv. Xanthi NC	-/-	-/-	*Cucumis sativus*	-/-	-/-
N. tabacum cv. Ky-57	CRL/-	-/-	*Citrullus vulgaris*	-/-	-/-
Gomphrena globosa	-/-	NS/M,MAL	*Cucumis melo*	-/-	-/-
Physalis floridana	CL/SM	CS/CS,M	*Crysanthemum coronarium*	-/-	-/-
Datura stramonium	NRL/-	CRS/CRS	*Capsicum annuum*	CS/CS,M	-/-
Tetragonia expansa	CRL/-	CS/CS,MAL	*Spinach oleracea*	-/-	-/-
Vicia faba	NRS/NRS	-/-	*Solanum melongena*	-/-	CS/VC,M
Pisum sativum	NL/VB	-/M,Y	*Phaseolus vulgaris* cv. Top crop	-/-	-/-
Glycine max	NL/-	-/-			

[a]The isolate of PE-ASA3 was identified from *Perilla frutescens*.

[b]The isolate of P-K2 was identified from *Pisum sativum*.

[c]CS: Chrolotic spot, CL: Chlorotic local, CRL: Chlorotic ring local, CRS: Chlorotic ring spot, M: Mosaic, MAL: Malform, NL: Necrotic local, NRL: Necrotic ring local, SM: Severe mosaic, VC: Vein clearing, VN: Vein necrosis.

Table 2. Comparison of the reactions on the indicator plants inoculated mechanically with BBWV isolates

Indicator plants	Reactions[a] for the isolate of				
	S-Y3[b]	PE-ASA3[c]	P-K2[d]	RP-BS3[e]	RP-22[f]
Chenopodium amaranticolor	CL/SM,MAL	CL/SM,MAL	CL/SM,MAL	CL/SM,MAL	CL/CS,MAL
Capsicum annuum	CL/M	CL/CS	-/-	-/M	CL/M
Lycopersicon esculentum	-/-	-/-	-/-	-/-	*
Nicotiana glutinosa	NL/-	NL/SM	CRS/CRS	CL/-	NL/-
N. tabacum cv. Ky-57	CL/-	CL/-	-/-	-/-	NL/-
Pisum sativum	NL/M	NL/M	NL/M	NL/SM	NL/M
Perilla flutescens	-/-	VN/VN	-/-	-/M,MAL	*
Solanum melongena	CL/SM,MAL	-/-	-/-	*	*
Vicia faba	CS/M	NRS/NRS	-/M	NL/SM	CL/M,D
Vigna sesquipedalis	NL/M	NL/M	NL/M	-/-	CL/M

[a]CL: Chlorotic local, CRS: Chlorotic ring spot, NL: Necrotic local, M: Mosaic, MAL: Malformation, SM: Severe mosaic, -: Negative reaction, Inoculated leaves/Upper leaves.

[b]S-Y3 was isolated from spinach.

[c]PE-ASA3 was isolated from *Perilla frutescens*.

[d]P-K2 was isolated from *Pisum sativum*.

[e]RP-BS3 and [f]RP-22 were isolated from *Capsicum annuum*.

*Not tested.

'Ky-57'.

Pea infected with BBWV in the field could produce mosaic and necrotic streak in culms and leaves. The P-K2 isolate of BBWV from pea could infect systemically on 11 indicators out of 29 tested plants (Table 1). In *C. amaranticolor*, severe mosaic and malformation were induced at two weeks after the mechanical inoculation (Plate 1-9). The virus killed *C. quinoa*. Mosaic and yellow discoloration were produced on *Pisum sativum* (Plate 1-10). Top necrosis and wilt were occurred on *Vicia faba* (Plate 1-11). *Vigna sesquipedalis* produced mosaic after showing necrotic spots on the inoculated

leaves (Plate 1-12).

Virulence of BBWV isolates : The isolates of S-Y3, P-K2, PE-ASA3, RP-BS3 and RP-22 were identified from spinach, pea, perilla and red peppers of last two, orderly. Ten indicator plants including *C. amaranticolor* were chosen from 29 tested plants for the comparison of biological characteristics (Table 2).

BBWV isolates could systemically infect *C. amaranticolor* and *V. faba*, and not infect *Lycopersicon esculentum*. Seven indicator plants including *Nicotiana glutinosa* were infected locally or systemically depending upon the virus isolates. In Table 3, five BBWV isolates from 4 selected indicator plants were compared. The weakest virulent isolate was RP-BS3 and three isolates, S-Y3, RP-22 and PE-ASA3, had wider host ranges. The virulence of five BBWV isolates on the natural host plants was also different (Table 4). The two isolates of RP-BS3 from red pepper and PE-ASA3 from perilla had almost the same virulence.

Ultrastructures of the infected cells

Comb structure : The comb structures were round, linear, angular and band shape in ultra-thin sections. Round comb was observed in cells infected with RP-BS3 from red pepper and PE-ASA3 from perilla. Round (Plate 1-13), angular (Plate 1-14), linear (Plate 2-1) and band (Plate 2-2) comb structures were observed in the cells infected with isolate of RP-BS3 and RP-22 from red pepper, and S-Y3 from spinach. All of the four comb structures were composed of several layers of hexagons, which were electron denser and made of packed virus particles. Depending on the number of hexagon layers, the size of comb structures was various. In the linear comb, the stratum was mostly of 4 or 5 layers and the length was various. Linear comb coexisted with the round comb depending on the sectional orientation. The inner structures of angular and banded comb were same with those of round and linear combs.

Table 3. Differentiation of virulence in the indicator plants inoculated mechanically with BBWV isolates

Indicator plant	Reactions[a] for the isolate of				
	S-Y3[b]	RP-22[c]	PE-ASA3[d]	P-K2[e]	RP-BS3[f]
Chenopodium amaranticolor	+/+	+/+	+/+	+/+	+/+
Pisum sativum	+/+	+/+	+/+	+/+	+/+
Vigna sesquipedalis	+/+	+/+	+/+	+/+	+/+
Nicotiana tabacum 'Ky-57'	+/-	+/-	+/-	+/-	+/-

[a] +: Positive reaction, -: Negative reaction, Inoculated leaves/Upper leaves.
[b] S-Y3 was isolated from spinach.
[c] PE-ASA3 was isolated from *Perilla frutescens*.
[d] P-K2 was isolated from *Pisum sativum*.
[e] RP-BS3 and [f] RP-22 were isolated from *Capsicum annuum*.

Table 4. Differentiation of virulence in the natural hosts of BBWV by mechanical inoculation

Natural host	Reactions[a] for the isolate of				
	RP-BS3[b]	PE-ASA3[c]	S-Y3[d]	RP-22[e]	P-K2[f]
Vicia faba	+	+	+	+	+
Pisum sativum	+	+	+	+	-
Capsicum annuum	+	+	+	+	-
Perilla frutescens	+	+	-	-	-

[a] +: Positive reaction, -: Negative reaction.
[b] S-Y3 was isolated from spinach.
[c] PE-ASA3 was isolated from *Perilla frutescens*.
[d] P-K2 was isolated from *Pisum sativum*.
[e] RP-BS3 and [f] RP-22 were isolated from *Capsicum annuum*.

The angular comb had several figures of triangle, rectangle or pentagon. Banded comb was composed of 8-9 layers for hexagon and the inner structure was also virus particles.

Tube structure : The tubes sectioned vertically (Plate 2-3) and obliquely or horizontally (Plate 2-4) were observed in the cytosol infected with the isolate of RP-22 from red pepper. A tube was 170~180 nm in diameter and composed of 19~22 virus particles, which were not arranged in a line in sections. The smaller circular tube was made of nine virus particles of BBWV when sectioned transversely. The virus particles arranged clearly and regularly in a helical line.

Membrane proliferation : The typical membrane proliferation was observed in the cytoplasm of cells infected with P-K2 and PE-ASA3 isolates (Plate 2-5). The cytoplasmic membranes were packed in the cytosol by viral infection.

Crystal of virus particle : Isolated virus particles were scattered in the cytosol. Virus particles of S-Y3 were crystallized in cytosol (Plate 2-6, 2-7). There were plenty of virus particles in a degenerated cell infected with P-K2 (Plate 2-8).

DISCUSSION

Broad bean wilt fabavirus (BBWV) was pathogenically divided into two groups by the symptoms for necrosis of type strain, and mosaic and necrotic spots of pea streak strain on *V. faba* (Tayler & Stubbs, 1972). Two isolates, PE-ASA3 and RP-22 out of 5 isolates, were grouped in the type strain. However, those isolates can be divided into 4 groups by the symptomatology for the 4 selected host plants of *C. amaranticolor, P. sativum, V. sesquipedalis* and *N. tabacum* 'Ky-57', and by the symptoms in natural hosts of BBWV as *V. faba, C. annuum, P. sativum, P. frutescens* and *S. melongena.*

The four kinds of cytological ultrastructures of BBWV were reported as circular tube (Tayler & Stubbs, 1972; Vega et al., 1980), rectangular tube (Vega et al., 1980), hexagonally packed sheet (Tayler & Stubbs, 1972) and membrane proliferation (Francki et al., 1985). Those ultrastructures were specifically observed in the cells infected with BBWV isolates. The hexagonally packed sheets were referred as comb structures in this study. The comb structure was divided into four groups of round, angled, linear and band combs. They were produced in the cells infected by S-Y3, RP-BS3 and RP-22 isolates. Round comb was reported as the specific ultrastructure of BBWV for petunia ringspot strain (Taylor & Stubbs, 1972). It was reported newly that angular and banded combs were the one kind of specific ultrastructures of BBWV.

Vega et al. (1980) reported the three specific ultrastructures of membranous inclusion, rectangular tube and circular tube in an Argentine isolate. These tubular structures adhered closely to the tonoplast. Large tubes were made by 15 to 30 circular tubes. The circular tubes reported by Tayler & Stubbs (1972), and Vega et al. (1980) were relatively smaller in diameter (80 nm) than observed in this study. In the RP-22 isolate, the specific ultrastructures of circular tube and four kinds of comb structures were observed simultaneously. The circular tube of RP-22 isolate didn't made large tubes reported by Vega et al. (1980) and had little affinity to the tonoplast. The round comb and membrane proliferation were also observed together in the cells infected with BBWV PE-ASA3 isolate. The P-K2 isolate induced only membrane proliferation and packed virus particles characteristic in degraded cells. The membrane elongation is known as a kind of specific ultrastructural changes induced by BBWV (Francki et al., 1985; Jang & Jung, 1987).

The diversity of cytological ultrastructures for BBWV was confirmed again in this study. The number of virus particles and width of the tube for Korean BBWV isolates was about two folds than those of the circular tube reported by Tayler & Stubbs (1972) and Vega et al. (1980) and had little affinity with the tonoplast.

As a conclusion, broad bean wilt viruses in Korea could be classified as a subgroup I with comb structures (S-Y3, RP-BS3), a subgroup II with tube structures (RP-22) and a subgroup III with membrane proliferation (P-K2, PE-ASA3).

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<국문초록>

잠두위조바이러스(broad bean wilt virus) 5종의 분리주에 대하여 명아주 등 29종의 지표식물반응에 의한 병원성을 분류하였다. 이들 분리주에 감염된 세포에서 3종류의 서로 다른 특이한 미세구조가 관찰되었다. 첫번째 구조는 바이러스입자로 된 1~2층의 원형관(tube)이고, 둘째는 6각형의 벌집구조(comb)로서 이것은 외부모양이 원형 또는 각으로 된 구조로 구분되었으며, 셋째는 세포원형질 내에 존재하는 다량의 막구조(membrane proliferation)이었다.

FIGURE LEGENDS

Plate 1. Broad bean wilt virus was isolated firstly from *Perilla frutescens* showing yellow mosaic symptom in field (1-1). The isolate of PE-ASA3 from perilla could infect systemically on red pepper producing mild mosaic symptom (1-2), *Chenopodium amaranticolor* producing chlorotic spots (1-3), *C. quinoa* producing bud necrosis (1-4), and *Physalis floridana* producing mosaic (1-5). BBWV from perilla induced local lesions of multilayered spots on *Nicotiana tabacum* 'Ky-57' (1-6) and *Datura stramonium* (1-8), and necrotic spots *N. glutinosa* (1-7). The isolate of P-K2 isolated from pea produced systemic chlorotic spots and malformation on C. *amaranticolor* (1-9), mosaic on pea (1-10), leaf necrosis on *Vicia faba* (1-11) and necrotic spots on *Vigna sesquipedalis* (1-12). The comb structures of hexagonal hole were formed amorphous crystals of round shape (1-13) and multi-angular shape (1-14). The empty shells of crystals were empty protein shells and the black shells were entire virus particles. Bars represent 200 nm.

Plate 2. The comb structures of linear shape (2-1) were the crystal composed of 4 to 5 layers. Band comb (2-2) was also induced and the length of band comb was not determined. The O-rings made by virus particles of BBWV shown in the enlarged O-ring on the upper right side were a slice of tube sectioned crossly (2-3). The width of the tubes was about 160-180 nm. The lengths of tubes were various (2-4). The virus particles of the layers were arranged helically but not in a line. The membrane elongation was induced severely in cells infected with BBWV isolates of P-K2 and PE-ASA3 isolated from pea and perilla, respectively (2-5). The BBWV virus particles presented by crystals in vacuoles (2-6) and degraded cell (2-7). The large amount of virus particles presented in a degenerated cell infected with BBWV from pea (2-8). Bars in photo 3 and 8, and other photoes represent 400 nm and 200 nm, respectively.

Plate 1

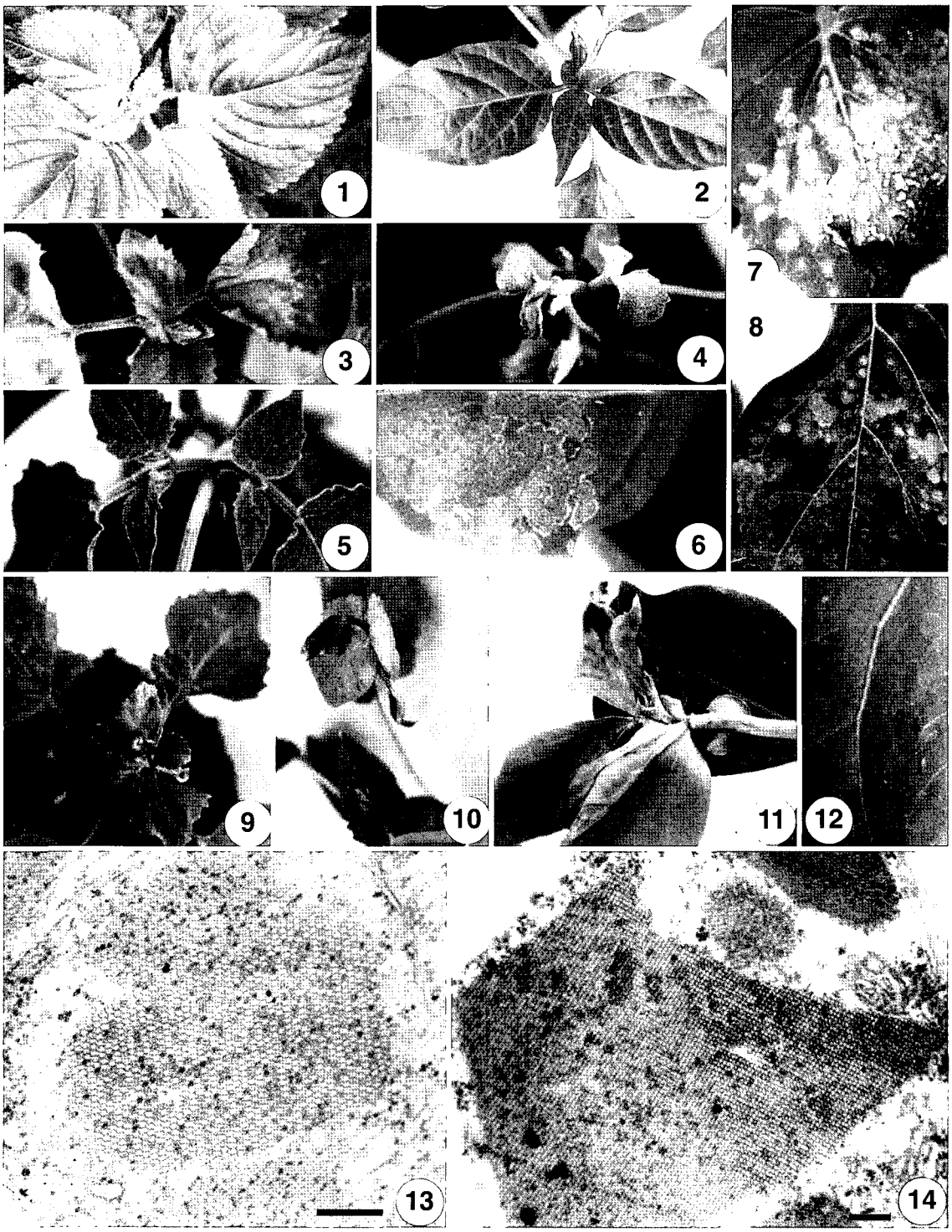

Plate 2