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Petunia Asteroid Mosaic Virus Isolated from *Petunia hybrida* Vilm.

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페츄니아에서 분리한 Petunia Asteroid Mosaic Virus

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ABSTRACT : A virus was isolated from petunia (*Petunia hybrida* Vilm.) plants showing chlorotic ring spots on the leaves and color breaking on the flowers, and was identified as petunia asteroid mosaic virus (PAMV). Identification of the PAMV was established by host range test, electron microscopy, serological reaction, and physical properties of the virus. In the host range test, *Nicotiana glutinosa*, *N. rustica*, *N. clevelandii*, *P. hybrida*, *Gomphrena globosa*, and *Chenopodium amaranticolor* were systemically infected with the virus. The virus produced local lesions on inoculated leaves of *N. tabacum* 'Samsun', *N. tabacum* 'Xanthi nc', *Datura stramonium*, *Vigna unguiculata* 'White eye', *C. quinoa*, *Capsicum annuum*, *Vicia faba*, and *Lycopersicon esculentum* 'Rutgers'. However, *Cucurbita sativus* and *C. moschata* did not show any symptoms. PAMV particles were isometric with 30 nm in diameter. The crude sap from *G. globosa* infected with the virus reacted positively with antiserum to tomato bushy stunt virus (TBSV) in agar gel double diffusion test. Thermal inactivation point of the virus was 80°C and the virus retained its infectivity at the dilution of 10^{-4}. Longevity *in vitro* of the virus was estimated longer than 35 days.

Key words **: petunia asteroid mosaic virus, *Petunia hybrida* Vilm., host range, serology, physical property.**

Petunia asteroid mosaic virus (PAMV) is a member of tombusvirus group (3). PAMV was first reported by Loviosolo (8) in 1957. The virus has been described to be a causal agent of a disease affecting the petunia plant growth, inducing color breaking of the flowers and stellate yellowish ring spots on the leaves, accompanied by puckering and malformation of the leaf blades (8). Some isolates of PAMV have also been described in hop plants with yellow mottling and malformation of the leaves and grapevine with vein necrosis and stunting (11). Some biological properties for PAMV including the manner of soil-borne or fungal vector transmission have been reported from several European countries (2, 6, 9). However, detail biological, biochemical and serological properties of PAMV have not been well characterized until now.

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In 1994 we found petunia plants showing chlorotic ring spots on the leaves and color breaking on the flowers in Chuncheon, Korea. This paper describes some biological and serological properties of PAMV isolated from the petunia plants.

MATERIALS AND METHODS

Virus source. Petunia (*Petunia hybrida* Vilm.) plants naturally showing chlorotic ring spots and color breaking were used as an initial inoculum (Fig. 1). Isolation of the virus source was made by three successive single lesion transfers on *Gomphrena globosa* and the 3rd single lesion was propagated on healthy petunia plants.

Host range test. Plants representing 16 species from five families were mechanically inoculated with sap of systemically infected *P. hybrida* leaves extracted

in 10 volumes of 0.01 M phosphate buffer, pH 7.0. The inoculated plants were maintained at 25°C in a greenhouse and observed daily for symptom development. Twenty days after inoculation, the sap of the tested plants was back-inoculated on healthy petunia plants to determine the presence of the virus.

Electron microscopy. The virus particles were observed by direct dip method (5) after negative staining with 2% potassium phosphotungstate, pH 6.0. Electron micrographs were taken with a Zeiss EM 109 electron microscope.

Serology. Serological reactions were examined using the agar gel double diffusion method (12). Double diffusion tests were made in a medium consisting of 0.8% agarose, 0.2% sodium azide and 0.85% sodium chloride in 0.01 M phosphate buffer, pH 7.0. The crude sap from systemically infected leaves of *G. globosa* or *Nicotiana rustica* were used as the antigens of the virus. Antiserum (PVAS 163) of the type strain of TBSV supplied by American Type Culture Collection (ATCC) was used for serological test of the virus.

Physical properties of the virus. All of the following tests were conducted using homogenized sap from the infected *N. rustica* leaves with 0.01 M phosphate buffer, pH 7.0 (w : v=1 : 10), and the preparations then were tested on healthy *N. glutinosa* plants. For thermal inactivation point (TIP) of the virus, two milliliter aliquots were incubated for 10 min at temperatures ranging between 60°C and 95°C. Infected *N. rustica* sap was serially diluted (10^{-1} to 10^{-6}) with the buffer and was used for determination of the dilution end point (DEP). For determination of the longevity *in vitro* (LIV) of the virus, the plant sap was incubated at room temperature for 1 to 35 days.

RESULTS

Host range. Results in the reactions of the test plants to the virus infection are shown in Table 1. Host range and symptomatology are as follows. *N. glutinosa*, *N. rustica*, *N. clevelandii*, *N. tabacum* 'Samsun',

Table 1. Host range and symptoms of petunia asteroid mosaic virus on test plants

Host	Symptoms[a]
Nicotiana glutinosa	RS/RS, M, N
N. rustica	RS/RS, M, N
N. clevelandii	RS/RS
N. tabacum 'Samsun'	RS/–
N. tabacum 'Xanthi nc'	RS/–
Petunia hybrida	RS/RS, M
Gomphrena globosa	NS, N/M, N
Chenopodium amaranticolor	NS/M
C. quinoa	NS/–
Datura stramonium	NS/–
Capsicum annuum 'Kumtap'	NS, F/–
Lycopersicon esculentum 'Rutgers'	NS/–
Vigna unguiculata 'White eye'	NS/–
Vicia faba	NS/–
Cucurbita sativus	–/–
C. moschata	–/–

[a] Abbreviations : Symptoms on inoculated leaves/Symptoms on systemically infected upper leaves; M, mosaic; NS, necrotic spot; RS, ring spot; N, necrosis; F, leaf falling; –, no infection.

Fig. 1. Chlorotic ring spots on the leaf (a) and color breaking on the flower (b) of *P. hybrida* naturally infected with PAMV. Healthy flower shows on the right side in (b).

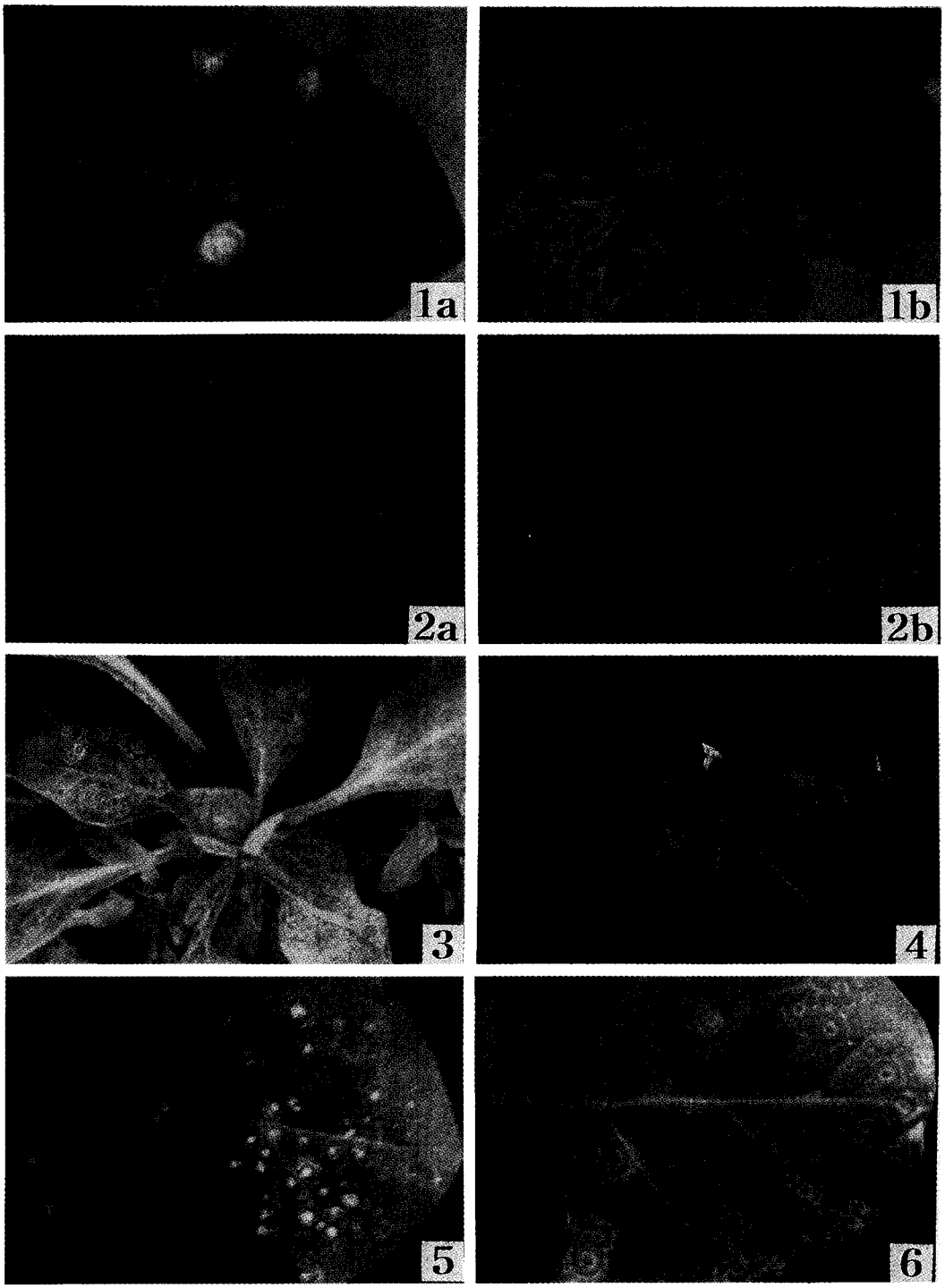

Plate 1. 1. Necrotic ring spots on an inoculated leaf (1a) and systemic chlorotic ring spots on upper leaves (1b) in *Nicotiana glutinosa*. 2. Necrotic ring spots on an inoculated leaf (2a) and mosaic symptom on the upper leaf (2b) in *N. rustica*. 3. Systemic chlorotic ring spots or asteroid symptom in *Petunia hybrida*. 4. Systemic chlorotic ring spots in *N. clevelandii*. 5~6. Chlorotic ring spots on inoculated leaves in *N. tabacum* 'Samsun' (5) and 'Xanthi nc' (6).

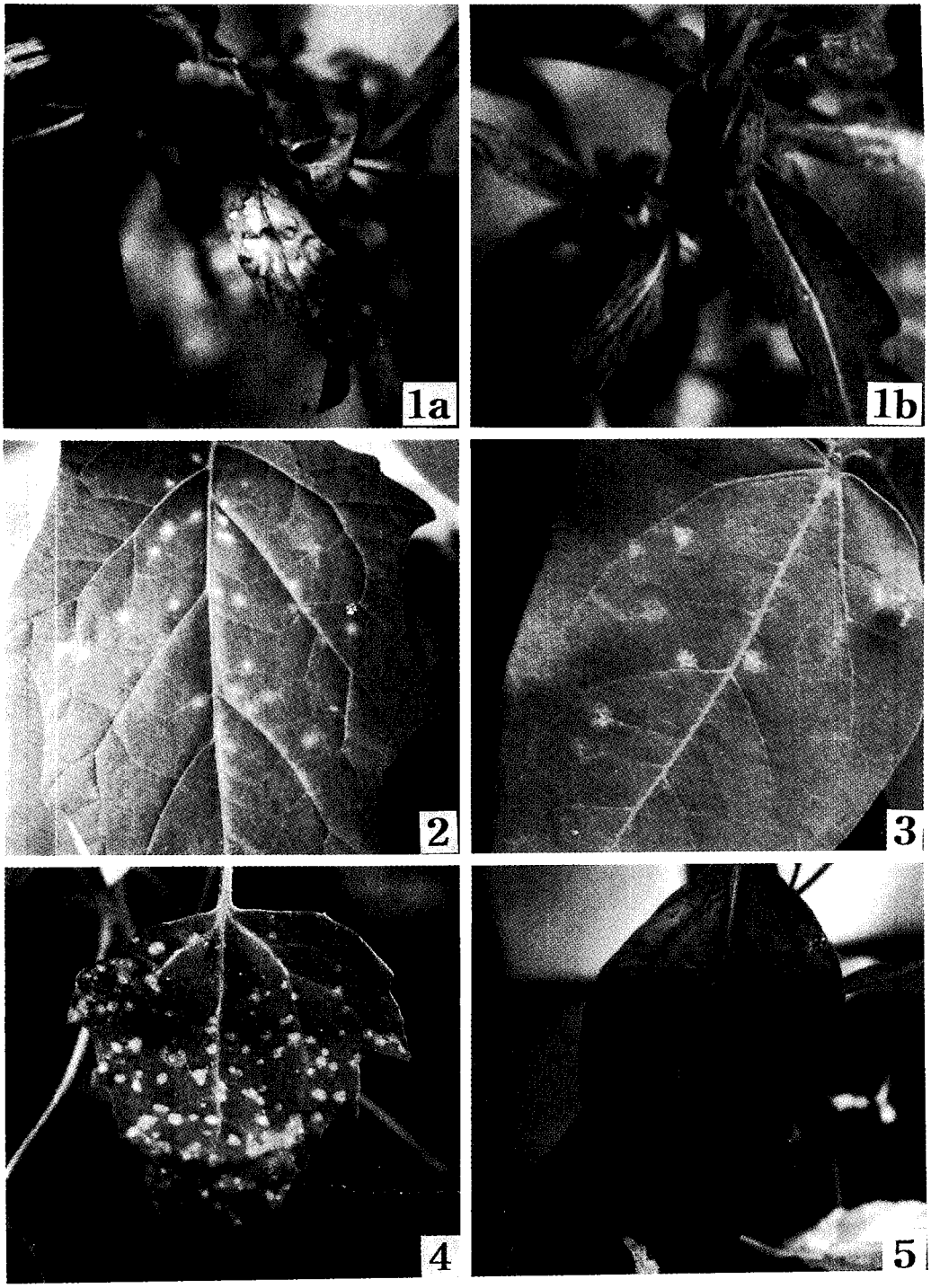

Plate 2. 1. Necrotic spots and necrosis on inoculated leaves (1a) and mosaic or vein necrosis on upper leaves (1b) in *Gomphrena globosa*. 2~3. Chlorotic local lesions on inoculated leaves in *Datura stramonium* (2) and *Vigna unguiculata* 'White eye' (3). 4~5. Necrotic local lesions on inoculated leaves in *Chenopodium quinoa* (4) and *Capsicum annuum* 'Kumtap' (5).

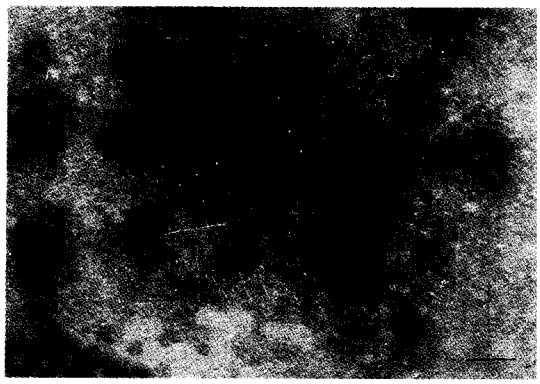

Fig. 2. Particles in leaf dip preparation of infected petunia leaves stained with 2% potassium phosphotungstate. Bar represents 100 nm.

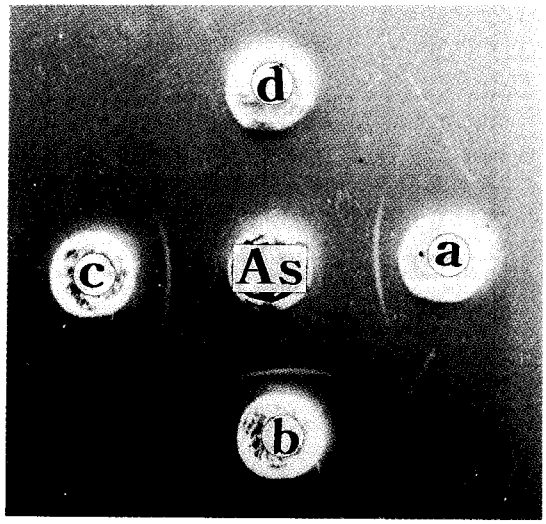

Fig. 3. Agar gel double diffusion test using antiserum to tomato bushy stunt virus (TBSV) (As). Antigens are PAMV of this study (a), PAMV (ATCC PV193) (b), TBSV (ATCC PV163) (c) and healthy petunia sap (d).

N. tabacum 'Xanthi nc' and *P. hybrida* showed ring spots on the inoculated leaves (Plate 1). Of them, *N. glutinosa*, *N. rustica*, *N. clevelandii* and *P. hybrida* were systemically infected, and produced ring spot, mosaic or necrosis on the upper leaves. Whereas in *G. globosa*, *Datura stramonium*, *Vigna unguiculata* 'White eye', *Vicia faba*, *Chenopodium amaranticolor*, *C. quinoa*, *Capsicum annuum* and *Lycopersicon esculentum* 'Rutgers', necrotic spots were produced on the inoculated leaves (Plate 2), and mosaic and necrotic spots were shown on the upper leaves of *G. globosa* and *C. amaranticolor*. *Cucurbita sativus* and *C. moschata* were not infected by the mechanical inoculation of the virus.

Table 2. Physical properties of petunia asteroid mosaic virus examined by using the crude sap of infected *N. rustica*

Virus	Thermal inactivation point (°C)	Dilution end point	Longevity *in vitro* (days)
PAMV	80	10^{-4}	> 35
TBSV[a]	90~95	10^{-6}	28~35

[a] Data of TBSV were described by Martelli *et al.*, 1971 (10).

Electron microscopy. Electron micrographs of negatively stained virus taken from leaf dip preparations revealed isometric particles (Fig. 2). The virus particles showed typical size of 30 nm in diameter.

Serology. The virus reacted positively with TBSV antiserum in agar gel double diffusion test (Fig. 3). No precipitates were observed when tested against healthy plant extract.

Physical properties of the virus. The thermal inactivation point of the virus was 80°C, and the virus retained its infectivity up to 10^{-4} solution. The infectivity of the virus was maintained even after 35 days at room temperature (Table 2).

DISCUSSION

Although PAMV and its isolates have been reported from several European countries (6, 7, 8, 11), only limited information on the virus is available.

PAMV isolated in this study revealed a wide host range, inducing mainly ring spot symptoms on most of the tested plants, which coincided with that of PAMV in previous reports (6, 7). In our host reaction tests, symptoms of the virus were very similar to those of a type strain of PAMV (ATCC PV193) except that the virus in this experiment did not produce top necrosis on *N. clevelandii* (data not shown). Moreover, the virus was definitely reacted with TBSV antiserum, which is considered serologically identical to the TBSV strain (ATCC PV163). Physical properties of the virus were also similar to those of TBSV (10).

The tombusvirus group was established in 1971 (3). However, the definition with respect to the membership of the group is not clear. For example, tombusviruses such as TBSV, PAMV, pelargonium leaf

curl virus (PLCV), carnation Italian ringspot virus (CIRV) and artichoke mottled crinkle virus (AMCV) are serologically related to one another (4). From these serological relationships, PAMV has been described as a strain of TBSV (1, 2, 4). Furthermore, Koenig and Kunze (6) reported that classification of the tombusviruses on the basis of host responses was more difficult. Therefore, further biological, serological and/or biochemical data of PAMV are needed for the characterization of the virus.

요 약

1994년 잎에 겹둥근무늬와 꽃에 얼룩무늬를 나타내는 페츄니아를 발견하고, 여기에서 petunia asteroid mosaic virus(PAMV)를 분리하였다. 이 PAMV의 동정은 지표식물 검정, 전자현미경 관찰, 혈청학적 성질 및 물리적 성질의 검정을 통하여 실시하였다. 이 바이러스는 *Nicotiana glutinosa*, *N. rustica*, *N. clevelandii*, *Petunia hybrida*, *Gomphrena globosa* 및 *Chenopodium amaranticolor*에 전신감염되었다. 한편 *N. tabacum* 'Samsun', *N. tabacum* 'Xanthi nc', *Datura stramonium*, *Vigna unguiculata* 'White eye', *C. quinoa*, *Capsicum annuum*, *Vicia faba* 및 *Lycopersicon esculentum* 'Rutgers'의 접종엽에는 국부병반을 형성하였으나, *Cucurbita sativus*와 *C. moschata*에는 감염되지 않았다. 전자현미경 관찰 결과 직경 30 nm의 구형 바이러스입자가 관찰되었다. 바이러스를 증식시킨 *G. globosa*의 즙액을 이용하여 agar gel double diffusion법으로 검정한 혈청실험은 TBSV의 항혈청과 양성반응을 나타냈다. 한편 이병 즙액의 물리적 성질은 내열성 80℃, 내희석성 10^{-4}, 내보존성 35일 이상으로 나타났다.

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