Etiology

Characterization and Comparison of Passionfruit Mottle Virus, a Newly Recognized Potyvirus, with Passionfruit Woodiness Virus

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ABSTRACT

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A virus, designated passionfruit mottle virus (PaMV), was isolated from plants of passionfruit cultivar Tainung No. 1, which showed mild mottling. Unlike the previously described passionfruit woodiness virus (PWV), PaMV does not induce severe foliage mosaic and woody, misshapen fruits. It was identified as a potyvirus on the basis of particle morphology, aphid transmissibility, and the ability to induce cytoplasmic cylindrical inclusions (CI) in infected cells. Of 25 plant species inoculated, 11 (*Nicotiana benthamiana*, four passiflora species, and six leguminous species) developed differential reactions. Two bean cultivars (i.e., Sutter pink and Dubbele witte) resistant to PWV were susceptible to PaMV, whereas another cultivar, Black turtle, which is resistant to PaMV, was systemically infected with PWV. PaMV induced bundle-shaped CIs in the cytoplasm of infected cells; PWV induced short platelike CIs. Purified virions and the cylindrical inclusion proteins (CIPs) of PaMV were obtained by isopycnic centrifugation and preparative electrophoresis, respectively. Antisera against intact virions and CIPs of PaMV and PWV were prepared for the comparison of antigenic properties. In reciprocal sodium dodecyl sulfate immunodiffusion tests and direct enzyme-linked immunosorbent assays, PaMV was shown to be antigenically related to, but distinct from, PWV, bean common mosaic, blackeye cowpea mosaic, watermelon mosaic 2, and soybean mosaic viruses, but was unrelated to 12 other potyviruses tested in the study.

Golden passionfruit (*Passiflora edulis* Sims. f. *flavicarpa* Degener) has been commercially cultivated in Taiwan for more than 25 yr. Virus disease, however, was not a major limiting factor for growing this crop until 1980 when an outbreak of passionfruit woodiness virus (PWV) occurred (9). Since then, surveys for virus diseases were routinely conducted in the passionfruit-growing areas across Taiwan. In addition to PWV, cucumber mosaic virus (CMV) was also sporadically found in several passionfruit-planting areas on the island (9). Infection of the most widely grown passionfruit cultivar, Tainung No. 1 (TN1) (*P. edulis* × *P. edulis* f. *flavicarpa*), with PWV resulted in severe systemic mosaic and rugose foliar symptoms. Infected plants were greatly reduced in vigor and produced severely malformed and woody fruits (23). CMV-infected TN1 normally develop bright yellow spots on young leaves and sometimes showed acute chlorosis and tip blighting on young shoots (9).

During a regular survey in 1987, some TN1 plants in Puli areas showed only mild mottling symptoms with no woody and misshapen fruits. The symptoms differed from those caused by PWV and CMV. In enzyme-linked immunosorbent assays (ELISA) and sodium dodecyl sulfate (SDS) immunodiffusion tests, antigenic differences between PWV and the newly found virus isolate were also detected (5). The virus isolate is currently designated passionfruit mottle virus (PaMV). This paper reports the characteristics of PaMV and compares them with those of PWV.

MATERIALS AND METHODS

Viruses. Leaf and twig samples were collected from TN1 passionfruit plants showing mottling symptoms at Fengshan Tropical Horticultural Experiment Station in Kaoshiung. The twigs were grafted onto healthy golden passionfruit rootstocks and maintained as original virus cultures. Sap extracted from diseased leaves was used to mechanically inoculate *Chenopodium amaranticolor* Coste and Reyn. After three successive single lesion transfers through *C. amaranticolor*, the virus was back-inoculated to golden passionfruit seedlings. Infected seedlings with typical mottling symptoms were later used as a stock culture of PaMV throughout this study. The PWV isolate (9,23) used in this study was maintained similarly in golden passionfruit seedlings.

Host range tests. Host range trials were conducted by mechanical inoculation. At least four plants of each species or cultivar were tested. Inoculum was prepared by grinding leaf tissues with potas-

sium phosphate buffer (100 mM, pH 7.0) in a mortar. Inoculated plants were kept in a screenhouse for at least 1 mo for observation. Virus infection was determined by the reading of symptom expression, by virus recovery from test plants with golden passionfruit seedlings, and also by serological tests.

Aphid transmission tests. Aphid transmission trials were conducted with green peach aphids (*Myzus persicae* Sulzer) as previously described (8), except that aphids were allowed to stay on healthy or PaMV-infected golden passionfruit leaves for 30 min to do access feeding. Groups of five aphids were subsequently transferred to each golden passionfruit seedling. After an inoculation feeding period of 4–5 h, aphids were removed and plants were kept in a screenhouse for observation. The transmission was conducted in duplicate trials each with 15 golden passionfruit seedlings.

Electron and light microscopy. Virus particles in crude sap of PaMV-infected golden passionfruit or TN1 leaves were negatively stained in 2% potassium phosphotungstate (PTA), pH 6.5, containing 0.1% (w/v) bovine serum albumin (11) and observed with a Hitachi 7000 electron microscope. Epidermal strips of healthy or virus-infected golden passionfruit leaves were treated with Triton X-100, stained either with calcomine orange and Luxol brilliant green (O-G) or with azure A, and examined by light microscopy for virus-induced inclusions (10).

TABLE 1. Comparison of host reactions between passionfruit mottle (PaMV) and passionfruit woodiness (PWV) viruses in Taiwan[a]

Test plant	Reactions[b]	
	PaMV	PWV
Passifloraceae		
Passiflora edulis	m, TB	m
P. edulis f. sp. *flavicarpa*	CS, M	M, R, TB
P. edulis × *P. edulis* f. sp. *flavicarpa*	m, mF	M, R, WF
P. suberosa	m	m
P. incarnata	...	...
P. warmingii	m	m
P. coccinea	SLI	SLI
P. foetida	m	YW
Chenopodiaceae		
Chenopodium quinoa	CL	CL
C. amaranticolor	CL	CL
Leguminosae		
Pisum sativum 'Alaska'	...	...
P. sativum 'Taichung 11'	...	...
Phaseolus vulgaris 'Sutter pink'	NL, M (s)[c]	NL (r)
P. vulgaris 'Dubbele witte'	NL, M (s)	NL (r)
P. vulgaris 'Black turtle'	NL (r)	LI (s)
Vigna radiata	...	...
V. mungo	...	...
V. unguiculata subsp. *unguiculata*	...	CL, SLI
V. unguiculata subsp. *sesquipedalis*	...	...
V. angularis	m	...
Arachis hypogaea 'TN 4'	...	...
Cassia occidentalis	m	...
Solanaceae		
Nicotiana benthamiana	M	m
N. tabacum 'Havana'	...	...
N. debneyi	...	...
Lycopersicon esculentum	...	...
Datura stramonium	...	...
Amaranthaceae	...	...
Gomphrena globosa	...	...
Cucurbitaceae	...	...
Cucumis sativus	...	...
C. melo	...	...
Citrullus vulgaris	...	...
Momordica charantia	...	...
Luffa cylindrica	...	...

[a]Plants were inoculated mechanically on the leaves at seedling stage. At least four plants of each species or cultivar were tested. Inoculated plants were kept in a screenhouse for at least 1 mo for observation. Confirmations of infection were conducted by recovery of inoculated and systemic leaves on *C. amaranticolor* and by serological tests as described in the text.

[b]CL = chlorotic lesion; CS = chlorotic spotting; LI = lethal infection; M = mosaic; m = mottling; mF = mottling fruit; NL = necrotic lesion; R = rugose; SLI = symptomless infection; TB = tip blight; WF = woody fruit; YW = yellow wilting; ... = no infection.

[c](s) = Susceptible response; (r) = resistant response. Inoculated plants showing systemic symptoms are considered susceptible to test virus, whereas those expressing only localized symptoms without any systemic infection are considered resistant to test virus.

Purification of virus and cylindrical inclusion protein. PaMV and PWV were propagated in golden passionfruit, and virions were purified by the following procedures. Leaf tissue collected about 14 days post-inoculation was homogenized in a blender for 2 min with 2 ml of potassium phosphate buffer (500 mM, pH 7.5, containing 0.1% [v/v] thioglycolic acid and 10 mM Na_2EDTA) and 1 ml of a chloroform and carbon tetrachloride mixture (1:1) per gram of tissue. The homogenate was clarified by low-speed centrifugation (3,500 *g*, 5 min), and the supernatant was filtered through two layers of cheesecloth. The filtrate was centrifuged at 13,200 *g* for 10 min, and the pellet was retained for isolating cylindrical inclusions (CIs) as previously described (6,8). The supernatant was centrifuged at 85,000 *g* for 1.5 h, and the resulting pellet was resuspended in borate buffer (50 mM, pH 8.2) containing 10 mM EDTA. After a low-speed centrifugation (13,200 *g*, 10 min) for removing insoluble material, the supernatant was subjected to an isopycnic centrifugation in $CsSO_4$ (8). Virions in the opalescent zone were collected and subjected to another cycle of differential centrifugation. Both cylindrical inclusion proteins (CIPs) induced by PaMV and PWV were further purified from the crude inclusion preparations obtained above by preparative SDS-polyacrylamide gel electrophoresis as previously described (6).

Antiserum preparation. Immunization schemes for preparation of antisera against virions and virus-induced CIP of PaMV and PWV were carried out as described by Purcifull and Batchelor (17). For antiserum against virions, three injections each with 1 mg of intact purified virions were administered. For antiserum against CIP, four injections each with 1 mg of purified CIP were given. Antisera collected 1–3 mo after the last injection were used in this study.

Serological tests. Serological relationships of PaMV with PWV and other potyviruses were studied by ELISA and SDS-immunodiffusion tests. The direct ELISA test developed by Clark and Adams (12) was used. Antigens in infected plant extracts and antisera were used against the following viruses in ELISA: bean yellow mosaic (BYMV) (3); blackeye cowpea mosaic (BlCMV) (2); cucumber mosaic (CMV) (2); maize dwarf mosaic (MDMV) (8); papaya ringspot type W (PRV-W) (18); peanut stripe (PStV) (8); potato virus Y (PVY) (17); soybean mosaic (SMV) (3); watermelon mosaic 2 (WMV2) (18); and zucchini yellow mosaic (ZYMV) (16).

The SDS-immunodiffusion test was conducted as described by Purcifull and Batchelor (17). For comparison, the reactants were arranged so that homologous antigens were placed in wells adjacent to the heterologous ones. Antigens and antisera of the following potyviruses, not tested in ELISA, were included: bean common mosaic (BCMV) (3); clover yellow vein (CYVV) (6); papaya ringspot virus type P (PRV-P) (18); pea seed-borne mosaic (PSbMV) (4); peanut mottle (PMoV) (3); and tobacco etch (TEV) (17). Intragel cross-absorption trials, as described by Purcifull and Batchelor (17), were conducted in SDS-immunodiffusion tests for further detection of serological differences among closely related viruses. Heterologous SDS-treated antigens for absorption were added to the wells and incubated at 25 C for 8 h before replacement of the well contents with antisera.

Peptide mapping by partial digestion with protease. The purified capsid protein (CP) and CIP induced by PaMV and PWV were partially digested with *Staphylococcus aureus* V8 protease. The resulting peptides were compared as previously described (6).

Electrophoresis of viral proteins. Purified viral proteins were analyzed by discontinuous SDS-polyacrylamide gel electrophoresis as described previously (8).

Seed transmission tests. To test the possibility that PaMV and PWV are transmitted through seeds of infected passionfruit, each virus was inoculated separately on two golden passionfruit plants

at the seedling stage. The seeds collected from the fruits about 6 mo after inoculation were germinated on sand benches in a screenhouse. Virus infections were determined by indexing the seedlings with ELISA.

RESULTS

Host range and symptomatology. Twenty-five species in six plant families were inoculated with PaMV and PWV for comparisons. Symptoms were usually observed about 7–10 days after inoculation. PaMV induced reactions distinct from those induced by PWV in plants of four passiflora species, six leguminous species, and *Nicotiana benthamiana* Domin. (Table 1). Both viruses induced foliar mosaic symptoms on golden passionfruit; however, PaMV also consistently produced characteristic chlorotic spotting on fully developed leaves. This symptom was never observed on PWV-infected golden passionfruit plants. Plants of *P. foetida* developed mild mottling symptoms when infected with PaMV, whereas these plants expressed yellowing and wilting of the whole plant after inoculation with PWV. Passionfruit cultivar TN1, when infected with PaMV, developed mild mottling on leaves and fruits, but the fruits were of normal appearance (Fig. 1). In contrast, PWV-infected TN1 plants expressed strong mosaic and rugose foliar symptoms and produced severely malformed and woody fruits (Fig. 1). Inoculation of *N. benthamiana* with PaMV always produced severe mosaic symptoms, whereas PWV induced only mild mottling. Adzuki bean (*Vigna angularis* (Willd.) Ohwi & H. Ohashi) and *Cassia occidentalis* L. became infected with PaMV and developed foliar mottling symptoms but were not infected with PWV. On the contrary, PWV, but not PaMV, infected blackeye cowpea (*V. unguiculata* subsp. *unguiculata*) and produced chlorotic lesions on inoculated leaves. Cultivars of common bean (*Phaseolus vulgaris* L. 'Sutter pink' [Sp] and 'Dubbele witte' [Dw]) were susceptible to PaMV and developed systemic mosaic symptoms, but they were resistant to PWV and developed only localized lesions on inoculated leaves. In contrast, another bean cultivar, Black turtle, developed systemic mosaic when inoculated with PWV, but it was resistant to PaMV in that it showed only localized necrotic lesions without systemic infection.

Aphid transmission trials. In two replicate trials used to test the aphid transmissibility of PaMV and PWV, 21 and 24 of 30 golden passionfruit seedlings, respectively, became infected.

Electron and light microscopy. Flexuous rod-shaped particles were consistently observed in negatively stained TN1 or golden passionfruit leaf extracts infected with PaMV by electron microscopy. Particles with similar morphology and size were also observed in samples after virion purification. A total of 104 particles were measured; the mean length was 736 nm.

Cytoplasmic CIs were observed by light microscopy in epidermal cells of PaMV- and PWV-infected TN1 passionfruit processed with O-G staining (Fig. 2). The CIs induced by either virus were not stained by azure A. PaMV-induced CIs were seen as large witches'-broom-like structures (Fig. 2A), which could be readily distinguished from those observed as short platelike aggregates (Fig. 2B) in PWV-infected tissue. No nuclear or amorphous inclusions were observed in PaMV- or PWV-infected tissues stained either with O-G or with azure A.

Purification of virions and viral proteins. Purified virion samples of PaMV and PWV had an ultraviolet absorption spectrum typical of potyviruses with maximum and minimum absorption peaks at 260 and 247 nm, respectively. Without light-scattering corrections, the ratio (260:280) was 1.23–1.25 for purified PaMV and PWV virion samples. With an extinction coefficient of 2.4 for potyviruses, the estimated yields of purified PaMV and PWV virions were 1.5–6.0 and 1.7–5.5 mg per 100 g of infected tissue, respectively. Higher yields of both viruses were obtained by changing the incubation conditions of propagation plants from a regular greenhouse to a growth chamber with a constant temperature of 30 C.

Purified PaMV and PWV viruses (CP) and cytoplasmic CIPs were analyzed in 12% polyacrylamide gels to determine the purities and protein species. All four viral proteins each contained a single species of protein monomer, and no contaminating proteins were detected (Fig. 3). The relative masses (M_r) for CPs of PaMV and PWV were estimated as 38 and 37 kDa, and for CIPs of

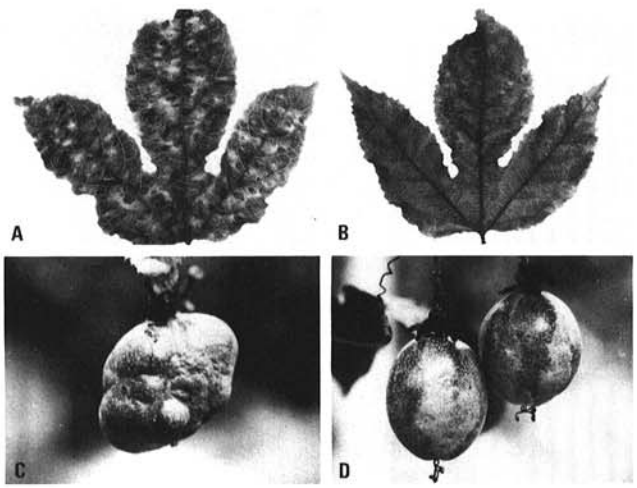

Fig. 1. Reactions of passionfruit cultivar Tainung No. 1 (TN1) to passionfruit mottle virus (PaMV) and passionfruit woodiness virus (PWV). **A,** Severe mosaic induced by PWV. **B,** Mild mottling developed by PaMV. **C,** Malformed and woody fruit of TN1 caused by PWV. **D,** Normal-shaped fruit but with skin mottling induced by PaMV.

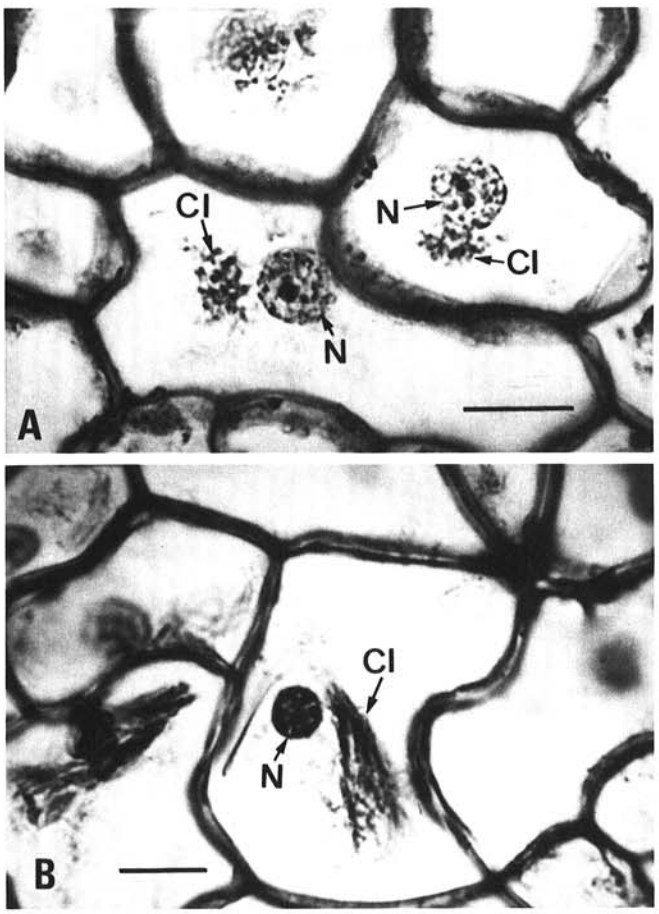

Fig. 2. Light micrographs of epidermal tissue of golden passionfruit stained with Luxol brilliant green and calcomine orange. **A,** Short platelike structures of cylindrical inclusions (CI) accumulated beside the nucleus (N) in epidermal cells sampled from plants 30 days after inoculation with passionfruit woodiness virus. **B,** Large bundle-shaped CI in epidermal cells sampled from plants 30 days after inoculation with passionfruit mottle virus.

PaMV and PWV as 79 and 76 kDa, respectively.

Serology. Four antisera against virions and electrophoretically purified CIPs of PaMV and PWV were prepared in this study for the determination of the serological relationships of PaMV with PWV and other potyviruses. Reciprocal SDS-immunodiffusion tests and ELISA were conducted, and the results are summarized in Tables 2 and 3. All four antisera prepared in this study reacted strongly with their respective homologous antigens but not with healthy plant extracts (Fig. 4). In SDS-immunodiffusion tests, antiserum against intact PaMV virions reacted with antigens of PWV, BCMV, BlCMV, WMV2, and SMV, but the homologous precipitation lines clearly spurred over the heterologous ones (Fig. 4; Table 2). PaMV antiserum did not react with antigens of the following potyviruses: BYMV, CYVV, PSbMV, PStV, PMoV, TEV, ZYMV, PRV-W, PRV-P, and PVY. When PWV virion antiserum was tested, homologous precipitation lines

TABLE 2. Summary of serological relationships of capsid and cylindrical inclusion proteins (CIP) among passionfruit woodiness virus, passionfruit mottle virus, and seven other potyviruses as determined by sodium dodecyl sulfate (SDS) immunodiffusion test[a]

	Cross-reactivities to SDS-treated antigens[b,c]								
Antiserum[d]	PWV	PaMV	BlCMV	BCMV	PStV	WMV2	SMV	ZYMV	PSbMV
PWV	I	S	S	S	S	S	S	S	S
PaMV	S	I	S	S	S	S	S	...	...
PWV-CIP	I	S	...	...	...	...	...	...	...
PaMV-CIP	S	I	...	...	...	...	...	...	...
PStV	S	S	S	S	I	S	S	...	...
PStV-CIP	...	...	*[e]	*	I	...	...	...	...
BlCMV	S	S	I	S	S	S	*	*	S
BlCMV-CIP	...	...	I	*	*	*	*	*	*
BCMV	S	S	S	I	S	*	*	*	
BCMV-CIP	...	...	*	I	*	*	*	*	...
WMV2	...	...	S	S	S	I	*	*	...
SMV	S	S	*	*	*	*	I	*	...
ZYMV	S	S	*	*	*	*	*	I	...
PSbMV	...	...	S	...	...	...	...	...	I

[a]SDS-immunodiffusion tests were done as described by Purcifull and Batchelor (17).
[b]BCMV = bean common mosaic virus isolate US1 from the United States; BlCMV = blackeye cowpea mosaic virus isolate T from Taiwan; PaMV = passionfruit mottle virus from Taiwan; PRPV = passionfruit virus from Puerto Rico; PSbMV = pea seed-borne mosaic virus isolate Ps8 from Taiwan; PStV = peanut stripe virus isolate Ts from Taiwan; PWV = passionfruit woodiness virus from Taiwan; SMV = soybean mosaic virus isolate T1 from Taiwan; WMV2 = watermelon mosaic virus from Florida, U.S.A.; ZYMV = zucchini yellow mosaic virus isolate 1119 from Florida.
[c]Results of cross-reactivities are the reactions between homologous and heterologous antigens: I = precipitin lines of homologous and heterologous reactions fused without spur formation; S = homologous reactions spurred over heterologous reactions; ... = no reaction.
[d]Antiserum indicated with virus names followed by CIP are those against cylindrical inclusion protein of the viruses. Antisera against WMV2 and ZYMV were provided by D. E. Purcifull. All other antisera were produced in my laboratory.
[e]Not tested in this study.

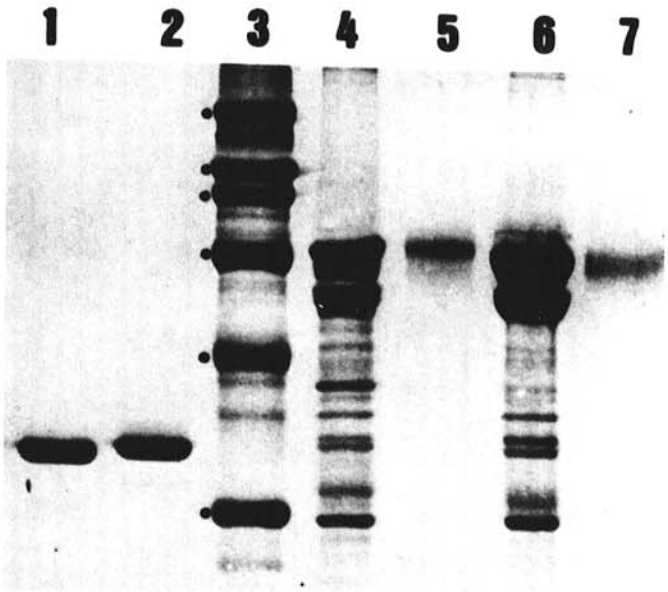

Fig. 3. Analysis of purified capsid (CP) and cylindrical inclusion proteins (CIP) induced by passionfruit mottle virus (PaMV) and passionfruit woodiness virus (PWV) by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Lane 1, purified PWV CP with estimated relative mass (M_r) of 37 kDa; lane 2, purified PaMV CP with M_r of 38 kDa; lane 3, six protein markers that are marked by dark circles on the left side; lane 4, semipurified PaMV CIP preparation; lane 5, purified PaMV CIP with estimated M_r of 79 kDa; lane 6, semipurified PWV CIP preparation; lane 7, purified PWV CIP with estimated M_r of 76 kDa. Marker proteins are (from bottom to top) carbonic anhydrase (29 kDa), ovalbumin (45 kDa), bovine serum albumin (66 kDa), phosphorylase B (98 kDa), galactosidase (116 kDa), and myosin (200 kDa).

TABLE 3. Serological relationships of passionfruit mottle and passionfruit woodiness viruses to nine other viruses as determined by direct enzyme-linked immunosorbent assay (ELISA)[a]

	Absorbance A_{405nm}[b]							
	Infected sap							
	Homologous		PaMV		PWV		Healthy sap	
Antiserum[c]	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}	10^{-2}	10^{-3}
PaMV	1.89	1.21	←[d]	←	0.25	0.13	0.15	0.09
PWV	2.72	2.56	0.39	0.14	←	←	0.04	0.02
BlCMV	2.76	2.63	0.43	0.02	0.40	0.20	0.03	0.02
PStV	2.94	0.86	0.03	0.01	0.04	0.02	0.04	0.02
SMV	2.83	1.80	0.10	0.06	0.16	0.08	0.05	0.04
WMV2	2.83	1.76	0.02	0.00	0.00	0.00	0.01	0.00
ZYMV	2.80	2.50	0.18	0.07	0.01	0.06	0.14	0.09
MDMV	2.83	2.76	0.03	0.02	0.02	0.03	0.03	0.03
BYMV	1.24	0.83	0.09	0.05	0.06	0.04	0.04	0.05
PVY	0.83	0.41	0.05	0.00	0.00	0.00	0.09	0.04
PRV-W	2.85	2.56	0.02	0.02	0.01	0.01	0.01	0.03
CMV	1.48	0.67	0.08	0.07	0.12	0.08	0.07	0.06

[a]Direct ELISA test was conducted as described in the text. Immunoglobulins (IgG) to each virus were used at a concentration of 1 μg/ml. The alkaline-phosphatase-conjugated IgGs from each virus antiserum were used at a dilution of 1:1,000. Virus-infected tissues were ground in 0.05 M phosphate buffer, pH 7.2, at a ratio of 10 ml/g. The sap obtained was taken as 10^{-1} dilution.
[b]Absorbance values were an average of three replicate wells.
[c]BlCMV = blackeye cowpea mosaic; BYMV = bean yellow mosaic; CMV = cucumber mosaic; MDMV = maize dwarf mosaic; PaMV = passionfruit mottle; PRV-W = papaya ringspot virus type W; PStV = peanut stripe; PVY = potato virus Y; PWV = passionfruit woodiness; SMV = soybean mosaic; WMV2 = watermelon mosaic virus 2; ZYMV = zucchini yellow mosaic. Antisera against PVY, WMV2, and ZYMV were provided by D. E. Purcifull. All other antisera were produced in my laboratory.
[d]Absorbance readings identical to those shown in homologous reactions.

spurred over the precipitation lines formed between the antiserum and heterologous antigens of PaMV, BCMV, BlCMV, PStV, PSbMV, SMV, WMV2, and ZYMV (Table 2). Reciprocally, among 13 different antisera tested, only those against BlCMV, PStV, and BCMV reacted, with spur reactions, against PaMV and PWV antigens (Fig. 4; Table 2). Serological distinctions between PaMV and PWV were further detected by intragel cross-absorption tests in which heterologous reactions could be completely removed while the homologous ones were retained (Fig. 4).

On the other hand, when the CIP antisera were tested in SDS-immunodiffusion tests, spur reactions, although not as clear as those obtained by virion antisera, were found between PaMV and PWV antigens (Fig. 4). PaMV and PWV CIP antisera, however, did not react with any of the 13 potyviruses tested (Fig. 4; Table 2). In reciprocal tests, CIP antisera of PStV, BlCMV, and BCMV did not react with PaMV and PWV antigens (Table 2). Heterologous reactions between PWV antigen and PaMV CIP antiserum were removed, whereas the homologous reaction of PaMV was retained when the antiserum was cross-absorbed with PWV antigen (Fig. 4).

In direct ELISA with antisera against intact virions, absorbance values between PaMV antiserum and PWV antigen were lower than two times the healthy control readings, which were considered the thresholds for positive reactions in this study. In reciprocal tests, however, absorbance values between PWV antiserum and PaMV antigen were significantly above the thresholds (Table 3). Differing from the results obtained in SDS-immunodiffusion tests, BlCMV antiserum, but not the other nine antisera, reacted with antigens of PaMV and PWV (Table 3).

Peptide mapping comparison of the viral proteins. Because M_r similarities and serological relatedness were detected between PaMV- and PWV-induced CP and CIP, further comparisons of the viral proteins were made by peptide mapping. After a digestion period of 30 and 45 min with V8 protease, the digested peptides were analyzed in a 12% polyacrylamide gel. Peptide patterns of PaMV CP and CIP were clearly different from those of the respective PWV viral proteins, especially in the high molecular weight regions (Fig. 5). Those in the low molecular weight regions, however, were similar between PaMV- and PWV-induced viral proteins. These results evidently supported that at least two gene products (i.e., CP and CIP) encoded by PaMV and PWV were not only serologically but also chemically distinct.

Seed transmission. One month after germination, no seedlings from 235 and 198 seeds harvested from PaMV- and PWV-infected golden passionfruit plants, respectively, were indexed as virus-infected. All seedlings were further indexed by ELISA, and no virus was detected after another 2-mo incubation in a screenhouse.

DISCUSSION

The five elongated viruses reported to infect passionfruit include PWV (19), passionfruit ringspot (PRV) (14), passionfruit latent (PLV) (1), passionfruit mosaic (15), and *P. foetida* chlorotic spot (13). Among these, only PLV, a carlavirus (1), PWV and PRV, potyviruses (19, 14), have been sufficiently characterized to allow a valid comparison with more recently isolated viruses. The PaMV isolated from Taiwan should be grouped into potyviruses on the basis of its aphid transmissibility, particle morphology, and the ability to induce cytoplasmic CIs as shown in this study. One other potyvirus infecting passionfruit, PRV, is no longer available for a direct comparison with PaMV. However, in the original report by De Wijs (14), PRV induced ringspotting on the foliage without any effect on the fruits of golden passionfruit plants, which is different from those symptoms induced by PaMV. In addition, PRV also reacted differently with other host plants from PaMV. For example, PRV induced symptomless infections on *N. benthamiana*, which when infected with PaMV produced severe systemic mosaic. Although further studies are required to conclusively determine the relationships between PRV and PaMV, they seem to be distinct viruses.

Another potyvirus, PWV, has long been recognized as the most important virus in worldwide passionfruit production areas (19). Properties of PWV have been fully described by various workers (9,19–21,23). Our comparative studies showed that PaMV differed from PWV in many respects, including pathogenicity and symptomatology in passionfruits and other host plants; morphology of CI; serological properties of CPs and CIPs; and chemical nature

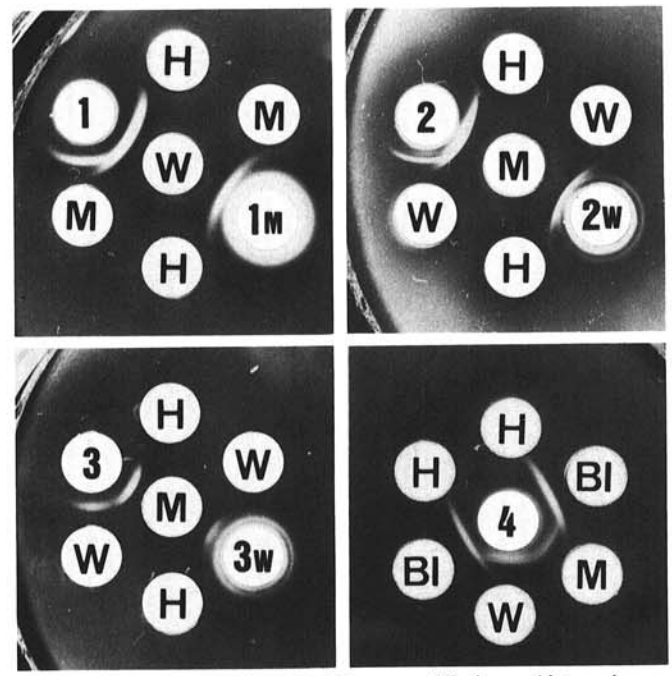

Fig. 4. Sodium dodecyl sulfate (SDS) immunodiffusion and intragel cross-absorption tests with passionfruit mottle virus (PaMV), passionfruit woodiness virus (PWV), and blackeye cowpea mosaic virus (BlCMV). Healthy passionfruit (H), BlCMV (Bl), PaMV (M), and PWV (W) were used as antigens to react with undiluted antisera against PWV virion (1), PaMV virion (2), PaMV-induced cylindrical inclusion protein (3), and BlCMV virion (4). Intragel cross-absorption tests were performed by incubating SDS-treated antigen in wells at 25 C for 8 h before addition of antiserum. Wells of cross-absorption tests were designated by the number of testing antiserum (e.g., 1–3) followed by the letter representing antigens used for cross-absorption (e.g., M or W).

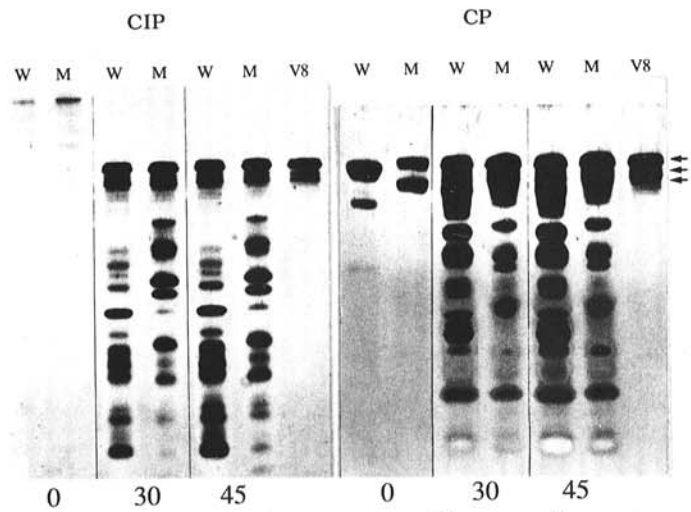

Fig. 5. Comparison of *Staphylococcus aureus* V8 protease digest patterns of passionfruit mottle virus (PaMV) (M) and passionfruit woodiness virus (PWV) (W) induced capsid (CP) and cylindrical inclusion (CIP) proteins. The protease-digested peptides were separated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and visualized by silver staining. Peptide pattern comparisons between PWV- and PaMV-induced CIPs are shown on the left of the figure, whereas those of the CPs are on the right. Identities of proteins are indicated at the top of each lane. Numbers at the bottom indicate digestion period in minutes. Arrows indicate components of V8 protease consistently observed in each lane.

of CPs and CIPs. With these obvious differences, it is reasonable to recognize PaMV as a distinct potyvirus from PWV.

The results obtained from SDS-immunodiffusion tests showed that PaMV was serologically closely related to, but distinct from, PWV in the antigenicities of CP and CIP. However, on the basis of ELISA data, these two viruses were only distantly related to each other. This discrepancy was possibly because the major antigenic determinants reactive in these two serological tests were different. In SDS-immunodiffusion tests, the reactive antigenic determinants are those known as cryptotopes, which reside in dissociated protein subunits of viral proteins (22). In contrast, in direct ELISA, the neotopes residing on the surface of intact virions react with antibodies (22).

The use of the direct ELISA in this study allowed us not only to index but also to readily distinguish PaMV from PWV. By this technique, the distribution of PaMV in the passionfruit-growing areas in Taiwan has been determined to be almost the same as that of PWV (C. A. Chang, *unpublished report*). Mixed infections of both viruses in the same plants were commonly found, indicating the lack of cross-protection effects between these two viruses in nature. This is further evidence showing the biological distinction between PaMV and PWV.

Similar to PWV, PaMV is not seed-borne but can be easily disseminated by aphid transmission and by the use of infected scions. Leaf symptoms of PaMV usually are mild and easily escape visual detection by growers. Without proper serological indexing, scions taken from these plants are possibly misjudged as virus-free and used for propagation. PWV, however, causes severe mosaic on the foliage, which is unlikely to escape visual diagnosis. Therefore, PaMV has a greater probability than PWV to be disseminated through infected seedlings. This is a possible reason for the rapid spread of PaMV in passionfruit-growing areas across Taiwan in recent years. Although PaMV does not produce distorted foliage and woody fruits as PWV does, the vigor of the infected plants can be gradually reduced to an unprofitable level within 1 yr. For this reason, the importance of PaMV to the passionfruit industry in Taiwan is no less than that of PWV. An extensive virus-free passionfruit seedling propagation program has been implemented to deal with this new challenge (7).

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