Comparative Studies on Host Range and Serology of Papaya Ringspot Virus and Watermelon Mosaic Virus 1

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ABSTRACT

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A total of nine isolates of papaya ringspot virus (PRV) were obtained from Taiwan, Hawaii, Florida, and Ecuador. The host ranges of these isolates included members of Chenopodiaceae, Cucurbitaceae, and Caricaceae. Variations in symptoms were observed, but there were no significant differences among the host ranges of the isolates. Three species of Cucurbitaceae (*Cucumis metuliferus* (Acc. 2459), *Cucumis anguria* var. *anguria*, and *Cucumis anguria* var. *longipes*) were found to be valuable hosts for the propagation of PRV. Three isolates of watermelon mosaic virus 1 (WMV-1), one each from New York, Virginia, and Florida were used for comparison. The major difference between PRV isolates and WMV-1 isolates was that the former infected *Carica papaya* (papaya) and the latter did not. *Cucumis metuliferus* (PI 292190), *Cucumis melo* line B66-5, and *Cucumis sativus* 'Surinam,' which possess genes resistant to WMV-1, reacted identically to all isolates of WMV-1 and PRV. All the isolates of PRV and WMV-1 tested were serologically indistinguishable as determined by agar immunodiffusion tests with antisera to PRV and WMV-1. The similarities in resistant-susceptible host reaction and in serology strongly indicate that PRV and WMV-1 are very closely related. Our data also indicate that PRV isolates from widely separated geographic regions of the world have very similar biological and serological properties.

Papaya ringspot virus (PRV) causes one of the most destructive diseases of papaya (*Carica papaya* L.) (4,17), a fruit tree that is grown throughout the tropical and subtropical areas. PRV has been reported to be a major limiting factor for papaya production in Hawaii (10,12), Caribbean countries (1,10,22), India (2,21), South America (9,10), and Florida (3,5). In the late 1970s the virus spread throughout Taiwan island and destroyed most of the commercial papaya orchards (25). PRV is transmitted by aphids in a nonpersistent manner and has been placed in the potyvirus group (6,8). Species of three dicotyledonous families, Caricaceae, Chenopodiaceae, and Cucurbitaceae, have been reported to be hosts of this virus (17).

Watermelon mosaic virus (WMV), a member of the potyvirus group, is a pathogen of great importance wherever cucurbits are grown (24). Based on the failure of cross-protection tests, host range tests, and serological differences, WMV isolates have been classified into two distinct groups, WMV-1 and WMV-2 (13,19,20,28). Purcifull and Hiebert (19) showed that two isolates of PRV from Florida were serologically closely related to WMV-1 but were not related to WMV-2. With antisera to PRV, Gonsalves and Ishii (7) also reported that PRV isolates from Hawaii and Florida were serologically indistinguishable from WMV-1 but were not related to WMV-2.

Despite the worldwide importance of PRV and WMV-1, direct comparison between these viruses have been done with only a few isolates from the United States (7,19). To study the characteristics and relationships of PRV and WMV-1 from different geographic origins, nine PRV isolates from Taiwan, Hawaii, Florida, and Ecuador; and three WMV-1 isolates from New York, Virginia, and Florida were secured. Here we report the results of host range and serological comparisons of those isolates.

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MATERIALS AND METHODS

Source and culture of virus. Five virus isolates from Taiwan (Su-mm, Su-sm, Su-smn, T-Chen, and T-Wang) have been partially characterized and identified as PRV (11,25,29). Two isolates from Hawaii (HA and HB) and one isolate from Florida (F-340) were also reported as PRV (7). One isolate from Ecuador (ED) is first described in this report. All isolates were derived by serial passage of single lesions through *Chenopodium quinoa* Willd. and maintained in *Carica papaya* 'Kapoho Solo.' To avoid the inhibition from papaya latex (14) during host range and serology studies, all isolates were propagated in *Cucumis metuliferus* (Naud.) Mey. (Acc. 2459) which is an extremely sensitive host for PRV (15). Three isolates of WMV-1 from New York (WMV-1 NY), Virginia (WMV-1 VG), and Florida (WMV-1 F) were used for comparison. The WMV-1 isolates were passed through differential hosts (19) and a dilution series to determine their homogeneity and were maintained in zucchini squash *Cucurbita pepo* L. 'President.'

Host range. Tissue from *Cucumis metuliferus* and zucchini squash infected with PRV and WMV-1, respectively, was ground in 0.01 M potassium phosphate buffer, pH 7.0, and extracts were rubbed on test plants dusted with 42-μm (600-mesh) corundum. In host range trials, at least two plants of each species or cultivar were inoculated with isolates of PRV or WMV-1. In cases that yielded negative or uncertain results, the tests were repeated at least twice. The plants were kept in a greenhouse at 24–28 C with supplemental fluorescent lights to maintain a day length of at least 15 hr and observed for 3 wk or longer after inoculation. A modified double-antibody sandwich ELISA test (7) was used in addition to symptomology to check for the presence of PRV or WMV-1. Cheesecloth dipped in Vangard® fungicide was hung in the greenhouse to prevent powdery mildew development (23).

Serology. Antisera produced to intact particles and to dissociated coat protein of PRV HA (7) were used for serological tests. Antiserum to intact particles of WMV-1 was kindly provided by D. E. Purcifull (University of Florida, Gainesville 32611). Antiserum to intact virus of PRV HA (7) was used in ELISA tests, whereas antisera to dissociated coat protein of PRV HA (7) and to

intact virus of WMV-1 (19) were used in agar immunodiffusion tests. The agar medium consisted of 0.8% Ionagar, 1.0% sodium azide, and 0.5% SDS (18). Virus antigens were prepared by grinding 1 g of freshly harvested tissue in 1 ml of distilled water followed by the addition of 1 ml of 3% SDS. The samples were expressed through cheesecloth and used immediately at room temperature. PRV antigen and WMV-1 antigen were prepared from *Cucumis metuliferus* (Acc. 2495) and zucchini squash, respectively.

RESULTS

Symptoms on papaya. On papaya cultivar Kapoho Solo, the isolates of PRV caused foliar symptoms ranging from mild mottling to severe mottling, mosaic, leaf distortion, and shoestringing (Table 1). Most of the isolates incited their own combination of symptoms on the leaves (Fig. 1), but they all caused watersoaked streaks on the stem and extreme retardation of plant growth. In the winter months, all isolates except Su-mm caused severe distortion and shoestringing leaf symptoms. In contrast to PRV, all three isolates of WMV-1 did not infect papaya.

TABLE 1. Symptoms caused by isolates of papaya ringspot virus on *Carica papaya* 'Kapoho Solo' when grown under greenhouse conditions

Origin and isolates designation	Foliar symptoms on papaya[a]	Source
Taiwan		
Su-mm	Mild mottling	H. J. Su
Su-sm	Severe mottling	H. J. Su
Su-smn	Severe mottling, systemic necrosis, and wilting	H. J. Su
T-Chen	Mottling and mosaic	M. J. Chen
T-Wang	Mottling and mosaic	H. L. Wang
Hawaii		
HA	Mosaic and leaf distortion	Gonsalves and Ishii
HB	Mosaic and leaf distortion	Gonsalves and Ishii
Florida		
F-340	Mosaic and shoestringing	Purcifull
Ecuador		
ED	Mosaic and shoestringing	Gonsalves

[a] In the winter months all isolates except Su-mm showed severe symptoms of leaf distortion and shoestringing.

Host range. The host reactions to PRV and WMV-1 are listed in Table 2. All isolates of PRV produced local lesions on *Chenopodium quinoa* and *Chenopodium amaranticolor* Coste et Reyn., and systemically infected most cucurbitaceous plants. All symptoms developed 10–20 days after inoculation. PRV isolates appeared to induce milder symptoms on cucurbitaceous plants than those caused by WMV-1 isolates. However, this difference was not sufficient to distinguish PRV from WMV-1. Isolates PRV HA, PRV F-340, PRV Su-sm, PRV Su-mm, PRV Su-smn, and PRV ED incited more severe mottling or mosaic than did isolates PRV HB, PRV T-Wang, and PRV T-Chen in the curcurbitaceous plants. PRV Su-mm caused slow-developing, diffused local lesions on *Chenopodium quinoa* in contrast to the fast-developing, distinct local lesions of the other PRV isolates.

Among the cucurbitaceous plants tested, *Cucumis metuliferus*, (Acc. 2459), *Cucumis anguria* L. var. *anguria*, and *Cucumis anguria* L. var. *longipes* were the most sensitive hosts. Each isolate of PRV produced prominent mosaic symptoms on these plants within 10 days after inoculation. All PRV and WMV-1 isolates could not be recovered by infectivity assay or detected by ELISA testing of *Cucumis metuliferus* (PI 292190) or *Cucumis melo* L. line B66-5. *Cucumis sativus* L. 'Surinam,' which possesses a single recessive gene for resistance to WMV-1 (R. Provvidenti, *unpublished*), showed transient systemic mottling confined to one or two leaves followed by symptomless growth when inoculated with WMV-1 isolates. Although it remained symptomless when inoculated with PRV isolates, a weak ELISA reaction revealed that the systemic infection was confined to one or two leaves. The WMV-1 susceptible genotypes of these *Cucumis* species were also susceptible to the PRV isolates (Table 2). WMV-1 F, but not WMV-1 NY or WMV-1 VG, produced local lesions on *Chenopodium amaranticolor* and *Chenopodium quinoa*. The major difference between WMV-1 isolates and PRV isolates was that the former could not infect papaya.

All isolates of WMV-1 and PRV produced systemic symptoms on *Cucumis melo* 'Gold Star,' *Cucumis meeusii* Jeffrey, *Cucumis*

TABLE 2. Host reactions of isolates of papaya ringspot virus and watermelon mosaic virus 1[a]

	WMV-1			PRV								
Host	VG	NY	F	HA	HB	F-340	Su-mm	Su-sm	Su-smn	T-Chen	T-Wang	ED
Carica papaya 'Kapoho Solo'	–	–	–	S	S	S	S	S	S	S	S	S
Chenopodium quinoa	–	–	L	L	L	L	L	L	L	L	L	L
Chenopodium amaranticolor	–	–	L	L	L	L	L	L	L	L	L	L
Curcurbita pepo 'President' zucchini	S	S	S	S	S	S	S	S	S	S	S	S
Cucumis anguria var. *anguria*	S	S	S	S	S	S	S	S	S	S	S	S
var. *longipes*	S	S	S	S	S	S	S	S	S	S	S	S
Cucumis sativus 'Marketer'[b]	S	S	S	S	S	S	S	S	S	S	S	S
'National Pickling'[b]	S	S	S	S	S	S	S	S	S	S	S	S
Cucumis sativus 'Surinam'[c]	±	±	±	±	±	±	±	±	±	±	±	±
Cucumis metuliferus (Acc. 2459)[b]	S	S	S	S	S	S	S	S	S	S	S	S
Cucumis metuliferus (PI 292190)[c]	–	–	–	–	–	–	–	–	–	–	–	–
Cucumis melo 'Iroquois'[b]	S	S	S	S	S	S	S	S	S	S	S	S
Cucumis melo Line B66-5[c]	–	–	–	–	–	–	–	–	–	–	–	–

[a] Minuses (–) = not infected as determined by symptom observations and ELISA tests; ± = transient systemic mottling or symptomless, weak ELISA positive; S = systemic symptoms; and L = local lesions.
[b] Susceptible genotypes.
[c] Resistant genotypes.

dipsaceous Spach., *Cucumis dinteri* Cogn., *Cucumis hardwickii* Royle, *Citrullus lanatus* (Thunb.) Matsum. & Nakai 'Sugar Baby,' *Cucurbita moschata* Duch., and *Luffa acutangula* Roxb. None of the PRV or WMV-1 isolates infected *Nicotiana benthamiana* Domin., *Brassica campestris* L. ssp. *chinensis*, *Pisum sativum* L. 'Ranger,' or *Phaseolus vulgaris* L. 'Red Kidney.'

The readings from ELISA were consistent with symptom observations. All the plants showing symptoms following inoculation with WMV-1 isolates or PRV isolates gave positive reactions in ELISA; whereas the symptomless plants, except those of cultivar Surinam of *Cucumis sativus*, gave negative reactions.

Serology. In SDS-immunodiffusion tests, infected papaya tissue

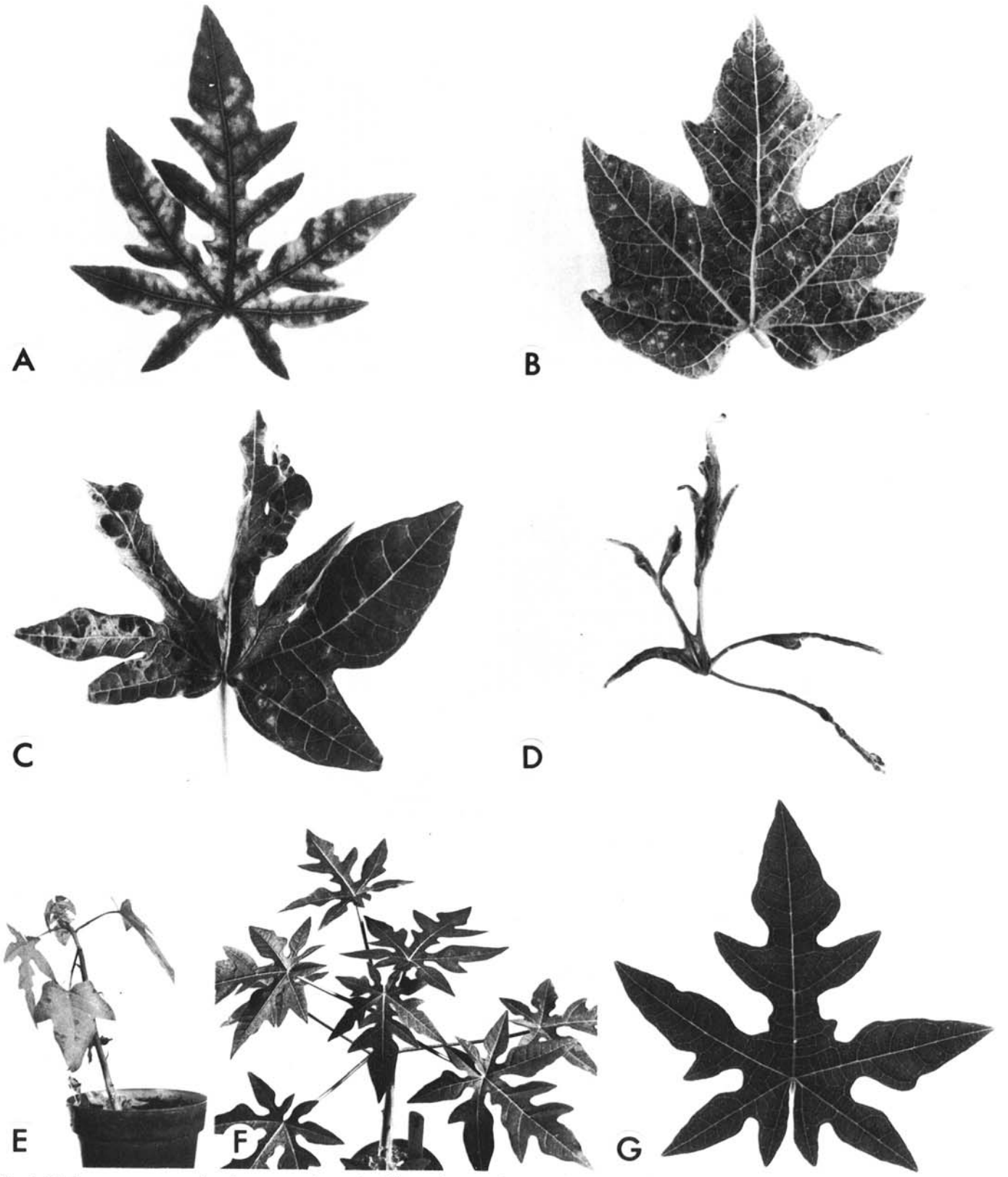

Fig. 1. Distinct symptoms produced on papaya leaves by different isolates of papaya ringspot virus (PRV): **A,** mild mottling by PRV Su-mm; **B,** severe mottling and mosiac by PRV T-Chen, PRV T-Wang, and PRV Su-sm; **C,** mosaic and distortion by PRV HA and PRV HB; **D,** distortion and shoestringing by PRV F-340 and PRV ED; **E,** systemic necrosis and wilting by PRV Su-smn; **F,** healthy papaya plant; and **G,** healthy papaya leaf.

was initially used as a source of crude PRV antigen, but no precipitin lines could be detected. When infected zucchini squash tissue was used, only PRV HA, PRV Su-sm, PRV Su-smn, and PRV Su-mm gave distinct precipitin lines but the other isolates gave either faint reaction lines or no reaction. However, a reliable and consistent reaction was obtained by using infected *Cucumis metuliferus* (Acc. 2459) as a crude-antigen source for PRV, and infected zucchini squash for WMV-1. The reactions determined by using either antiserum to dissociated coat protein of PRV or antiserum to intact particles of WMV-1 indicated that all isolates of PRV and the three isolates of WMV-1 were serologically indistinguishable (Fig. 2). Previous studies showed antiserum to intact particles of PRV gave a weak reaction in agar immunodiffusion tests (7). However, a second antiserum produced to intact particles of PRV gave excellent reactions, similar to the antiserum to dissociated coat protein of PRV or to intact particles of WMV-1.

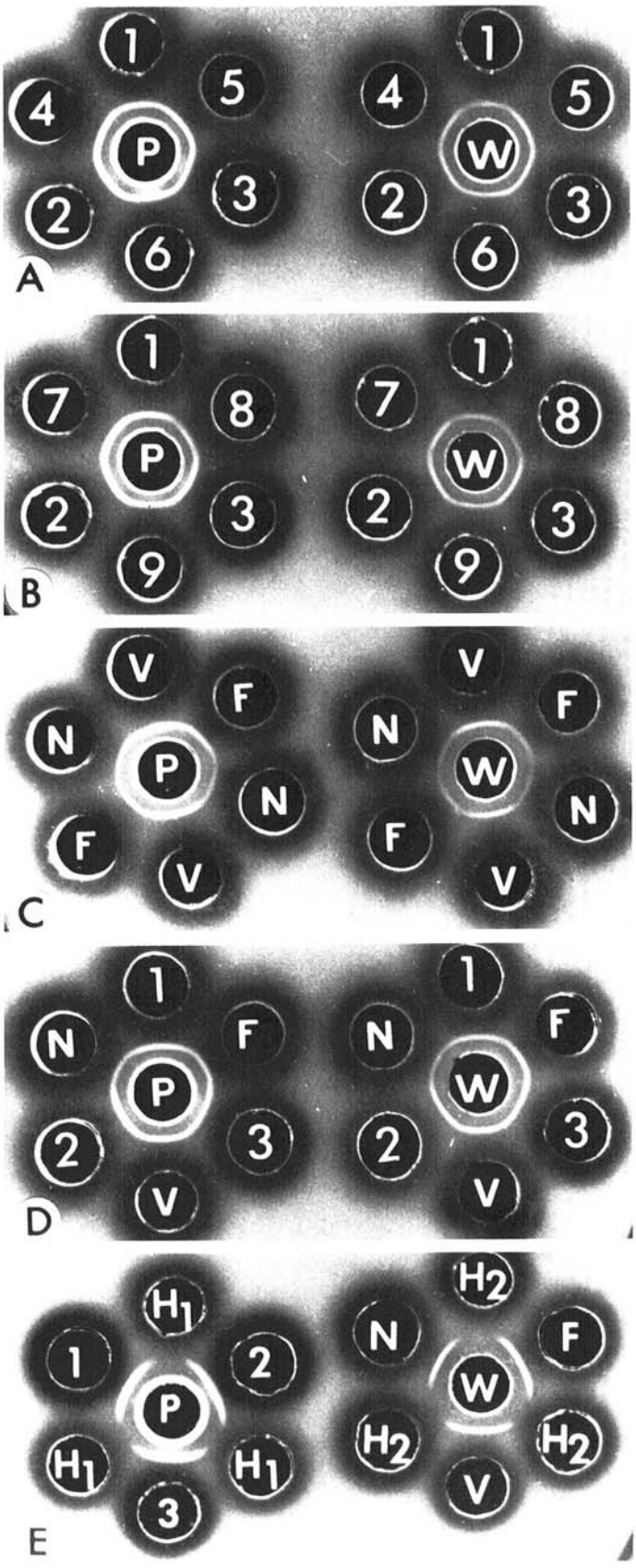

Fig. 2. Reactions in sodium dodecyl sulfate (SDS)-immunodiffusion tests of antisera to capsid protein of papaya ringspot virus (PRV) and to watermelon mosaic virus 1 (WMV-1) against nine isolates of PRV and three isolates of WMV-1. Central wells were filled with antisera and peripheral wells with crude sap antigens from *Cucumis metuliferus* (Acc. 2459) infected with PRV or from zucchini squash with WMV-1. **A and B** show the relationships among PRV isolates. **C** shows the relationships among WMV-1 isolates. **D** shows the relationships among PRV and WMV-1 isolates. **E** shows that antisera did not react with saps from uninfected plants. Note that reactions of serological identities were observed in all combinations. Wells contain: P = PRV antiserum, W = WMV-1 antiserum; 1 = PRV HA, 2 = PRV HB, 3 = PRV F-340, 4 = PRV Su-mm, 5 = PRV Su-sm, 6 = PRV Su-smn, 7 = PRV T-Wang, 8 = PRV T-Chen, 9 = PRV ED; N = WMV-1 NY, V = WMV-1 VG, F = WMV-1 F; H_1 = sap of healthy *C. metuliferus*, and H_2 = sap of healthy squash.

DISCUSSION

According to host reactions of PRV isolates, we confirmed that PRV only infects species in three families, Caricaceae, Chenopodiaceae, and Cucurbitaceae (17,25). Most of the cucurbitaceous plants tested were susceptible to PRV. *Cucumis hardwickii, Cucumis anguria* var. *anguria, Cucumis dipsaceus, Cucumis anguria* var. *longipes, Cucumis meeusii, Cucumis dinteri*, and *Cucurbita moschata* are the new hosts experimentally infected. Although there were variations in the severity of symptoms, ie, mild mottling to severe mosaic, we could not detect significant differences in the host ranges of our PRV isolates. Thus, even though the isolates were obtained from different regions of the world, they seem to belong to the same biological group.

A major problem in purifying PRV was the lack of a suitable host with a high virus titer (7), since papaya latex makes it difficult to purify directly from papaya. The results from this study indicate that *Cucumis metuliferus* (Acc. 2459), *Cucumis anguria* var. *anguria*, and *Cucumis anguria* var. *longipes* might be excellent propagative hosts for the purpose of purification and serology of PRV. In fact, PRV is now routinely purified from *Cucumis metuliferus* (Acc. 2459) in our laboratory.

Based on host reactions, we concluded that WMV-1 NY and WMV-1 VG infect only Cucurbitaceae, but WMV-1 F can infect Cucurbitaceae and Chenopodiaceae. The results support the observation of previous studies (19,20) that some strains of WMV-1 cause local lesions on *Chenopodium quinoa* and *Chenopodium amaranticolor*. Apparently, inability to produce local lesions on these two species should not be used as criteria to distinguish WMV-1 from WMV-2. However, local-lesion production on *Chenopodium quinoa* and *Chenopodium amaranticolor* may be useful criteria to distinguish strains of WMV-1. The major difference between PRV and WMV-1 is that PRV can infect *Carica papaya* whereas WMV-1 can not infect this species.

Provvidenti and Gonsalves (15) have shown that the single dominant resistance gene *Wmv* in *Cucumis metuliferus* (PI 292190) which confers resistance to WMV-1 (16) also apparently confers resistance to three isolates of PRV (HA, HB, and F-340). Webb (26,27) reported that the immunity of *Cucumis melo* line B66-5 to WMV-1 is controlled by a single dominant gene, *Wmv*-1. The resistance (virus confined to one or two uninoculated leaves) in cultivar 'Surinam' of *Cucumis sativus* to WMV-1 is controlled by a single recessive gene (R. Provvidenti, *unpublished*). These three resistant genotypes of *Cucumis* species reacted identically to all

WMV-1 and PRV isolates tested. Our data coupled with previous works (15,16,26,27) strongly indicate that the resistance to WMV-1 and to PRV might be conditioned by identical or closely linked genes.

The PRV isolates from Hawaii and Florida were considered serologically indistinguishable from WMV-1 in previous studies (7,19). We have shown that PRV isolates from widely separated geographic regions of the world are serologically indistinguishable from the WMV-1 isolates from the United States. The similarity in resistant-susceptible host reactions and the identity in serology strongly indicate that PRV and WMV-1 are very closely related. Reclassification of PRV and WMV-1 as strains of the same virus rather than as two different viruses should be considered.

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