Comparison of Diagnostic Hosts and Serological Tests for Four Cucurbit Potyviruses

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ABSTRACT

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Four cucurbit potyviruses, i.e. zucchini yellow mosaic virus (ZYMV), type W isolate of papaya ringspot virus (PRV-W), watermelon mosaic virus-2 (WMV-2) and melon vein-banding mosaic virus (MVbMV), were compared by their reactions on six diagnostic hosts, SDSimmunodiffusion and ELISA tests. Results of host reactions indicated that isolates of PRV-W group (PRV-W FL, PRV-W T and PRV-Wv) did not infect Cucumis metuliferus PI "292190", but other three viruses did. Both Phaseolus vulgaris "Black Turtle 2" and Nicotiana benthamiana were only infected by WMV-2. In Luffa cylindrica, ZYMV induced severe mosaic and rugose symptoms, MVbMV mild mosaic, PRV-W latent infection or mild mosaic, while WMV-2 did not infect this plant. Another diagnostic host, Lagenaria siceraria, generally produced conspicuous mosaic symptoms when infected with MVbMV but latent or mild mosaic when infected with isolates of PRV-W group. This plant did not react to WMV-2 and ZYMV. Chenopodium quinoa separated MVbMV from other three viruses by producing systemic local lesions in contrast to localized lesions of other viruses. In SDS-immunodiffusion tests, antisera to virus particles (VP) of each virus were specifically reactive to their homologous antigens. However, antiserum to PRV-W FL VP did not react with PRV-Wv. Conversely, antiserum to PRV-Wv VP produced spur precipitin bands with PRV-W FL. Antisera to cylindrical inclusion (CI) proteins were specific for the homologous viruses except that antiserum to MVbMV CI which produced spur precipitin lines with WMV-2 antigen. In ELISA tests, antiserum to PRV-W FL VP did not detect PRV-Wv, and antiserum to PRV-Wv VP reacted only weakly with PRV-W FL antigen when compared with the homologous antigen/antibody reactions.

Key words: Cucurbit potyvirus, ZYMV, PRV-W, WMV-2, MVbMV.

INTRODUCTION

Cucurbits have become an important vegetable and fruit crops in Taiwan. About 45,000 ha are planted with different cultivars each year (1). The major cultivars are watermelon (Citrullus vulgaris Schard.), melon (Cucumis melon L.), cucumber (Cucumis sativus L.) and sponge gourd (Luffa cylindrica [L.] Bailey). Virus diseases have been considered to be one of the limiting factors affecting the cucurbit production. To date, six viruses have been identified (3,6,7,10,31). Surveys of the viruses in the cucurbit producing areas indicated that zucchini yellow mosaic virus (ZYMV) was the most prevalent, followed by type W isolate of papaya ringspot virus (PRV-W)(formerly watermelon mosaic virus-1, WMV-1) and cucumber mosaic virus (CMV)(3,7). However, the melon vein-banding mosaic

virus (MVbMV), a newly identified cucurbit potyvirus, is also common especially in sponge gourd, bottle gourd and melon plants (10).

For a virus identification, it is generally based on host reactions, physical properties, insect vector, serology and electron microscopic observation (6,13,17). In this study we reported the use of selected diagnostic hosts, sodium dodecyl sulfate (SDS)-immunodiffusion test and enzyme-linked immunosorbent assay (ELISA) for diagnosis of four cucurbit potyviruses.

MATERIALS AND METHODS

Viruses

Four cucurbit potyviruses used were zucchini yellow mosaic virus (ZYMV), type W isolate of papaya

ringspot virus (PRV-W), watermelon mosaic virus-2 (WMV-2)(provided by D. E. Purcifull, University of Florida), and melon vein-banding mosaic virus (MVbMV). In the PRV-W group, a Florida isolate (PRV-W FL, provided by D. E. Purcifull), a Taiwan isolate (PRV-W T) and a variant of PRV-W (PRV-Wv) were included. All viruses were maintained on Cucumis pepo L. var. "zucchini" squash in screenhouse.

Diagnostic hosts

The diagnostic plant species were bottle gourd (Lagenaria siceraria Standl.), sponge gourd, Cucumis metuliferus (Naud.) Mey. Acc. "2459" (line 35) and PI "292190" (line 37), Phaseolus vulgaris L. "Black Turtle 2", Nicotiana benthamiana Domin., and Chenopodium quinoa Willd. All plants were grown at temperatures 25-30 C under screenhouse conditions. Inoculum of each virus was prepared from zucchini leaves inoculated with each virus. For each species, groups of 3 plants were mechanically inoculated with virus and the plants were observed for symptom development for 3 wks. Back inoculation to zucchini squash was carried out if inoculated plants produced no clear symptoms.

SDS-immunodiffusion test

Antisera to virus particles (VP) and cylindrical inclusion (Cl) proteins of each virus were prepared in our laboratory. The agar medium for SDSimmunodiffusion tests was prepared as described

eleswhere (8,10). Crude antigens for each virus were extracted from fresh leaves of infected zucchini plants. Generally, the leaf tissues were ground in distilled water (1 g/ml), followed by adding 1 ml of 3% SDS and then filtered through cheesecloth. The formation of precipitin bands in agar gel was observed after a 24 hr incubation at room temperature.

ELISA tests

Antisera to VP of ZYMV, WMV-2, PRV-W, MVbMV and PRV-Wv were used. The procedures for the purification and conjugation of immunoglobulin (IgG) for double antibody ELISA (direct ELISA) were similar to those described by Clark and Adams (4), and Lister (15). Dilutions of coating and conjugated IgG were 0.67 ug/ml and 1/1500 for each virus, respectively. Leaves were harvested from zucchini plants 8 days for ZYMV, 10 days for WMV-2, PRV-Wv and MVbMV. and 20 days for PRV-W FL after inoculation at temperatures 25-30 C under screenhouse conditions. Antigens prepared from those infected leaves were extracted in 0.1 M phosphate buffer, pH 7.5, and serially diluted to 10¹, 10², 10³, 10⁴ and 10⁵. Absorbance A405 was read by a Bio-Tek ELISA reader, Model EL 310 (Bio-Tek Instruments, Winooski, VT, USA) after incubation at room temperatures (25-30 C) for 15 or 25 min depending on absorbance values of the reactions from different viruses.

TABLE 1. Reactions of six plant species to the inoculation of four cucurbit potyviruses

Test plants	Symptoms					
	PRV-W					
	FL	T	V	WMV-2	ZYMV	MVbMV ^τ
Chenopodium quinoa Willd.	2	LL		LL	LL	SLL ²
Lagenaria siceraria Standl.						
Sib 82-12	*****		MM		_	M, F, MO
89-904	_	_				M, F, MO
82-13		_	SL	_	_	M, F
Luffa cylindrica (L.) Bailey		MM	MM	_	SM, R	MM
Cucumis metuliferus (Naud.) Mey.						
Acc. 2459 (Line 35)	M	M	M	M	SM, S	M, S
PI. 292190 (Line 37)			_	M	SM, S	M M
Phaseolus vulgaris L. var.						
Black Turtle 2			_	M	_	_
Nicotiana benthamiana Domin	_		_	M	_	_

¹ PRV-W: type W isolate of papaya ringspot virus (FL: Florida isolate, T: Taiwan isolate, V: Variant of PRV-W); WMV-2: watermelon mosaic virus-2; ZYMV: zucchini yellow mosaic virus; MVbMV: melon vein-banding mosaic virus.

² —: no infection; M: mosaic; MM: mild mosaic; SM: severe mosaic; R: rugose; S: stunting; F: flecking; MO: mottle; SL: symptomless; LL: local lesion; SLL: systemic local lesion.

RESULTS

Diagnostic host reactions

The results of inoculation of ZYMV, WMV-2, MVbMV, and PRV-W isolates on six diagnostic hosts were summarized in Table 1. Symptoms induced by different viruses in selected diagnostic hosts were in Fig.

1. It showed that PRV-W group did not infected C. metuliferus PI "292190", whereas the other three viruses induced clear mosaic or rugostic symptoms. This plant could be used to differentiate PRV-W from other viruses. Both P. vulgaris "Black Turtle 2" and N. benthamiana inoculated with WMV-2 produced mosaic symptoms, but the other three viruses did not. These two plants could be used for diagnosis of WMV-2.

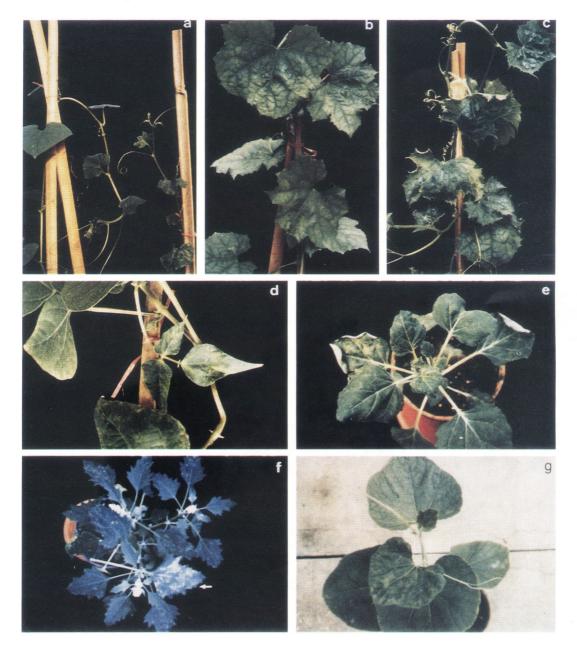


Fig. 1. Symptoms of different diagnostic plants induced by one of the four cucurbit potyviruses after inoculation. (a) no symptom in Cucumis metuliferus PI "292190" 2-3 weeks after inoculation with type W isolate of papaya ringspot virus (Left); severe mosaic and stunting induced by zuccchini yellow mosaic virus (ZYMV)(Right). (b) mild mosaic in Luffa cylindrica by melon vein-banding mosaic virus (MVbMV). (c) severe mosaic and rugose symptoms in L. cylindrica by ZYMV. (d) mosaic in Phaseolus vulgaris "Black Turtle 2" by watermelon mosaic virus-2 (WMV-2). (e) mosaic in Nicotiana benthamiana by WMV-2. (f) systemic local lesions in Chenopodium quinoa by MVbMV; local lesions on inoculated leaf (arrow). (g) mosaic in Lagenaria siceraria by MVbMV.

Generally, ZYMV produced severe mosaic or rugose symptoms on sponge gourd but did not infect bottle gourd. However, MVbMV induced mild mosaic symptoms on sponge gourd, and mosaic, flecking or mottling on bottle gourd. In addition, MVbMV produced systemic local lesions in *C. quinoa*. Thus, ZYMV and MVbMV could be distinguished by above three diagnostic hosts.

SDS-immunodiffusion tests

The reactions of four cucurbit potyviruses were serologically distinguishable in SDS-immunodiffusion tests (Fig. 2). Antiserum to VP of each virus only produced precipitin band with its homologous antigen (Fig. 2A,C,D,E). However, antiserum to PRV-W FL VP did not react with PRV-Wv antigen (Fig. 2A), but antiserum to PRV-Wv VP produced spurred precipitin bands between homologous and PRV-W antigens (Fig. 2B). Antiserum to CI proteins of ZYMV or WMV-2 only produced precipitin band with respective antigen (Fig. 2C,D), but antiserum to MVbMV CI protein formed spurred bands between homologous and WMV-2 antigens (Fig. 2E). Of the PRV-W virus group, antisera to Cl protein of both PRV-W T and PRV-Wv reacted specifically with viruses in this group and could be used to separate PRV-W from other three viruses (Fig. 2A,B).

ELISA tests

Results of ELISA tests are shown in Fig. 3. Generally, IgG purified from antisera to individual virus was only specific to its antigen or to different

isolates/strains of same virus. In PRV-W group, the serological reactivity was stronger in homologous than in heterologous antigens. Interestingly, IgG purified from PRV-W FL antiserum had no serological reactivity to PRV-Wv (Fig. 3a). Conversely, PRV-Wv antiserum reacted with PRV-W FL antigen although the absorbance value of PRV-W FL was much lower $(\Lambda_{405}=0.4)$ than that of PRV-Wv $(A_{405}=1.21)$ (Fig. 3b).

DISCUSSION

Previously we reported the occurrence of two cucurbit potyviruses, namely ZYMV and PRV-W, in the cucurbit production areas in Taiwan (3,7). Although more than 3000 samples were collected from virus infected cucurbits and tested by ELISA, we did not detect the presence of WMV-2 (3,7). However, a new cucurbit potyvirus, melon vein-banding mosaic virus (10), and a type W variant of papaya ringspot virus were isolated (9).

The use of diagnostic hosts to distinguish different potyviruses from cucurbits have been reported (14,21,25, 26). Webbs (30) showed that *Luffa acutangula*, which is susceptible to PRV-W but immune to WMV-2, is a good host for separating PRV-W from WMV-2. Milne *et al.* (18) reported that WMV-2 strains could be readily identified by their ability to induce local lesions on *C. amaranticolor*, but PRV-W strains did not. However, strains of PRV-W inducing local lesions on *C. amaranticolor* have been reported (25). We have also found that several Taiwan isolates of PRV-W induced

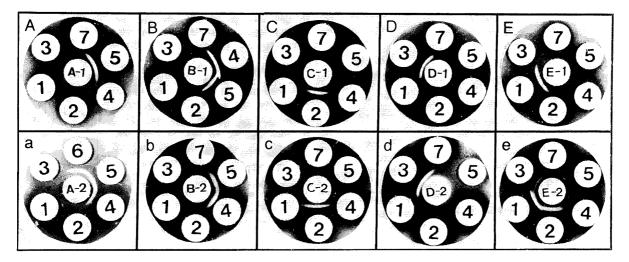


Fig. 2. Scrological reactions of four cucurbit potyviruses in SDS-immunodiffusion tests. Central wells contained antisera to virus particles: A-1 = type W isolate of papaya ringspot virus, a Florida isolate (PRV-W FL), B-1 = a variant of PRV-W (PRV-Wv), C-1 = watermelon mosaic virus-2 (WMV-2), D-1 = zucchini yellow mosaic virus (ZYMV), E-1 = melon vin-banding mosaic virus (MVbMV); antisera to cylindrical inclusion proteins: A-2 = PRV-W Taiwan isolate (PRV-W T), B-2 = PRV-Wv, C-2 = WMV-2, D-2 = ZYMV, E-2 = MVbMV. Outer wells contained SDS-treated antigens: 1 = MVbMV, 2 = WMV-2, 3 = ZYMV, 4 = PRV-Wv, 5 = PRV-W FL, 6 = PRV-W T and 7 = healthy extracts from zucchini leaf tissues.

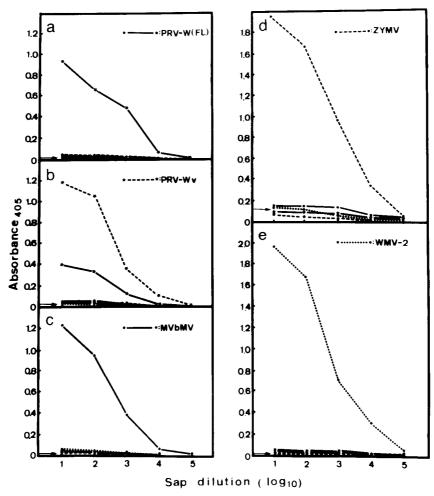


Fig. 3. ELISA tests of four cucurbit potyviruses. Immunoglobulin purified from antisera to: a=type W isolate of papaya ringspot virus, a Florida isolate (PRV-W FL); b=type W variant of papaya ringspot virus (PRV-Wv); c=melon vein-banding mosaic virus (MVbMV); d=zucchini yellow mosaic virus (ZYMV) and e=watermelon mosaic virus-2 (WMV-2). Absorbance A₄₀₅ for different viruses were: * \longrightarrow = PRV-W FL; *----* = PRV-Wv; • \longrightarrow = MVbMV; •---- = ZYMV; • • = WMV-2. Arrows indicate control values from healthy zucchini leaf tissues.

local lesions on both C. amaranticolor and C. quinoa (Huang et al., unpublished). Therefore, C. amaranticolor is insufficient to separate PRV-W from WMV-2. C. metuliferus PI "292190" is resistant to type P and W isolates of PRV (20,25), but susceptible to WMV-2 and ZYMV (21). Phaseolus vulgaris "Black Turtle 2" and Nicotiana benthamiana were reported to be systemically infected by WMV-2, but not or only locally infected by PRV-W and ZYMV (5,19,21). We have confirmed that C. metuliferus PI "292190" is resistant to PRV-W, but susceptible to other 3 viruses (Table 1). In addition, both P. vulgaris "Black Turtle 2" and N. benthamiana were not infected by PRV-W, ZYMV and MVbMV, but WMV-2 did. Thus, the above 3 species are useful for differentiating PRV-W, WMV-2 and ZYMV. In comparing ZYMV with MVbMV, we found that the

former produced severe mosaic or rugose, while the latter induced mild mosaic symptoms in L. cylindrica. In addition, MVbMV produced systemic chlorotic local lesions in C. quinoa, but ZYMV only induced lesions on the inoculated leaves. Another selected host, Lagenaria siceraria was also susceptible to the infection of MVbMV. Therefore, these six selected hosts are good for diagnosis of the four cucurbit potyviruses under this study, but they are not suitable for distinguishing the isolates/strains of PRV-W.

The SDS-immunodiffusion technique is useful for detecting and distinguishing several isometric and anisometric viruses (22). Mokkouk and Gumpf (17) reported that the five PVY strains could be devided into two groups by agar-gel double diffusion tests using purified SDS-degraded virus preparations as antigens. In cucurbit potyviruses, Purcifull et al. (23,24) reported that PRV-W, WMV-2, WMV-M (a Moroccan isolate of WMV) and ZYMV were distinguished by SDSimmunodiffusion tests although WMV-2 antiserum collected at late bleedings formed definite spur precipitin bands between WMV-2 and ZYMV 1119 isolate. We have confirmed that PRV-W, WMV-2, ZYMV, and MVbMV antisera can be used to distinguish the respective antigens by SDSimmunodiffusion tests. However, to recognize the strains or variant of a virus it would require the use of different antisera to VP, CP and CI proteins of a virus. Purcifull et al. (25) reported that PRV-W and PRV-P show different biological and physical properties but possess serologically indistinguishable capsid proteins. Another PRV isolate (PRV-T) had biological and physical properties similar to those of PRV-W, but its CP was distinct from the PRV-W CP (27,28). Our results indicated that antiserum to PRV-W FL VP did not react with PRV-Wv antigen, but antiserum to PRV-Wv VP produced spur precipitin bands between PRV-W FL and PRV-Wv antigen wells. Nevertheless antisera to PRV-Wv or PRV-W CI proteins reacted with both virus isolates were serologically identical.

Both direct and indirect ELISA for detection of plant viruses has been widely used. Regenmortel et al. (29) showed that indirect ELISA is better than direct ELISA for detecting serological distinct strains of tobacco mosaic virus, whereas other reports indicated that direct ELISA is extremely strain specific (2,12,16, 29). Therefore, we used direct ELISA for this study and demonstrated that four cocurbit potyviruses were readily distinguished by direct ELISA. However, in PRV-W group ELISA values are influenced by different isolates/strains. Interestingly, PRV-W FL antiserum did not react with PRV-Wv antigen. Conversely, PRV-Wv antiserum did react with PRV-W FL even at a low absorbance value. Huang et al. (11) reported that antiserum to ZYMV-7 reacted with homologous (ZYMV-7) antigen produced a higher ELISA values than those of three isolates of ZYMV from Taiwan and one ZYMV isolate from Florida under same conditions. Thus, to clarify these differences more research is needed.

In summary, the four cucurbit potyviruses described above can be readily diagnosed by six diagnostic hosts. ELISA or SDS-immunodiffusion tests.

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摘 要

黄秋雄、梁素琴、鄧汀欽、許秀惠. 1993. 四種屬於 PVY 群之瓜類病毒在診斷寄主與血清反 應特性之比較. 植病會刊 2:169-176. (台中縣 台灣省農業試驗所植物病理系)

四種病毒包括矮南瓜黃化嵌紋病毒 (zucchini yellow mosaic virus, ZYMV), 木瓜輪點病毒-西瓜系統 (type W isolate of papaya ringspot virus, PRV-W), 西瓜嵌紋病毒-2(watermelon mosaic virus-2, WMV-2)及甜瓜脈綠嵌紋病毒 (melon vein-banding mosaic virus, MVbMV)。從 供試的六種診斷寄主反應結果獲知, PRV-W不感染 Cucumis metuliferus "PI 292190", 但其他 三種病毒可。 Phaseolus vulgaris "Black Turtle 2"和 Nicotiana benthamiana僅被 WMV-2 感染。 利用 Luffa cylindrica 為診斷寄主, ZYMV 可引起嚴重嵌紋與葉片皺縮之病徵, MVbMV 引起 輕微嵌紋, PRV-W引起輕微嵌紋或潛伏, 但WMV-2不會感染該植物。另外一種寄主, Lagenaria siceraria,如果感染 MVbMV 則產生明顯嵌紋病徵,而 PRV-W 僅引起輕微嵌紋或潛 伏感染, 該植物不被 WMV-2或 ZYMV感染。從目前所得結果, MVbMV可利用 Chenopodium quinoa分開,因爲 MVbMV 在該植物上產生系統性單斑,而其他三種病毒僅在 接種葉形成單斑。在血清反應特性上,四種病毒之抗血清 (antisera to virus particles)在 SDSimmunodiffusion tests上均可區別之。若以內含體抗血清 (antisera to cylindrical inclusion (CI)), 同樣可以分別出。然 MVbMV CI 抗血清會與 WMV-2 反應, 但有交叉現象 (spur precipitin lines)

。利用 ELISA tests, 抗體與同源抗原 (homologous antigen) 反應之 ELISA 吸收值相當高,對不 同病毒抗原均不反應。因此同樣可以利用 ELISA 將四種不同病毒區別之。然而相同病毒之不 同分離株或系統 (virus isolate/strain),不論利用 SDS-immunodiffusion test或 ELISA, 反應結果 可能略有差異,必須視 isolate 或 strain 作進一步之研究。

關鍵詞: 瓜類 PVY 病毒, ZYMV, PRV-W, WMV-2, MVbMV。