Serological and Biological Variability Among Papaya Ringspot Virus Type-W Isolates in Florida

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ABSTRACT

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Six distinct monoclonal antibodies (MAbs) were obtained to the viral structural protein of a Florida isolate of papaya ringspot virus type-W (PRSV-W). These MAbs were distinguished by their reactivity in indirect ELISA with 10 Florida isolates of PRSV-W, five additional isolates of PRSV-W, and with single isolates of the papaya strain of PRSV (PRSV-P) and a strain of PRSV from Guadeloupe (PRSV-T). MAb-1 and MAb-2 reacted with all 15 of the PRSV-W isolates and with PRSV-P. MAb-1 reacted with PRSV-T but MAb-2 did not. MAb-3 and MAb-4 reacted with eight of the 10 Florida isolates and with the other five isolates of PRSV-W. Neither MAb-3 nor MAb-4 reacted with PRSV-T. MAb-4 reacted with PRSV-P but MAb-3 did not. MAb-5 reacted with seven of the 10 Florida isolates of PRSV-W, the other five isolates of PRSV-W, with PRSV-P, and with PRSV-T. MAb-6 reacted only with the PRSV-W isolate used for immunization. When the PRSV isolates in this study were inoculated to different melon (*Cucumis melo*) cultivars, three biological variations were seen among the three isolates of PRSV-W that did not react with MAb-5. Isolate 2030, like the 12 other isolates of PRSV-W, gave compatible reactions in Edisto 47 and Vedrantais. Isolate 2052 gave incompatible reactions in Edisto 47 and compatible reactions in Vedrantais. Isolate 2040, like PRSV-T, gave incompatible reactions in both Edisto 47 and Vedrantais. The Florida isolates 2030, 2052, and 2040 also did not react with the Agdia "potyvirus group" MAb. The remaining PRSV-W isolates did react with this MAb. Our results indicate that the Florida population of PRSV-W is antigenically and biologically variable. This information has implications for the diagnosis and control of this virus in Florida.

Additional keyword: watermelon mosaic virus-2.

Three strains of the potyvirus papaya ringspot virus (PRSV) have been described (18). The papaya strain (PRSV-P) is an important pathogen of papayas in most areas where the crop is grown, and it can also infect cucurbits (18,30). The watermelon strain (PRSV-W), also called watermelon mosaic virus-1, is a pathogen of worldwide importance in cucurbits. Although PRSV-P and PRSV-W are closely related serologically (18,30), PRSV-W does not infect papaya. A strain of PRSV isolated from squash in Guadeloupe (PRSV-T) also does not infect papaya; it is antigenically related to, but different from, PRSV-W (23). Quiot-Douine et al (22) have detected biological differences

between PRSV-W and PRSV-T by inoculation to melon (*Cucumis melo* L.) lines differing in their resistance to PRSV. They also detected antigenic variability among PRSV isolates with polyclonal antisera by using intragel absorption in double radial immunodiffusion tests (22).

Although PRSV-W has been an important pathogen of cucurbits in Florida for many years, only limited studies have been conducted to assess variation among its isolates. This study reports biological variation detected by inoculation of different melon lines with 10 Florida isolates of PRSV-W and serological variation detected by the use of monoclonal antibodies to PRSV-W in indirect enzyme-linked immunosorbent assays (I-ELISA). The antigenic and biological variation detected among Florida isolates of PRSV-W has implications for the diagnosis and control of this virus in Florida. A preliminary account of the results with the monoclonal antibodies has been published (2).

MATERIALS AND METHODS

Virus purification. An isolate of PRSV-W from Florida (19) was used for virus purification. This isolate (W-1A) was maintained in *Cucurbita pepo* L. 'Small Sugar' pumpkin by manual inoculation. The virus was purified by the procedure of Purcifull et al (18). For the preparation of capsid protein, some of the purified virus was dissociated in an equal volume of Laemmli dissociating solution (12), heated, and electrophoresed in sodium dodecyl sulfate preparatory 10% polyacrylamide gels (SDS-PAGE). The protein bands were detected by soaking the gels in cold 0.2 M KCl, excising them, and crushing them with a mortar and pestle. The proteins were removed from the gel by methods for the amorphous inclusion protein described by de Mejia et al (6). Protein yields were estimated by spectrophotometry at A_{280nm} (10), and the protein purity was determined by analysis in SDS-PAGE. The dialyzed protein was freeze-dried and stored at −17 C. Purified virus was stored at −80 C.

Polyclonal rabbit antisera. A New Zealand white rabbit (1125) was immunized with 1 mg/ml of purified capsid protein emulsified with 1 ml of Freund's complete adjuvant. A second injection in incomplete adjuvant was given a week later and was followed the next week by a third injection. For each injection, 0.15 ml was given in a toe pad and the remainder was injected into a thigh muscle. The first bleeding was taken a month after the first injection, and the rabbit was bled at weekly intervals for several months. The rabbit was given a 1 mg/ml booster in Freund's incomplete adjuvant 8 mo after the first injection and was again bled weekly for several months.

Monoclonal antibodies. Two immunization procedures were used for the generation of monoclonal antibodies (MAbs) to the whole virus and the capsid protein of W-1A. A preimmunized in vitro technique (28), modified (S. Zam, *personal communication*) from that described by Boss (4), was used to generate hybridoma cells F3C-C10 (MAb-5), F22A-B9 (MAb-2), F22A-E8 (MAb-3), and F22A-C8 (MAb-6). Monoclonal antibodies F21D-E10 (MAb-1) and F21C-E4 (MAb-4) were generated by the more common in vivo technique (8). The immunogen used for MAb-5 was purified capsid protein. The immunogen for the other MAbs was purified virus.

For each fusion, two parts spleen cells were combined with one part Sp2/0-Ag14 (Sp2/0) (26) cells and fused (8) with the aid of polyethylene glycol (PEG, 1,500 M_r). As colonies of hybridoma cells developed, their supernatants were tested for antibody production by I-ELISA (plate-trapped and/or antibody-trapped). They were tested against purified capsid protein and/or extracts of Small Sugar pumpkin infected with W-1A and extracts of healthy pumpkin (Hssp).

Antibody-producing primary hybridomas were cloned twice by limiting dilution (31), and the supernatant of each clone was tested for antibody production. The primary hybridomas and selected clones were frozen in liquid nitrogen. Ascitic fluid was produced from one clone of each hybridoma by injection of 1×10^7 cells in sterile phosphate-buffered saline (PBS) into BALB/c mice injected 1–2 wk previously with 0.5 ml of 2,6,10,14-tetramethylpentadecane. The supernatant was used to isotype each monoclonal antibody with a MonoAb EIA kit (Zymed Laboratories, Inc., San Francisco, CA).

Polyclonal antibodies produced in mice. BALB/c mice, preinoculated with capsid protein or whole virus, were injected with nonfused Sp2/0 cells for the production of polyclonal mouse ascitic fluid (24). Other mice were injected with nonfused Sp2/0 cells to produce ascitic fluid free of PRSV-W antibodies for use as a negative control in both antibody and plate-trapped I-ELISA.

Virus isolates. In addition to W-1A, nine other Florida isolates of PRSV-W, and one each from Jordan (19), Greece (19), New York (19), California (19), and the American Type Culture Collection (ATCC PV-23) were used. Also included were an isolate of PRSV-T (23) and an isolate of PRSV-P (9).

In addition to PRSV, single isolates of seven other viruses that infect cucurbits were used: the Moroccan isolate of watermelon mosaic virus (WMV-M) (7), zucchini yellow mosaic virus (ZYMV) (16), watermelon mosaic virus-2 (WMV-2), squash mosaic virus (SqMV), cucumber mosaic virus (CMV), a virus from *Trichosanthes* (TV) (20), and zucchini yellow fleck virus (ZYFV) (27). These isolates were also maintained in Small Sugar pumpkin.

Differential reactions of PRSV isolates inoculated to eight melon lines. The melon lines used in this study and a brief description of their characteristics are listed in Table 1. Each of the primary PRSV isolates, either from fresh or dried material, was initially inoculated mechanically to Small Sugar pumpkin. Twenty-four days later, infected leaves of Small Sugar pumpkin from each of the isolates were ground in 0.02 M potassium phosphate buffer, pH 7.5–7.6, with 600-mesh Carborundum. Cheesecloth pads were dipped in extracts and used to inoculate the eight melon cultivars and 10 plants of Small Sugar pumpkin. A second set of plants was inoculated a month later. Each isolate was also inoculated to three plants of *Carica papaya* L. (papaya).

Serology. SDS-immunodiffusion tests were done by the procedure of Purcifull and Batchelor (17). Each isolate was tested against the homologous antigen (W-1A) and Hssp with polyclonal rabbit antiserum to the capsid protein (RPAb-1125) and preimmune rabbit serum.

For the plate-trapped I-ELISA tests, 100 μl of antigen in carbonate buffer (pH 9.6) (5) was added directly to appropriate duplicate wells of Immulon II flat-bottom plates (Dynatec Laboratories

TABLE 1. Melon (*Cucumis melo*) lines used in the papaya ringspot virus

Melon line	Description
Vedrantais	French cultivar (Vilmorin Co., La Ménttré, Beaufort-en-Vallée, France) widely cultivated, compatible to papaya ringspot virus (PRSV)-W ("Charentais" type).
VAC	Old French cultivar (Vilmorin Co.). ("Charentais" type).
Ouzbeque	Iranian cultivar developing mosaic-type symptoms after inoculation by any of the PRSV- or PRSV-related isolates tested.
PI 161375	Korean cultivar "tolerant" to some isolates of PRSV. Also resistant to common strains of cucumber mosaic virus and to *Aphis gossypii* Glov. ("Conomon" type).
PI 414753	Indian cultivar possessing a single dominant gene (probably *Prv*2) for resistance to PRSV-W. Also resistant to Zucchini yellow mosaic virus (ZYMV) and to *A. gossypii*.
72025	Breeding line possessing the single dominant gene *Prv*2 for resistance to PRSV-W; derived from PI 180283 ("Charentais" type) (1,15).
WMR-29	Breeding line possessing the single dominant gene *Prv*1 for resistance to PRSV-W; derived from PI 180283 ("Charentais" type) (3).
Edisto 47	American cultivar grown in Florida (PetoSeed Co., Woodland, CA).
Supermarket	American cultivar grown in Florida. Both Supermarket and Edisto 47 are "cantaloupe" types and show good levels of resistance to powdery mildew.

Inc., Chantilly, VA). For antibody-trapped I-ELISA, the plates were first coated with 100 μl of RPAb-1125, diluted 1:1,000 in carbonate buffer. (Rabbit polyclonal antisera to WMV-2 and ZYMV were also used as trapping antibodies for WMV-2 and ZYMV, respectively, in some antibody-trapped I-ELISA tests.) After washing, 50 μl of antigen in potassium phosphate buffer (0.25 M, pH 7.2) (9) was added to duplicate wells. In both types of tests, antigen consisted of tissue ground 1:10 (w/v) in the appropriate buffer and strained through cheesecloth. After washing, the antigen step was followed by 50 μl of antibody diluted in 0.2 M Tris-HCL–0.15 M NaCl (pH 7.2). Bound antibodies were detected by goat-anti-mouse IgG or IgM, or goat-anti-rabbit IgG antibodies conjugated to alkaline phosphatase (Sigma Chemical Co., St. Louis, MO). For each step, the plates were incubated at 37 C for 1–2 h. Enzyme reactions were developed 30–60 min after the addition of 50 μl of substrate (*p*-nitrophenyl phosphate, disodium [Sigma] at 1 mg/ml in 9.7% diethanolamine buffer, pH 9.8). Absorbance values were determined (A_{405nm}) on a Biotek automated microplate reader, model EL 309 (Bio-Tek Instruments Inc., Winooski, VT).

Extracts made from plants singly infected with each of the 17 isolates of PRSV, WMV-M, ZYFV, ZYMV, WMV-2, SqMV, CMV, or TV in Small Sugar pumpkin were tested in plate- and antibody-trapped I-ELISA against all six MAbs. Both types of I-ELISA were done at the same time with the same sources of antigen. Rabbit polyclonal antiserum 1125 (plate-trapped) and mouse polyclonal antiserum (antibody-trapped) were included in each test as positive controls. Preimmune serum (plate-trapped), Sp2/0 ascites, and tissue culture fluid (both plate- and antibody-trapped) were included as negative controls. Preimmune rabbit serum, Sp2/0 ascites, ascites of MAb-4, -5, and -6 were diluted 1:10,000; RPAb-1125 was diluted 1:100,000; ascitic fluids of MAb-1 and -2 were diluted 1:2,000; tissue culture fluid of MAb-3 and Sp2/0 cells were diluted 1:4; polyclonal mouse ascitic fluid to the capsid protein was diluted 1:5,000; and whole virus ascitic fluid was used at a 1:2,000 dilution. Criteria for judging a reaction either as positive or negative are indicated in Table 2, footnote c.

Sap from the inoculated papayas and an uninoculated papaya were tested for virus infection in plate-trapped I-ELISA with the RPAb-1125 and preimmune serum.

RESULTS

SDS-immunodiffusion tests. Polyclonal antiserum to the capsid protein (RPAb-1125) gave a reaction of identity in SDS-immunodiffusion with extracts from pumpkin infected with PRSV-W (W-1A) and PRSV-P. A reaction of partial identity was seen when PRSV-W (W-1A) was compared with PRSV-T, WMV-2, WMV-M, and ZYFV. No reaction was seen with extracts from healthy pumpkin or pumpkin infected with CMV, SqMV, TV, or ZYMV.

Indirect ELISA. All of the isolates of PRSV that reacted with RPAb-1125 in SDS-immunodiffusion also reacted with RPAb-1125 in plate-trapped I-ELISA (Fig. 1A); none reacted with preimmune serum. The RPAb-1125, taken after the last boost, also reacted with WMV-M, ZYMV, ZYFV, and WMV-2. The absorbance readings were lower for these viruses than for the PRSV-W isolates. RPAb-1125 did not react with SqMV, CMV, TV, or Hssp.

In the I-ELISA test of the inoculated papayas, only the positive controls (W-1A and PRSV-P in Small Sugar pumpkin) and the papaya inoculated with PRSV-P reacted with RPAb-1125. None of the extracts reacted with normal serum.

In antibody-trapped I-ELISA, both of the mouse polyclonal antisera (capsid protein or whole virus) reacted with all the PRSV-W isolates and PRSV-P, but not with WMV-M, ZYFV, ZYMV, WMV-2, SqMV, CMV, TV, or with Hssp (Fig. 1B). The mouse polyclonal antiserum to the capsid protein reacted

TABLE 2. Summary of the reactivities and isotypes of six monoclonal antibodies (MAb) to the capsid protein of papaya ringspot virus type-W (PRSV-W)

Antigen	Antibody/type of indirect ELISA test used[a]					
	MAb-1/AT	MAb-2/AT	MAb-3/AT	MAb-4/AT	MAb-5/PT	MAb-6/AT
PRSV-W						
One isolate[b]	+ (0.36)[c]	+ (0.27)	+ (0.44)	+ (1.29)	+ (0.95)	+ (2.34)
Nine isolates[d]	+ (0.23)	+ (0.25)	+ (0.29)	+ (1.19)	+ (1.14)	− (0.02)
Three isolates[e]	+ (0.40)	+ (0.29)	+ (0.67)	+ (1.25)	− (0.00)	− (0.02)
Two isolates[f]	+ (0.25)	+ (0.25)	− (0.04)	− (0.03)	+ (1.27)	− (0.02)
PRSV-P	+ (0.14)	+ (0.17)	− (0.05)	+ (1.06)	+ (0.33)	− (0.02)
PRSV-T	+ (0.22)	− (0.05)	− (0.03)	− (0.03)	+ (1.25)	− (0.02)
WMV-2[g]	− (0.07)	+ (0.11)	− (0.03)	− (0.03)	+ (0.23)	− (0.01)
Other viruses[h]	− (0.08)	− (0.06)	− (0.03)	− (0.03)	− (0.01)	− (0.01)
Hssp[i]	− (0.06)	− (0.04)	− (0.07)	− (0.01)	− (0.00)	− (0.02)
Test type						
PT	−	−	+	+	+	+
AT	+	+	+	+	+	+
Isotype	IgG1κ	IgMκ	IgMκ	IgG2aκ	IgG1κ	IgMκ

[a] AT = antibody-trapped indirect ELISA (I-ELISA) and PT = plate-trapped I-ELISA.

[b] W-1A, the isolate of PRSV-W used for immunization.

[c] The + and − represent the results of at least two tests of each type of I-ELISA. Numbers in parentheses represent the average of the absorbance readings in two wells for one of these AT or PT I-ELISA tests taken 30 min after substrate addition. The results for the other type of test done at the same time with the same antigen source are summarized as: for MAb-1, the average of all antigens in the plate-trapped test was 0.007; for MAb-2, the average of all antigens in the plate-trapped test was 0.07; for MAb-3, average absorbance readings in plate-trapped I-ELISA for each of the nine categories of antigens listed were 0.46, 0.59, 0.47, 0.07, 0.05, 0.02, 0.02, 0.02, and 0.04 respectively; for MAbs-4 and -5, see Figure 1C and D for the antibody-trapped data; and for MAb-6, the absorbance reading for W-1A was 1.46 and <0.06 for all others. For MABs-1, -2, and -3, which had relatively low absorbance readings, results were considered positive if an absorbance value was greater than two times the absorbance reading of uninoculated tissue (Hssp). For the other MAbs, values greater than three times the Hssp reading were considered positive. Plates were zeroed on a well that contained substrate in substrate buffer.

[d] Florida PRSV-W isolates 1637, 1870, 2038, and 2201, the PRSV-W isolates from California, New York, Jordan, and Greece, and an American Type Culture Collection isolate (ATCC PV-23).

[e] Florida PRSV-W isolates 2030, 2040, and 2052.

[f] Florida PRSV-W isolates 2169 and 2207.

[g] Only MAb-2 reacted with WMV-2 when WMV-2 rabbit polyclonal antiserum was used as the trapping-antibody. The absorbance reading shown is from an antibody-trapped I-ELISA test with rabbit polyclonal antiserum 1125 as the trapping antibody. The corresponding absorbance value with rabbit polyclonal antiserum to WMV-2 as the trapping antibody was 0.261. MAb-5 reacted with WMV-2 only in plate-trapped I-ELISA.

[h] Includes the Moroccan isolate of watermelon mosaic virus, zucchini yellow mosaic virus, and zucchini yellow fleck virus.

[i] Uninoculated Small Sugar pumpkin.

weakly with PRSV-T in antibody-trapped I-ELISA (Fig. 1B). In plate-trapped I-ELISA, both mouse polyclonal antisera reacted with PRSV-T. The mouse polyclonal antiserum to the capsid protein also reacted with WMV-M, WMV-2, ZYMV, and ZYFV in the plate-trapped test. The ascitic fluid made with the injection of nonhybridized Sp2/0 cells did not react with any of the antigens.

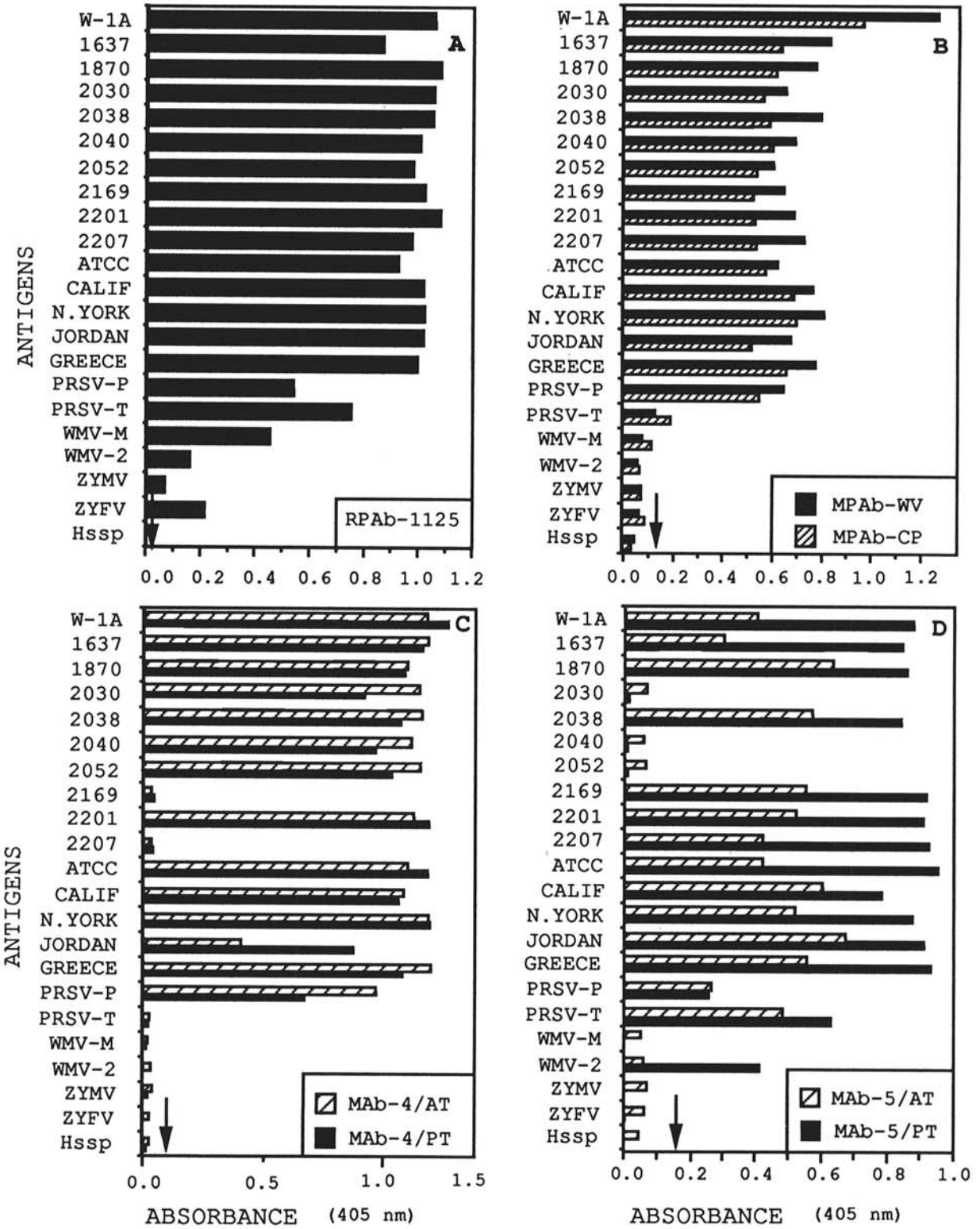

Fig. 1. Reactivities of polyclonal antisera and two monoclonal antibodies to papaya ringspot virus (PRSV), four other cucurbit potyviruses and healthy Small Sugar pumpkin (Hssp). **A,** Reactivity of rabbit polyclonal antiserum 1125 (RPAb-1125) in plate-trapped indirect (I) ELISA. **B,** Reactivity of mouse polyclonal ascites to capsid protein (MPAb-CP) and to whole virus (MPAb-WV) in antibody-trapped I-ELISA. **C,** Reactivity of MAb-4 in antibody-trapped (AT) and plate-trapped (PT) I-ELISA. **D,** Reactivity of MAb-5 in AT and PT I-ELISA. W-1A is the homologous isolate of papaya ringspot virus type-W (PRSV-W). The other Florida isolates of PRSV-W are represented by collection numbers. ATCC is an American Type Culture Collection isolate of PRSV-W (ATCC PV-23). Jordan, Greece, New York, and California are isolates of PRSV-W. PRSV-P is a papaya isolate of PRSV. PRSV-T is the Tigre isolate of PRSV from Guadeloupe. WMV-M is the Moroccan watermelon mosaic virus. WMV-2 is watermelon mosaic virus-2. ZYMV is zucchini yellow mosaic virus. ZYFV is zucchini yellow fleck virus, and Hssp is uninoculated Small Sugar pumpkin. The tests were done at the same time and with the same antigen sources. Values to the right of the arrow are considered positive; those to the left are considered negative.

MAb-1 (F21D-E10), an IgG1, κ, only reacted in antibody-trapped I-ELISA (Table 2). It reacted positively with all 17 isolates of PRSV tested so far and it did not react with WMV-2, WMV-M, ZYMV, ZYFV, CMV, SqMV, TV, or with Hssp.

MAb-2 (F22A-B9), an IgMκ also only reacted in antibody-trapped I-ELISA (Table 2). It reacted with all 15 PRSV-W isolates tested in antibody-trapped I-ELISA and with PRSV-P. It did not react with PRSV-T, WMV-M, WMV-2, ZYMV, ZYFV, CMV, SqMV, TV, or Hssp. However, when WMV-2 was trapped with rabbit polyclonal antiserum to WMV-2 instead of RPAb-1125, MAb-2 did react with WMV-2. The absorbance values were similar for WMV-2 and PRSV-W when each was trapped by its homologous antiserum. When ZYMV was trapped with polyclonal antiserum to ZYMV, no reaction was seen with MAb-2. None of the other MAbs reacted with either ZYMV or WMV-2 when the respective homologous antiserum was used as the trapping antibody.

MAb-3 (F22A-AE8), an IgMκ reacted in both plate- and antibody-trapped I-ELISA (Table 2). In both tests, it reacted with 13 PRSV-W isolates, but it failed to react with PRSV-W isolates 2207 and 2169, PRSV-P, PRSV-T, and WMV-M. It also did not react with ZYMV, ZYFV, WMV-2, SqMV, CMV, TV, or Hssp.

MAb-4 (F21C-E4), an IgG2aκ reacted in both plate- and antibody-trapped I-ELISA (Table 2). It reacted with all but two of the PRSV-W isolates tested (2207, 2169) (Fig. 1C). It also reacted with PRSV-P, but it did not react with PRSV-T, WMV-M, WMV-2, ZYMV, ZYFV, SqMV, CMV, TV, or Hssp.

MAb-5 (F3C-C10), an IgG1κ reacted in both plate- and antibody-trapped I-ELISA (Table 2). Of the isolates shown to be infected with PRSV-W in SDS-immunodiffusion and I-ELISA tests with RPAb-1125, three (2030, 2040, 2052) did not react with this monoclonal antibody (Fig. 1D). The remaining isolates of PRSV-W reacted positively with this antibody as did PRSV-P and PRSV-T. The WMV-M, ZYMV, ZYFV, SqMV, CMV, and TV isolates and Hssp did not react with this monoclonal antibody. The WMV-2 isolate reacted with this MAb in plate-trapped I-ELISA, but not in antibody-trapped I-ELISA (Fig. 1D). The absorbance value for the WMV-2 reaction in plate-trapped I-ELISA was always lower than that of W-1A or any of the other PRSV-W isolates that reacted with this MAb.

MAb-6 (F22A-C8), an IgMκ reacted in both I-ELISA tests, but it reacted only with the immunogen (W-1A) (Table 2).

The Florida isolates of PRSV-W, WMV-2, and ZYMV were also tested with the Agdia potyvirus group MAb (Agdia Inc., Elkhart, IN) in plate-trapped I-ELISA. The same three Florida isolates of PRSV-W (2040, 2030, and 2052) that did not react with MAb-5 also did not react with the Agdia potyvirus group MAb. The other PRSV-W isolates, WMV-2, and ZYMV reacted positively. The average absorbance for the majority of PRSV-W isolates was 0.415, with a range of 0.296–0.614. For isolates 2030, 2040, and 2052, the absorbance readings were 0.007, 0.002, and 0.006, respectively. For Hssp, the absorbance reading was 0.006 and for WMV-2 and ZYMV it was 0.564 and 0.635, respectively. Absorbance was measured 45 min after the addition of substrate. As in previous tests, all the PRSV-W isolates, WMV-2, and ZYMV reacted with RPAb-1125.

Melon line inoculation tests. A summary of the symptoms caused in the melon lines inoculated with the 17 original isolates is given in Table 3. In general, all isolates induced a compatible reaction in Ouzbeque and Supermarket. Incompatible reactions occurred in PI 414753, 72025, and WMR-29, lines that are known to possess PRSV-resistant genes (Table 1). The major differences were found with isolates 2040, 2052, PRSV-T, and WMV-M. These four isolates caused incompatible reactions in VAC and Edisto 47, but isolate 2030 and the remaining isolates induced compatible reactions in these melon lines. In addition, 2040, PRSV-T, and WMV-M induced incompatible reactions in Vedrantais and PI 161375. The remaining isolates, including 2052 and 2030, caused compatible reactions in Vedrantais and PI 161375.

Additional PRSV isolates. Because of the results of the melon line inoculation study and I-ELISA tests with MAb-5, 37 PRSV-infected cucurbit samples, collected in the spring of 1988 during a survey of the distribution of cucurbit viruses in Florida (20), were also tested for reactivity to the MAb-5 and rabbit polyclonal antiserum 1125 (RPAb-1125) in plate-trapped I-ELISA. Eight of these PRSV isolates, all of which reacted with RPAb-1125, did not react with MAb-5. These eight isolates and 10 that reacted with both MAb-5 and RPAb-1125 were inoculated to four of the melon lines (Supermarket, Edisto 47, Vedrantais, and WMR-29). All 18 isolates induced a compatible reaction (mosaic) in

TABLE 3. Reactions of nine melon lines to papaya ringspot virus isolates (PRSV) and to a watermelon mosaic virus from Morocco (WMV-M)[a]

	Reactions to isolates[b]								
Melon line	W-1A[c]	1637	1870	2030	2038	2040	2052	2169	2201
Ouzbeque	VB	VC	VC	VC	VC	VC	VC	VC	VB
Supermarket	VB	VC	VC	VC	VC	VC	VC	VC	VB
Vedrantais	VB	VC	VC	VC	VC	TN	VC	VC	VB
VAC	VB	VC	VC	VC	MO	TN	TN	VC	VB
Edisto 47	VB	VC	VC	VC	MO	TN	TN	VC	VB
PI 161375	M	MO	MO	MO	MO	TN	M	MO	M
PI 414753	TN	TN	TN	TN	TN	L	L/SNS	TN	TN
72025	SNS/TN[d]	TN	O/SNS	SNS/TN	L/SNS	L	SNS	TN	TN
WMR-29	O	L/SNS	O	L/SNS	L/O	L	L	O	O

	Reactions to isolates[b]								
Melon line	2207	ATCC	California	New York	Jordan	Greece	PRSV-P	PRSV-T	WMV-M
Ouzbeque	VC	VC	VC	VC	VB	VC	VC	VC	VC
Supermarket	VC	VC	VC	VC	VB	VC	VC	VC	VC
Vedrantais	VC	VC	VC	VC	VB	VC	M	SNS	TN
VAC	VC	VC	VC	VC	VB	VC	M	TN	TN
Edisto 47	VC	VC	VC	VC	VB	VC	M	O/SNS	TN
PI 161375	M	M	MO	MO	MO	M	M	SNS	TN
PI 414753	TN	TN	TN	TN	TN	TN	TN	L	TN
72025	TN	TN	TN	TN	TN	L/SNS	L/SNS	L	SNS
WMR-29	L	L/SNS[d]	L	L	SNS/TN	O	L	L	O

[a] Isolates 2030, 2040, 2052, and WMV-M did not react with MAb-5.
[b] Compatible reactions: VC = vein clearing, yellowing; VB = vein banding; MO = mosaic; M = mottle. Incompatible reactions: TN = top necrosis; SNS = systemic necrotic spots; L = local lesions; O = no symptoms.
[c] The W-1A and the numbered isolates are all from Florida.
[d] Reactions with two symptom types (e.g., L/SNS) represent variations in the incompatibility-type reaction within the two trials.

Supermarket and incompatible reaction (local lesions) or no reaction in WMR-29. As seen previously, three different reactions were seen among the eight isolates that did not react with MAb-5. Like isolate 2040, four of these eight isolates induced incompatible reactions in both Edisto 47 and Vedrantais. Like isolate 2052, three of the eight isolates caused incompatible reactions in Edisto 47 and compatible reactions on Vedrantais. Like isolate 2030, one of the eight isolates induced a compatible reaction in both Edisto 47 and Vedrantais. As before, all the isolates that reacted with MAb-5 induced compatible reactions in Vedrantais and Edisto 47.

DISCUSSION

Our results demonstrate both serological and biological variability heretofore unknown in Florida isolates of PRSV-W. They also show that monoclonal antibodies can distinguish PRSV-W from other strains (i.e., PRSV-T) and from several other potyviruses that infect squash.

Only two of the PRSV-W MAbs reacted with PRSV-T, indicating that PRSV-W has at least three epitopes that were not detectable on PRSV-T. Four of the five MAbs defined epitopes present on the majority of PRSV-W isolates that were also present on the isolate of PRSV-P used in this study. This confirms the close relationship of PRSV-P with PRSV-W, but also shows that there is a serological difference of at least one epitope in the capsid proteins of PRSV-W and this isolate of PRSV-P. More isolates of PRSV-P will have to be tested to determine if this epitope difference is characteristic of PRSV-P isolates in general. However, this difference is not surprising in light of the fact that there are eight amino acid differences in the capsid proteins of PRSV-W (W-1A) and PRSV-P (21). A single amino acid change in an antigenic site can alter its reactivity to an antibody (14). At least three of the amino acid changes are located in the N-terminus portion of the protein (21) and thus they could be involved in antibody stimulation (25).

Two MAbs (MAb-2 and MAb-5) reacted with WMV-2, another potyvirus commonly found in cucurbits. The reactivities of MAbs-2 and -5 with WMV-2 are not evident when these MAbs are used in antibody-trapped I-ELISA with rabbit polyclonal antiserum to PRSV-W as the trapping antibody. Thus, either MAb-2 or MAb-5 could be used in diagnosis of PRSV-W, as could MAbs-1, -3, and -4. However, MAbs-3, -4, and -5 do not react with all PRSV-W isolates and MAb-1 and -2, which appear to be PRSV-specific, react weakly and produce positive absorbance values that are too low and background readings that are too high for use in routine diagnosis. However, a combination of MAbs-4 and -5 could recognize all the PRSV isolates tested. Such a mixture has been successfully used in antibody-trapped I-ELISA together with MAbs specific to WMV-2 and ZYMV (29) to distinguish cucurbit potyviruses in Florida (G. C. Wisler, *unpublished data*).

Eleven (23%) of all the Florida PRSV-W isolates tested failed to react with MAb-5. Nine of these 11 isolates gave reactions in the melon lines that differed from those of the isolates that reacted with MAb-5. While studies similar to those done for biological variants of TMV (11) need to be done to confirm or refute any correlation of the serological and biological variations found in this study, it is certain that both serological and biological differences exist in the Florida population of PRSV-W. The biological differences should be taken into account when breeding for resistance to PRSV-W in melon. For example, the *Prv*1 gene from WMR-29 appears to be more suitable to control PRSV-W in Florida than the *Prv*2 allele from 72025 or PI 414753. In addition, some isolates of PRSV-W in Florida induce incompatible reactions in cultivars not known to possess resistance genes to standard PRSV-W isolates (i.e., isolate 2040 in Vedrantais, VAC, and Edisto 47).

The serological variability among Florida isolates of PRSV-W detected by MAb-5 was corroborated by the reactivity of the Florida PRSV-W isolates to the Agdia potyvirus group MAb. This study and one other (13) indicate that the epitope recognized by the potyvirus group MAb is variable. These data also indicate that there are risks in using a single MAb for the diagnosis of PRSV-W in Florida and in using only one or two isolates of a given potyvirus to screen the reactivity of a particular MAb.

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