Serological and Biological Variability of Virus Isolates Related to Strains of Papaya Ringspot Virus

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We gratefully acknowledge Drs. M. Bar-Joseph and A. Franck for providing an Israel isolate, A. Beyries for providing Tahiti isolates, D. Gonsalves for providing PRSV-P antisera and *Cucumis metuliferus* Acc. 2459 seeds, V. Lisa for providing the ZYFV strain, R. Provvidenti for providing *C. metuliferus* PI 292190 and *Phaseolus vulgaris* seeds, D. E. Purcifull for providing WMV-M, WMV-2 FL, and ZYMV FL antisera and PRSV-W, PRSV-P, and WMV-M strains, J. C. Thouvenel for providing Côte d'Ivoire and Niger isolates, D. G. A. Walkey for providing Yemen isolates, and M. Jacquemond for her participation in the preparation of the antiserum against amorphous inclusions.

Accepted for publication 5 September 1989.

ABSTRACT

Quiot-Douine, L., Lecoq, H., Quiot, J. B., Pitrat, M., and Labonne, G. 1990. Serological and biological variability of virus isolates related to strains of papaya ringspot virus. Phytopathology 80:256-263.

Three papaya ringspot virus strains (PRSV-W FL, PRSV-P HA, and PRSV-T), 15 potyvirus isolates related to PRSV, and four other cucurbit potyviruses (watermelon mosaic virus 2, WMV-2; watermelon mosaic virus from Morocco, WMV-M; zucchini yellow mosaic virus, ZYMV; and zucchini yellow fleck virus, ZYFV) were compared for seven properties. The PRSV strains and the 15 isolates were clearly differentiated from WMV-2 and ZYMV. Five criteria discriminated between isolates or PRSV strains. Among them, the serological properties of the capsid distinguish nine groups, the serological properties of the amorphous inclusion protein distinguish two groups, and the symptoms induced on a set of muskmelon lines distinguish four groups or pathotypes. These results were compared through a factorial analysis of multiple correspondences. It appeared that the extent of the variability observed among the PRSV isolates enlarges those already known for PRSV and for WMV-M. Moreover, the similarities between these two groups suggest that PRSV and WMV-M are related. Similar pathotypes have been observed also between some of isolates of PRSV and ZYFV. In spite of the limited number of PRSV isolates that have been studied, some conclusions can be drawn from an analysis of their geographical origin: the common strain of PRSV has been identified in America, in Africa, in Europe, and in Oceania. By contrast, the four isolates related to WMV-M have been collected only in Africa or in its vicinity. PRSV-T, collected in the West Indies, has some similarities with an isolate collected in West Africa.

Three main potyviruses cause major diseases of cucurbits throughout the world: papaya ringspot virus type W (PRSV-W) (28), watermelon mosaic virus type 2 (WMV-2) (30), and zucchini yellow mosaic virus (ZYMV) (19). Two other potyviruses have been also isolated from cultivated cucurbits: watermelon mosaic virus from Morocco (WMV-M) (10) and zucchini yellow fleck virus from Italy (39). PRSV, WMV-2, and ZYMV are now considered distinct on the basis of their serological and biological properties. WMV-M is serologically related to, but distinct from PRSV-W (19,20,28,30).

PRSV-W (formerly watermelon mosaic virus-1, WMV-1) is considered to be one of the five most important viruses in field-grown vegetables (36). It is commonly encountered in tropical or subtropical areas and occasionally in temperate areas (28). WMV-1 is sufficiently similar to PRSV to be grouped with this virus. Consequently, it has been renamed PRSV-W, and the former PRSV is now named PRSV-P (28). PRSV-P and PRSV-W are serologically indistinguishable but present two different pathotypes: one (PRSV-P) can infect papaya, the second one (PRSV-W) cannot. Another strain (PRSV-T) nonpathogenic on *Carica papaya* L. was differentiated from PRSV-W mainly by its serological properties (33).

In a survey on the geographical distribution of viruses infecting cucurbits, several isolates from different continents were observed to be biologically or serologically more or less closely related to PRSV. We have attempted to estimate the extent of the relationships between these isolates from different parts of the world to determine whether they should be considered as strains of this virus.

MATERIALS AND METHODS

Virus origin and propagation. Four viruses (WMV-M, ZYFV, WMV-2, and ZYMV), three previously described strains of PRSV (HA, FL, and T), and 15 potyvirus isolates clearly differentiated from WMV-2 and ZYMV were compared for several properties. The viruses, strains, and isolates are listed with their country of origin in Table 1.

For simplification, the word "type" has been used in this paper as a general name for viruses, strains, and isolates.

The purity of the viruses was checked by sodium dodecyl sulfate (SDS)-immunodiffusion tests or by inoculation to differential hosts to detect possible coinfection with other mechanically transmitted viruses known to infect cucurbits. After multiplication in zucchini squash (*Cucurbita pepo* L. 'Diamant F1'), viruses were preserved in leaves desiccated at 4 C over calcium chloride.

Diagnostic species. Different lines of *Cucumis melo* L. were used in diagnostic tests: 'Ouzbèque' (origin: Uzbekistan, U.S.S.R.), 'Vedrantais' (Vilmorin, France), 'Charentais T' (Vilmorin, France), 'Voatango' (Madagascar), 'PI 161375' (Songwhan Charmi, Korea), '72025' resistant to PRSV-W (31), 'PI 414723' (India), and 'WMV 29' (6).

Other hosts were: *Carica papaya* L. 'Solo', *C. pepo* 'Diamant F1', *Cucumis metuliferus* (Naud.) Mey. 'Acc. 2459' and 'PI 292190' (25), *Chenopodium amaranticolor* Coste et Reyn., *Lavatera trimestris* L., *Phaseolus vulgaris* L. 'Black Turtle 1', and 'Black Turtle 2' (23), and two wild cucurbits growing in southern France: *Ecballium elaterium* L. and *Bryonia dioïca* Jacquin.

Host range assays. Viruses in dried leaf material were cultured in zucchini squash before inoculation to other hosts. One gram of tissue from infected zucchini squash was ground in 4 ml of 0.03 M sodium phosphate buffer, pH 8.5, containing 0.2% sodium diethyldithiocarbamate; 0.1 g of Carborundum 400 mesh was

added and extracts were rubbed on test plants. For each host species, all virus types were inoculated at the same time and all plants put in the same conditions (insect-proof greenhouse or growth chamber). When symptoms were indistinct, infection of plants was checked by biological or serological assays.

Aphid transmission. Aphid transmission of virus types was from squash to squash by *Aphis gossypii* Glov. as previously described (34). For each type, three tests were performed using two different plants of squash as sources to inoculate a set of 50 test plants with one aphid per test plant.

Serology. Antisera As E2, As 861, and As H4 prepared against intact virus particles of isolates 3, 12, and 13, respectively, were prepared in our laboratory. Viruses were purified as described by Purcifull et al (28) for PRSV-W (isolate 12) or as described by Lecoq and Pitrat (17) with an additional high-speed centrifugation before the cesium sulfate gradient (isolates 3 and 13).

Immunization was conducted as described by Purcifull and Batchelor (27): the thigh muscles of rabbits were injected initially with 1 mg of virus emulsified in Freund's complete adjuvant followed 2 or 3 wk later by an injection of 1 mg emulsified in Freund's incomplete adjuvant. Blood was collected weekly, starting 1 wk after the second injection.

The antiserum As HELP was prepared against amorphous inclusion protein (AIP) of isolate 3, which was purified as described by Hiebert et al (12). Immunization followed the procedure described above.

Additional antisera used in these studies were prepared against capsid protein of several viruses and types: common type HA (As 129) and severe type HA (As 107) (11); PRSV-T (As 1062) (33); WMV-M (As 955) (2,4); ZYMV from Florida (As 1028) (26) and from France (As E9); WMV-2 from Florida (As 868) (29) and from France (As WMV-2); isolate 3 (As E2); isolate 12 (As 861); and isolate 13 (As H4). Two other antisera were used: the first one against PRSV-T cylindrical inclusion protein (As 1078) (33), the second one against amorphous inclusion protein of isolate 3 (As HELP).

SDS-immunodiffusion in agar and intra-gel cross-absorption tests were conducted, using crude extracts, as described by Purcifull and Batchelor (27), in 4-mm-diameter and 3-mm-depth wells (six peripheral antigen wells 4 mm from the central serum well) (15). Antisera were usually preabsorbed with healthy crude extracts.

Determination of molecular weight of capsid proteins. The molecular weight of capsid proteins was determined from crude sap of infected squash. Capsid proteins were first separated from other proteins by SDS-polyacrylamide electrophoresis (9) for 1 hr at 40 mA through a 5% stacking gel (1 × 8.5 × 0.15 cm) and a 10% separating gel (5.5 × 8.5 × 0.15 cm) (14). They were then detected by electro-blot immunoassay.

Electrophoretic transfer onto nitrocellulose membranes was performed immediately after electrophoresis as described by Towbin et al (37), at 200 mA for 1–16 hr. Nitrocellulose membranes were used immediately or dried and kept at 4 C up to 1 wk before use.

Immunological detection of viral coat protein bands was then performed using the procedure of Bode et al (5). The antiserum used was a mixture of antisera against capsid of PRSV-T strain, isolate 3, isolate 12, and isolate 13, each at a dilution of 1:200.

The coat protein molecular weight of each isolate was estimated from at least four measurements by comparison with a set of standards: myosin (205K), β galactosidase (116K), phosphorylase B (97.4K), bovine serum albumin (66K), ovalbumin (45K), and carbonic anhydrase (29K).

Observation of viral inclusions. Cylindrical and amorphous inclusions were observed by light microscopy in epidermal strips of *C. pepo* 'Diamant F1' as described by Christie and Edwardson (8).

Statistical analysis. Eight properties of viruses and virus isolates were compared using a factorial analysis of multiple correspondences using the STATITCF program. It allows the selection of a set of axes optimized for presenting the variability of individuals described by several variables that do not need to be quantitative.

RESULTS

Serological relationships of the viruses. With the set of antisera used, serological reactions of ZYFV, ZYMV, and WMV-2 were clearly differentiated from those of the 18 PRSV types and from

TABLE 1. Viruses, strains, and isolates studied[a]

Name	Country of origin	Climate of origin	Plant origin	References
Strains of PRSV				
HA: PRSV-P	Hawaii	Tropical	Papaya	Gonsalves & Ishii 1980 (11)
FL: PRSV-W	Florida USA	Tropical	Squash	Purcifull & Hiebert 1979 (29)
T: PRSV-T	Guadeloupe FWI	Tropical	Squash	Quiot-Douine et al 1986 (34)
Isolates related to PRSV				
1	Côte d'Ivoire	Tropical	Squash	Thouvenel (unpublished)
2	Israel	Mediterranean	Papaya	Bar-Joseph (unpublished)
3	France	Temperate	Muskmelon	Lecoq et al 1982 (16)
4	France	Temperate	Muskmelon	Lecoq 1986 (unpublished)
5	Senegal	Tropical	Squash	FAO 1979 (unpublished)
6	Tahiti	Tropical	Squash	Beyries 1987 (unpublished)
7	Tahiti	Tropical	Pumpkin	Beyries 1987 (unpublished)
8	Guadeloupe FWI	Tropical	Cucumber	Quiot et al 1971 (31)
9	Guadeloupe FWI	Tropical	Squash	Quiot 1982 (unpublished)
10	Guadeloupe FWI	Tropical	Substrain from isol. 9	Labonne 1983 (unpublished)
11	Spain	Temperate	Muskmelon	Lecoq 1985 (unpublished)
12	Niger	Desert	Pumpkin	Thouvenel et al 1986 (35)
13	Algeria	Mediterranean	Squash	Bousalem & Lecoq 1986 (unpublished)
14	Yemen	Desert	Squash	Walkey 1985 (unpublished)
15	Yemen	Desert	Squash	Walkey 1985 (unpublished)
Other potyviruses of cucurbits				
WMV-M	Morocco	Mediterranean	Squash	Fischer & Lockhart 1974 (10)
ZYFV	Italy	Mediterranean	Squash	Vovlas et al 1981 (38)
WMV-2 FR	France	Temperate	Squash	Luis Arteaga et al 1976 (21)
ZYMV E15	France	Temperate	Muskmelon	Lecoq et al 1981 (18)

[a]PRSV: papaya ringspot virus; WMV-M: watermelon mosaic virus from Morocco; ZYFV: zucchini yellow fleck virus; WMV-2: watermelon mosaic virus-2; ZYMV: zucchini yellow mosaic virus.

WMV-M. Indeed, ZYFV, the ZYMV isolate E15, and WMV-2 FR did not react in SDS-immunodiffusion tests with any of the antisera prepared against the capsid of the types: HA, T, 3, 12, 13, or WMV-M. Conversely, none of the 15 isolates, the PRSV strains, WMV-M, or ZYFV reacted with the antisera against ZYMV or with the antisera against WMV-2.

Thirteen antigenic determinants of the capsid were deduced from the serological reactions of the 19 types of PRSV and WMV-M observed with the seven antisera used following cross-absorption in SDS-immunodiffusion tests (Table 2). They are described below and presented in Table 3.

Three antisera reacted alike: those against isolate 3, HA common, and HA severe. With 11 types (HA, FL, 2, 3, 4, 5, 6, 7, 8, 9, and 10), they developed a precipitin line that did not appear when the antisera were previously absorbed with any of these 11 types. These reactions indicated that the 11 types had

TABLE 2. Serological reactions of virus types with antisera raised to capsid proteins (sodium dodecyl sulfate immunodiffusion tests)

	Virus types																				
Sera	T	1	2	HA	FL	3	4	5	6	7	8	9	10	M	11	12	13	14	15	ZYFV	ZYMV E15	WMV-2 FR
As anti isolate 13	0[a]	0	0	0	0	0	0	0	0	0	0	0	0	+b	+b	+b	+a	0	0	0	0	0
As Is 13 * M or 11 or 12[b]														0	0	0	+					
As Is 13 * 13														0	0	0	0					
As anti isolate 12	0	0	0	0	0	0	0	0	0	0	0	0	0	+a	+a	+a	0	+b	0	0	0	0
As Is 12 * 14														+a	+a	+a		0				
As Is 12 * M or 11														0	0	+		0				
As Is 12 * 12														0	0	0		0				
As anti M	0	0	0	+c	+c	+c	+c	+c	+c	+c	+c	+c	+	+a	+a	+b	+c	0	0	0	0	0
As M * FL or 10 or 13					0								0	+a	+a	+b	0					
As M * 12					0								0	+a	+a	0	0					
As M * M or 11														0	0	0						
As anti HA severe	0	0	+a	+	+	+a	+	+	+	+	+	+a	+	0	0	0	0	0	0	0	0	0
As anti HA common	0	0	+a	+a	+a	+a	+a	+a	+a	+	+a	+a	+a	0	0	0	0	0	0	0	0	0
As anti isolate 3	0	0	+a	+a	+a	+a	+a	+a	+a	+a	+a	+a	+	0	0	0	0	0	0	0	0	0
As Is 3 * FL or 2 or 3 or 6 or 9			0		0	0			0			0										
As anti T	+a	+b	+b	+b	+b	+b	+b	+b	+b	+b	+b	+b	+b	+c	+c	+c	+c	+	+	0	0	0
As T * 15	+a	+	+		+b	+			+b			+b		+c	+c	+	+	+	0			
As T * 14	+a	+b	+b		+b									+b	+b	+b	+b	0	+b			
As T * 13	+a	+b	+b		+b	+			+b			+b		+c	+c	+c	0	0	+c			
As T * M or 11 or 12	+a	+b	+b		+b	+	+	+		+	+		+	0	0	0	0	0	0			
As T * HA or FL or 1 to 10	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
As T * T	0																					

[a]Type of reaction: 0 = no reaction; + = positive reaction; the letter next to the "+" indicates the presence or the absence of spur between isolates when they are tested side by side: the isolates that have the same letter (a, b, or c) do not form spur between them, the isolates which have an "a" form a spur over the isolates that have a "b" and the isolates which have a "b" form a spur over the isolates which have a "c".

[b]* Indicates cross-absorption of the serum with the isolate(s) whose number(s) follows.

TABLE 3. Coat protein antigenic determinants and serological reactions with the amorphous and cylindrical inclusion protein antisera of the 19 papaya ringspot virus related types grouped according to their geographical origin

		Continents																		
		Oceania			America					Europe			Africa					Asia		
Antigenic properties		HA	6	7	FL	8	9	10	T	3	4	11	M	13	12	1	5	2	14	15
Coat protein antigenic determinants	A	+[a]	+	+	+	+	+	+		+	+						+	+		
	B	+	+	+	+	+	+	+	+	+	+					+	+	+		
	F	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+		+
	G	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
	C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
	K	+	+	+	+	+	+	+		+	+	+	+	+	+		+			
	L											+	+	+	+					
	M											+	+		+				+	
	J											+	+		+					
	E											+	+							
	I														+					
	H													+						
	D								+											
As anti amorphous inclusion protein of isolate 3		+	+	+	+	+	+	+		+	+						+	+		
As anti cylindrical inclusion protein of PRSV-T		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a]+ The virus type that is indicated above gives, in sodium dodecyl sulfate-immunodiffusion tests, a positive reaction with the antiserum against amorphous or cylindrical inclusion protein that is indicated on the left or has on its coat protein the antigenic determinant that is indicated on the left. The antigenic determinants have been deduced from results obtained in SDS-immunodiffusion tests with seven antisera. The virus types already described are: PRSV-P (HA), PRSV-W (FL), PRSV-T (T), and WMV-M (M).

at least one common antigenic determinant that we named 'Ad A'.

The serum against strain T reacted with the 19 types of PRSV and WMV-M. When absorbed with its homologous antigen, the serum no longer reacted with any of the types. When absorbed with strains HA or FL, or with any of the isolates 1 to 10, this serum still reacted with strain T only, showing that strain T had a specific antigenic determinant 'Ad D'. When absorbed with WMV-M, or with isolates 11 or 12, this serum still reacted with 13 types (T, HA, FL, and 1 to 10), showing that they had an antigenic determinant 'Ad B'. When absorbed with isolate 13, this serum still reacted with 17 types (T, HA, FL, and 1 to 12, plus isolate 15, and WMV-M) showing that they all had the antigenic determinant 'Ad F'. When absorbed with isolate 14, this serum still reacted with 18 types (T, HA, FL, and 1 to 13, plus isolate 15, and WMV-M). These reactions could correspond to the antigenic determinants 'Ad C' and 'Ad F'. When absorbed with isolate 15, this antiserum still reacted with all other types showing that they had the antigenic determinant 'Ad G'.

The serum against WMV-M reacted with WMV-M, and with types HA, FL, and 3 to 13. When absorbed with its homologous antigen WMV-M or with the isolate 11, this serum no longer reacted with any of these types, showing that isolate 11 has all the antigenic determinants of WMV-M reacting with the serum. When absorbed with isolate 12, this serum still reacted with WMV-M and isolate 11, showing that both had the antigenic determinant 'Ad E'. When absorbed with isolate 13 or one of the types HA, FL, or 3 to 10, this serum still reacted with WMV-M, and with isolates 11 and 12 showing that they had the antigenic determinant 'Ad J'. The antigenic determinant common to WMV-M and to types HA, FL, and 3 to 13 was named 'Ad K'.

The serum against isolate 13 reacted with types 13, 11, 12, and WMV-M. When absorbed with its homologous antigen, this serum no longer reacted with any of these types. When absorbed with types 11, 12, or WMV-M, this serum still reacted with isolate 13, showing that it had the specific antigenic determinant 'Ad H'. The antigenic determinant common to types 11, 12, 13, and WMV-M was named 'Ad L'.

The serum against isolate 12 reacted with types 12, 11, 14, and WMV-M. When absorbed by its homologous antigen, this serum no longer reacted with any of these types. When absorbed with either WMV-M or isolate 11, this serum still reacted only with isolate 12, showing that it had one specific antigenic determinant 'Ad I'. When absorbed with isolate 14, this serum still reacted with types 11, 12, and WMV-M. The antigenic determinant involved in this reaction could be 'Ad J'. The antigenic determinant common to the three isolates and WMV-M was named 'Ad M'.

When spurs occurred, they confirmed the results obtained by the cross-absorption tests but, in our conditions, spurs did not always develop when expected.

The distribution of these 13 antigenic determinants on the 19 types of PRSV and WMV-M demonstrated the existence of three main serological groups (Table 3). The largest group, relatively homogeneous, is mainly characterized by the presence of Ad A and B antigenic determinants (A^+B^+) and the absence of Ad L and M antigenic determinants (L^-M^-). It includes 10 types (HA, FL, 3, 4, 5, 6, 7, 8, 9, and 10) plus isolate 2, which lacks the antigenic determinant Ad K. A second group is characterized by $A^-B^-J^+L^+M^+$ and includes WMV-M and isolate 11, plus isolate 12, which is differentiated from the first two by E^-I^+. The third group includes the other types defined either by their specific antigenic determinant or by the fact that they do not have all of the commonest antigenic determinants: T, 1, 13, 14, and 15, plus ZYFV.

These three groups were established using antigenic determinants deduced from reactions of types with a particular set of antisera. No conclusions about the degree of relationship between groups can be deduced from these results alone because other antigenic determinants could appear if other antisera were used and this would modify the distributions.

Serological properties of the amorphous inclusion protein. Eleven PRSV types (HA, FL, 2, 3, 4, 5, 6, 7, 8, 9, and 10) reacted with the antiserum prepared against amorphous inclusion protein of isolate 3. Seven other PRSV types (T, 1, 11, 12, 13, 14, and 15) did not react with this serum (Table 3). WMV-M, ZYFV, ZYMV E15, and WMV-2 FR also did not react.

Serological properties of the cylindrical inclusion protein. All types of PRSV and WMV-M reacted, in SDS-immunodiffusion, with the antiserum prepared against the cylindrical inclusion protein of strain T (Table 3). The viruses ZYFV, ZYMV, and WMV-2 did not react.

Symptomatology in muskmelon. Eight lines of muskmelon were mechanically inoculated concurrently with each of the 22 virus types.

The inoculated plants showed either a compatible reaction expressed as a systemic mottle or mosaic, or a reaction of incompatibility expressed by no infection or necrotic symptoms. The kind of reaction induced by a virus type on a muskmelon line was repeatable in three assays. However, the hypersensitive reaction could be more or less intensive according to the virus type: it could be expressed either by necrotic local lesions and no systemic symptoms, necrotic local lesions and translocation of the virus (systemic necrotic spots), or no local symptoms but a clear reaction of systemic necrosis or apical necrosis (Table 4).

WMV-2 and ZYMV were clearly differentiated from the 20 other types by their ability to induce mosaic symptoms on all the lines (except ZYMV, whose local reaction on PI 414723 was previously described [22]). All PRSV types, WMV-M, and ZYFV induced a mosaic on Ouzbèque, but isolate 15 was the only PRSV type able to induce a mosaic also on the line WMV 29 and so to produce a reaction identical to those of WMV-2 and ZYMV on this line. These results show that isolate 15 was compatible with seven of the eight lines tested; seven PRSV types were compatible with five lines; five PRSV types were compatible with only two lines, and five PRSV types plus WMV-M and ZYFV were compatible with only one line.

On the basis of disease reactions on muskmelon, the 18 PRSV types, WMV-M, and ZYFV were divided into four main groups. The first one includes isolate 15 only; the second one includes seven virus types: FL, 2, 3, 4, 5, 6, and 7; the third one includes five virus types: HA, 8, 9, 10, and 14; and the fourth one includes five virus types: T, 1, 11, 12, and 13, and WMV-M plus ZYFV.

Comparative host range. Reactions of the 18 PRSV types, WMV-M, ZYFV, ZYMV, and WMV-2 on several hosts revealed some differences. Only HA and isolate 2 that were obtained from naturally infected papaya were found able to infect papaya.

C. metuliferus 'Acc. 2469' and *C. pepo* 'Diamant' were both susceptible to all virus types. Nevertheless, symptoms on Diamant were different according to the type inoculated: a mottle was produced by isolates 3, 4, and 5; a mosaic alone by WMV-2 and types HA, FL, T, 1, 2, 10, 12, and 14; a mosaic with shoe stringing by ZYMV E15, WMV-M, and isolates 6, 7, 8, 9, 11, and 13; and yellow flecks by ZYFV and isolate 15.

C. metuliferus PI 292190 was susceptible only to the isolate 12, which induced a hypersensitive reaction (systemic necrosis). This *C. metuliferus* line could be infected also by WMV-2 or ZYMV (24,25) but not by WMV-M or ZYFV.

None of the 18 PRSV types, WMV-M, or ZYFV could infect *E. elaterium* or *B. dioica,* whose seeds were collected in southern France.

Reactions of virus types on *C. amaranticolor*, *L. trimestris*, and *P. vulgaris* 'Black Turtle 1' (BT1) and 'Black Turtle 2' (BT2) were classed in two groups characterized by no infection or local lesions. The viruses inducing local lesions on *C. amaranticolor* were WMV-2, ZYMV, WMV-M, isolates 2, 7, 11, 12, 13, and 15, and sometimes, HA and ZYFV. On *L. trimestris*, WMV-2 FR and isolate 13 induced local lesions and systemic necrotic spots. For *P. vulgaris*, WMV-M and isolate 11 only caused local lesions on BT1; ZYMV E15 and WMV-2 FR induced local lesions and a systemic mosaic on BT2. Also, isolate 13 occasionally induces local lesions on BT2.

Transmission by aphids. The percentages of transmission by *A. gossypii* varied from 3 to 86% according to the virus type assayed (Table 5). Three tests were successively performed for

each virus or type. They gave consistent results except for types FL and 2, which presented some inconsistencies (between 3 and 17% and between 18 and 40% of successful infection, respectively).

Molecular weights of the capsid proteins. The values obtained for the 18 PRSV types, WMV-M, and ZYFV varied from 35,400 to 38,800 (Table 6) with coefficients of variation ranging from

TABLE 4. Reactions induced by 22 virus types on different lines of muskmelon

Virus types	Muskmelon lines							
	Ouzbèque	Védrantais	Charentais T	Voatango	161375	72025	414723	WMR 29
WMV-2 FL[a]	0[b] / Mo	0 / Mo	0 / Mo	0 / Mo	0 / M	0 / Mo	0 / Mo	0 / Mo
ZYMV E15	0 / Mo	0 / Mo	0 / Mo	0 / Mo	0 / Mo	0 / Mo	ll / 0	0 / Mo
15	0 / Mo	ll / Mo	ll / Mo	ll / Mo	ll / Mo	ll / Mo	0 / AN	ll / Mo
FL	0 / Mo	0 / Mo	0 / Mo	0 / Mo	0 / M	ll / NSp	ll / NSp	0 / 0
3	0 / Mo	0 / Mo	0 / Mo	0 / Mo	0 / M	ll / AN	ll / AN	ll / 0
4	0 / Mo	0 / Mo	0 / Mo	0 / Mo	0 / M	ll / NSp	ll / AN	0 / 0
5	0 / Mo	0 / Mo	0 / Mo	0 / Mo	0 / M	ll / NSp	ll / NSp	0 / 0
6	0 / Mo	0 / Mo	0 / Mo	0 / Mo	0 / M	ll / 0 (NSp)	ll / NSp	0 / 0
7	0 / Mo	0 / Mo	0 / Mo	0 / Mo	0 / M	ll / NSp	ll / NSp	0 / 0
2	0 / Mo	0 / Mo	0 / Mo	0 / Mo	0 / M	ll / NSp	ll / NSp (AN)	0 / 0
HA	0 / Mo	0 / M	0 / AN	ll / 0	0 / 0	ll / 0	ll / 0 (NSp)	0 / 0
8	0 / Mo	0 / Mo	0 / N	0 / N	ll / N	ll / 0 (NSp)	ll / 0	ll / 0
9	0 / Mo	0 / Mo	0 / N	ll / N	0 / N	ll / 0 (NSp)	ll / 0	ll / 0
10	0 / Mo	0 / M	ll / N	ll / N	ll / N	ll / 0 (NSp)	ll / 0	0 / 0
14	0 / Mo	0 / Mo	ll / N	ll / N (AN)	0 / N	ll / NSp	ll / NSp	ll / 0
T	0 / Mo	ll / NSp	ll / NSp	ll / NSp (0)	ll / NSp (0)	ll / 0	ll / 0	ll / 0
I	0 / Mo	ll / N	0 / N	ll / 0	0 / N	ll / 0	ll / 0	ll / 0
WMV-M	0 / Mo	ll / AN	ll / AN	ll / N	ll / AN	ll / AN (NSp)	ll / NSp	ll / NSp (0)
11	0 / Mo	0 / AN	0 / AN	ll / N	0 / AN	ll / AN (NSp)	ll / NSp	ll / NSp (0)
12	0 / Mo	ll / N	ll / AN	ll / NSp (AN)	ll / AN	ll / NSp (AN)	ll / NSp (AN)	ll / NSp (0)
13	0 / Mo	0 / AN	0 / AN	0 / NSp (0)	ll / NSp	ll / NSp (0)	ll / 0	ll / 0
ZYFV	0 / Mo	0 / AN (N)	0 / AN (N)	ll / N	ll / N	ll / NSp (N)	ll / 0 (NSp)	ll / NSp (0)

[a] WMV-2 = watermelon mosaic virus type 2; ZYMV = zucchini yellow mosaic virus; WMV-M = watermelon mosaic virus from Morocco; and ZYFV = zucchini yellow fleck virus.

[b] Local reaction/systemic reaction; symptoms indicated in parentheses are also obtained sometimes; ll = local lesions; M = mottle; Mo = mosaic; N = necrosis or necrotic; Sp = spot; AN = apical necrosis; 0 = no infection.

0.84 to 3.95. Types T and 1 differed from the other isolates by the larger molecular weight of their coat proteins.

The mixture of antisera used in electro-blot experiments detected the capsid protein of PRSV, WMV-M, ZYFV, WMV-2, and ZYMV. Such heterologous reactions were observed in electro-blot by Burgermeister and Koenig (7) and in enzyme-linked immunosorbent assay tests by Katul and Makkouk (13). No protein was detected in crude sap of healthy plants with these antisera. No protein was detected with normal serum in crude sap of infected plants.

Cytoplasmic inclusions. Cylindrical and amorphous inclusions were easily observed by light microscopy in plant infected with WMV-M, ZYFV, and all PRSV types except isolate 15, for which several preparations presented very poorly stained aggregates.

DISCUSSION

From these results, it appears that all the isolates studied could be differentiated from WMV-2 and ZYMV by their serological and biological properties. In SDS-immunodiffusion, no cross-reaction was observed using antisera prepared against the capsid of WMV-2, ZYMV, or several PRSV types. The antiserum prepared against the cylindrical inclusion protein of strain T did not react with the proteins of WMV-2 or ZYMV as well as the antiserum prepared against the amorphous inclusion protein of isolate 3. Similarly, the set of muskmelons and *C. metuliferus* PI 292190 that contain genes for resistance to PRSV were infected by our isolates of WMV-2 and ZYMV with the exception of PI 414723, which contains a gene for hypersensitivity to ZYMV (22).

The relationships between the PRSV types and ZYFV have also been evaluated, but the results are incomplete because no ZYFV antiserum was used. No relationship was observed with the antisera prepared against the capsid of several PRSV types, the cylindrical inclusion protein of strain T, and the amorphous inclusion protein of isolate 3. However, Baker and Purcifull (1) observed serological relationships between PRSV-W and ZYFV using polyclonal antisera prepared against the SDS-PAGE purified capsid and cylindrical inclusion proteins of PRSV-W. The possible relationship between ZYFV and PRSV types is also justified by similarities in biological properties such as pathotypes on *C. metuliferus* PI 292190 and on the set of muskmelons.

Common properties have been found among the 15 isolates, the PRSV strains (FL, HA, and T), and WMV-M but differential responses to several criteria are also observed that justify the use of an analysis of multiple correspondences to allow a better vizualisation of the relationships between all these types.

The statistical analysis considered as variables the criteria for which differential responses were observed. These criteria are the molecular weight and 13 antigenic determinants of the coat protein, the reaction to the amorphous inclusion protein antiserum, and symptoms on six differential hosts and on six varieties of muskmelon. The aphid transmission frequency was not used because of a possible bias influencing the measured values due to the variable periods, the types had been maintained by mechanical transmissions, which can lead to a loss of aphid transmissibility.

The results show that 83% of the total inertia is explained by the five first axes. The 19 types of PRSV and WMV-M form two main groups (Fig. 1). The first contains 11 types including FL and HA. The second contains five other types including WMV-M. The strain T and the isolate 1 on the one hand, and the isolate 15 on the other hand, are linked to the isolate 14 that belongs to the second group.

In the first group, the types have similar antigenic determinants on their capsid and all react with the amorphous inclusion protein antiserum. Although five of these isolates (3, 4, 5, 6, and 7) cannot be differentiated from the reference PRSV-W FL by any of the properties studied, five others have some biological differences: the ability to infect papaya (HA and isolate 2) and/or the pathotype on muskmelon (HA, and isolates 8, 9, and 10).

The second group includes the types WMV-M, 11, 12, 13, and 14. The types in this group present some serological discrepancies on their coat proteins (except isolate 11, which is indistinguishable from WMV-M). But none of them reacts with the amorphous inclusion protein antiserum and all, except isolate 14, have the same pathotype on muskmelon, especially a necrosis reaction on Vedrantais, which is controlled by a particular gene (32).

TABLE 5. Transmission percentage of each virus type by *Aphis gossypii*

Virus type	Transmission (%)	Virus type	Transmission (%)
15	22[a]	10	16
FL	8	14	31
3	76	T	31
4	84	1	66
5	59	WMV-M	31
6	86	11	54
7	67	12	38
2	28	13	56
HA	3	ZYFV	7
8	55	WMV-2	...[b]
9	65	ZYMV	...

[a]Average of three tests using two different plants of squash as sources, one aphid per test plant, and 50 test plants per virus type.
[b]... = not tested.

TABLE 6. Molecular weight of the coat protein of the virus types

Virus type	Mol. wt.	Virus type	Mol. wt.
T	38,500[a]	9	36,600
1	38,800	10	37,100
2	36,900	WMV-M	37,600
HA	35,400	11	36,900
FL	37,200	12	36,800
3	35,700	13	35,700
4	37,000	14	36,000
5	36,600	15	37,200
6	36,300	ZYFV	35,700
7	36,500	WMV-2	...[b]
8	36,900	ZYMV	...

[a]Average of at least four measurements made on as many different electro-immunoblots.
[b]... = not tested.

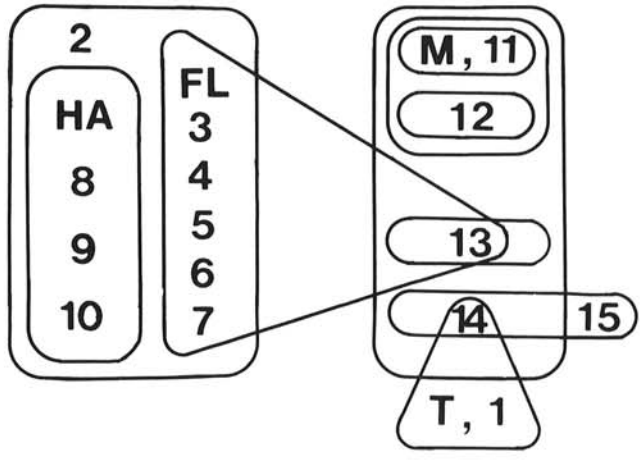

Fig. 1. Grouping of 18 PRSV strains and isolates and WMV-M (ZYFV, WMV-2, and ZYMV were omitted in this analysis) as determined by a multi-factorial analysis of correspondences using 27 variables: the molecular weight and 13 antigenic determinants of the coat protien, the reaction to the amorphous inclusion protein antiserum, and the symptomatology on six differential hosts and on six varieties of muskmelon. The viruses already described are: PRSV-P (HA), PRSV-W (FL), WMV-M (M), and PRSV-T (T).

Strain T and isolate 1 do not react with the amorphous inclusion protein antiserum and have the same muskmelon pathotype as the other types of the second group (isolate 14 excepted), but the serological properties and molecular weight of their coat protein show some differences.

In this analysis, isolate 15 is linked to the second group through isolate 14 because both lack the characteristics of other types. Nevertheless, isolate 15 has at least one of the most common coat protein antigenic determinants and it reacts with the cylindrical inclusion protein antiserum used. It has a peculiar pathotype on muskmelon.

These data demonstrated that five isolates cannot be differentiated from FL, the PRSV-W reference, and one cannot be differentiated from WMV-M. Four isolates differ slightly from FL but are easily associated with HA, the PRSV-P reference, three present some similarity with WMV-M, one presents some similarity with strain T, and one appears only distantly related to all these PRSV strains (Fig. 1). It is apparent also that PRSV-P (HA) or PRSV-W (FL), PRSV-T and WMV-M have similar degrees of relationship. Some relationships among these strains were previously described (2,3,29,33).

Under our conditions (mechanical inoculation to young plants maintained in greenhouse or growth chambers), some of the types induce a systemic or severe apical necrosis on muskmelon lines. It could be important to establish if such symptoms are encountered in field conditions. Isolate 15, which is able to overcome the resistance of the lines WMV 29 and 72025, needs more extensive study.

In spite of the limited number of types that have been studied, some conclusions can be drawn about the geographical distribution of the variants. Some isolates indistinguishable from FL (the reference of PRSV-W) are present in different continents: North America (FL), Oceania (isolates 6 and 7), West Africa (isolate 5), and Europe (isolates 3 and 4). Some other isolates closely related to PRSV-W FL and PRSV-P HA (isolates 2, 8, 9, and 10) have been found also in different parts of the world and numerous reports suggest that PRSV-W and PRSV-P have a distribution that can include all the tropical countries.

By contrast, isolates of WMV-M group have been found, until now only in Africa or nearby. WMV-M was originally described in Morocco in 1974 (10). An isolate related to WMV-M has been described recently in South Africa, where it could have been present for several years (38). We have also found isolate 11 from Spain, which is indistinguishable from WMV-M by our criteria. In other parts of Africa (Algeria and Niger) or in the vicinity (Yemen Arab Republic), some other isolates more or less closely related to WMV-M were found.

PRSV-T and isolate 1, which show some similarities, have been isolated from two different continents: Central America and West Africa, both in humid tropical lowlands.

The difference of geographical distribution between PRSV and WMV-M groups present some similarities with the situation observed for WMV-2 and ZYMV during recent years. WMV-2 had a well-known worldwide distribution, whereas ZYMV, initially discovered in northern Italy and in southern France, was found eventually to have a quasi-worldwide distribution.

It could be interesting to establish if the WMV-M group also has a geographical distribution larger than currently documented and, if not, to try to understand what limits its distribution.

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