

# Relationship Between Watermelon Mosaic Virus (WMV-1) and Watermelon Mosaic Virus 2 (WMV-2) on the basis of Nucleotide Sequence Homology

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**Key words:** Potyvirus; immune electron microscopy; molecular hybridization analysis.

## ABSTRAK

*Kedua ahli subkumpulan 'watermelon mosaic virus', WMV-1 dan WMV-2 dianggap sebagai virus-virus yang berlainan berdasarkan kepada bidang hos dan serologi. Immun mikroskop elektron dengan penunjuk pembezaan indeks 2 ke 3 mengesahkan perbezaan serologi ini. Analisis hibridisasi molikul dengan menggunakan DNA berkoplomen yang disediakan oleh transkripsi berbalik RNA WMV-1 berprima rawak, menunjukkan tiada homologi di antara WMV-1 dan WMV-2 dan DNA berkoplomen juga menunjukkan tiada homologi dengan RNA dari beberapa 'potyvirus' yang menjangkiti kekacang dan 'potato virus Y' (PVY). Analisa hibridisasi molikul selanjutnya mengesahkan yang WMV-1 dan WMV-2 adalah virus-virus yang nyata berbeza.*

## ABSTRACT

*The two members of the watermelon mosaic virus subgroup, WMV-1 and WMV-2, were considered to be different viruses on the basis of host range and serology. Immune electron microscopy with serological differentiation indices of 2 to 3 confirmed this serological difference. Molecular hybridization analysis using complementary DNA prepared by reverse transcription of randomly primed WMV-1 RNA showed no homology between WMV-1 and WMV-2 and the complementary DNA from a range of legume infecting potyviruses and potato virus Y (PVY). Molecular hybridization analysis further confirmed that WMV-1 and WMV-2 are distinct viruses.*

## INTRODUCTION

Watermelon mosaic virus — 1 (WMV-1) and watermelon mosaic virus — 2 (WMV-2) have been differentiated on their biological and serological properties. Using agar double-diffusion tests, Milne and Grogan (1969) found a close serological relationship between various isolates of WMV-1 and WMV-2 and concluded that they were strains of WMV even though their host ranges differed.

Purcifull and Hiebert (1979) indicated that by SDS-immunodiffusion tests the Florida

isolates of WMV-1 and WMV-2 were serologically distinct as no cross-reactions were detected.

The Queensland isolates of WMV-1 and WMV-2 were found to have some distinct host range differences. WMV-2 Q has a much wider host range as compared to WMV-1 Q (Greber, 1978). They were serologically distinct from one another but closely related to Florida isolates of the respective types using the SDS-immunodiffusion tests with crude sap (Greber, 1978). Physical properties of these WMV isolates and electron microscopic examination of the virus particles

and inclusions were found to be similar to the other isolates reported elsewhere.

Makkouk and Lesemann (1980) found that their WMV-1 reacted with the Florida isolate of WMV-1 but not with WMV-2 Florida isolate antiserum when tested in SDS-immunodiffusion tests and with the decoration technique of immune electron microscopy.

Inconsistencies in the reported relationships between WMV-1 and WMV-2 probably resulted from the use of different strains and techniques.

In this paper, the relationship between WMV-1 and WMV-2 was reexamined by molecular hybridization analysis (MHA) which discriminates isolates and strains of the potyvirus, bean yellow mosaic virus (BYMV) (Abu-Samah, 1982; Abu-Samah & Randles, 1981; Abu-Samah & Randles, 1983). Molecular hybridization analysis (MHA) allows more sensitive discrimination and provides a semi-quantitative estimate between closely related isolates. But MHA is too specific to show relationships between distantly related strains or different viruses. Therefore in this study serological relationships were also examined by immune electron microscopy (IEM).

## MATERIALS AND METHODS

### *Sources of Virus*

Isolates of WMV-1 and WMV-2 were supplied by a government department\* in Indooroopilly, Queensland and maintained in the glasshouse on *Cucumis sativus* cv. Polaris. In this paper, these isolates are referred to as WMV-1 Q and WMV-2 Q.

### *Purification of WMV-1 Q and WMV-2 and Their RNAs*

Purification of WMV-1 Q and WMV-2 Q was from systemically infected leaves of *C. sativus* cv. Polaris harvested 2–3 weeks after inoculation. Both viruses were purified by

methods based on those of Purcifull and Hiebert (1979) with modifications of the steps after the polyethylene glycol (PEG 6000) concentration step.

For WMV-1 Q, the PEG pellets were resuspended in 0.05 M potassium phosphate buffer, pH 7.5. The virus was centrifuged through 1 ml of a 30% buffered sucrose cushion in a Spinco 65 rotor at 78,000 g for 105 min. The pellet was resuspended in water.

For WMV-2 Q, after the precipitation step with PEG and sedimentation by high speed centrifugation as above, the virus pellet was resuspended in 0.05 M potassium phosphate buffer, pH 7.5. The resuspended material was subjected to centrifugation in a CsCl gradient ( $\rho = 1.28$  g/ml) using the SW 50 rotor at 40,000 rpm for 18 hrs. The virus containing zone was removed, diluted with buffer and was further subjected to high speed centrifugation at 78,000 g in the Spinco 65 rotor for 120 min. The pellet was resuspended in water.

The RNAs were extracted from the virus suspension by the pronase procedure as described for BYMV-RNA (Abu-Samah & Randles, 1981). Only WMV-1 was purified further through two cycles of sucrose density gradients (Abu-Samah & Randles, 1981).

### *Comparison by Immune Electron Microscopy (IEM)*

The antisera of the Florida isolate of WMV-1 (WMV-1 Fl) and WMV-2 (WMV-2 Fl) used in this study were kindly provided by Dr. D.E. Purcifull, University of Florida, Gainesville. The Florida antisera were used in antibody decoration and dilution end-point clumping IEM tests to compare WMV-1 Q and WMV-2 Q (Milne and Luisoni, 1977).

### *Molecular Hybridization Analysis (MHA)*

The random primer method of Taylor *et al.*, (1976) as used for BYMV-RNA (Abu-Samah

\*Supplied by Mr. R.S. Greber of the Department of Primary Industries.

& Randles, 1981) was used to synthesize complementary DNA (cDNA) to WMV-1. The hybridization solution contained 0.01 M Tris-HCl, pH 7.0, 0.18 M NaCl, 1 mM EDTA and 0.05% SDS (Gould & Symons, 1977; Abu-Samah & Randles, 1981).

Hybridization was carried out in siliconized glass test tubes, approx.  $7 \times 50$  mm with reaction mixtures overlaid with paraffin oil. To 40  $\mu$ l of the appropriately diluted RNA solution was added 2  $\mu$ l of [ $^3$ H] cDNA (c. 2000 cpm). The reaction mixtures were immersed in boiling water for 2–3 mins and then incubated at 65–66°C. Hybridizations were terminated by chilling the tubes, removing 30  $\mu$ l aliquots, and adding them to 300  $\mu$ l of a low salt  $S_1$  assay buffer (0.03 M sodium acetate, 0.05 M NaCl, 1 mM  $ZnSO_4$ , 5% glycerol, pH 4.6) containing 40  $\mu$ g/ml of denaturated calf thymus DNA. Two samples, each 150  $\mu$ l, were taken and to one was added 2 units of  $S_1$  nuclease; the other was left as a control. After incubation of both samples at 45°C for 30 mins, nuclease resistance was determined by comparing the duplicates incubated either with or without enzymes, as described by Gould & Symons (1977).

The  $T_m$  of DNA-RNA hybrid was set up in a total vol. of 100  $\mu$ l and incubated at 65°C to a  $R_o$  value exceeding  $1.0 \text{ mol sec liter}^{-1}$ . The mixture was chilled and placed in a waterbath in which the temperature was raised by approx. 1°C per min. At the appropriate temperatures, 5  $\mu$ l portions were transferred to 150  $\mu$ l of cold  $S_1$  buffer and the percentage hybrids remaining at each temperature was determined by  $S_1$  nuclease resistance.

## RESULTS

### Comparison by IEM

In the decoration IEM test at an antiserum dilution of 1/10, both WMV-1 Q and WMV-2 Q particles were heavily decorated with the antibody of WMV-1 Fl and WMV-2 Fl respectively. Therefore the Queensland isolates of WMV-1 and WMV-2 were closely related to their Florida

counterparts using IEM (Figs. 1 and 2). These results were consistent with those of Greber (1978) who showed a serological relationship of the Q isolates with the Florida type isolates by SDS-immunodiffusion tests.

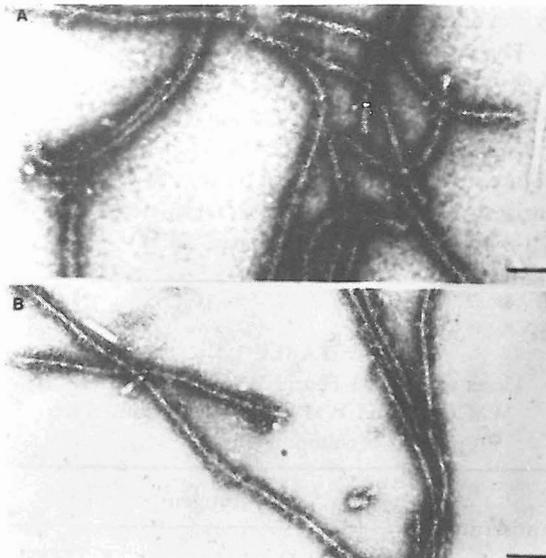


Fig. 1. Serological relationship between WMV-1 Q and WMV-2 Q by decoration IEM. WMV-1 Q against antisera to (A) WMV-1 Fl and (B) WMV-2 Fl at a 1/10 dilution. Bar represents 500 nm.

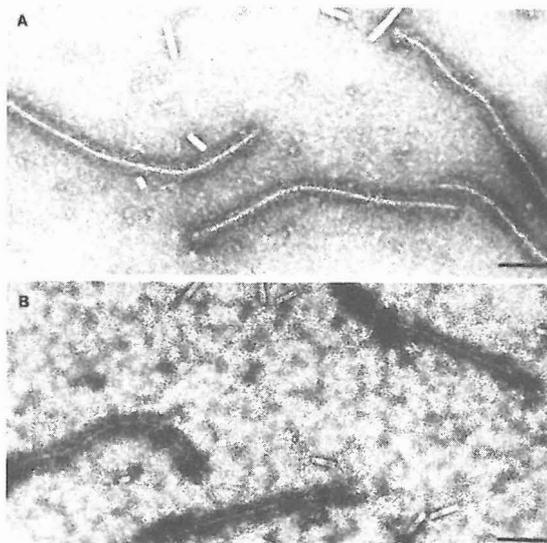


Fig. 2. Serological relationship between WMV-2 Q and WMV-1 Q by decoration IEM. WMV-2 against antisera to (A) WMV-2 Fl and (B) WMV-1 Fl at a 1/10 dilution. Bar represents 500 nm.

At the antiserum dilution used, cross-reactions were detected in reciprocal tests, although with much less decoration for the heterologous tests. The dilution end-points of both antisera with both WMV-1 Q and WMV-2 Q by the clumping IEM test are presented in Table 1. The serological differentiation index between WMV-1 Fl antiserum and WMV-2 Q was 2 and between WMV-2 Fl antiserum and WMV-1 Q was 3. These antisera failed to decorate the bean isolate of tobacco mosaic virus (B-TMV) and antiserum to U1 isolate of tobacco mosaic virus (U1-TMV) failed to decorate WMV-1 Q or WMV-2 Q.

TABLE 1  
Titres of WMV-1 Fl and WMV-2 Fl antisera to WMV-1 Q and WMV-2 Q as determined by clumping IEM

Antiserum	Antigens		
	WMV-1 Q	WMV-2 Q	B-TMV
WMV-1 Fl <sup>a</sup>	2048	512	0
WMV-2 Fl <sup>a</sup>	512	4096	0
U <sub>1</sub> - TMV <sup>b</sup>	0	0	0

Antisera kindly supplied by Dr. D.E. Purcifull<sup>(a)</sup>, and Dr. R.I.B. Francki<sup>(b)</sup>.

Figures in the Table are reciprocal titres of antisera.

*Comparison by Molecular Hybridization Analysis (MHA)*

Reverse transcription of WMV-1 RNA gave cDNA with  $2 \times 10^6$  count/min (13.4 ng) for an input of 2 µg of RNA. The specificity of the cDNA is illustrated in Table 2; hybridization of the cDNA with other viral RNAs or with nucleic acid extracted from healthy cucumber was not observed.

Homologous and heterologous hybridization kinetics between WMV-1 Q cDNA and WMV-1 Q and WMV-2 Q RNAs are presented in Fig. 3. The WMV-1 Q cDNA showed a maximum percentage hybridization of approximately 90% with homologous RNA. The single phase curve up to the maximum  $R_o t$  tested indicates that the RNA used was uncontaminated (Gould and Symons, 1977) with other RNAs and probably had few reiterated sequences.

The  $R_o t_{1/2}$  value for the homologous hybridization was  $1.2 \times 10^{-2}$  mol sec liter<sup>-1</sup> similar to that obtained by the BYMV-RNAs (Abu-Samah & Randles, 1981). Thermal denaturation of the homologous hybrid showed a sharp transition and a high  $T_m$  of 84°C under the conditions used (Fig. 4). This indicated that hybrid formation was specific with no evidence of mismatching.

TABLE 2  
Percentage sequence homologies of WMV-1 Q and WMV-2 Q RNAs with WMV-1 Q complimentary DNA

RNA	RNA concentraion (ug/ml)	$R_o t$	Percent hybridization
No RNA	—	—	5.7
Healthy cucumber total nucleic acid	96.8	104.5	2.0 <sup>a</sup>
TMV-RNA	75.2	81.2	2.1 <sup>a</sup>
Yeast RNA	400.0	432.0	0 <sup>b</sup>
WMV-1 RNA	4.0	4.3	89.1 <sup>a</sup> ± 5.6 <sup>b</sup>
WMV-1 RNA	4.0	4.3	0

<sup>a</sup>Hybridization percentages were corrected for self-hybridization of cDNA.

<sup>b</sup>Standard errors of the mean of three replicates.

COMPARISON OF WMV-RNA

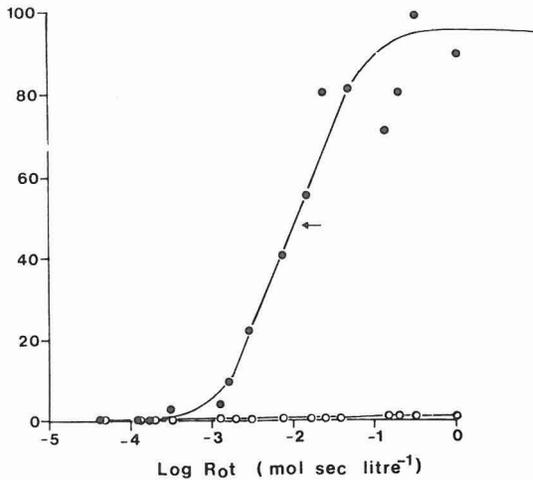


Fig. 3. Kinetics of hybridization of WMV-1 cDNA with WMV-1 RNA (●) and WMV-2 RNA (○). The  $R_0 t_{1/2}$  value is indicated by an arrow.

Test of Relationship Between WMV-1 and a Range of Potyviruses

The relationship of WMV-1 Q to a range of potyviruses from South Australia was examined by MHA using cDNA synthesized to WMV-1 Q RNA. Potyvirus RNAs from partially purified preparations concentrated by polyethylene 6000 (PEG), were phenol extracted and ethanol precipitated (Abu-Samah & Randles, 1981). The results in Table 3 show that WMV-1 Q had no detectable homology with any of the legume-infecting potyviruses tested or with PVY.

TABLE 3  
Percentage sequence homologies between WMV-1 Q cDNA and RNAs of a range of legume infecting potyviruses and PVY

RNA <sup>b</sup>	Percentage homology <sup>a</sup> with WMV-1 cDNA
PVY	0 ± 0.77
LMV	0 ± 0.45
PMV	0 ± 0.68
SoMV	0 ± 0.08
SPMV	0 ± 0.53
CAMV	0 ± 0.31
BCMV	0 ± 0.81
BYMV-G	0 ± 1.1
BYMV-Q	0 ± 1.6
BYMV-S	0 ± 1.9
Homologous	91.6 ± 5.4

<sup>a</sup>Hybridizations were done to a  $R_0 t$  of 10.8 mol sec litre<sup>-1</sup>. Standard errors of the mean of three replicates are given for each hybridization reaction.

<sup>b</sup>PVY = potato virus Y; LMV = lettuce mosaic virus; PMV = pea mosaic virus; SoMV = soybean mosaic virus; SPMV = sweet pea mosaic virus; CAMV = cowpea aphid-borne mosaic virus; BCMV = bean common mosaic virus; BYMV-G = G isolate of bean yellow mosaic virus; BYMV-Q = Q isolate of bean yellow mosaic virus; BYMV-s = S isolate of bean yellow mosaic virus.

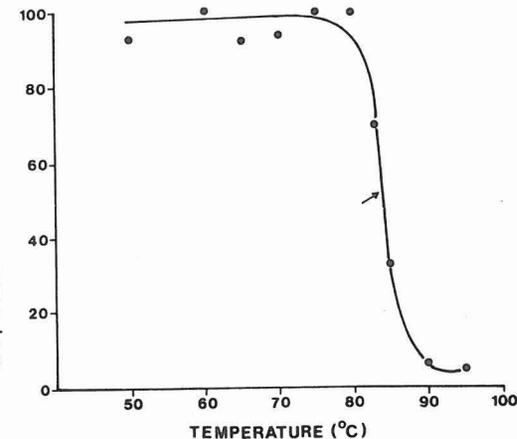


Fig. 4. Thermal stability of the hybrid of WMV-1 Q cDNA with homologous RNA. The  $T_m$  value (84°C) is indicated by an arrow.

DISCUSSION

Host reactions can differentiate WMV-1 Q and WMV-2 Q (Greber, 1978). WMV-2 Q infected cucurbits, legumes and chenopodiaceae

but WMV-1 Q infected only cucurbits. There was an apparent similarity of the wide-host-range type of WMV-2 and the limited-host-range type of WMV-1 worldwide. This was in agreement with Milne and Grogan (1969); Purcifull and Hiebert (1979) and Makkouk and Lesemann (1980).

Serological data for the two viruses by IEM indicate that they were distantly related. This, however, is not surprising considering that distant serological relationships are widespread among members of the potyvirus group (Hollings and Brunts, 1981a, b). Cross-contamination between WMV-1 Q seemed unlikely since the SDI values were different and would have been detected in hybridization analysis experiments. The inability to detect cross-reactions between WMV-1 and WMV-2 in SDS-immunodiffusion tests (Greber, 1978; Purcifull & Hiebert, 1979; Makkouk & Lesemann, 1980) could be due to the insensitivity of the method as a result of the alteration of the antigenic specificity between SDS-treated virus and the untreated virus which had been used in preparing antiserum (Shalla & Shepard, 1970; Shepard & Shalla, 1970; Shepard & Secor, 1972).

From previous work (Abu-Samah & Randles, 1981, 1983) significant homology was demonstrable only between potyviruses that were closely related. For example, the G, Q and S isolates of bean yellow mosaic virus (BYMV), which are closely related serologically and biologically, were distinguished quantitatively by their percentage homology. The present study shows that WMV-1 Q and WMV-2 Q, which are biologically and serologically distantly related to each other (Greber, 1978; Purcifull & Hiebert, 1979; Makkouk & Lesemann, 1980), had no detectable base sequence homology in common. These data confirm that WMV-1 Q and WMV-2 Q cannot be considered as strains and are distinct under the conditions used for analysis.

Therefore these data imply that distantly related strains do not show homology, but with TMV (Palukaitis & Symons, 1980; Palukaitis *et al.*, 1981) further relationships were demonstrated under less stringent conditions. Randles *et*

*al.*, (1981) showed that under conditions of low stringency for MHA, low homology was detected in several TMV isolates which showed no homology under stringent conditions. Since MHA between WMV-1 Q and WMV-2 Q was carried out under conditions of high stringency (Abu-Samah & Randles, 1981), conditions of lower stringency might detect some relationships between the viruses.

As all attempts to synthesize cDNA to WMV-2 were unsuccessful, the relationship between WMV-1 and WMV-2 was determined by using the cDNA synthesized to WMV-1 only. It has been shown previously that the percentage homology was the same in reciprocal tests between three isolates of BYMV (Abu-Samah & Randles 1981). Heterologous hybridizations between WMV-2 Q RNA and WMV-1 Q cDNA showed no detectable homology (*Fig. 3*) under stringent conditions which allowed reactions among strains of BYMV (Abu Samah & Randles, 1981).

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