Serological Relationships Among Potyviruses: Maize Dwarf Mosaic Virus, Tobacco Etch Virus, and Turnip Mosaic Virus

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ABSTRACT

Serological relationships of maize dwarf mosaic virus strain-B (MDMV), turnip mosaic virus (TurMV), and tobacco etch virus (TEV) were studied using antisera produced against the intact viruses. Microprecipitin tests showed that MDMV, TurMV, and TEV are distantly related serologically. This relationship provides further evidence for placing MDMV in the potyvirus group of plant viruses.

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Members of the potyvirus plant virus group exhibit either distant, or no, serological relatedness (1, 2, 3, 4, 5, 6, 10, 11, 12, 14, 15, 17, 18, 19). Serological relationships between virions of the three most well-characterized viruses in this group, maize dwarf mosaic virus (MDMV), turnip mosaic virus (TurMV), and tobacco etch virus (TEV) have not been well established. The data we report demonstrate serological relationships between these three viruses. Shepard et al. (16) have recently shown cross-reactivity between dissociated capsid proteins of several potyvirus group members including MDMV, TurMV, and TEV.

MATERIALS AND METHODS.—TEV (ATCC-PV69) was purified according to Damirdagh and Shepherd (7) except 8% (w/v) polyethylene glycol (MW 6,000-7,500), instead of 4% was used, and the suspension was incubated overnight at 4 C before the virus was collected by centrifugation. TurMV (ATCC-PV134) and MDMV, strain B (ATCC-PV53), were purified as described by Hill et al. (8) and Hill and Shepherd (9). Virus concentrations were estimated using the extinction coefficient for TEV of 2.4 $cm^2\ mg^{-1}$ at 261 nm (13).

Intact virus antigens were dialyzed overnight at 4 C against 50 mM sodium borate buffer, pH 8.2, and emulsified (1:1, v/v) with Freund's incomplete adjuvant. Adult rabbits were immunized by weekly intramuscular injections with 1.5, 1.0, 1.0 and 1.5 mg of virus, and serum was collected by cardiac puncture 7 days after the final injection. Two rabbits were immunized against each virus. Serum from each animal was processed and tested separately.

Microprecipitin tests in plastic petri dishes were incubated for 2 hours at 25 C, followed by 21 hours at 4 C, after which readings were made. Antisera dilutions were made in 50 mM sodium borate-buffered saline, pH 7.4, and antigen dilutions (initial concentrations–1.0 mg/ml) in 0.05 M sodium-potassium phosphate buffer, pH 7.4. Controls were purified tobacco mosaic virus (TMV) and healthy sap antigens (prepared from *Zea mays* L. 'Golden Bantam' sweetcorn, *Nicotiana tabacum* L. 'Havana 425', or *Brassica campestris* L. 'Tendergreen' mustard) and clarified by centrifugation at 10,000 *g* for 10 minutes. All tests were repeated twice, and when results differed, a range was reported.

The higher-titered of the two antisera produced for each virus was used to prepare cross-absorbed antisera. Antisera were cross-absorbed by addition of four volumes of heterologous antigen (0.5 mg/ml), as determined by absence of precipitate in the microprecipitin test after cross-absorption.

RESULTS AND DISCUSSION.—Homologous and heterologous titers of antisera prepared to intact virus

TABLE 1. Homologous and heterologous titers of intact virus antisera as determined by microprecipitin tests (titers are expressed as the reciprocal of the highest dilution giving a positive reaction)

Antiserum	Rabbit no.	Antigen		
		MDMV[a]	TEV[a]	TurMV[a]
MDMV[a]	1	256-512	32	32
	2	512-1,024	64	64
TurMV[a]	1	4-8	64	1,024-2,048
	2	8-16	32	1,024
TEV[a]	1	8-16	1,024	64
	2	16-32	1,024	16-32

[a]MDMV, TEV, and TurMV refer to maize dwarf mosaic, tobacco etch, and turnip mosaic viruses respectively.

TABLE 2. Homologous and heterologous titers of cross-absorbed intact virus antisera as determined by microprecipitin tests (titers are expressed as the reciprocal of the highest dilution giving a positive reaction)

Antiserum	Cross absorbed with	Antigen		
		MDMV[b]	TEV[b]	TurMV[b]
MDMV[b] (2)[a]	TurMV	128	16-32	0
	TEV	128-256	0	32
TurMV[b] (1)[a]	MDMV	0	16-32	1,024
	TEV	2	0	1,024
TEV[b] (1)[a]	MDMV	0	512	16
	TurMV	16-32	512-1,024	0

[a]Refers to rabbit no. in Table 1.
[b]MDMV, TEV, and TurMV refer to maize dwarf mosaic, tobacco etch, and turnip mosaic viruses respectively.

(Table 1) were sufficiently high to suggest distant serological relationships. When two rabbits were immunized against the same intact virus, consistent results were obtained between the two antisera produced.

Titers of cross-absorbed antisera further indicated distant serological relationships (Table 2). Results shown in Tables 1 and 2 are directly comparable, since the titers reported in Table 2 are adjusted for antiserum dilution due to the cross-absorption process.

No reactions were observed between normal serum and any of the intact virus antigens. Clarified sap from healthy host plants showed no reactions when tested against antisera to their respective viruses. Similarly, buffer and saline controls in each microprecipitin plate were negative. In addition, when TMV was used as a control antigen in tests with each of the antisera, no specific reactions were observed.

The results reported here were not unexpected since the work of Shepard et al. (16) showed that the depolymerized coat proteins of these viruses have antigenic determinants in common. This work indicates that at least some of the common antigenic sites of these three viruses are exposed on the virion.

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