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Serological Interrelationships in the Turnip Yellow Mosaic Virus Group

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The serological interrelationships between eleven viruses in the turnip yellow mosaic virus group (tymoviruses) were studied with 286 antisera from 48 rabbits. Antisera from different rabbits and from different bleedings of the same rabbit showed considerable differences in their homologous and heterologous reactivities. A classification based on average serological differentiation indices is proposed. There is a continuous range of serological relationships in the tymovirus group and subdivision into a turnip yellow mosaic virus and an Andean potato latent virus subgroup does not seem justified.

INTRODUCTION

Until recently the tymovirus group was believed to consist of two serologically unrelated subgroups (Gibbs, 1969; Harrison *et al.*, 1971). The first subgroup is composed of turnip yellow mosaic (TYMV), wild cucumber mosaic (WCuMV), and cocoa yellow mosaic viruses (CoYMV) (TYMV subgroup), the second one of Andean potato latent (APLV), belladonna mottle (BMV), dulcamara mottle (DMV), eggplant mosaic (EMV), and ononis yellow mosaic viruses (OYMV) (APLV subgroup). The first evidence of a serological relationship of viruses in the APLV subgroup with TYMV was given by Bercks and Querfurth (1971). Somewhat later (Bercks *et al.*, 1971), it was found that Scrophularia mottle virus (ScrMV) provided a serological link between TYMV and the viruses in the APLV subgroup. Recently, two other viruses with the typical properties of tymoviruses and a serological relationship to TYMV have been described, i.e., okra mosaic virus (OkMV) (Givord and Hirth, 1973) and desmodium yellow mottle virus (DeYMV) (Walters and Scott, 1972; Scott and Moore, 1972). The present study was undertaken to elucidate the systematic position of these newly described viruses in the tymovirus group and to give an inclusive account of the serological interrelationships in this group.

MATERIALS AND METHODS

Isolates of tymoviruses were kindly provided by Drs. A. A. Brunt (CoYMV), A. J. Gibbs (DMV, OYMV), B. D. Harrison (APLV, EMV), M. N. Short (WCuMV), and H. A. Scott (DeYMV). The isolates of BMV, ScrMV, TYMV, and OkMV were those described in previous papers from the Braunschweig and Abidjan laboratories (Bercks *et al.*, 1971; Givord and Hirth, 1973). TYMV and WCuMV were purified with bentonite according to Dunn and Hitchborn (1965). The method recommended for TYMV was also successfully applied to the other viruses. In tests with antisera to the host proteins of 17 different plants, including all the hosts used in the present study, no contaminations with host proteins were found in any of the virus preparations. Rabbits were usually immunized by two intramuscular injections spaced 1 week apart of virus adjusted to a titer of 1:512 and emulsified in Freund's adjuvant. Bleedings were usually taken at 2-week intervals. Double diffusion tests were

done with 0.85% Difco special Noble agar containing 0.85% sodium chloride and 0.01 *M* Tris·HCl buffer, pH 8. The reactant wells, 4 mm in diameter, were spaced 2.5 mm apart. Serial dilutions of antisera were tested against virus antigens with titers adjusted to 1:16 and 1:4. Readings were taken after incubation for 2 days at room temperature.

RESULTS AND DISCUSSION

The homologous and heterologous titers of selected antisera to the eleven tymoviruses are listed in Table 1. Altogether 3146 tests were done with 286 bleedings from 48 rabbits. It is evident that two serological phenomena which are well documented for other groups of plant viruses (Bercks, 1963; Tremaine and Wright, 1967; Koenig and Bercks, 1968; Allen, 1968; van Regenmortel and von Wechmar, 1970) also apply to the tymovirus group; i.e., the antisera from different rabbits immunized with the same virus show considerable individual differences in their homologous and especially their heterologous reactivities (Table 1, antisera to BMV, DeYMV, ScrMV, and TYMV). In addition, the ratios of these reactivities change with bleedings taken from the same animal at different stages of the immunization process. Homologous and heterologous reactivities may show a great variety of apparently more or less independent developmental tendencies.

To summarize:

1. At the beginning of the immunization process, homologous and heterologous titers increase at about the same rate (APLV antisera 99: APLV and EMV; DMV antisera 176: DMV and EMV; ScrMV antisera 222: ScrMV and WCuMV).

2. After being initially relatively low, heterologous titers increase more rapidly than homologous ones (BMV antisera 58: APLV and DMV; DMV antisera 176: BMV; ScrMV antisera 222: OYMV). This is frequently the case with closely related viruses (DMV—BMV, OYMV—ScrMV) which can be better differentiated with antisera from early than from late bleedings.

3. Heterologous titers increase more slowly than homologous ones (APLV antisera 99: WCuMV; ScrMV antisera 222: BMV; TYMV antisera 89: ScrMV).

4. Early during the immunization process heterologous titers are relatively high, but they do not increase as the homologous titer increases, (DeYMV antisera 457: OkMV).

5. Early during the immunization process heterologous titers are relatively high, but they soon drop as immunization proceeds. This has been found quite frequently with antisera to potexviruses (Koenig and Bercks, 1968). With tymoviruses we found this less frequently (APLV antisera 99: BMV; TYMV antisera 89: OkMV, WCuMV).

6. Late in immunization the heterologous titers decrease at about the same rate as the homologous ones (BMV antisera 58: APLV; TYMV antisera 91: OkMV).

7. Late in immunization the heterologous titers drop more slowly than the homologous ones (BMV antisera 58: EMV, OYMV).

8. Late in immunization heterologous titers remain constant or increase while the homologous titers decrease (EMV antisera 68: APLV, BMV, DMV).

The type of pattern which develops is neither unique for the antigen (Table 1, heterologous reactivities of TYMV antisera 89 and 91 with OkMV) nor for the rabbit (heterologous reactivities of APLV antisera 99, BMV antisera 58, DeYMV antisera 457, TYMV antisera 89). If under the conditions (1) to (3) the difference between homologous and heterologous reactivities is high, it may well be that a heterologous reactivity cannot be detected before the homologous titer has reached a certain level (DeYMV antisera 457: OYMV, ScrMV, TYMV; ScrMV antisera 222: DMV and EMV) or immunization has proceeded for some time (EMV antisera 68: OYMV, ScrMV). No general statement can be made as to whether early or late bleedings are better suited for the detection of distant relationships.

The few examples in Table 1—which are selected from a much larger number of similar data—illustrate once more how unreliable conlusions on the degree of serological relationships can be as long as they are based on tests with one or a few antisera. The question arises whether estimates on the close-

TABLE 1

HOMOLOGOUS AND HETEROLOGOUS REACTIVITIES OF ANTISERA TO TYMOVIRUSES[a]

Serial No. of animal	Immunized with	Number of days after the first injection	APLV	BMV	CoYMV	DeYMV	DMV	EMV	OkMV	OYMV	ScrMV	TYMV	WCuMV
99	APLV	7	*64*	8	1	0	8	1	0	2	0	0	1
99		28	*512*	2	1	0	8	8	0	1	0	0	2
99		49	*1024*	0	1	0	32	16	0	0	0	0	2
99		250	*1024*	0	0	0	64	32	0	0	0	0	4
60	BMV	309	1024	*4096*	0	0	2048	32	0	8	0	0	0
926		224	128	*1024*	0	0	1024	64	0	16	0	0	0
855		420	256	*1024*	0	0	512	16	1	32	8	0	0
122		35	8	*256*	0	0	256	2	0	1	8	0	0
58		7	4	*256*	0	0	32	4	0	0	0	0	0
58		21	64	*256*	0	0	128	8	0	2	0	0	0
58		35	256	*512*	0	0	512	8	0	4	0	0	0
58		91	512	*1024*	0	0	512	16	0	16	0	0	0
58		270	64	*128*	0	0	128	8	0	8	0	0	0
458	DeYMV	83	0	0	128	*8192*	0	0	64	0	0	2	0
459		82	0	0	128	*2048*	0	0	32	0	0	4	1
—[b]		?	0	0	64	*2048*	0	0	128	0	0	8	0
457		13	0	0	4	*256*	0	0	16	0	0	0	0
457		27	0	0	32	*2048*	0	0	16	2	0	2	0
457		41	0	0	128	*2048*	0	0	16	4	4	4	0
457		83	0	0	128	*4096*	0	0	16	4	2	8	0
176	DMV	14	8	32	0	0	*256*	4	0	0	0	0	0
176		28	16	1024	0	0	*2048*	32	0	2	1	0	0
176		135	64	1024	0	0	*1024*	32	0	16	8	4	1
176		360	32	64	0	0	*128*	16	0	8	8	0	4
68	EMV	21	16	2	0	0	2	*256*	0	0	0	0	0
68		35	64	4	0	0	4	*256*	0	0	0	0	0
68		112	64	8	0	0	16	*64*	0	4	4	0	0
221	ScrMV	290	128	32	0	0	64	4	2	1024	*2048*	16	8
109		530	256	8	0	0	64	8	0	2048	*2048*	16	128
284		84	64	2	0	0	8	8	0	1024	*1024*	2	16
335		329	512	8	0	0	128	1	0	2048	*2048*	64	16
558		56	32	4	0	0	8	0	0	512	*1024*	8	4
222		7	1	1	0	0	0	0	0	8	*64*	2	2
222		35	4	2	0	0	4	0	0	512	*1024*	8	32
222		63	16	4	0	0	4	1	0	512	*1024*	16	16
222		290	16	2	0	0	4	1	0	512	*1024*	16	8
89	TYMV	7	1	0	0	0	0	0	16	0	1	*16*	4
89		21	0	0	0	0	0	0	2	0	1	*256*	1
89		35	0	0	0	0	0	0	0	0	2	*256*	0
89		49	0	0	0	0	0	0	0	0	1	*256*	0
91	TYMV	7	0	0	1	1	0	0	4	0	1	*512*	1
91		49	0	0	0	0	0	0	8	0	0	*2048*	0
91		240	0	0	0	0	0	0	4	0	0	*256*	0
91		320	0	0	0	0	0	0	1	0	0	*128*	0

[a] Reciprocal values of titers.

[b] Antiserum kindly provided by H. A. Scott.

ness of a serological relationship are at all possible. van Regenmortel and von Wechmar (1970) working with tobacco mosaic virus and cucumber virus 4 deny that such estimates can be obtained whereas Koenig and Bercks (1968) working with the potexvirus group believed that approximate estimates of the degree of serological relatedness are possible. The present data confirm this. Some relationships in the tymovirus group are definitely much closer than others. Thus, BMV and DMV are so closely related that a distinction between them is almost impossible with most of their homologous antisera, except for those from very early bleedings (Table 1). The same is true for the distinction between OYMV and ScrMV with antisera to ScrMV (Table 1). Other relationships are so distant that in our tests cross reactivities were rarely or never observed. Intermediate cases are the definite heterologous reactivities (Table 1) of all DeYMV antisera with OkMV and CoYMV or of all ScrMV antisera with WCuMV. These heterologous reactivities have nevertheless much lower titers than the homologous reactivities.

In Fig. 1 we have arranged the eleven tymoviruses in the order of the approximate closeness of their serological relationships. This was estimated on the basis of the average number of 2-fold dilution steps separating homologous and heterologous titers, i.e. the average serological differentiation indices (SDI) (van Regenmortel and von Wechmar, 1970). Individual SDIs were usually not more than two or three units below or above the average SDI. Only, in some extreme cases, their range was much larger (Table 1, heterologous reactivity of TYMV antisera 89 with OkMV). It has to be stressed that the number of antisera we tested, though much higher than any studied before with tymoviruses, is still rather limited. Thus, future research with even greater numbers of antisera may necessitate slight changes in the proposed order.

It is obvious from Fig. 1 that there are continuous ranges of serological relation ships in the tymovirus group. Viruses

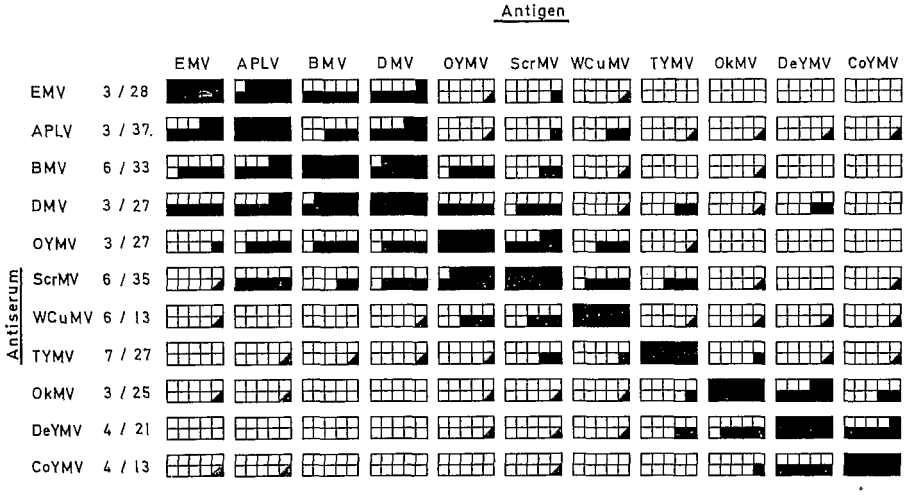

FIG. 1. Serological interrelationships in the tymovirus group. The first number following each antiserum indicates the number of rabbits immunized; the second indicates the number of bleedings tested. The viruses are arranged according to the approximate closeness of their serological relationships. The white squares indicate the average number of 2-fold dilution steps by which the heterologous titers of the antisera tested were below the homologous ones (all squares black), e.g., ▬ signifies that the heterologous titer was on the average five 2-fold dilution steps below the homologous titer; i.e., if the homologous titer was 1:4096 the average heterologous titer was 1:128; if the homologous titer was 1:512 the average heterologous titer was 1:16. ⊞ signifies that the heterologous titer was on the average nine 2-fold dilution steps below the homologous titer. Frequently, only a few antisera showed this reactivity. ⊞ signifies that no heterologous reaction was found.

which directly between themselves are only distantly related, are stepwise interconnected by others which are more closely related. Thus, the significance of the distant relationship between OkMV and CoYMV is emphasized by their mutual relationship with DeYMV. EMV is only distantly related to ScrMV and OYMV, but these viruses are interconnected via APLV, BMV, and DMV. ScrMV links the original APLV subgroup not only to TYMV (Bercks *et al.*, 1971), but also to WCuMV. Actually, ScrMV and OYMV seem to be more closely related to WCuMV than to EMV. The position of TYMV in our tests is rather isolated. Surprisingly, only a few antisera indicated a weak relationship between TYMV and WCuMV. MacLeod and Markham (1963) and Brunt *et al.* (1965), who apparently used the same antiserum to WCuMV, found higher SDIs than we did. Possibly by chance they used a very cross-reactive antiserum, or the serological properties of their isolate of TYMV differed from those of ours. In any case, it is not unreasonable to expect that in the future viruses will be found that will bridge the gap between TYMV and the other viruses of the group. By using a great number of antisera we found many cross reactions between members of the two subgroups which had not been described before (e.g., of TYMV antisera with DMV, OYMV, and CoYMV and of WCuMV antisera with DMV and OYMV).

Figure 1 illustrates also another feature which may be relevant to serological differentiations between viruses. Frequently, heterologous antisera are better suited for the differentiation of closely related viruses than homologous ones. Thus APLV and EMV are better differentiated with most antisera to ScrMV, OYMV, and BMV than with antisera to EMV; BMV and DMV are better differentiated with many antisera to APLV and ScrMV than with their homologous antisera; ScrMV and OYMV are better differentiated with many antisera to BMV and TYMV than with those to ScrMV (see also Table 1).

The serological observations depicted in Fig. 1 make it appear very doubtful whether a subdivision of the tymovirus group in a APLV and a TYMV subgroup is still justified. Recent data on the base compositions of the RNAs (Fig. 2), too, do not support

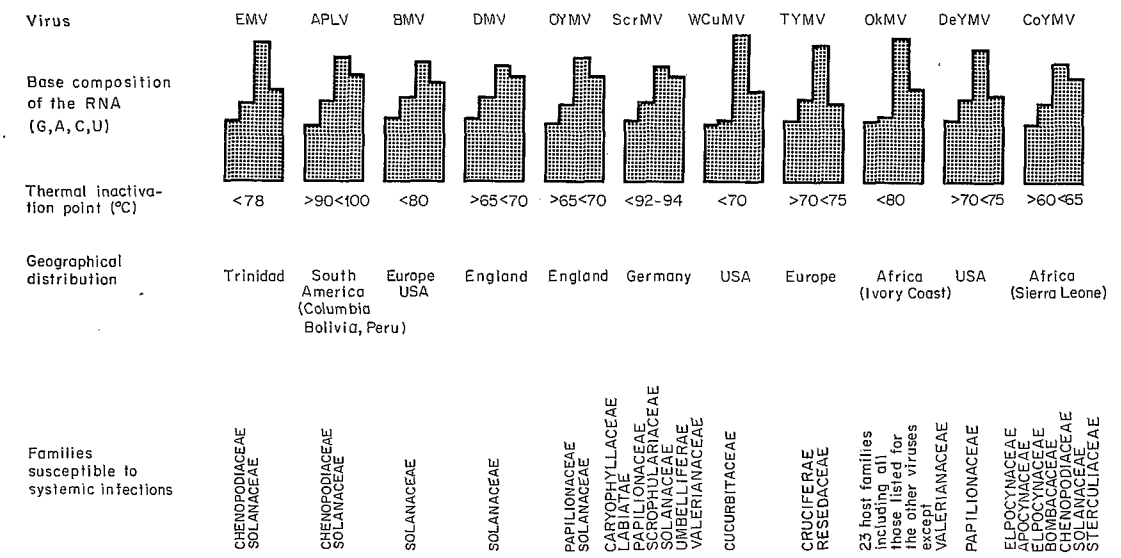

FIG. 2. Correlation of the serological order in Fig. 1 with other distinguishing data on tymoviruses. The data are from Bercks *et al.* (1971), Brunt (1970), Brunt *et al.* (1965), Gibbs and Harrison (1969), Gibbs *et al.* (1966), Givord and Hirth (1973), Hein (1959), Matthews (1970), Moline and Fries (1972), Paul (1971), Scott and Moore (1972), van Regenmortel (1972), Walters and Scott (1972).

this subdivision. On the basis of its high cytidylic acid content, EMV (Bercks *et al.*, 1971) should belong to the TYMV subgroup whereas CoYMV (Brunt, 1970) should belone to the APLV subgroup. However, very different base compositions have been published by Brunt (1970) and Gibbs *et al.* (1966) for the RNA of CoYMV. With TYMV strains, differences in base composition in the RNA are known (Symons *et al.*, 1963).

Attempts have also been made to correlate the proposed classification order in Fig. 1 with other distinctive properties (Fig. 2). There is no correlation with the thermal inactivation points and with the geographical distribution. It is interesting, for instance, that the two viruses found in the western part of Africa (OkMV, CoYMV) are serologically linked by DeYMV which has been isolated in the USA. However, considering the world-wide traffic of agricultural commodities these days it may often be rather fortuitous where a virus is first detected. Thus BMV, which originally had been reported only from Europe (Paul, 1971), has recently been found in the USA (Moline and Fries, 1972). There are also few correlations between the serological relationships and the reported host ranges (Fig. 2). The breadth of the reported host ranges, however, may be rather dependent on the effort of the individual investigator on this matter. Host range studies are currently being extended by one of us (L. G.). In view of problems of evolution it will be interesting to look for further differentiating properties which reflect other genetic information than the coat protein.

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REFERENCES

Allen, W. R. (1968). Tomato bushy stunt virus from *Prunus avium*. II. Serological typing and the characterization of antibody types and activities. *Can. J. Bot.* **46**, 229–233.

Bercks, R. (1963). Untersuchungen über individuelle Unterschiede von Antiseren gegen Kartoffel-X-Virus bei Reaktionen mit verwandten Viren. *Phytopathol. Z.* **47**, 301–313.

Bercks, R., and Querfurth, G. (1971). The use of the latex test for the detection of distant serological relationships among plant viruses. *J. Gen. Virol.* **12**, 25–32.

Bercks, R., Huth, W., Koenig, R., Lesemann, D., Paul, H. L., and Querfurth, G. (1971). Scrophularia mottle virus: Charakterisierung und Vergleich mit anderen Viren der turnip yellow mosaic virus Gruppe. *Phytopathol. Z.* **71**, 341–356.

Brunt, A. A. (1970) Cacao yellow mosaic virus. C.M.I./A.A.B. descriptions of plant viruses. No. 11.

Brunt, A. A., Kenten, R. H., Gibbs, A. J., and Nixon, H. L. (1965). Further studies on cocoa yellow mosaic virus. *J. Gen. Microbiol.* **38**, 81–90.

Dunn, D. B., and Hitchborn, J. H. (1965). The use of bentonite in the purification of plant viruses. *Virology* **25**, 171–192.

Gibbs, A. J. (1969). Plant virus classification. *Advan. Virus Res.* **14**, 263–328.

Gibbs, A. J., and Harrison, B. D. (1969). Eggplant mosaic virus, and its relationship to Andean potato latent virus. *Ann. Appl. Biol.* **64**, 225–231.

Gibbs, A. J., Hecht-Poinar, E., Woods, R. D., and McKee, R. K. (1966). Some properties of three related viruses: Andean potato latent, Dulcamara mottle, and Ononis yellow mosaic. *J. Gen Microbiol.* **44**, 177–193.

Givord, L., and Hirth, L. (1973). Identification, purification and some properties of a mosaic virus of okra (Hibiscus esculentus). *Ann. Appl. Biol.* **74**, 359–310.

Harrison, B. D., Finch, J. T., Gibbs, A. J., Hollings, M., Shepherd, R. J., Valenta, V., and Wetter, C. (1971). Sixteen groups of plant viruses. *Virology* **45**, 356–363.

Hein, A. (1959). Beiträge zur Kenntnis der Viruskrankheiten an Unkräutern. V. Ein Virus von Scrophularia nodosa L. *Phytopathol. Z.* **36**, 290–301.

Koenig, R., and Bercks, R. (1968). Änderungen im heterologen Reaktionsvermögen von Antiseren gegen Vertreter der potato virus X-Gruppe im Laufe des Immunisierungsprozesses. *Phytopathol. Z.* **61**, 382–398.

MacLeod, R., and Markham, R. (1963). Experimental evidence of a relationship between turnip yellow mosaic virus and wild cucumber mosaic virus. *Virology* **19**, 190–197.

Matthews, R. E. F. (1970). Turnip yellow mosaic

virus. C. M. I./A.A.B. descriptions of plant viruses. No. 2.

MOLINE, H. E., and FRIES, R. E. (1972). A virus isolated from *Physalis* sp. in Iowa serologically related to belladonna mottle virus. *Phytopathology* **62,** 1109 (Abstract).

PAUL, H. L. (1971). Belladonna mottle virus. C.M.I./A.A.B. descriptions of plant viruses. No. 52.

SCOTT, H. A., and MOORE, B. J. (1972). A comparison of certain properties of desmodium yellow mottle and turnip yellow mosaic viruses. *Virology* **50,** 613–614.

SYMONS, R. H., REES, M. W., SHORT, M. N., and MARKHAM, R. (1963). Relationships between the ribonucleic acid and protein of some plant viruses. *J. Mol. Biol.* **6,** 1–15.

TREMAINE, J. H., and WRIGHT, N. S. (1967). Cross reactive antibodies in antisera to two strains of southern bean mosaic virus. *Virology* **31,** 481–488.

VAN REGENMORTEL, M. H. V. (1972). Wild cucumber mosaic virus. C.M.I./A.A.B. descriptions of plant viruses. No. 105.

VAN REGENMORTEL, M. H. V., and VON WECHMAR, M. B. (1970). A reexamination of the serological relationship between tobacco mosaic virus and cucumber virus 4. *Virology* **41,** 330–338.

WALTERS, H. J., and SCOTT, H. A. (1972). Host range and some properties of Desmodium yellow mottle virus. *Phytopathology* **62,** 125–128.