

SEROTYPES OF RED CLOVER NECROTIC MOSAIC VIRUS II. TYPING OF 34 ISOLATES

M. MUSIL, O. LEŠKOVÁ, J. GALLO

Institute of Virology, Slovak Academy of Sciences, 817 03 Bratislava, Czechoslovakia

Summary. — Examination of 34 isolates of red clover necrotic mosaic virus (RCNMV) from Czechoslovakia in agar gel double diffusion precipitation tests and immunoelectrophoresis in agarose gel showed that 16 isolates belonged to serotype B, 6 isolates to serotype C and 4 isolates to serotype A; 3, 1 and 4 isolates represented mixtures of serotypes A + B, A + C and B + C, respectively. The distribution of the individual RCNMV serotypes in Czechoslovakia is not bound to definite geographic areas; two serotypes were even involved in mixed infections of single plants. Isolates of a given serotype were serologically and electrophoretically identical with the respective type isolate, i.e. there was a correlation between antigenic properties and electrophoretic mobility.

Key words: red clover necrotic mosaic virus; serotypes; immunodiffusion; immunoelectrophoresis

Introduction

Previously (Musil and Gallo, 1982) we characterized three distinct serological types of red clover necrotic mosaic virus (RCNMV). The results of a serological analysis of 34 isolates of this virus from Czechoslovakia are presented below.

Materials and Methods

Serological typing of 34 RCNMV isolates from different localities in Czechoslovakia (Table 1) was done by agar gel double diffusion precipitation (further on immunodiffusion — ID) tests and immunoelectrophoresis as described (Musil and Gallo, 1982). The isolates were propagated in bean (*Phaseolus vulgaris* L. cv. Saxa) plants and purified as described (Musil and Gallo, 1982) and the purified preparations were used in the tests. Antisera against reference type isolates, namely TpM34 (serotype A), TpM48 (serotype B) and 63/70 (serotype C) were used for typing along with the homologous antigens for comparison. In ID tests we used either non-absorbed antisera (for determination of the type and of the degree of serological relationship between isolates), or antisera absorbed with heterologous antigens, or antisera diluted 1 : 50 (TpM34 and TpM48) or 1 : 150 (63/70) which gave a specific reaction only with antigens of the respective serotype (see Musil and Gallo, 1982).

Table 1. RCMV isolates tested

Iso- late No.	Locality (district)	Serotype		
		A	B	C
1	Andělská Hora (Karlovy Vary)		+	+
2	Bor u Tachova (Tachov)		+	
3	Kostelec (Tachov)		+	+
4	Velký Malahov (Domažlice)			+
5	Březí (Domažlice)	+	+	
6	Annín (Klatovy)			+
7	Rožmitál p. Třemš. (Příbram)		+	
8	Želnavá (Prachatice)	+		
9	Klení (Český Krumlov)		+	
10	Obrataň (Pelhřimov)			+
11	Horní Lhota (Benešov)	+		+
12	Zákupy (Česká Lípa)	+		
13	Březina (Mladá Boleslav)		+	
14	Žlábek (Semily)		+	+
15	Dršťekryje (Jičín)	+		
16	Bukvice (Jičín)		+	
17	Bartoušov (Jičín)		+	
18	Hlušičky (Hradec Králové)		+	
19	Sezemice (Pardubice)		+	
20	Boršice (Uherské Hradiště)	+		
21	Horní Němčí (Uherské Hradiště)		+	
22	Skotnice (Nový Jičín)		+	
23	Kozmice (Opava)		+	
24	Vojkovice (Frýdek—Místek)			+
25	Hnojník (Frýdek—Místek)		+	
26	Mosty u Jablunk. (Frýdek—Místek)		+	+
27	Terchová (Žilina)			+
28	Oravská Lesná (Dolný Kubín)			+
29	Krušetnica (Dolný Kubín)	+	+	
30	Babín (Dolný Kubín)	+	+	
31	Oravský Podzámok (Dolný Kubín)		+	
32	Ždiar (Poprad)		+	
33	Nižná Polianka (Bardejov)		+	
34	Lutila (Žiar n. Hronom)		+	

Results and Discussion

In the ID tests we confirmed that isolates of a given serotype react only with absorbed or diluted antiserum against the same serotype by a clear-cut single precipitation line coalescing with the precipitation line formed against homologous (type) antigen (Figs. 1a, b, c). Isolates of a given serotype formed with non-absorbed antisera against this serotype precipitation lines that coalesced with the precipitation line formed against the type antigen of the given serotype, as distinct from a spur formed against antigens of another serotype. The precipitation lines formed by isolates of a given serotype with the respective antiserum were stronger than the precipitation lines formed by antigens of another serotype. In some isolates, absorbed or diluted antisera revealed the presence of 2 serotypes. These isolates represent mixtures of

populations of virions belonging to two distinct serotypes (Figs 2a, b, c). This conclusion was confirmed experimentally like in the case of isolate TpM50 (Musil, 1969).

Isolates of a given serotype showed the same electrophoretic mobility as the respective type isolate. This means that all the teste isolates of serotypes A and C moved in veronal buffer like the type isolates TpM34 and

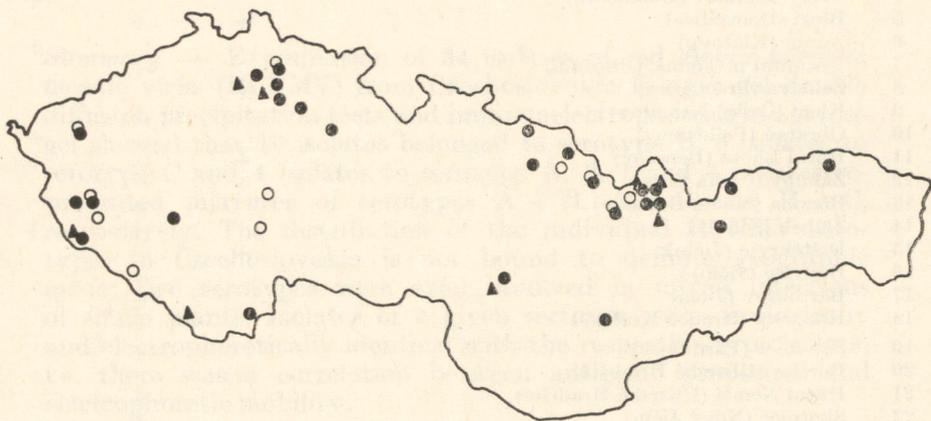


Fig. 4.

Distribution of RCNMV serotypes (▲ - A, ● - B, ○ - C) in Czechoslovakia

63/70, respectively, namely more rapidly than isolates of serotype B (including the type isolate TpM48; Fig. 3). Also in Tris buffer all isolates of a given serotype moved similarly to the type isolate, i.e. isolates of serotype A moved from the cathode to the anode more quickly than isolates of serotypes B and C. On immunoelectrophoresis in either environment, the isolates representing a mixture of two serotypes were separated and specific precipitation lines formed at a distance corresponding to the respective serotypes (see Fig. 3).

On immunoelectrophoresis of variously concentrated purified virus suspensions, the mobility depending on the antigenic properties (pertinence to a given serotype) remained unchanged. The results obtained on the 34 isolate confirmed that immunoelectrophoresis can be used for typing (differentiation) of RCNMV isolates (see Musil and Gallo, 1982).

The ID tests showed that of the 34 RCNMV isolates studied 16 belonged to serotype B, 6 to serotype C and 4 to serotype A; 3, 1 and 4 isolates represented mixtures of serotypes A + B, A + C and B + C, respectively (Table 1). It appears that the distribution of the individual isolates in Czechoslovakia is not bound to certain geographic areas (Fig. 4). An examination of a greater number of isolates from individual localities would be necessary to make

possible a definite evaluation of the present findings concerning the difference in incidence of serotypes among isoates in the known localities and concerning the distribution of the serotypes in Czechoslovakia.

Acknowledgement. We thank Miss M. Augustínová and Mrs. G. Havlová for their technical assistance.

References

- Musil, M. (1969): Serological properties of certain isolates of red clover necrotic mosaic virus. *Acta virol.* **13**, 226—234.
- Musil, M. & Gallo, J., (1982): Serotypes of red clover necrotic mosaic virus I. Characterization of three serotypes. *Acta virol.* **26**, 497—501.

Explanation of Figures (Plates LII—LIII):

- Fig. 1.* ID tests of RCNMV isolates with diluted antisera against serotypes A, B and C. Central wells — antisera: a — TpM34 (dil. 1 : 50); b — TpM48 (dil. 1 : 50); c — 63/70 (dil. 1 : 150). Peripheral wells — antigens: A = TpM34; B = TpM48; C = 63/70; the other figures correspond to the numbering of isolates in Table 1.
- Fig. 2.* ID tests of RCNMV isolates with undiluted and non-absorbed antisera against serotypes A, B and C. Arrangement and designation of wells as in Fig. 1.
- Fig. 3.* Immunoelectrophoregrams of RCNMV isolates. Antigens designated as in Fig. 1. Antisera: a — TpM34, b — TpM48, c — 63/70. Agarose gel buffered with 0.04 mol/l sodium barbiturate — HCl buffer at pH 8.6.