SEROLOGICAL TYPING OF RED CLOVER NECROTIC MOSAIC VIRUS ISOLATES BY ENZYME-LINKED IMMUNOSORBENT ASSAY

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Summary. – Enzyme-linked immunosorbent assay (ELISA) enabled a more precise serological typing of isolates of red clover necrotic mosaic virus (RCNMV) originating from Slovak and Czech Republics, Poland, Sweden and Great Britain. We found 5 isolates of serotype A, 14 isolates of serotype B, and 6 isolates of serotype C. Some isolates represented mixtures of serotypes, namely A+B (1 isolate), A+C (1), B+C (7), and A+B+C (5). ELISA was found to be a more suitable method of serotype identification of RCNMV isolates than the double diffusion agar gel test for its higher sensitivity and greater selectivity.

Key words: red clover necrotic mosaic virus; serotypes; ELISA

Introduction

The serological analysis enables not only a detection of new isolates of RCNMV (dianthovirus group), but due to antigenic or serological differences among them also their classification to individual serotypes (serotyping). From so far described RCNMV serotypes (Musil, 1969; Musil and Gallo, 1982; Rao *et al.*, 1987) three have been found on the territory of Slovak and Czech Republics, occuring either individually or in mixtures. A selective reaction of individual RCNMV serotypes with properly diluted serotype-specific IgG in ELISA (Hiruki *et al.*, 1984; Musil and Gallo, 1990) pre-determines this method for their serotyping.

In the present study the serotype of available RCNMV isolates originating from Slovak and Czech Republics, Poland, Sweden and Great Britain was verified by means of ELISA.

Materials and Methods

RCNMV isolates. We determined the serotype of 36 isolates from Slovak and Czech Republics (Musil *et al.*, 1982), 2 from Great Britain, 1 from Poland, and 1 from Sweden (Musil *et al.*, 1983). Purified suspensions of individual isolates (nucleoprotein concentration 1 mg/ml), diluted 1:1000 were used for ELISA.

ELISA. The double antibody sandwich ELISA (Clark and Adams, 1977) was used. IgG fractions were prepared from rabbit antisera against isolates TpM_{34} (serotype A), TpM_{48} (serotype B) and No. 6 (serotype C). Conjugates with alkaline phosphatase and other reagents were prepared and used according to Musil and Gallo (1990).

Results and Discussion

The serotype of purified suspensions of individual RCNMV isolates, determined previously by immunodiffusion (ID) test (Musil *et al.*, 1982, 1983) was verified by ELISA with the following results (Table 1).

The serotype A of all four isolates (ID test) was confirmed also by ELISA. From 17 isolates of serotype B (ID test) only 13 were confirmed, and from 8 isolates of serotype C (ID test) only 6 were confirmed.

From 4 isolates of mixed serotype A+B (ID test) only 1 was confirmed and 3 were found of serotype A+B+C by ELISA. The mixed serotype A+C (ID test) was confirmed also by ELISA.

On the other hand, the serotype B+C was found by ID test in 4 isolates, but by ELISA in 7 isolates. The mixed serotype A+B+C was found altogether in 5 isolates, but just by use of ELISA. In ID test, these isolates were of serotype B (No. 9), or C (No. 24), or A+B (No. 5, 29, 30).

These results show that using ELISA we were able to demonstrate in 4 and 2 isolates, respectively, instead of single serotype (found by ID test) a mixture of two sero-

Table 1. Reaction of RCNMV isolates with serotype-specific IgG in ELISA

RCNMV isolates[a]	A_{405} with IgG		
	anti-TpM_{34}	anti-TpM48	anti-No.6
Serotype A			
TpM_{34}	1.34	0.04	0.05
No.8, 12, 15, 20	1.10 – 1.75	0.03 – 0.05	0.04 – 0.05
Serotype B			
TpM_{48}	0.04	1.30	0.02
No.2, 7, 13, 16, 17, 18, 19, 21, 23, 31, 33, 34, 1307 (GB)	0.04 – 0.06	0.95 – 1.35	0.02 – 0.04
Serotype C			
No.6	0.04	0.03	1.45
No.4, 10, 27, 28, S (GB), SW 63/70 (S), PL (Poland)	0.04 – 0.05	0.04 – 0.05	1.20 – 1.60
Serotype A+B			
No.25	0.81	1.10	0.06
Serotype B+C			
No.1, 3, 14, 22,26, 27, 32	0.04 – 0.05	0.15 – 1.30	0.68 – 1.70
Serotype A+C			
No.11	0.90	0.04	1.54
Serotype A+B+C			
No.5, 9, 24, 29, 30	0.28 – 1.32	0.28 – 1.10	0.20 – 1.60

[a] Isolates are designated according to Musil *et al.* (1982).

types, and in 3 isolates instead of a mixture of two serotypes (found by ID test) a mixture of three serotypes. In the case of mixed serotypes determined by ELISA, one serotype was usually in higher concentration than another one or two others. E.g. A_{405} of the isolate No. 11 (serotype A+C) with anti-TpM_{34} and anti-No. 6 IgG had values of 0.9 and 1.5, respectively, and A_{405} of the isolate No. 24 (serotype A+B+C) with anti-TpM_{34}, anti-TpM_{48} and anti-No. 6 IgG amounted to 0.9, 0.4 and 1.6, respectively. We assume that the occurence of some serotypes in purified suspensions of some RCNMV isolates in a lower concentration was the cause of their detection by ELISA, but not by ID test.

It can be concluded that ELISA, as compared to the ID test is a more suitable method for serotyping of RCNMV isolates, because it enables to detect also minor serotypes present in mixtures of serotypes in virus isolates. The ID test employed previously (Musil *et al.*, 1982) did not possess that high sensitivity and selectivity. Nevertheless, all the available data indicate that some plants in fields are evidently infected by several different serotypes of RCNMV.

References

Clark, M.F., and Adams, A.M. (1977): Characteristics of the microplate method of enzyme-linked immunosorbent assays. *J. gen. Virol.* **47**, 509–512.

Hiruki, C., Rao, L.N., Furuya, Y., and Figueiredo, G. (1984): Serological studies of Dianthoviruses using monoclonal and polyclonal antibodies. *J. gen. Virol.* **65**, 2273–2275.

Musil, M. (1969): Serological properties of certain isolates of red clover necrotic mosaic virus. *Acta virol.* **13**, 226–234.

Musil, M., and Gallo, J. (1982): Serotypes of red clover necrotic mosaic virus. I. Characterization of three serotypes. *Acta virol.* **26**, 497–501.

Musil, M., and Gallo, J. (1990): Determination of serotypes of red clover necrotic mosaic virus by enzyme-linked immunosorbent assay. *Acta virol.* **34**, 410–422.

Musil, M., Lešková, O., and Gallo, J. (1982): Serotypes of red clover necrotic mosaic virus. II. Typing of 34 isolates. *Acta virol.* **26**, 502–505.

Musil, M., Micinski, B., Lešková, O., and Pruszynska, M. (1983): Serological properties of some isolates of red clover necrotic mosaic virus from Czechoslovakia, Poland and Sweden. *Zeszyty Problemowe Post. nauk Roln.* **291**, 227–232 (in Polish).

Rao, A.L.N., Lakshman, D.K., Ohki, S.T., and Hiruki, C. (1987): Identification of a new serotype and antigenic relationships among six strains of red clover necrotic mosaic virus. *Phytopatology* 77, 995–999.