

ISOELECTRIC POINTS OF RED CLOVER NECROTIC MOSAIC VIRUS SEROTYPES

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Summary. — Isoelectric focusing in agarose gel was used to determine the isoelectric points of red clover necrotic mosaic virus serotypes. The isoelectric points of serotypes A, B and C were in the pH ranges from 5.03—5.06, from 4.82—4.84 and from 4.60—4.62, respectively.

Key words: red clover necrotic mosaic virus; serotypes: isoelectric focusing; isoelectric points

The basic properties of three red clover necrotic mosaic virus (RCNMV) serotypes and the possibilities of their differentiation by serological methods were described previously (Musil and Gallo, 1982; Musil *et al.*, 1982; Gallo and Musil, 1984). To our knowledge the isoelectric points of RCNMV have not yet been reported (see Hollings and Stone, 1977). We used isoelectric focusing to determine the isoelectric points of three RCNMV serotypes.

We used purified suspensions (Musil and Gallo, 1982) of three RCNMV serotypes (serotype A — isolate TpM34; serotype B — isolate TpM48; and serotype C — isolate No. 6 (see Musil *et al.*, 1982). One per cent agarose (Agarose C, Pharmacia, Uppsala) containing 12% sorbitol (Difco-Bacto) and 6.3% Pharmalyte 3-10 (Pharmacia) was poured on a glass slide in a layer 2 mm thick and measuring 5 × 8 cm. Wells were cut into the gel and filled with 10 µl of purified suspensions of the individual serotypes (singly or in various combinations). Electrophoresis lasted for 18 hr at a constant current of 20 V. Under these conditions the focusing was complete. One, 0.5 or 0.02 mol/l NaOH was used as the cathode solution and 0.5, 0.25 or 0.01 mol/l H₂SO₄ as anode solution. After the end of isoelectric focusing the gels were fixed for 2 hr with a solution of 15% trichloroacetic acid and 4.5% sulphosalicylic acid, followed by washing for 6—18 hr with a 10 : 45 : 45 mixture of acetic acid, methanol and distilled water. The gels were stained for 4 hr in 0.04% SERVA Blau R (Serva, Heidelberg) dissolved in the washing solution and then washed in the washing solution until the gel background became clear. The destained gels were maintained in 7% acetic acid. The pH gradient in the gels after focusing was determined as follows: before fixation a 1-cm wide strip of agarose was cut from the gel along its whole length. This 5 cm long strip was cut into twenty 0.25 cm wide fractions. Each of the

latter was immersed for 24 hr into 1 ml of twice distilled water and the pH of the solution was measured.

The formation of the pH gradient in agarose gels was considerably influenced by the selection of electrode solutions. With 1 mol/l NaOH and 0.5 mol/l H₂SO₄ the pH gradient in the well was from 2.92 to 6.82, with

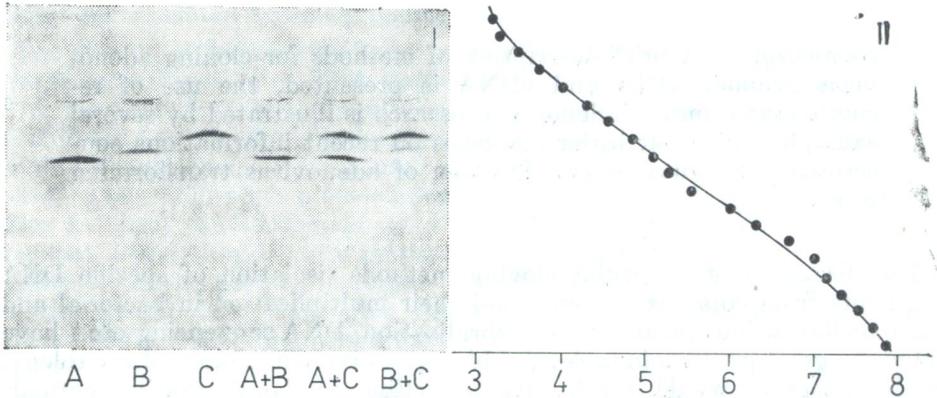


Fig. 1.

Isoelectric focusing of the three RCNMV serotypes

I — A, B, and C refer to serotypes A, B and C, respectively. II—Abscissa: pH; ordinate: gel length.

0.5 mol/l NaOH and 0.25 mol/l H₂SO₄ it was from 2.96 to 7.26 and with 0.02 mol/l NaOH and 0.01 mol/l H₂SO₄ it was from 3.28 to 7.89. The latter combination was used in all further experiments, because the pH gradient was linear (Fig. 1-II). The isolates, representatives of three RCNMV serotypes, had different isoelectric points, which was confirmed by testing of serotype mixtures (Fig. 1-I). The isoelectric points found were pH 5.03—5.06 for serotype A (isolate TpM34), pH 4.82—4.83 for serotype B (isolate TpM48) and 4.60—4.62 for serotype C (isolate No. 6).

The results obtained could be used as a marker in the differentiation of RCNMV serotypes.

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