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DETERMINATION OF SEROLOGICAL DIFFERENTIATION INDEX VALUES OF TURNIP YELLOW MOSAIC VIRUS (TYMV) STRAINS BY MEANS OF ROCKET IMMUNOELECTROPHORESIS

MLADEN KRAJAČIĆ, ĐORĐE MAMULA and NIKOLA JURETIĆ

(Department of Botany, Faculty of Science, University of Zagreb)

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An attempt was made to apply rocket immunoelectrophoresis (RIE) technique for the determination of serological differentiation index (SDI) values between three British strains of turnip yellow mosaic virus (TYMV) based on the height of »rocket« precipitates. The experiments were conducted in 1% agar gel containing antiserum to Edinburgh (E) strain of the virus, the holes being charged separately with purified E and two other strains. Following experiments with E strain in several subsequent twofold dilutions, a calibration graph showing linear proportionality was drawn in a coordinate system determined by steps of virus dilutions and logarithm of »rockets« heights. By interpolation procedure SDIs of two heterologous strains against E were found fairly comparable with SDI values obtained by double radial immunodiffusion technique.

Introduction

In the determination of serological differentiation index (SDI) showing degree of serological relatedness of two viruses, the viruses are compared on the basis of antiserum (or virus) titre in the homologous and heterologous reactions (Van Regenmortel and Von Wechmar 1970; Hamilton et al. 1981). This has been performed in precipitin tests, either in liquid or in gel medium, in the tube or on the slide, the

number of twofold dilution steps separating the usually higher homologous titre and the heterologous one giving SDI value (Van Regenmortel 1982). The difference between the equivalent homologous and heterologous reaction should give the same value.

The above mentioned techniques are generally accepted in virus research, however the titre of the equivalent reactions cannot always be determined quite precisely. Jaeglé and Van Regenmortel (1985) and Krajačić et al. (1990) demonstrated more precise determining SDIs using ELISA and single radial immunodiffusion (SRID) techniques, respectively. This paper presents an attempt to use rocket (quantitative) immunoelectrophoresis (RIE) technique (Laurell 1966) for measuring SDI. RIE has been successfully used for antigen quantitation in biochemistry, medicine, botany and plant virology (Havránek 1978a, Reichenbächer et al. 1979, Tobias et al. 1979).

Materials and Methods

Three British strains of turnip yellow mosaic virus (TYMV), »Edinburgh« (E, type), »Northumberland« (N) and S (Matthews and Ralph 1966, Mamula 1985) were investigated using antiserum homologous to the E strain which had a titre of 1/512. The viruses were partially purified from systemically infected turnip (*Brassica rapa* L. cultivar. *rapa*) leaves by clarification with chloroform (2.5 : 1, v : v) and two cycles of differential centrifugation (90,000 g/90 min and 4,000 g/15 min).

A glass plate 5 x 5 cm was layered with 5 ml of phosphate buffered agar gel (0.066 M pH 7.0, 1% Noble agar, 0.1% sodium azide) after mixing 4.75 ml of the agar solution with 0.25 ml of the antiserum at 50°C. Virus samples were applied to the antigenic holes and electrophoresis was performed overnight at 20 V/cm of the gel. The twofold buffer molarity in the apparatus vessels ensured better electrophoretic movement of virus particles. The height of the »rockets« vas measured in millimetres on twice enlarged photographs starting from the middle of the hole towards the anodic end of the »rocket«.

Results and Discussion

The quantitative immunoelectrophoresis is represented by the simultaneous electrophoretic migration and immunoprecipitation of antigens in antibody-containing gel. The developed immunoprecipitin pattern depends on the relationship between the concentrations of the reactants in the system (Weeke 1973) the quantitation of antigens being performed through the height of the »rockets«. As Havránek (1978b) points out, there is no linear relationship between the relative virus concentration and the »rocket« height but the logarithmic expression of the data significantly linearizes the whole dilution curve.

Our attempt is based upon the presumption that equally concentrated virus strains, like different concentrations of the same virus isolate, produce quantitatively different patterns. According to this assumption the »rocket« height produced by related viruses should be proportional to the degree of their antigenic homology.

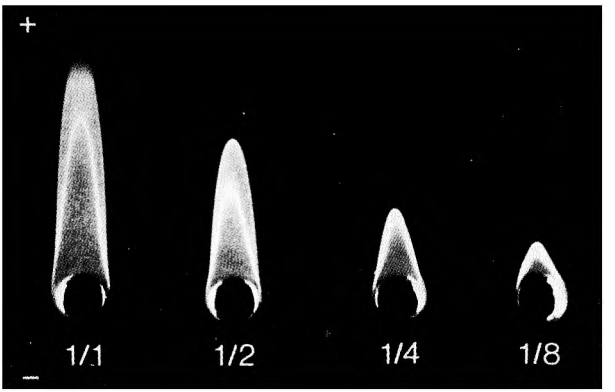

Fig. 1. Precipitin patterns (»rockets«) of twofold dilutions of TYMV-E strain obtained by rocket (quantitative) immunoelectrophoresis (RIE) in gel containing homologous antiserum. 1/1 = 1 mg/ml. Twice enlarged.

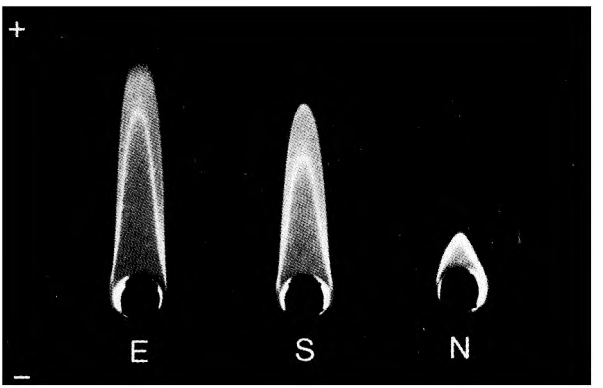

Fig. 2. Precipitin patterns of equally concentrated (1 mg/ml) TYMV strains E, N and S obtained by RIE in gel containing antiserum to E strain. Twice enlarged. (conf. Fig. 1)

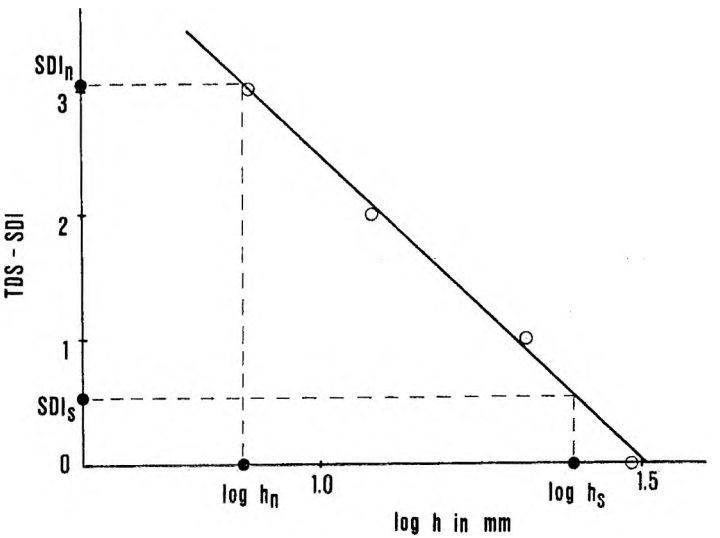

Fig. 3. Linear relationship between twofold dilution step (TDS) of TYMV-E strain (ordinate) and logarithm of »rocket« height (log h; abscisse) in RIE experiments. SDI = serological differentiation index; log h_n, log h_s = log »rocket« height of heterologous TYMV strains N and S; SDI_n, SDI_s = SDI of strains N and S. (conf. Fig. 1)

The homologous virus preparation (E) was diluted with the neutral phosphate buffer to obtain a series of dilutions comprising the following dilution ratios: 1/1 (the starting undiluted sample 1 mg/ml), 1/2, 1/4 and 1/8. These were applied into the holes in the gel and subjected to the electric field. Figure 1 demonstrates the patterns obtained in this experiment. When steps of twofold dilutions instead of relative concentration were plotted (ordinate) against logarithm of the »rockets« height (abscisse), the graph revealed linear relationship between the two parameters (Fig. 3). Following »rocket« height of the heterologous viruses N and S (Fig. 2) it was possible to read out on the ordinate (Fig. 3) the respective SDI values which were 3 (SDI_n) and 0.5 (SDI_s). In comparison with these results the SDI of 3 (N) and 1 (S) were obtained in double radial immunodiffusion (DRID) experiments, and also those of 3.2 and 0.9, respectively, in SRID experiments (Mamula 1985, Krajačić et al. 1990).

The results of our experiments demonstrated, to some extent, a consistency with the aforestated assumption. However, although the RIE like ELISA and SRID enables calculating SDIs in fraction number, we find it in this performance somewhat less accurate than the two and even DRID techniques. In comparison with SRID and DRID, the RIE involves an additional experimental factor, i.e. the influence of the electric current, which often acts differently upon different viruses and strains of the same virus. Besides, a clear influence of the degree of virus particles aggregation on the results of RIE has been demonstrated (Reichenbächer et al. 1981). However, these disadvantages can be overcome in RIE performance with reverse role of the reactants (virus sample in the gel and antiserum in the holes), so that homologous and heterologous antibodies are allowed to migrate electrophoretically from the starting wells. Besides, in the latter type of experiment serological relationships of elongated viruses could be examined, as they are unable to migrate through the gel.

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SAŽETAK

ODREĐIVANJE INDEKSA SEROLOŠKE RAZLIKE MEĐU SOJEVIMA VIRUSA ŽUTOG MOZAIKA POSTRNE REPE (TYMV) TEHNIKOM »RAKETNE« IMUNOELEKTROFOREZE

Mladen Krajačić, Đorđe Mamula i Nikola Juretić

(Botanički zavod Prirodoslovno-matematičkog fakulteta Sveučilišta u Zagrebu)

Indeks serološke razlike (SDI), jedinica koja pokazuje stupanj serološke razlike između virusa i njihovih sojeva, može se manje ili više precizno odrediti pomoću nekih standardnih seroloških tehnika. U toku ovog rada pokušali smo taj indeks izmjeriti »raketnom« (kvantitativnom) imunoelektroforezom (RIE) na osnovi veličine (visine) imunoprecipitata oblika rakete. U pokusima smo koristili purificirane sojeve E (tipični soj), N i S virusa žutog mozaika postrne repe (turnip yellow mosaic virus; TYMV) koji potječu iz V. Britanije i imuni serum protiv soja E koji je bio pomiješan s 1% agarskim gelom. Na osnovi pokusa sa sojem E u nekoliko uzastopnih dvostrukih razrjeđenja (1 do 1/8 mg/ml) našli smo linearnu ovisnost između stupnja razrjeđenja virusa i logaritma visine »raketa«. Interpolacijskim postupkom našli smo da su SDI vrijednosti heterolognih sojeva bile prilično podudarne s onima koje su bile izmjerene tehnikom dvostruke radijalne imunodifuzije.

Dr. Mladen Krajačić
Botanički zavod
Prirodoslovno-matematičkog fakulteta
Sveučilišta u Zagrebu
Maruličev trg 20/II
YU-41000 Zagreb (Jugoslavija)