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A SIMPLE METHOD FOR QUANTITATIVE SEROLOGICAL ASSAY FOR PLANT VIRUSES

The level of virus concentration in plant tissues is one of the most important characteristics of virus synthesis. There are several possibilities to estimate the virus concentration in plants. A rapid indication of the number of rod-shaped virus particles can be obtained by electron microscopy. The relative concentration of infective virions may be determined by infectivity tests using local lesion indicator plants. For the detection of virus antigen, several serological methods are used. Most of them give data about the existence or nonexistence of certain virus antigens in the material analysed. The new, recently elaborated serological methods aim at determining the minutest amounts of virus antigens in plants. There are only few methods giving information about the values of the concentration of the virus antigen in plants. Those are immunoelectrophoresis, serologically specific electron microscopy and enzyme-linked immunosorbent assay (ELISA). The method for a quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies (Laurell, 1966) was the first quantitative serological assay of plant viruses. The determination of the amount of antigen is based upon measuring the height or the area of precipitation. This method is suitable for determining spherical viruses. Modification is necessary for working with rod-shaped viruses. Another method used for characterizing the quantity of plant virus antigens is a serologically specific electron microscopy or immunoelectron microscopy presented by K. S. Derrick (1973) and modified by R. G. Milne and E. Luisoni (1975). That method is based on counting, with the help of an electron microscope, the virus particles with an altered appearance caused by binding of the antigen or by a halo of excessive antibody surrounding the particles. The ELISA test is the latest method elaborated for a quantitative characterization of antigen-antibody reaction. That method was earlier used for determining various human and animal diseases. The first attempts at applying ELISA for the detection of plant viruses were made by A. Voller (Voller et al., 1976) and M. F. Clark and A. N. Adams (1977). The ELISA technique is now applied with some modifications, three of which are used in most cases (Бобкова, Чирков, 1983). The intensity of the antigen-antibody reaction (one of them bound with an enzyme) is assessed quantitatively by measuring the absorption at 405 nm with a spectrophotometer.

All the methods described yield exact data about the relative concentration of the viral antigen in the plants examined. These methods are micromethods, and for getting the desired results one must have special equipment (apparatus, chemicals) and a relatively long time (24—48 hours).

For diagnosing plant viruses, a widely used method is the drop-agglutination test, elaborated in the 1930s by K. S. Chester (1935) and M. S. Dunin and N. N. Popova (Дунин, Попова, 1937). In the agglu-

tination test a drop of the antiserum is mixed with freshly extracted crude plant sap on the microscope plate. A visible precipitate is formed between the antibody and the antigen. The intensity of the precipitation reaction depends on the concentration of the virus antigen in the leaf sap and on the titre of the antiserum used. The intensity of the visible precipitate (the agglutination value) is determined by observing the drops under a stereo-binocular microscope or magnifying glass and recording the amount of the precipitate with crosses (from one to four). The values of those data are of a subjective nature, depending on the experience of the researcher.

In this paper the author recommends a simple quantitative serological assay based on combining the ordinary drop-agglutination test with microcentrifugation in capillary tubes. That method gives the possibility to provide quantitative indices of the amount of the precipitate (the agglutination value) by measuring the height of the precipitate column in the capillary tubes.

Material and procedure

Nicotiana tabacum L. 'Samsun' plants infected with potato X virus (PXV) and *Lycopersicon esculentum* L. 'Peremoga' plants infected with potato M virus (PMV) were used as virus antigen sources. PXV antiserum with

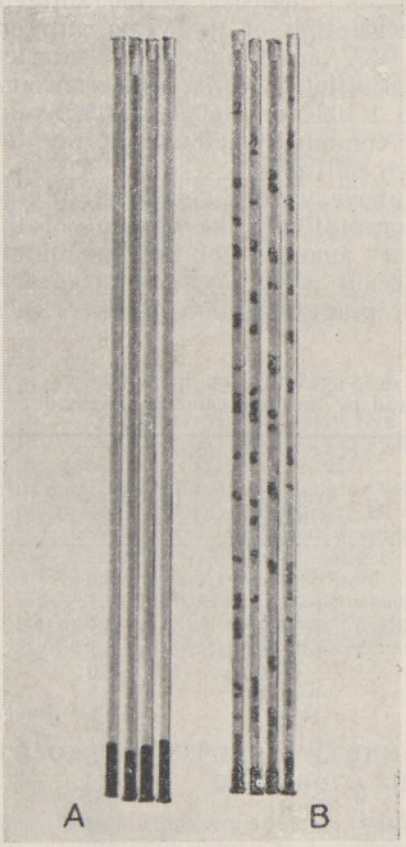

Fig. 1. Capillary tubes filled with the suspension of PXV antiserum and PXV infected tobacco plants sap before (B) and after (A) microcentrifugation.

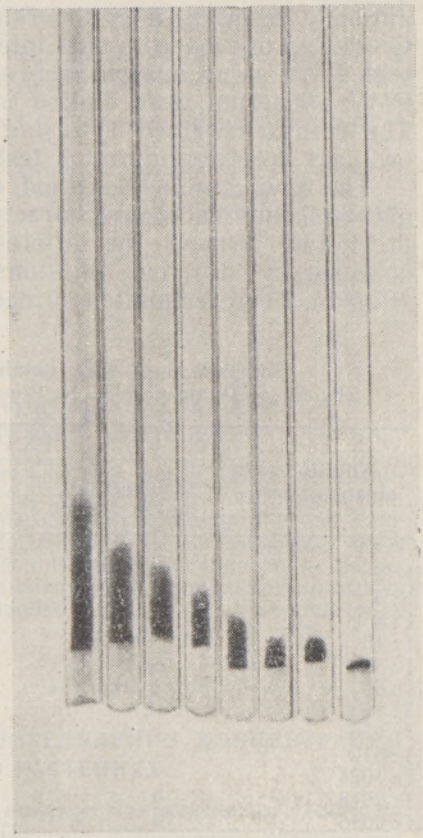

Fig. 2. Precipitate columns formed by microcentrifugation in capillary tubes using different dilutions of PXV antiserum (x2.6).

a precipitation titre of 1:4096 and PMV antiserum with a precipitation titre of 1:2048 were prepared by injecting rabbits intravenously. Microcentrifugation was carried out with a microcentrifuge МЦГ-8 used by clinical laboratories for detecting the hematocrit value of the blood.

Ten drops of the antiserum were mixed with crude plant sap on a plastic slide. The relation of the plant sap/antiserum was ca 1:10. Capillary glass-tubes with the inner diameter of 0.75 mm and the length of 75 mm were filled with antiserum and plant sap suspension immediately after mixing. The capillary tube was dipped into the suspension taking a sample of each drop. One end of the tube was then pressed into a special mixture for closing the tube, while the other end was closed with the forefinger. Two capillary tubes could be filled with ten drops. The tubes were kept at a temperature of 22—24 °C for two hours and then centrifuged for 2 minutes (1200 rev/min). The height of the precipitate column was measured by using the scale of the measuring ocular of the microscope МВС-2 (Ломо) with a magnification of 8×2 (Fig. 1). The background must be white. It might be possible to use calibrated capillary tubes. The time period needed for all the procedures does not exceed three hours.

Results and discussion

The method described above has been used to examine the possibility of getting quantitative data characterizing the intensity of the antigen-antibody reaction, i. e. the relative concentration of the virus antigen. Crude sap of tobacco plants infected with PXV and tomato plants infected with PMV as an antigen were used without dilution. PXV antiserum and PMV antiserum were diluted from 1:2 to 1:1024 or 1:4096, respectively. The results obtained by using the new combined method and by the ordinary drop-agglutination test are shown in Table.

The new combined method described above yielded the possibility to get mathematical data characterizing quantitatively the amount of the precipitate formed; the differences in the amounts of the precipitate obtained by different dilutions of antiserum were quite considerable (Fig. 2). The precipitates formed by the drop-agglutination test were of a

Comparison of data obtained by the drop-agglutination method (by M. S. Dunin and N. N. Popova, 1937) and by the new combined method

Dilution of antiserum	PXV		PMV	
	drop-agglutination method	combined method	drop-agglutination method	combined method
1 : 2	+++	5.5*	++++	8.0
1 : 4	+++	5.0	+++	7.7
1 : 8	+++	4.3	+++	7.0
1 : 16	++(+)	3.5	+++	6.7
1 : 32	++	3.0	++(+)	6.0
1 : 64	+(+)	2.3	++	5.3
1 : 128	+	1.7	+(+)	4.5
1 : 256	(+)	1.0	+(+)	3.7
1 : 512	–	0.5**	+	3.0
1 : 1024	–	0.5	(+)	2.2
1 : 2048	0	0	(+)	1.3
1 : 4096	0	0	–	0.7**

* Data from the scale of the microscope ocular.

** The precipitate columns formed by mixing healthy tobacco sap with PXV antiserum were of the height of 0.5 scale units, while those of healthy tobacco sap + PMV antiserum showed the height of 0.7 scale units.

rather similar value in the first three or four dilutions and were all marked with three crosses at visual examination.

Capillary tubes were earlier used in the latex test by O. F. Marcussen and T. Lundsgaard (1975). I have added microcentrifugation to that principle, making possible not only to characterize the precipitate but also to measure its amount. The new combined method recommended here is not a micromethod; its sensitivity is similar to the sensitivity of the drop-agglutination test. The new combined method can be used in experiments for a quantitative determination and comparison of the antigen concentration of plant viruses if the degree of the antigen concentration is sufficient for a formation of a visible precipitate using the drop-agglutination test. The detection of the antigen-antibody precipitate amount by the new method is quick and simple: it involves a minimum of labour, and the results can be obtained in a short time.

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LIHTNE SEROLOOGILINE MEETOD VIIRUSLIKU ANTIGEENI KONTSENTRATSIOONI MÄÄRAMISEKS TAIMEDES

Artiklis on tutvustatud uut, lihtsat seroloogilist meetodit, mis annab võimaluse kvantitatiivselt iseloomustada reaktsiooni antigeen—antikeha. Meetod põhineb seroloogias üldtuntud tilkaglutinatsioonimeetodi kombineerimisel mikrotsentrifuugimisega. Meetodi kasutamine võimaldab reaktsiooni antigeen—antikeha intensiivsuse iseloomustamisele lisaks anda reaktsioonile ka matemaatiliselt väljendatava kvantitatiivse hinnangu, mille aluseks on antigeeni—antiseerumi suspensiooni mikrotsentrifuugimisel kapillaartorus tekkiva sademesamba kõrguse mõõtmine. Meetodi väljatöötamisel on kasutatud kartuliviirusi X ja M ning neile vastavaid antiseerumeid.

Мильви АГУР

НЕСЛОЖНЫЙ СЕРОЛОГИЧЕСКИЙ МЕТОД ОПРЕДЕЛЕНИЯ КОНЦЕНТРАЦИИ ВИРУСНОГО АНТИГЕНА В РАСТЕНИЯХ

Новый метод охарактеризования реакции антиген-антитело изложен на базе общепринятого капельного метода М. С. Дунина и Н. Н. Поповой, дополненного микроцентрифугированием. Наряду с визуальным определением метод позволяет дать и математическую оценку реакции антиген-антитело на основе данных измерения высоты столбиков осадка, образующихся в капиллярных трубках при центрифугировании. При разработке метода использовали X- и M-вирусы картофеля и соответствующие им антисыворотки.