CP 031 CP031

1275

Tob. Res. 7 (1): 14 - 17, 1981.

SPECIAL ARTICLE :-

RECENT DEVELOPMENTS IN SEROLOGY AND ELECTRON MICROSCOPY FOR THE DIAGNOSIS OF PLANT VIRUS DISEASES*

Dr. D. V. R. REDDY**

Introduction :

One of the important prerequisites to the management of virus diseases is the precise identification of the causal agent. Diagnosis of viral diseases now demands a wide variety of skills which are constantly being improved as a result of rapid technological advances. By the introduction of increasingly sensitive serological methods and transmission electron microscopy, it is now possible to detect viruses that are present in plant tissues in extremely low concentrations. Many early reports on the occurrence of viral diseases have been largely based on visual symptoms and host range. External symptoms of diseases associated with spiroplasma, rickettsiae, mycoplasma-like organisms and even nutrient deficiency symptoms have been confused with viral diseases. In addition, external symptoms produced by viruses can be greatly influenced by such factors as genotype, plant age, environment and strain of virus present.

Various steps involved in the diagnosis of viral diseases (Bos, 1976; Reddy, 1980) are given in Table I. For sap transmissible viruses it is essential to achieve consistant mechanical transmission. This would facilitate diagnosis on the basis of specific symptoms in selected indicator plants. Results of these tests should be verified by serology and electron microscopy. Although the value of serology for the detection of plant viruses has been recognised for many year, serology has not been fully utilized. Serological tests which are relatively simple, rapid and very sensitive are described in this communication. Highly sensitive methods of diagnosis are indispensable for viruses in low concentration and for detecting viruses in their vectors.

Serology :

If virus antisera are available, serological techniques (Ball, 1974; Van Regenmortel, 1978) offer effective means of identification. They are rapid and can easily be standardised for the detection of specific viruses. Several methods are now available for showing specific combination between the particles of viruses and their antibodies. They differ in convenience and sensitivity and the majority of

Principal Virologist, Groundnut Improvement Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru P. O. Andhra Pradesh, 502 324, India.

Submitted as Conference Paper No. Cp-31 by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Presented at the Fourth National Symposium on Tobacco January 19-22, 191, Rajahmundry, India.

techniques can be used to measure antigen and antibody concentrations. Tube precipitin test, microprecipitin test, precipitin ring test and Ouchterlony's agar gel double-diffusion test (AGD) are simple to perform. However, they are less sensitive than the passive hemagglutination (PHA) test and the more recently developed Enzyme Linked Immunosorbent Assay (ELISA) and Radioimmunoassay. The AGD, PHA and ELISA tests are described in detail because they are expected to replace some of the conventional serological techniques now employed in India.

The AGD test was devised nearly 30 years ago by Ouchterlony (1958) and has been widely employed in the detection of plant viruses. Antigen and antibody are allowed to diffuse into the agar. A positive reaction results in the appearance of a thin white band where antigen and antibody meet. The test is easy to perform and requires no specialized equipment (Ball, 1974). It can be used to test several reactants at the same time. The test can be used successfully for the detection of short rod shaped and spherical viruses. More recently the test has been modified by the incorporation of 3,5-diiodosalycilic acid into agar for the detection of long rod shaped viruses (Sreenivasulu *et al.* In press)

The PHA test, one of the most sensitive serological techniques, has not been widely used for the detection of plant viruses because of non specific agglutination and the requirement and processing of fresh red blood cells (RBC) for each testing. The PHA test has been simplified and modified to prevent non-specific agglutination (Rajeshwari *et. al.* In press). A method to fix red blood cells with glutaraldehyde has been developed to obviate the need for processing fresh red blood cells for each testing. Glutaraldehyde fixed RBC, after treatment with tannic acid, are coated with antibody. The optimal quantity of antibody required to sensitize RBC has to be determined empirically for each system. Antibody sensitized red blood cells are then added to various dilutions of test solutions. The test is performed in lucite plates containing 'U'-shaped wells and, in a positive reaction, the red cells agglutinate forming a smooth mat with a serrated margin at the bottom of the well. In a negative reaction, red cells form a discrete ring at the periphery of the well. The PHA test is extremely sensitive, easy to perform, and requires much less antisera than the AGD test. The PHA technique can be used to detect viruses in crude plant extracts.

The ELISA test is by far the most sensitive and specific serological technique now available for the detection of plant viruses (Clark and Adams 1977; Voller *et al.* 1976). The procedure is simple and rapid. The γ-globulins extracted from antisera are adsorbed to wells of a special microtiter plate. Test samples, which could be crude plant extracts, purified viruses, or extracts from seed are added to the wells. If the test sample contains specific viral antigens, these are bound to the γ-globulins coated on the inner surface of the well. The test samples are washed off and alkaline phosphatase – conjugated globulins are added to the wells. The labelled antibodies bind to the viral antigens already bound to the γ-globulins coated on the plastic surface. Finally p-nitrophenyl phosphate, a substrate for alkaline phosphatase, which was used earlier for conjugation of γ-globulins, is added to the wells. The

intensity of the yellow color that develops in the substrate is directly proportional to the amount of enzyme present, which in turn is proportional to the viral antigen concentration. The two major limitations of ELISA are the need for high titered antisera and specialized reagents and plates for performing the test. Several commercial firms are manufacturing equipment for the automation of ELISA and with this several hundred plant samples could be screened for the presence of one or more viruses in a day.

Electron Microscopy :

Electron microscopy is an essential technique for the detection and identification of plant viruses.

If concentration of the virus is adequate in crude plant extracts, leaf dip preparations could be negatively stained with 2% phosphotungstic acid or 1% uranyl acetate for detecting virus particles. Similarly, virus, particles in purified preparations could be detected following negative staining.

Methods of virus detection have been improved considerably by combining specificity of serological reactions with ability of the electron microscope to detect virus particles. Dilute solutions of antisera are placed on the surface of an eletron microscope grid. The edge of the cut surface of an infected leaf is dipped into the drop of antiserum. The grids are then dried and negatively stained. A specific reaction between the antibody and the virus particles can be observed in the electron microscope. Virus particles with similar morphology but unrelated serologically can be detected if specific antisera are used. Viruses occurring in low concentrations in crude plant extracts can be detected (Ball, 1971). The method has been improved by coating the girds with antisera (Derrick, 1973) which facilitates the attachment of serologically related viruses. The number of virus particles that attach to a microscope grid can further be increased by rendering the supporting film hydrophilic bymeansofa high voltage glow discharge (Milne and Luisoni, 1975), and by precoating the grid surface with protein A before coating with antibody (Shukla and Gough, 1979). Virus particles should be washed thoroughly to remove host contaminants, salts and other impurities to facilitate a clean image of virus particles coated wifh antibodies. These methods permit detection of extremely low concentrations of virus particles.

Conclusions :

The PHA test can be performed without the need for specialized equipment or reagents. Thus it is a better choice among conventional serological tests for routine diagnosis of virus diseases.

ELISA has been applied in cases where the previous methods could not be used an example of this being the detection of viruses in seed. The serological specificity of ELISA has been utilized to distinguish precisely virus serotypes and strains. Even with limited experience in plant virus serology it is possible to perform the test. In addition if plates were coated with γ-globulins and supplied together with the necessary reagents and conjugates, ELISA could be carried out in laboratories which would normally lack the resources to do this work.

References

1. BALL, E. M. 1971. Leaf dip serology. *In Methods in Virology.* ed. K. Maramorosch, H. Koprowski. Academic Press, New York 5 : 445-450.

2. BALL, E. M. 1974. Serological tests for the identification of plant viruses. *American Phytopathological Society. St. Paul.* pp 1-30.

3. BOS, L. 1976. Research on plant virus diseases in developing countries : Possible ways for improvement. *FAO Plant Protection Bulletin.* 24 : 109–118.

4. CLARK, M. F. and ADAMS, A. N. 1977. Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology.* 34 : 475 483.

5. DERRICK, K. S. 1973. Quantitative assay for plant viruses using serologically specific electron microscopy. *Virology* 56 : 652–653.

6. MILNE, R. G., and LUISONI, E. 1975. Rapid high resolution immune electron microscopy of plant viruses. *Virology.* 68 : 270-274.

7. OUCHTERLONY, O. 1958. Diffusion-in-gel methods for Immunological analysis. In progress in allergy. S. Karger, Basel, New York 5 : 1 78.

8. RAJESHWARI, R., REDDY, D.V.R. and IIZUKA, N. Improvements in the passive hemagglutination technique for serological detection of plant viruses. *Phytopathology* (In press)

9. REDDY, D.V.R. 1980. International aspects of groundnut virus research. *Proceedings of the International Workshop on groundnuts,* 13-17 October, 1980, Patancheru, A.P.India.

10. SHUKLA, D.D., and GOUGH, K.H., 1979. The use of protein A, from *Staphylococcus aureus,* in immune electron microscopy for detecting plant virus particles. *Journal of General Virology.* 45: 533-536.

11. SREENIVASULU, P., IIZUKA, N., RAJESHWARI, R., REDDY, D.V.R. and NAYUDU, M.V. Peanut green mosaic-a member of the potato virus Y group infecting groundnut *(Arachis hypogaea)* in India. *Annals of applied biology.* (In press).

12. VAN REGENMORTEL, M.H V.1978. Application for plant Virus Serology. *Annual Review of Phytopathology.* 16: 57-81.

13. VOLLER, A.M., BIDWELL, D.E. and BARTLETT, A. 1976. Enzyme immunoassays in diagnostic medicine. *Bulletin of World Health Organization* 53: 55 65.