

APPLICATION OF SERO-DIAGNOSIS FOR FIELD VIRUS INSPECTION IN JAPAN

Ken-ichiro-Shohara*

ABSTRACT

Advantage of immunological methods for the rapid and accurate diagnosis of plant viruses is well recognized. The Japan Plant Protection Association has started to promote sero-diagnosis programs for the control of virus diseases since 1981. Including the major viruses in grasses, vegetables and fruit trees, 25 kinds of antisera have been prepared and distributed all over the country.

Simplified double diffusion test in agar gel, ring interface and microprecipitin tests can be applied for detecting common vegetable viruses. Highly sensitive techniques, such as latex flocculation (LF). Passive hemagglutination test (PHA) and ELISA were adopted for field inspection for a very small amount of antigen with practical modifications.

In addition to polyclonal (rabbit) antibodies, attempts were made to promote the production and application of monoclonal(mouse hybridoma) antibodies for virus diagnostics due to the specificity of the serological reactions. Serological assays, such as LF and PHA, as well as the (simplified) ELISA method, have been successfully employed every spring season for routine mass-detection of rice stripe virus in individual planthoppers and have also been applied to citrus mosaic virus surveys in scions of mandarin orange.

Introduction

No agricultural chemicals are able to control plant viruses directly. Rapid diagnosis of the virus diseases, therefore, in the early stage of symptom expression is very important to take efficient measures for preventing the spread of the diseases.

Several methods for virus diagnosis have been applied including electronmicroscopic observations and biological assays on the indicator plants. Among them, the immunological techniques are generally recognized as superior methods for the detection of virus antigen, due to their relative simplicity and the specificity of serological reactions.

The Japan Plant Protection Association (JPPA), which is a semi-official organization established to promote plant protection activities, has started to implement the sero-diagnosis program of the Ministry of Agriculture, Forestry and Fisheries for the control of virus diseases in Japan. In order to prepare and distribute the virus antisera for field use, a virus laboratory was built in the research farm of JPPA in Ushiku City by the governmental subsidiary in 1981.

Production and distribution of antisera and monoclonal antibodies

Fundamentally, virus antisera have been produced by immunizing rabbits with purified virus antigen except for monoclonal antibody preparation for special purpose (Kohler and Hilstein, 1975).

Starting with the production of satsuma dwarf virus(SDV) antiserum in 1981, antiserum against rice stripe virus(RSV) was then prepared.

Satsuma dwarf disease had spread by grafting scions of mandarin orange infected with the virus, and caused severe lesions on the orange fruits in the western part of Japan. In order to prevent the spread of virus, examination for viruliferous scions was indispensable. Since the concentration of the virus was very low even in the newly developing leaves, it was necessary to develop a highly sensitive immunological technique and virus antiserum for the detection of the disease. Rice stripe is

* Plant Pathologist, Institute of Japan Plant Protection Association, Ushiku, Ibaraki, 300-12 Japan.

the most harmful disease of rice in Japan. The virus is transmitted by the small planthopper (*Laodelphax striatellus*) in a persistent manner, and is also transovarially transmitted in a high percentage. The incidence of the disease is correlated with the percentage of viruliferous insects. Therefore, routine field detection of RSV in individual insects by serological assays has been performed continuously in every spring season.

Thereafter, antisera against main vegetable viruses, for example, tobacco mosaic (TMV), cucumber mosaic (CMV) and potato viruses (PVX, PVY) were successively prepared. Including the major viruses in grasses, vegetables and fruit trees, 25 kinds of antisera and monoclonal antibodies have been prepared and today they are available nationwide. All antisera including the antibodies stored are listed in Table 1.

At the beginning, the serological field tests were conducted mainly at the Prefectural Agriculture and/or Fruit Tree Experiment Stations. Presently they are carried out for practical field inspection and for research purposes at institutes from private companies and universities as well.

Table 1 List of virus antisera and monoclonal antibodies

Antisera (rabbit polyclonal)

satsuma dwarf virus, citrus mosaic virus, citrus tristeza virus, grapevine fanleaf virus, rice stripe virus, rice dwarf virus, barley yellow mosaic virus, cucumber mosaic virus, cucumber green mottle mosaic virus (watermelon strain and cucumber strain), tobacco mosaic virus (ordinary mosaic strain, tomato strain, pepper strain and wasabi strain), turnip mosaic virus, potato virus X, potato virus Y, beet necrotic yellow vein virus, soybean mosaic virus, soybean dwarf virus, lily symptomless virus, odontoglossum ring-spot virus

Monoclonal antibodies

rice stripe virus, tobacco mosaic virus, cucumber green mottle mosaic virus

Serological techniques for practical field virus inspection

At least more than 15 serological techniques are used for the identification and diagnosis of plant viruses as a part of the virological research carried out in the laboratory. Some of these techniques are too complicated to be applied for the examination without appropriate training and special facilities. When the sero-diagnosis is aimed at practical field inspection or mass-indexing program for the control of plant viruses, the following characteristics are required (Omura *et al.*, 1984): 1) A simple method without sophisticated equipment, 2) Virus must be detected in crude sap, 3) Method sensitive enough to enable the detection of the virus in very small samples, 4) Many samples must be treated in a short time. Furthermore, the antisera or the reagents should be stable enough to tolerate the shocks and relatively high temperatures during the transportation (Shohara, 1988).

Several serological techniques which are actually used for field virus inspection in Japan are listed in Table 2.

1 Precipitin tests

The basic principle of a serological test is the mutual binding of virus antigens with antibodies. As a result of the antigen-antibody reaction, virus particles form large clumps which become visible with the naked eye. Methods 1-4 in Table 2 are applicable for the detection of major vegetable viruses with a relatively high concentration in the plant leaf sap, such as TMV, CMV and turnip mosaic virus.

When the leaf sap is mixed with or placed closely to the antiserum, the virus (if there is any)

Table 2 Characteristics of serological techniques

Method	Equipment	Antigen preparation	Relative sensitivity	End point of virus concentration (μ /ml)
1 slide method	glass slide	leaf sap	+	—
2 microprecipitin test	petri dish	leaf sap (centrifuged)	++	CMV ; 0.5
3 precipitin ring test	conical test tube	leaf sap (centrifuged)	++	TMV ; 4.0
4 (simplified) immunodiffusion test	agar gel plate	leaf sap	+	CMV ; 50
5 hemagglutination test	conical test tube	insect sap leaf sap	+++	RSV ; 0.02
6 latex flocculation test	conical test tube	insect sap leaf sap	+++	RDV ; 0.005
7 (simplified) ELISA	microtiter plate (spectrophotometer)	insect sap leaf sap	> +++	SDV ; 0.001
8 immunoelectron-microscopy	electron microscope	leaf sap	> +++	—
9 immunofluorescence technique	fluorescence microscope	tissue section	(++)	—

Source: Shohara, 1986.

precipitates in forming clumps in the mixture or forms a precipitin line in the area of contact. In most cases of precipitin tests, centrifugation for sample preparation is needed except for the simplified immunodiffusion (Shohara and Inouye, 1978) test and the glass slide method for PVX.

2 Agglutination (passive hemagglutination, PHA) and flocculation (latex flocculation, LF) tests

Sheep red blood cells or polystyrene latex particles are used as an antibody carrier material. When the carriers are mixed with antisera (or purified antibodies), antibodies are adsorbed on the surface of the carriers. The carrier materials sensitized with antibodies form a large network (PHA) or big clusters (LF) and the results can be seen with the naked eye, when the carriers are mixed with crude samples containing viruses.

Both methods are actually adopted for detecting a small amount of viruses with practical modifications (fixation and freeze-drying of red blood cells (Takahashi *et al.*, 1986), for instance), such as RSV or rice dwarf viruses in individual vector insects. In addition, the method for sample preparation is very simple. The results can be easily obtained within a short time (PHA; within 2 hr, LF; within 30 min) by adding carrier materials to the test tubes in which the individual insects are crushed simply with a glass rod.

3 Enzyme-linked immunosorbent assay (ELISA)

ELISA is the most reliable and sensitive method for detecting plant viruses in the field (Clark and Adams, 1967). The principle and the simple procedure of ELISA are illustrated in Fig. 1 (on the left handside is the standard ELISA). An antibody-coated microplate is used for the assay. The virus in the test sample is trapped by the adsorbed antibody. The presence of virus antigen is revealed as a color reaction by an enzyme-linked antibody (conjugate), when the substrate is digested.

Although the method is applicable for field inspection, the major constraint to the more widespread use of ELISA is the time involved (it generally requires 6-8 hr to be completed). The problem was solved by developing a simplified ELISA method (right in Fig.1) (Takahashi *et al.*, 1987) in which the conjugate was added to the plate with the test sample at the same time. The simplified

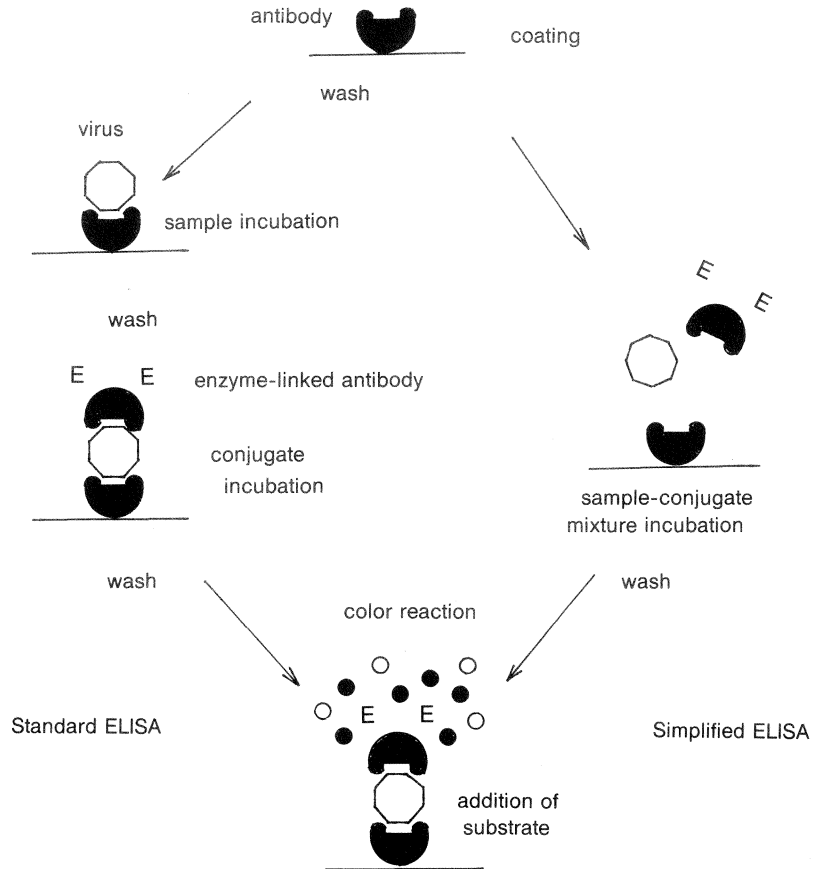


Fig. 1 Procedures for standard and simplified ELISA.

ELISA method has been applied successfully for the mass-inspection of RSV and some other vegetable viruses (TMV and soybean mosaic virus).

References

- 1) Clark, M.F. and Adams, A.N. (1967): Characteristics of microplate method of enzyme-linked immunosorbent assay for the detection of plant virus. *J. gen. Virol.*, 34, 475-497.
- 2) Kohler, G. and Milstein, C. (1975): Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature (London)*, 256, 495-497.
- 3) Omura, T., Hibino, H., Usugi, T., Inoue, H., Morinaka, T., Tsurumachi, S., Ong, C.A., Putta, M., Tsuchizaki, T., and Saito, Y. (1984): Detection of rice viruses in plants and individual insect vectors by latex flocculation test. *Plant Disease*, 68, 374-378.
- 4) Ohmura, T., Takahashi, Y., Shohara, K., Minobe, Y., Tsuchizaki, T., and Nozu, Y. (1986). Production of monoclonal antibodies against rice stripe virus for the detection of virus antigen in infected plants and viruliferous insect. *Ann. Phytopath. Soc. Japan*, 52, 270-277.
- 5) Shohara, K. and Inouye, T. (1978): Simplified immunodiffusion techniques in agar gel for

- detection and sero-diagnosis of cucumber mosaic virus. *Ann. Phytopath. Soc. Japan*, 44, 619-625.
- 6) Shohara, K. (1986): Sero-diagnosis of plant viruses and applications for immunological soil survey. *Soil Microorganisms*, 28, 17-20.
 - 7) Shohara, K. (1988): Serological diagnosis for plant viruses (3). Current status and potential application. *Shokubutsuboeki*, 42, 165-169.
 - 8) Takahishi, Y., Shohara, K., Oshima, N., and Araki, T. (1986): Stability of sheep erythrocytes sensitized with antibody. *Proceedings of the Kanto-Tosan Plant Protection Society*, 33, 40-42.
 - 9) Takahashi, Y., Ohmura, T., Shohara, K., and Tsuchizaki, T. (1987): Rapid and simplified ELISA for routine field inspection of rice stripe virus. *Ann. Phytopath. Soc. Japan*, 53, 254-257.