Serological Techniques for Plant Virus Indexing

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ABSTRACT

Effective disease control and sustaining agricultural productivity depend heavily on the prompt and accurate detection of plant viral infections. Our capacity to identify and track viral infections in agricultural crops has greatly increased because of recent developments in the detection of plant viruses. In order to minimize crop losses and stop viral infections from spreading to healthy plants, quick and accurate detection techniques are crucial. Additionally, the development of virus-free planting material, safe foreign travel and the screening of germplasm at quarantine facilities all depend heavily on diagnostic technologies. Due to its great sensitivity and accuracy, ELISA is one of the most important protein-based diagnostic techniques for plant pathogen identification. When many samples need to be examined simultaneously, it is extremely helpful. Thus, stakeholders would be able to select the best management methods against viral risks and guarantee global food security if they were aware of the key characteristics of the diagnostic methodologies now in use.

INTRODUCTION

Plant viruses are among the most damaging pathogens affecting agricultural and horticultural crops across the world. They infect a wide range of plant species and often result in serious yield losses and poor crop quality. The economic impact of these viral diseases is immense, with global losses running into several billion

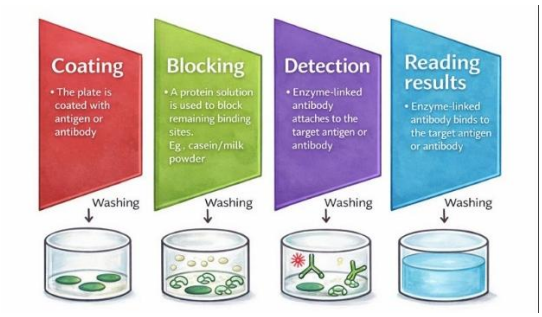
dollars every year. Due to their ability to spread rapidly and the limited availability of effective control measures, plant viruses remain a major concern for crop productivity and pose a continuous threat to global food security (Jones and Naidu 2019). Plant viruses use the host plant's cellular machinery and resources to multiply. This interference disturbs the normal physiological processes of the plant, resulting in stunted growth, poor quality of produce and reduced crop yield. Plant virus infections are often poorly managed or remain undetected because accurate diagnosis is difficult. As a result, rapid and reliable detection methods are essential to prevent the spread of viral diseases to healthy plants and to reduce crop losses. Diagnostic tools are also critically important for screening germplasm at quarantine facilities, ensuring safe international movement, and supporting the production of virus-free planting material. Among protein-based diagnostic methods, ELISA has played a major role in plant pathogen detection because of its high sensitivity and accuracy. It is especially useful when a large number of samples need to be tested at the same time.

Serological methods used for the rapid detection of plant pathogens rely on the specific interaction between antigens and their corresponding antibodies. The development of enzyme–antibody conjugates, formed through glutaraldehyde cross-linking, was a major breakthrough, as these conjugates retained both the binding specificity of immunoglobulin G (IgG) and the enzymatic activity required for signal detection. This advancement greatly enhanced the application of enzyme-linked immunosorbent assay (ELISA), establishing it as a rapid and highly sensitive technique for both qualitative and quantitative detection of viral infections in plants.

Serological Techniques

1. Enzyme Linked Immunosorbent Assay (ELISA)

ELISA is a solid-phase immunoassay that is commonly performed using 96-well microtiter plates made of polystyrene or polyvinyl chloride. The technique works on the specific interaction between antigens and antibodies. In the initial step, plant sap extract is added to the wells and allowed to incubate. This is followed by the addition of specific antibodies and washing steps. If the target virus is present in the plant sample, it binds to the antibodies. Unbound material is removed during washing, after which a secondary antibody that recognizes the primary antibody is added. The secondary antibody enables indirect detection of the virus because it carries a reporter molecule, most commonly an enzyme such as alkaline phosphatase (ALP) or horseradish peroxidase (HRP). When the appropriate substrate is added, the enzyme catalyzes a colour change, which can be observed visually or measured using a microtiter plate spectrophotometer (Webster *et al.,* 2004).



Advantages

- The wide commercial availability of different labelled secondary antibodies is a major advantage of this method.
- This is a versatile method since primary antibodies can be made in one species, and the same labelled secondary antibody can be used for detection.

- Maximum immunoreactivity of the primary antibody is retained.
- This method offers increased sensitivity because each primary antibody has multiple epitopes that can be recognized by the labelled secondary antibody, resulting in signal amplification.

Types of ELISA

ELISA tests are categorized into different types depending on the manner in which the analyte and antibodies are bound and used in the assay.

i) Double Antibody Sandwich ELISA (DAS-ELISA)

In the direct ELISA method, antibodies (the immunoglobulin G, IgG, fraction of the antiserum) are first coated onto the surface of the wells of a microtiter plate. These antibodies bind and capture the virus present in the test sample. After an incubation period, an enzyme-linked antibody specific to the virus is added. Following further incubation and washing, a suitable substrate is introduced, leading to colour development. This method is commonly known as the Double Antibody Sandwich ELISA (DAS-ELISA).

ii) Indirect ELISA - Double Antigen coated (DAC)

In an indirect ELISA, antibodies from two different plant species can be immobilized alongside the virus in the wells of the ELISA plate. This method is used to detect the presence of specific antibodies in a sample. The antigen of interest is first bound to the wells of the microtiter plate. When the sample is added, any antibodies present that are specific to the antigen will bind to it, allowing for their subsequent detection. After incubation, the wells are washed to remove any unbound antibodies. Enzyme-linked secondary antibodies are then added, which bind specifically to the primary antibodies. Following this, an appropriate substrate is introduced. The enzyme reacts with the substrate to produce a colour change, the intensity of which is directly proportional to the concentration of primary antibodies present in the serum sample.

iii) Triple Antibody Sandwich ELISA (TAS-ELISA)

Although this method is largely similar to DAS-ELISA, it involves an additional step in which a monoclonal antibody (MAb) is first applied, followed by an enzyme-conjugated, species-specific antibody. This approach was developed by Geering and Thomas (1996) for the detection of Banana bunchy top virus (BBTV) and has since been preferred over dot immunobinding assays due to its improved reliability and sensitivity.

iv) Competitive ELISA

This type of ELISA works on the principle of competition between the antigen present in the test sample and the same antigen already attached to the wells of the microtiter plate for binding to the primary antibody. In the first step, the primary antibody is mixed with the sample, allowing antigen–antibody complexes to form. The reaction mixture is then transferred to wells that have been previously coated with the identical antigen. Following incubation, excess antibodies are removed through washing. When a higher amount of antigen is present in the sample, a greater proportion of the primary antibodies bind to it during the initial reaction. Consequently, fewer primary antibodies remain available to attach to the antigen immobilized on the microtiter plate. An enzyme-linked secondary antibody is then added, and the reaction is visualized by adding a suitable substrate. The colour produced decreases as the concentration of antigen in the sample increases.

2. Immuno blotting

The antigen-antibody specific response is used in immunological blotting techniques to identify the target viral proteins among members of unrelated protein samples.

Types:

a. Dot Immuno blotting Assay (DIBA):

The Dot Immunoblotting Assay (DIBA) is a commonly used method for routinely diagnosing plant viruses in both plants and vectors. Because DIBA only needs a crude specific antiserum for each virus being tested for, it may be utilized in laboratories with inadequate equipment, making it a stronger approach than ELISA. It is largely comparable to ELISA, but it differs in that the plant extracts are spotted onto a membrane as opposed to the solid support or micro titre plate used in ELISA. In this case, the presence of the virus in the sample is confirmed by the visible colour precipitate that results from the hydrolysis of the chromogenic substrate.

b. Tissue Immuno blotting Assay (TIBA):

Tissue Immunoblotting Assay (TIBA) is based on transferring biological material directly onto a membrane by gently pressing freshly cut plant or insect tissues, such as leaf blades, stems, roots, tubers, or whole insects. The immobilized target is then detected using specific labelled antibodies. This technique allows rapid localization of pathogens within tissues without the need for complex extraction procedures. However, a major limitation of both DIBA and TIBA is that weak positive reactions may sometimes go undetected, particularly when intense sap coloration interferes with proper signal development.

CONCLUSION

Serological methods continue to play a central role in plant virus indexing programs. Although PCR-based techniques have gained increasing importance because of their simplicity and broad applicability, serology remains widely used. Detection and diagnosis of viral infections is, in fact, one of the most common applications of serological studies across all branches of virology. Serological cross-reactions provide a reliable basis for determining relationships among viruses, as serologically related viruses generally share similar morphological and biochemical features and therefore belong to the same taxonomic group. At present, serological techniques are extensively employed in plant virus indexing and certification schemes, where they effectively complement other established virus detection methods.

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