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Importance of Detection and Identification of Viral Diseases in Plant

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SUMMARY

In agriculture, plant viruses are still regarded as one of the main factors to economic losses. It has been estimated that plant viruses can cause as much as 50 billion euros loss worldwide, per year. This situation may be worsened by recent climate change events and the associated changes in disease epidemiology. The early and accurate diagnosis of viral diseases is critical for effective management of most crop systems. The presence of a virus in a plant cannot always be directly demonstrated. Although electron-microscopy has become a valuable advantage for the extension of our knowledge of viruses, and also has been very helpful in serological research, its application to clinical diagnosis of virus diseases of plants becomes too complicated. In many cases the serological diagnosis has rendered a satisfactory solution of the problems involved.

INTRODUCTION

Viruses cause significant yield and quality losses in a wide variety of cultivated crops. Hence, the detection and identification of viruses is a crucial aspect of successful crop production and of great significance in terms of world food security. The crop damages owing to viral diseases are difficult to predict, because it depends on host plant, virus strain, time of infection and geographical region. Viral diseases symptoms include mosaic, crinkling, browning of leaf tissues and necrosis. Sometimes, however, symptoms may not be visually detected because infection of plant viruses causes no symptoms. In addition, plants can also display virus-like symptoms when plants respond to nutritional imbalances, unfavourable weather, infection by other types of pathogens, damage caused by pests or abiotic agents and others (Van der Want and Dijkstra, 2006). Thus, viral disease diagnosis by symptoms is more difficult than other pathogens. The diagnosis is the basis to manage plant diseases and to predict the crop loss by infection of plant pathogens. Accurate diagnosis of virus diseases, is the first important step for crop management system. Because after virus infection, agrochemical treatments to plants do not lead to an effective control, viral diseases most effectively managed as control measures are applied before infection. In order to prevent plant viral diseases, it is important to figure out the causes and to distinguish diseased plants and unaffected plants that show virus– like symptoms (Pearson *et al.*, 2006). The methods for detection and identification of viruses are critical in virus disease management. Therefore, detection methods should be more convenient, effective, specific and permitted the use for detecting plant pathogens. Many methods have been developed to detect and identify plant viruses, such as microscopically observation, serological techniques, molecular methods and so on. Among them, a number of methods for the diagnosis of plant viral diseases are reviewed in the following two sections, serological method and nucleic acids procedures.

A. Serological Methods:

Serological detection systems use specific antibody developed in animals in respond to antigens. Viruses can be detected if viral antigens are used to develop antibody.

1. Enzyme-Linked Immunosorvent Assay (ELISA):

The use of enzyme linked immunosorbent assay (ELISA) for the detection of plant viruses is well standard and proved to be a very valuable detection tools for the plant viruses. Common ELISAs are performed in polystyrene plate capable of binding antibodies or proteins with association of the enzyme-substrate reaction. In order to get an accurate and reproducible result, the enzyme-substrate reaction needs to be optimized timing and development conditions. Level of infection is measured based on the optical density (the degree of coloration) of ELISA reaction.

Advantages of ELISA

- It is sensitive, a great number of samples can be examined at the same time, use little amount of antibody for the detection of diseases, the process can be semi automated
- Specific antiserum has been developed against the target virus.

Disadvantage of ELISA:

The major constraint of the method is the requirement for polyclonal or monoclonal antibody sera specific for each virus of interest that does not cross-react with plant proteins, but cross absorption with plant sap avoids this problem substantially.

2. Tissue Blot Immunoassay (TBIA):

TBIA has the same reliability to ELISA to detect plant viruses because the principle of TBIA is same with that of ELISA to which antibody is applied. However, polystyrene plate is used as platform of ELISAs whereas TBIA is performed on nitrocellulose and nylon membranes. So, this assay is called as TBIA or TIBA.

Advantage of TBIA

- TBIA has great benefits over ELISA in terms of detection time, cost, sensitivity and convenience
- The procedure is less labour-intensive than ELISA, simple (no virus extraction is required) and suitable for surveys of 1000 to 2000 samples per day, and the samples can be taken in the field and processed some time later.
- It has been applied for diagnosis of a number of viral diseases.

3. Quartz Crystal Microbalance Immunosensors (QCMI)

In this novel technique for plant virus detection, a quartz crystal disk is coated with virus-specific antibodies. Voltage is applied across the disk, making the disk warp slightly via a piezoelectric effect. Adsorption of virus particles to the crystal surface changes its resonance oscillation frequency in a concentration-dependent manner. It is therefore qualitative and quantitative.

Advantage QCMI

- High sensitivity, real time output, portability, label-free entities, and low cost of operation, fabrication, and maintenance becomes attractive alternatives to conventional analysis methods.
- If the analytical signal is too weak to detect target materials, the detection sensitivity can be increased by introducing the signal-enhancing step.
- QCMI shows high detection sensitivity for biological materials even viruses.
- QCM coated with virus-specific antibodies to detect plant viruses has long life span, it can be used for on site detection of plant viruses.

B. Nucleic Acids Procedures

1. Reverse Transcription–Polymerase Chain Reaction (RT–PCR) and PCR:

PCR is a scientific technique used to amplify, or create millions of identical copies of a particular DNA sequence. PCR has been used for other applications such as cloning, gene manipulation, gene expression analysis, genotyping, sequencing, and mutagenesis. In addition, PCR has also been used as a diagnostic tool to detect diseases. The effective diagnostic methods of viruses, PCR is able to process by the specificity of the primers. PCR is proceeded through three steps, denaturation above 94^{o}C, annealing of primers at 50-75^{o}C (depend on primers) and elongation at 72^{o}C. RT-PCR used for the detection of RNA viruses requires reverse transcriptase which is added at the step of reverse transcription before the regular PCR step. Since RT-PCR technique is sensitive, specific, and inexpensive compared to serological methods and is also more reliable than serological methods.

2. Multiplex RT–PCR

Multiple species or strains are detected in a single reaction by combining oligonucleotide primers specific for different viruses. It is important that the amplicons are of different lengths and that there is no cross-reactivity among them. This methods required several specific primers to detect over two viruses.

3. Fluorescence RT–PCR using Taqman™ technology

Two primers flank the sequence of interest and a third fluorescently labelled primer anneals between them. As the flanking primers extend, the labelled primer is released and fluorescence occurs. The advantages of this method are that no post-reaction processing is required to detect the reaction product and that it is quantitative.

4.Competitive fluorescence PCR (CF–PCR)

This is a variation on the above technique. It is used to simultaneously differentiate between virus strains and multiple virus infections.

5.Immunocapture PCR (IC–PCR)

This combines capture of virus particles by antibodies with amplification by PCR. In this method, the virus is adsorbed by the antibody bound to a surface, then removed by heating with a non ionic surfactant such as Triton X-100. The nucleic acids are then amplified using RT–PCR. This method is especially useful in concentrating virus particles from plant species where virus titre is low, or where compounds that inhibit PCR are present.

6.Nested PCR

The method is useful when the virus titre is very low, target gene is unstable, and can not be checked by electrophoresis due to low amplification product. The product from primary PCR amplification is used for second PCR amplification. However, the second reaction can be caused to face the risk of contamination. Problems mentioned above can be solved by Nested PCR. This nested PCR was combine with Immunocapture-RT PCR to increase sensitivity and to simplify preparation of sample.

7.Co-operational PCR (Co-PCR)

Both co-operational PCR and nested-PCR require a tetra primer set. However, co-operational PCR needs one external and three internal primers instead of two external and two internal primers associated with nested-PCR. Since co-operational PCR uses four primers like nested-PCR, this technique has some advantages over conventional PCR. Benefits include minimization of contamination risks, a single reaction, detection in real-time, high sensitivity similar to nested PCR and capability of coupling with dot blot hybridization. In addition, Co-operational PCR can avoid false positive shown at nested- PCR. The major obstacle to use conventional PCR is existence of PCR inhibitors. This problem can be overcome by co-PCR with diluted samples.

8.Real-time PCR

To monitor the amplification products of PCR in real-time, real-time PCR was developed as one of the technical methods and also allows accurate quantification of PCR products. Real-time PCR can be significantly reduced detection time and can be used for small concentration of target gene making possible to diagnose because of no need the gel electrophoresis for the confirmation. It also has been known that it is faster than conventional PCR with less risk of contamination. Although the real time monitoring curve was raised up as the DNA exponentially amplified, there are some drawbacks to use real-time PCR.

C. Isothermal Amplification

1.Loop-Mediated Isothermal Amplification (LAMP)

LAMP does not require a thermal cycler and requires a shorter amplification time than PCR but requires a purified DNA or RNA template. LAMP employs 4-6 primers which are difficult to design, further adding to the cost of the assay.

2.Recombinase Polymerase Amplification (RPA)

RPA is an isothermal gene amplification technique and has been considered as a rapid, sensitive, and cost-effective molecular diagnostic method of plant viruses. RPA is a simple technique and does not require a thermal cycler.

3.Microarray (Oligonucleotide Array):

Microarray is the evolved stage of the southern blotting technology. This technique used glass instead of nitrocellulose and nylon membrane as a supporter and was initially developed for differentiation of messenger RNA expression. Later, this technique demonstrated the potential to detect viral pathogens without amplification of viral RNA.

CONCLUSION

There was no commercialized chemical to manage plant viral diseases which caused heavy economic loss all over the world. Plant diseases caused by viruses can be successfully controlled when means of manage are applied at the initial step of viral disease development or by planting virus-free crops. This is reason why proper diagnosis is important. Molecular detection technology recently developed resulting in more effective, convenient and specific assays have opened the door to greater use of these tests for detecting plant pathogens. These assays will help to crop consultants, free growersand plant-health-care professionals from having to rely excessively on symptomatology and/or time-consuming diagnostic procedures, and permit early detection of pathogens. However, they should be viewed as management tools, to be used in combination with other diagnostic procedures, knowledge of the crop. and understanding of the biology of the pathogen and the ecology of the disease.

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