

EXERCISE # 3**DETECTION AND DIAGNOSIS OF PLANT VIRUSES**

The early and accurate detection of plant viruses is an essential component to control those. Because the globalization of trade by free trade agreement (FTA) and the rapid climate change promote the country-to-country transfer of viruses and their hosts and vectors, diagnosis of viral diseases is getting more important. Because symptoms of viral diseases are not distinct with great variety and are confused with those of abiotic stresses, symptomatic diagnosis may not be appropriate. From the last three decades, enzyme-linked immunosorbent assays (ELISAs), developed based on serological principle, have been widely used. However, ELISAs to detect plant viruses' decrease due to some limitations such as availability of antibody for target virus, cost to produce antibody, requirement of large volume of sample, and time to complete ELISAs. Many advanced techniques allow overcoming demerits of ELISAs. Since the polymerase chain reaction (PCR) developed as a technique to amplify target DNA, PCR evolved to many variants with greater sensitivity than ELISAs. Many systems of plant virus detection which includes immunological-based detection system, PCR techniques, and hybridization-based methods such as microarray. Some of techniques have been used in practical, while some are still under developing to get the level of confidence for actual use.

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. In fact, these kinds of techniques have been used for the routine diagnostic tool. Many serological methods have been reported including

1. Enzyme-linked immunosorbent assay (ELISA)
2. Tissue blot immunoassay (TBIA)
3. Quartz crystal microbalance immunosensors (QCMI)

B) MOLECULAR METHODS

Molecular methods can be applied for diagnosis of many viral diseases when genetic information of viruses is available. As an alternative method to serological one, it is most commonly used in the laboratory due to high accuracy and sensitivity.

1. Polymerase chain reaction (PCR).
2. Reverse transcription PCR (RT-PCR).
3. Multiplex RT-PCR:

4. Fluorescence RT-PCR using Taqman[®] technology
5. Competitive fluorescence PCR (CF-PCR):
6. Immunocapture PCR (IC-PCR):
7. Nested PCR.
8. Co-operational PCR (Co-PCR).
9. Real-time PCR.
10. Restriction fragment length polymorphism (RFLP)

C) ISOTHERMAL AMPLIFICATION

Generally speaking, the use of PCR variants is increasing for the disease diagnosis. In order to complete the first round of PCR, PCR is necessary of 3 different temperatures for denaturalization of double stranded DNA, primer annealing to target DNA, and extension of DNA synthesis. Thus it needs expensive instruments that can control temperature precisely. Polymerase, which can amplify DNA, at constant temperature was discovered. It called Isothermal PCR. Many isothermal PCRs exist but two are most important.

1. Nucleic acids sequence-based amplification (NASBA)
2. Loop-mediated isothermal amplification (LAMP) are described.

D) ARRAY

1. Microarrays (Oligonucleotide array)
2. Macroarrays

QUESTIONS

1. What is virus?
2. Enlist different nucleic acid procedure for virus detection?

Which are the serological method of virus detection?