

3. Techniques and Designs for Plant Virus Diagnosis. Jang Kyung Choi. Kangwon National University, Chunchon 200-701, Korea.

Diseases due to plant viruses cause losses estimated at about \$15 billion per year worldwide in both agricultural and horticultural crops. In attempts to control these viral diseases, the first essential step is to establish the diagnosis of the virus causing the disease and to determine its properties. Facilitating diagnosis has been the development of improved techniques for virus identification. The developments of refined serological and electron microscopic procedures, and the development of widely applicable methods using nucleic acid hybridization and polymerase chain reaction (PCR) have become particularly important. The main techniques currently available for the diagnosis of virus diseases in plants are serological procedures, especially various forms of enzyme-linked immunosorbent assay (ELISA) and reverse transcription and the PCR (RT-PCR). However, serological techniques can be used to detect the full range of virus strains (or isolates) differentiated with pathotype but distinction is difficult.

Because the nucleotide sequence of coat protein genes of the strains are practically similar, they cannot be differentiated by serological means. Although ELISA is able to discriminate between the virus strains, comparison requires the availability of the viruses and their specific antisera, and even so the methods may provide inconclusive results especially in double-infected samples. ELISA also is not always sensitive enough in the case of micropropagated plant materials. Therefore, the generally way to differentiate these strains is by bioassay methods which require the inoculation of appropriate indicator plants. Since this technique is tedious and inefficient for diagnostic purposes, a procedure based on RT-PCR was developed using virus (strain) specific-nucleotide sequence data. In addition, restriction enzyme analysis of the amplified PCR products produces distinct restriction patterns specific for each of the strains. Because this is a rapid, easy and highly sensitive method, it may prove useful for the diagnosis of the diseases caused by viruses in plants. This review presents several improved techniques and designs of RT-PCR for diagnosis of plant virus diseases.