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Citrus Tristeza Virus

Methods and Protocols

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Preface

Citrus tristeza virus (CTV) is one of the most destructive plant viruses that replicates in the cytoplasm of companion or phloem parenchyma cells of *Citrus*, *Poncirus*, and *Fortunella*. It causes a variety of symptoms depending on the host species, the cultivar, and the CTV isolate involved. Given that this virus is associated worldwide with citrus, many plant pathologists and virologists have been involved in the study of this complex virus and the diseases it causes.

Few, however, have spent as long as 50 years like Moshe Bar-Joseph. His pioneering efforts led to the development of new methods of CTV diagnosis (1970) based on the electron microscope observation of partially purified particles and enabled other groups to develop rapid serological assays (Chapter 1). With some modifications, his method was also useful for *Beet yellows virus* (BYV) particles that cause *Closterovirus*.

To demonstrate the genetic diversity of *CTV* isolates, Moshe Bar-Joseph developed strain-specific assays using CTV-VT cDNA fragments as hybridization probes. He also developed a CTV-dsRNA cloning method and used complementary oligonucleotides for cDNA synthesis and PCR amplification.

To honor his invaluable dedication and contribution to the study of the virus, 45 authors have contributed to this laboratory methods and protocols book on the *Citrus tristeza virus*, which is one of the most complex viruses, as well one of the most scientifically attractive research topics. Thanks to the highly sensitive and specific diagnostic procedures developed, knowledge of the molecular characteristics, expression strategies, genetic variability, and epidemiology of the virus have improved significantly. Since deep sequencing opened new doors to reconstructing viral populations in a high-throughput and cost-effective manner, many of the past grouping criteria have now been revisited.

Today, 67 complete sequences of CTV genomes from different countries are in the GenBank. Unfortunately, not all of them have been associated with a phenotypic profile. Reports from all over the world show that several destructive isolates of CTV, not dependent on sensitive rootstocks, may suddenly appear as a result of rearrangements or mutations of the genome. The rapid identification of the genetic diversity of the virus remains critical for surveying specific land areas.

This book provides methods and clear protocols for the various technologies available to detect, characterize, and study *CTV*, a member of the genus *Closterovirus* family *Closteroviridae* (Chapter 2). Despite the fact that new detection methods have strengthened the discrimination potential of genotypes of the isolates, biological indexing remains invaluable in order to phenotype the biological properties of isolates in terms of their aggressiveness on various hosts (Chapter 3). Enzyme immunoassays and PCR-based assays, which are frequently used in combination, have revealed the worldwide rapid diffusion of the virus, even in symptomless infected citrus trees. The relationships of vectors with the virus and its host plants, which are mostly based on host plant inoculation, have been highlighted by sensitive detection technologies (Chapter 4).

The potential of CTV detection by RT-PCR was strengthened after the development of direct systems of sample preparation and real-time RT-PCR (Chapter 5). Fast, reliable, and specific detection methods based on real-time PCR protocols have been designed to simultaneously detect CTV, HSVd, and CEVd (Chapter 6). The analysis of single-strand

conformation polymorphism of RT-PCR product by polyacrylamide gel or capillary electrophoresis is still now the most common and informative method to investigate the structure of CTV isolate populations (Chapter 7). Integrating molecular assays and biological tests has made it possible to identify RB CTV isolates which overcome the resistance of trifoliate orange and its hybrids (Chapter 8), whereas sequential RT-PCR and microarray hybridization, on a lab-on chip device, enable a fast characterization of virus genotypes (Chapter 9), which is very useful for the CTV surveillance of territories. At the same time, the fast and sensitive field detection of CTV has recently been achieved by reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay (Chapter 10). A strategy to clone the entire genome of CTV obtained from two RT-PCR amplified products has also been developed (Chapter 11). After high-throughput sequencing (HTS) was developed, bioinformatics started to be applied to analyze the genome of the virus (Chapter 12), to differentiate between isolates based on genotype composition which has been used to select candidate cross protective isolates (Chapter 13), and to study host RNA silencing and virus attack (Chapter 14). The study of proteins involved in CTV infection has been made possible by techniques such as proteomics (Chapter 15), whereas transient expression of the virus proteins is possible by biolistic bombardment (Chapter 16). Methods are now available for producing transgenic plants resistant to CTV (Chapter 17).

This book will be of interest to plant pathologists, plant virologists, molecular biologists, and graduate students, as a guide to performing qualitative and quantitative tests as well as recently developed diagnostic methods.

We hope the methods and protocols reported here will be helpful to find new solutions to improve the management of the disease, and we wish to thank all the authors who have contributed.

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