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Characterization of Plant Viruses

Methods and Protocols

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Humana Press

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ISSN 1949-2448 ISSN 1949-2456 (electronic)
Springer Protocols Handbooks
ISBN 978-1-0716-0333-8 ISBN 978-1-0716-0334-5 (eBook)
https://doi.org/10.1007/978-1-0716-0334-5

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The registered company address is: 233 Spring Street, New York, NY 10013, U.S.A.

Foreword

Characterization of Plant Viruses: Methods and Protocols written by Drs. Bhat and Rao provides a timely overview of all major protocols currently used in the discipline of plant virology. These include classic methods of indicator host inoculation, insect transmission, and virion isolation. Also addressed are serological and nucleic acid-based methods in widespread use. Inclusion of recently developed biotechnological approaches for plant virus diagnosis, characterization, and management brings the reader to the cutting edge of our field. Thus, this new book will be a valuable reference for students and practitioners of all levels with an interest in plant virus characterization.

USDA, ARS, USHRL, Fort Pierce, FL, USA ***Scott Adkins***

Preface

Viruses caused diseases in plants for many centuries before they were described and shown as the causal agents. After the first identification of the mosaic disease in tobacco caused by a virus in the early 1900s, many viral diseases on different crops are described. Intensive cultivation of crops coupled with changing climate scenario has made virus disease as one of the major production constraints in cereals, vegetables, fruits, and other crops. At present, more than 1300 viruses infecting different crops worldwide have been characterized. Enormous developments have been made in the diagnosis of plant viruses, and efficient detection tools are available for large number of viruses. Biological, physicochemical, protein, and nucleic acid-based methods are the broad methods used for diagnosis of viruses. The biological diagnosis methods such as symptoms, isolation, host range, and transmission play an important role in the preliminary identification of the viral pathogen. Serological (protein) and nucleic acid-based methods offer more reliable and sensitive methods for detection. Among serological methods, various forms of enzyme-linked immunosorbent assays (ELISA) have become very popular and are largely used for routine detection of viruses. Lateral flow assay is an onsite detection method that can be used by the cultivator himself in the field without the aid of either a laboratory or technical knowledge. Among the different nucleic acid-based methods, polymerase chain reaction (PCR) and real-time PCR are the most sensitive methods (10^2–10^5 times more than ELISA) available for detection and is important when viruses occur at low concentrations. It also has the potential to detect more than one pathogen in one reaction, and diagnosis is amenable to automation. Isothermal amplification methods such as loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), and recombinase polymerase amplification (RPA) are recently added assay methods that can be performed in laboratory with minimum facilities and equally or even more sensitive than PCR. When sufficient sequence information of pathogens is available, microarray technology can be used to detect large number of pathogens in a single reaction. Next-generation sequencing (NGS) is an unbiased approach for the identification of viruses when all other methods fail. There is a quite good advancement in the management of viruses especially production of virus-resistant transgenic crops using various approaches including the latest CRISPR-Cas system. Elimination of viruses and production of virus-free plants are possible using approaches such as somatic embryogenesis and meristem-tip culture.

The objective of this book on *Characterization of Plant Viruses: Methods and Protocols* is an attempt to update and describe the protocols for the biological, serological, and nucleic acid-based assays for detection, diagnosis, and management of plant viruses. The book contains 50 chapters, Appendix and Glosarry. Chapters 1 to 19 of the book deals with techniques used in the biological characterization of plant viruses such as symptoms, host range, transmission by mechanical, graft, and different vectors including insects, fungi, mites, and nematodes. Chapters 20 to 26 of the book deals with the protocols for purification of viruses, electron microscopy, coat protein molecular weight determination, and nature of viral nucleic acids. Chapters 27 to 30 consists of *in vitro* expression of coat protein, production of antibodies, and various serological assays. Isolation of nucleic acids, PCR assays, isothermal amplification assays, and next-generation sequencing approaches are dealt in Chaps. 31 to 41 of the book. Chapters 42 to 46 of the book deals with characterization of

the virus through cloning, sequencing, sequence analysis, and production of infectious clones while Chaps. 47 to 50 of the book provides biotechnological approaches for management of plant viruses. There are numerous colleagues to whom we are indebted for their valuable support in correction of the text and providing images used in the book. We most sincerely acknowledge and thank the publisher, Springer Nature for their help in various ways to publish this book in time. We strongly hope that this book will be useful to every laboratory, student, teacher, and researcher in plant virology, plant pathology, plant biology, and molecular biology and serve as a practical manual on identification, characterization, and management of plant viruses.

Kozhikode, India *Alangar Ishwara Bhat*
New Delhi, India *Govind Pratap Rao*

About the Book

The book on *Characterization of Plant Viruses: Methods and Protocols* gives detailed methodology used in the biological, serological, and nucleic acid-based assays for the plant virus detection, diagnosis, and management. The book contains 50 chapters, appendix, and glossary. Chapters 1 to 19 of the book deals with techniques used in the biological characterization of viruses such as symptoms, host range, transmission of viruses by mechanical, graft, and different vectors including insects, fungi, mites, and nematodes. Chapters 20 to 26 of the book deals with protocols for purification of viruses representing different species, properties of purified virus, and techniques for physicochemical properties such as determination of molecular weight of coat protein, isolation, and determination of nature of virus nucleic acid. Chapters 27 to 29 consists of *in vitro* expression of coat protein of viruses and production of polyclonal and monoclonal antibodies. Various serological assays from precipitin tests to ELISA, dot-blot, tissue blot, and electro-blot immunoassay, immunosorbent electron microscopy (ISEM), immunoflourescence, and lateral flow immuno assay (LFIA) are also dealt in Chap. 30 of the book. Isolation of DNA and RNA from virus-infected plants and nucleic acid-based assays such as dot-blot, polymerase chain reaction (PCR), real-time PCR, loop-mediated isothermal amplification (LAMP), rolling circle amplification (RCA), recombinase polymerase amplification (RPA), and next-generation sequencing approaches are dealt in Chaps. 31 to 41 of the book. Chapters 42 to 46 of the book deals with characterization of the virus through cloning, sequencing, sequence analysis, and production of infectious clones while Chaps. 47 to 50 of the book provides biotechnological approaches for management of viruses such as production of transgenic plants and use of CRISPR-Cas system for virus resistance; somatic embryogenesis and meristem-tip culture for production of virus-free plants. Appendix on DNA and protein data, online resources, and glossary are provided at the end. This book will be useful to every laboratory, student, teacher, and everyone interested in plant virology, plant pathology, plant biology, and molecular biology and serve as a practical manual on various aspects of plant viruses.

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About the Authors

Alangar Ishwara Bhat obtained his M.Sc. (Agri.) with specialization in Plant Pathology from University of Agricultural Sciences, Bangalore, and Ph.D. degree in Plant Pathology with specialization in Plant Virology from the ICAR-Indian Agricultural Research Institute, New Delhi. He joined Agricultural Research Service (ARS) in 1993; from 1994 to 2001 he worked as Scientist at the ICAR-Indian Agricultural Research Institute, New Delhi; from 2002 to 2008 as Senior Scientist and from 2009 onwards as Principal Scientist at the ICAR-Indian Institute of Spices Research, Kozhikode. His current area of research includes identification, characterization, development of diagnostics, and application of biotechnological approaches for management of viruses and phytoplasma infecting important spice crops such as black pepper, cardamom, ginger, and vanilla. His major contributions are on etiology, characterization, development of diagnostics, and application of biotechnological approaches for the management of plant viruses belonging to *Ampelovirus*, *Badnavirus, Cucumovirus, Ilarvirus*, *Nucleorhabdovirus*, *Panicovirus*, *Potyvirus*, and *Tospovirus* genera infecting different crop plants. Etiology of diseases such as necrosis disease in sunflower caused by *Tobacco streak virus* (TSV), bud blight of soybean and other legumes by *Groundnut bud necrosis virus* (GBNV), stunted disease in black pepper caused by *Piper yellow mottle virus* (PYMoV), chlorotic streak in cardamom caused by *Banana bract mosaic virus* (BBrMV), vein clearing disease in cardamom caused by *Cardamom vein clearing virus* (CdVCV), chlorotic fleck in ginger caused by *Ginger chlorotic fleck associated virus 1* (GCFaV-1), and GCFaV-2; mosaic disease in vanilla caused by *Cucumber mosaic virus* (CMV), *Cymbidium mosaic virus* (CymMV), *Bean yellow mosaic virus* (BYMV), and *Bean common mosaic virus* (BCMV) were identified for the first time. Cloning, molecular characterization, and sequencing of viruses infecting several crops were done to understand the possible origin and evolutionary relationship with other viruses. Complete genome sequencing of PYMoV and CMV infecting black pepper, BBrMV and CdVCV infecting cardamom, and GcFV-1 infecting ginger were done. Serological and nucleic acid-based diagnostics were developed for many viruses infecting different crops, such as multiplex PCR methodology for simultaneous detection of viruses in black pepper, cardamom, ginger, and vanilla; ELISA, RT-PCR/PCR/real-time PCR, and loop-mediated isothermal amplification (LAMP) methodology for the detection of viruses in black pepper, cardamom, ginger, and vanilla, TSV in sunflower and other crops; GBNV in legumes and tomato. Virus elimination through somatic

embryogenesis and meristem culture in black pepper and vanilla were developed. Protocols were developed for genetic transformation of black pepper and vanilla using viral sequences as transgenes to get virus-resistant plants.

During April 1998 to March 1999, Dr. Bhat was a visiting Scientist under the Department of Science and Technology, Govt. of India sponsored BOYSCAST Fellowship at University of Georgia, USA, and he was on deputation under NAIP sponsored training at the Food and Environment Research Agency, UK, during April to June 2011. He has guided nine Ph.D., one M.Phil., and eight M.Sc. students. He has handled ten externally funded (DBT, SERB, and ICAR) projects. Dr. Bhat has a total of 89 publications in reputed refereed journals. He has edited two books and contributed 28 book chapters, three technical bulletins, and was awarded with several national awards.

Govind Pratap Rao is working as a Principal Scientist (Plant Pathology) at Indian Agricultural Research Institute, New Delhi. He did his M.Sc. (Botany) in 1981 and Ph.D. in Plant Virology from Gorakhpur University in 1986. He did postdoc at the University of Urbana–Champaign, Illinois, USA, with Prof. R.E. Ford on characterization of sugarcane mosaic and maize dwarf mosaic viruses in 1994 and on sugarcane yellow leaf virus at Cedex, Montpellier, France, in 1998. Dr. Rao has 32 years of research experience on plant pathology especially on plant virology and phytoplasmas. He did significant contributions in characterization of viruses and phytoplasmas infecting sugarcane, vegetables, legumes, cucurbits, ornamentals, wheat, rice, maize, cucurbits, maize, and sorghum. He is the authority of phytoplasma research in India and characterized so far more than 50 new phytoplasma diseases on different crops and weeds in India and identified several insect vectors for the identified phytoplasma strains. He has published over 150 research publications and authored and edited 25 books to his credit. He has been awarded several prestigious awards to his credit. The most important ones are: National Biotechnology Associateship Award (1991–1992), DBT, Govt. of India; Young Scientist Award (1994–1995) from DST, Govt. of India; Overseas BOYSCAST Award (1996) from DST, Govt. of India; President Award, Society for General Microbiology, UK, 1998; Best U.P. Agriculture Scientist Award (UPCAR), Govt. of Uttar Pradesh in 2002; Vigyan Ratna Award by CST, Govt. of UP for the year 2003–2004; Jin Xiu Qiu Award in 2006 by People's Govt. of Guangxi Province, Nanning, China; Global Award of Excellence, IS 2008, Al-Arish, Egypt; Dr. Ram Badan Singh Vishisht Krishi Vaigyanik Puraskar–2014 by UPCAR, Lucknow, India, and Leadership Excellence Award in Sugarcane Crop Protection by Thailand Society of Sugar Cane Technologists, Bangkok. Dr. Rao is Editor-in-Chief of *Sugar Tech*

(an international journal of sugar crops and related industries) and *Phytopathogenic Mollicutes* (an international journal of phloem-limited microorganisms). Dr. Rao is also Secretary General of Indian Virological Society, New Delhi, and member of several prestigious scientific societies and organizations like APS, USA; ASM, USA; ISSCT, Mauritius; IAPSIT, China; IPWG, Italy: SSRP, New Delhi, and IPS, New Delhi. Dr. Rao has guided 5 M.Sc. and 15 Ph.D. students on different aspects of characterization, epidemiology, and management of plant viruses and phytoplasmas. He has handled ten externally funded (DST, DBT, SERB, and ICAR) research projects from Govt. of India. Besides, Dr. Rao has visited over 30 countries as visiting scientists, expert, for invited talk, postdoc fellow, research training, panel discussion, and for attending workshop and conferences. At present, Dr. Rao is working on characterization, epidemiology, and management of viruses infected cereal crops, millets, and maize and phytoplasmas infecting important agriculture and horticultural crops in India.

Abbreviations

A	Absorption
AAP	Acquisition access period
Ab	Antibody
AFP	Acquisition feeding period
AGPC	Acid guanidinium thiocyanate phenol chloroform
APS	Ammonium persulfate
BA	Benzyl adenine
BCIP	5-Bromo-4-chloro-3-indolyl-phosphate
BIP	Backward inner primer
BL	Backward loop
BLAST	Basic local alignment search tool
bp	Base pair
BPB	Bromo phenol blue
BSA	Bovine serum albumin
Bst	*Bacillus stearothermophilus*
CBB	Coomassie brilliant blue
cDNA	Complementary DNA
CDS	Coding regions
CFA	Complete Freund's adjuvant
Ci	Curie
cm	Centimeter
CP	Coat protein
CPMR	Coat protein-mediated resistance
CRISPR	Clustered regularly interspaced short palindromic repeats
crRNA	CRISPR RNA
Ct	Cycle threshold
CTB	Cetyl trimethyl ammonium bromide
cv	Cultivar
Da	Dalton
DAC-ELISA	Direct antigen coating enzyme-linked immunosorbent assay
DAS-ELISA	Double antibody sandwich enzyme-linked immunosorbent assay
DBBJ	DNA databank of Japan
ddNTP	dideoxy nucleoside triphosphate
DEP	Dilution end point
DIBA	Dot immunobinding assay
DIECA	Diethyl dithio carbamate
DIG	Digoxigenin
DMF	Dimethyl formamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribo nucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxy nucleoside triphosphate

dpm	Disintegrations per minute
DSB	Double-strand break
dsDNA	Double-stranded DNA
dsRNA	Double-stranded RNA
DTT	Dithiothreitol
E. coli	*Escherichia coli*
EBIA	Electro-blot immunoassay
EDTA	Ethylene diamine tetra methyl acid
eIF4G	Eukaryotic translation initiator factor 4G
ELISA	Enzyme-linked immunosorbent assay
EM	Electron microscopy
EMBL	European Molecular Biology Laboratory
EtBr	Ethidium bromide
$F(ab')_2$-ELISA	$F(ab')_2$-enzyme-linked immunosorbent assay
FCA	Freund's complete adjuvant
FIA	Freund's incomplete adjuvant
FIP	Forward inner primer
FL	Forward loop
g	Gram or Gravity
Gb	Giga base pair
gRNA	Guide RNA
GUS	Glucoronidase
h	Hour
HCl	Hydrogen chloride
HDR	Homology-directed repair
HEPES	*N*-2-hydroxylthylpiperazine *N'*-2-ethane sulfonic acid
His	Histidine
HP construct	Hairpin construct
HRP	Horseradish peroxidase
IAP	Inoculation access period
IC-PCR	Immunocapture polymerase chain reaction
IC-RT-PCR	Immunocapture reverse transcription polymerase chain reaction
IFA	Incomplete Freund's adjuvant
IFP	Inoculation feeding period
IgG	Immunoglobulin G
in	Inch
IP	Incubation period
IPTG	Isopropyl β-D-1-thiogalactopyranoside
ISEM	Immunosorbent electron microscopy
Kb	Kilo base pair
KDa	Kilodalton
kg	Kilogram
KN	Kinetin
L	Liter
LAMP	Loop-mediated isothermal amplification
LFA	Lateral flow assay
LFIA	Lateral flow immunoassay
LiCl	Lithium chloride

LIV	Longevity *in vitro*
LP	Latent period
m	Meter
M	Molar
mA	Milli ampere
mAb	Monoclonal antibody
Mb	Mega base pair
mCi	Milli Curie
mg	Milligram
min	Minute
mL	Milli liter
mm	Milli meter
mM	Milli molar
mPCR	Multiplex polymerase chain reaction
Mr	Relative molecular mass
mRNA	Messenger RNA
mRT-PCR	Multiplex reverse transcription polymerase chain reaction
MS medium	Murashige and Skoog medium
MSA	Multiple sequence alignment
MW	Molecular weight
NAA	Naphthalene acetic acid
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NBT	Nitro blue tetrazolium
NCBI	National Center for Biotechnology Information
NCM	Nitrocellulose membrane
ng	Nanogram
NGS	Next-generation sequencing
NHEJ	Non-homologous end joining
nm	Nanometer
nmole	Nanomole
NPT	Neomycin phosphotransferase
nt	Nucleotide
O.D.	Optical density
ORF	Open reading frame
pAb	Polyclonal antibody
PAGE	Polyacrylamide gel electrophoresis
PAM	Protospacer adjacent motif
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PDR	Pathogen-derived resistance
PEG	Polyethylene glycol
Pg	Picogram
pmole	Picomole
pNPP	*Para* nitro phenyl phosphate
ppm	Parts per million
PTGS	Post-transcriptional gene silencing
PVDF	Polyvinylidenedifluoride

PVP	Polyvinylpyrrolidone
qPCR	Quantitative polymerase chain reaction
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
RCA	Rolling circle amplification
rDNA	Recombinant DNA
Rf value	Relative distance of migration
RH	Relative humidity
RIA	Radioimmunoassay
RNA	Ribonucleic acid
RNAi	RNA interference
RPA	Recombinase polymerase amplification
rpm	Revolutions per minute
rRNA	Ribosomal RNA
RT-LAMP	Reverse transcription loop-mediated isothermal amplification
RT-PCR	Reverse transcription PCR
s	Sedimentation coefficient (expressed in Svedburg units)
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SE	Somatic embryo
s	Seconds
sgRNA	Single guide RNA
SH medium	Schenk and Hildebrandt medium
siRNA	Small interfering RNA
sRNA	Small RNA
SSB	Single stranded DNA binding protein
SSC	Saline sodium citrate
ssDNA	Single-stranded DNA
ssRNA	Single-stranded RNA
TAE	Tris-acetate-EDTA
Taq	*Thermus aquaticus*
TAS-ELISA	Triple antibody sandwich-ELISA
TBIA	Tissue blot immunoassay
TBS	Tris-buffered saline
T-DNA	Transfer DNA
TE	Tris EDTA
TEM	Transmission electron microscope
TEMED	*N*,*N*,*N*′,*N*′-tetramethylethylene diamine
Tm	Melting temperature
Tris	Tris (hydroxymethyl) amino ethane
UA	Uranyl acetate
UV	Ultraviolet
v/v	volume/volume
V	volt
var	Variety
VIGS	Virus-induced gene silencing

VSR	Viral suppressors of RNA silencing
w/v	weight/volume
w/w	weight/weight
WPM	Woody plant medium
x-gal	5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside
μL	Microliter
μm	Micrometer
μM	Micromolar
μg	Microgram
°C	Degree centigrade
%	Percent
<	Less than
>	More than
=	Equal