

Declaration

*I, Arup Karmakar hereby declare that the work embodied in my thesis entitled “**Studies on genetic variability and distribution of begomoviruses affecting economically important crops of Sub-Himalayan plains of North-east India**” has been carried out by me under the supervision of Prof. Aniruddha Saha, Department of Botany, University of North Bengal and Dr. Kajal Kumar Biswas, principal Scientist, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012 for the award of the Degree of Doctor of Philosophy in Botany. I also declare that, this thesis or any part thereof has not been submitted for any other degree /Diploma either to this or other university.*

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TO WHOM IT MAY CONCERN

This is to certify that the thesis entitled, "Studies on genetic variability and distribution of begomoviruses affecting economically important crops of Sub-Himalayan plains of North-east India" submitted by Mr. Arup Karmakar for the award of the degree of Doctor of Philosophy in Botany is based on the results of experiments carried out by him. Arup has worked under my supervision at Department of Botany, University of North Bengal and Co-supervision of Dr. Kajal Kumar Biswas, Principal Scientist, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012. I am forwarding his thesis for the Ph.D. degree (Science) in Botany of the University of North Bengal. He has fulfilled all requirements according to the rules of the University of North Bengal regarding the works embodied in his thesis. This thesis or any part thereof has not been submitted for any other degree / Diploma either to this or other University.

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It is certified that the thesis entitled, "Studies on genetic variability and distribution of begomoviruses affecting economically important crops of Sub-Himalayan plains of North-east India" submitted by Mr. Arup Karmakar for the Award of the Degree of Doctor of Philosophy in Botany is based on the results of experiments carried out by him. Arup has performed part of his thesis work under my Co-supervision at my Division of Plant Pathology, ICAR-Indian Agricultural Research Institute, New Delhi-110012 and under supervision of Dr. Aniruddha Saha at Department of Botany, University of North Bengal, Siliguri, West Bengal, India (734013). I am forwarding his thesis for the Ph.D. Degree (Science) in Botany of the University of North Bengal. He has fulfilled all the requirements accordingly the rules of the University of North Bengal regarding the works embodied in his thesis. This thesis or any part thereof has not been submitted for any other Degree/Diploma either to this or other University.

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Preface

Agriculture in India began several thousand years ago with the civilization of the Indus Valley. In 2018, as per Indian economic survey, agriculture employed 50% of the Indian work force and contributed 17–18% GDP of India. The contributions of agriculture in India have been increased over the years and share almost 20% of gross domestic product (GDP) for the first time in 17 years during the financial year 2020–2021. India is the 2nd largest producer of vegetable crops and first in net cropped area followed by the United States and China.

Among the various biotic and abiotic factors responsible for poor crop yield, viruses are one of the important pathogen group that cause significant losses in agriculture worldwide. They are responsible for global economic loss of about \$30 billion annually and nearly 50% of plant diseases, thus threatening global food security. Viral diseases can be disastrous in crops grown in areas where the socio-economic conditions are poor and inadequate with low technical support. Research should focus for improving food production and, therefore, health conditions especially for the groups that have low-income. On the basis of size, shape, chemical composition, structure of the genome and mode of replication, Viruses were grouped into several classes.

International Committee on Taxonomy of Viruses (ICTV) started to formulate and execute rules for naming and classification of viruses based on their genome structure. Among the plant viruses, *Begomovirus* is one of the largest genus of *Geminiviridae* family causes disease in several important crops such as cucurbitaceous, solanaceous, malvaceous vegetable and legume crops in most parts of the country. The most vulnerable affected crops are tomato, cucurbits, cotton, chilies, legumes, papaya, okra, and cassava. Till now, several begomoviral diseases have been described and identified from India and several other countries of the World.

The primary aims of disease management in plants are to reduce the economic and graceful damage caused by plant diseases. Application of chemical and pesticides that pollute both the crops and environment are being used less. Current management practices mostly depend on anticipating occurrence of disease and attacking weak points in the disease cycle. Therefore, disease management can be achieved by proper diagnosis of a disease that can help to identify the pathogen which is the actual target of the disease management program. In addition to this, proper plan of action need to be build up to permit with the strict regulations on chemical pesticide use. Various researchers throughout the world, working on disease management and focused their efforts to develop an alternative way instead of chemicals for controlling diseases.

The work of this thesis was started in the year 2015 with the objectives of understanding the occurrence of crop diseases caused by begomoviruses in sub-Himalayan plains of North-east India, about which very few reports of begomoviruses are known. Management of the diseases by inducing systemic resistance in plants by the application of chemical inducers and botanical extracts were studied. The thesis starts with an introduction where the objectives are given. A review on the present status of diagnosis and management of begomoviral disease in plants has been given in the next chapter. The materials and methods, results, discussion with reference to the present findings is represented in separate chapters. Bibliography and other supplementary details are given as appendix at the end of this thesis.

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Abbreviations

%	Percent
µg	Microgramme
AABA	α-amino butyric acid
AD	Anno Domini
APX	Ascorbate peroxidase
BABA	β-amino butyric acid
BLAST	Basic Local Alignment Search Tool
BLASTn	Nucleotide-BLAST
bp	Base pair
BTH	Benzothiadiazole
CaCl ₂	Calcium chloride
CAT	Catalase
ccc	Covalently closed circular
cc-ssDNA	Closed circular single stranded DNA
CD	Critical difference
Cm	centimetre
CP	Coat protein
CR	Common region
CRISPR	Clustered regularly interspaced palindromic repeats
CSR	Complementary strand replication
CTAB	Cetyl trimethyl ammonium bromide
DBIA	Dot blot immuno-binding assay
DIBA	Dot immunobinding assay
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded DNA
EBIA	Electro-blot immunoassay
EC	Enzyme Commission number
ELISA	Enzyme-linked immune sorbent assay
GABA	γ-aminobutyric acid
GPX	Guaiacol peroxidase
HDA	Helicase-dependent amplification

hr	Hour
IC-PCR	Immunocapture PCR
ICTV	International Committee on Taxonomy of viruses
IPTG	Isopropyl thiogalactoside / Isopropyl beta-D thiogalactopyranoside
IR	Intergenic region
ISEM	Immunosorbent electron microscopy
kb	Kilo-base pair
kDa	Kilodalton
LAMP	Loop-mediated isothermal amplification
LB	Luria-Berteni broth
MgCl ₂	Magnesium chloride
min	Minute
ml	Mililitre
MP	Movement protein
NASH	Nucleic acid spot hybridization
NGS	Next-generation sequencing
nm	nanometre
nt	nucleotide
ORFs	Open reading frames
OW	Old world
PAL	Phenylalanine ammonia lyase
PCR	Polymerase chain reaction
PDR	Pathogen-derived resistance
POD	Peroxidase
POX	Peroxidase
PPO	Polyphenol Oxidase
PTGS	Post transcriptional gene silencing
RCA	Rolling-circle amplification
RDR	Recombination dependent replication
REn	Replication enhancer protein
Rep	Replication-associated protein
RFLP	Restriction fragment length polymorphism

RIPs	Ribosome inactivating proteins
RPA	Recombinase polymerase amplification
rpm	Revolutions Per Minute
RT-PCR	Real-Time polymerase chain reaction
SCR	Satellite conserved region
SOD	Superoxide dismutases
ssDNA	Single-stranded DNA
TAE	Tris-Acetic acid-EDTA
TBIA	Tissue blotting immuno-binding assay
TEM	Transmission electron microscopy
TGS	Transcriptional gene silencing
TrAP	Transcriptional activator protein
X-Gal	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside
μl	Microlitre

Acronym of viruses

AEV	<i>Ageratum enation virus</i>
AYVV	<i>Ageratum Yellow Vein Virus</i>
BCMV	<i>Bean common mosaic virus</i>
BeYDV	<i>Bean yellow dwarf virus</i>
BGMV	<i>Bean golden mosaic virus</i>
BYVMV	<i>Bhendi Yellow Vein Mosaic Virus</i>
ChiLCAV	<i>Chilli leaf curl Ahmedabad virus</i>
ChiLCGV	<i>Chilli leaf curl Gonda virus</i>
ChiLCV	<i>Chilli leaf curl virus</i>
ChLCB	<i>Chili leaf curl betasatellite</i>
ChLCV	<i>Chilli leaf curl virus</i>
CLCuD	<i>Cotton leaf curl disease</i>
CLCuMB	<i>Cotton Leaf Curl Multan Betasatellite</i>
CLCuMV	<i>Cotton Leaf Curl Multan Virus</i>
CLCuV	<i>Cotton leaf curl virus</i>
CLKCoV	<i>Cotton leaf curl Kokhran virus</i>
CMV	<i>Cucumber mosaic virus</i>
CyTLCuD1	<i>Cyamopsis tetragonoloba leaf curl alphasatellite</i>
CYVMV	<i>Croton yellow vein mosaic virus</i>
DiAYVB	<i>Digera arvensis yellow vein betasatellite</i>
ELCV	<i>Enation leaf curl virus</i>
EuMVV	<i>Euphorbia mosaic Venezuela virus</i>
EuYMV	<i>Euphorbia yellow mosaic virus</i>
GMusSLA	<i>Gossypium mustelinium symptomless alphasatellite</i>
HYMV	<i>Horsegram yellow mosaic virus</i>
KLCuV	<i>Kenaf leaf curl virus</i>
MeCMV	<i>Melon chlorotic mosaic virus</i>
MeMV	<i>Merremia mosaic virus</i>
MeYVMV	<i>Mesta yellow vein mosaic virus</i>
MYMIV	<i>Mungbean yellow mosaic India virus</i>
MYMV	<i>Mungbean yellow mosaic virus</i>

MYVV	<i>Malvastrum yellow vein Virus</i>
PaLCV	<i>Papaya leaf curl virus</i>
PepGMV	<i>Pepper golden mosaic virus</i>
PepYLCTHV	<i>Pepper yellow leaf curl Thailand virus</i>
PeYVMLV	<i>Pepper yellow vein Mali virus</i>
PYLIV	<i>Pepper yellow leaf curl Indonesia virus</i>
PYMPV	<i>Potato yellow mosaic Panama virus</i>
PYMV	<i>Potato yellow mosaic virus</i>
PYVMD	<i>Pumpkin yellow vein mosaic disease</i>
PYVMV	<i>Pumpkin yellow vein mosaic virus</i>
RaLCV	<i>Radish leaf curl virus</i>
RgYMD	<i>Ridge gourd yellow mosaic disease</i>
RoLCuV	<i>Rose leaf curl virus</i>
SbCBV	<i>Soybean chlorotic blotch virus</i>
SbMMV	<i>Soybean mild mottle virus</i>
SiBYMV	<i>Sida bright yellow mosaic virus</i>
SiCMoV	<i>Sida chlorotic mottle virus</i>
SiGYSV	<i>Sida golden yellow spot virus</i>
SiMMV	<i>Sida micrantha mosaic virus</i>
SLCCNV	<i>Squash leaf curl China virus</i>
SLCCV	<i>Squash leaf curl China virus</i>
SLCV	<i>Squash leaf curl virus</i>
SPLCV	<i>Sweet potato leaf curl virus</i>
TbCSV	<i>Tobacco curly shoot virus</i>
TbLCTHV	<i>Tobacco leaf curl Thailand virus</i>
TbLCYNV	<i>Tobacco leaf curl Yunnan virus</i>
TGVV	<i>Tomato golden vein virus</i>
TLCCNV	<i>Tomato leaf curl China virus</i>
ToCLDV	<i>Tomato chlorotic leaf distortion virus</i>
ToDfLV	<i>Tomato dwarf leaf virus</i>
ToLCB	<i>Tomato leaf curl betasatellite</i>
ToLCBDB	<i>Tomato leaf curl Bangladesh betasatellite</i>
ToLCJAV	<i>Tomato leaf curl Java virus</i>

ToLCJoB	<i>Tomato leaf curl joydebpur betasatellite</i>
ToLCJV	<i>Tomato leaf curl Joydebpur virus</i>
ToLCKaV	<i>Tomato leaf curl Karnataka virus</i>
ToLCKunV	<i>Tomato leaf curl Kunene virus</i>
ToLCMLV	<i>Tomato leaf curl Mali virus</i>
ToLCNDV	<i>Tomato leaf curl New Delhi virus</i>
ToLCPaV	<i>Tomato leaf curl Palampur virus</i>
ToLCPV	<i>Tomato leaf curl palampur virus</i>
ToLCSiV	<i>Tomato leaf curl Sinaloa virus</i>
ToLCUV	<i>Tomato leaf curl Uganda virus</i>
ToLCV	<i>Tomato leaf curl virus</i>
ToLDeV	<i>Tomato leaf deformation virus</i>
ToMoWV	<i>Tomato mottle wrinkle virus</i>
ToSLCV	<i>Tomato severe leaf curl virus</i>
ToSRV	<i>Tomato severe rugose virus</i>
ToTLV	<i>Tomato twisted leaf virus</i>
ToVCLDeV	<i>Tomato vein clearing leaf deformation virus</i>
ToYLCRB	<i>Tomato yellow leaf curl Rajasthan betasatellite</i>
ToYSV	<i>Tomato yellow spot virus</i>
ToYVSV	<i>Tomato yellow vein streak virus</i>
TYLCCNV	<i>Tomato Yellow Leaf Curl China Virus</i>
TYLCIV	<i>Tomato yellow leaf curl Indonesia virus</i>
TYLCMLV	<i>Tomato yellow leaf curl Mali virus</i>
TYLCSV	<i>Tomato yellow leaf curl Sardinia virus</i>
TYLCSV-[Sic]	<i>Tomato yellow leaf curl Sardinia virus</i>
TYLCTHB	<i>Tomato yellow leaf curl Thailand betasatellite</i>
TYLCTHV	<i>Tomato yellow leaf curl Thailand virus</i>
TYLCV	<i>Tomato yellow leaf curl virus</i>
TYMLCV	<i>Tomato yellow margin leaf curl virus</i>
TYMoV	<i>Tomato yellow mottle virus</i>
VeYVV	<i>Vernonia yellow vein virus</i>
VeYVVB	<i>Vernonia yellow vein virus betasatellite</i>