USING PLANT MODEL SYSTEMS TO CHARACTERIZE GEMINIVIRUSES THAT REDUCE CROP PRODUCTIVITY

CYPRIAN ALOYCE RAJABU

DOCTOR OF PHILOSOPHY

(Plant Health Science and Management)

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

2018

Using Plant Model Systems to Characterize Geminivirus Infections that Reduce Crop Productivity

Cyprian Aloyce Rajabu

A thesis submitted in partial fulfillment for the degree of Doctor of Philosophy in Plant Health Science and Management in the Jomo Kenyatta University of Agriculture and Technology

2018

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

Signature: ……... Date: ……..

Cyprian Aloyce Rajabu

This thesis has been submitted for examination with our approval as the university supervisors

Signature: …….. Date: ……..

Prof. Elijah Miinda Ateka (PhD)

JKUAT, Kenya

Signature: …….. Date: ……..

Dr. Fred Tairo (PhD) 08/11/2018

Mikocheni Agricultural Research Institute, Tanzania

Signature: …… Date: ……..

Prof. Linda Hanley-Bowdoin (PhD)

North Carolina State University, USA

DEDICATION

This thesis is dedicated to my wife and my two sons whose affection, love, encouragement and prayers made me able to get such success and honor. Every challenging work needs self-effort as well as guidance of elders, I also dedicate this work to my sweet and loving mother and my late father who always loved me unconditionally and taught me to work hard for things I aspire to achieve in life since I was little boy.

ACKNOWLEDGEMENTS

I thank all who in one way or another contributed in the completion of this thesis. Thanks to merciful lord for all the countless gifts you have offered me, and thanks to my family for their love and support.

It is with immense gratitude that I acknowledge the support and help of my supervisors Professor Elijah Miinda Ateka of Jommo Kenyatta University of Agriculture and Technology (JKUAT) Kenya, Professor Linda Hanley-Bowdoin and Professor Jose Ascencio Ibanez of North Carolina State University (NCSU), Dr. Fred Tairo, Dr. Peter Sseruwagi and Dr. Joseph Ndunguru of Mikocheni Agricultural Research Institute (MARI), Dar es Salaam Tanzania for their helpful guidance, support and contributions.

I owe my deepest gratitude to NCSU MSc. student Jonatan Isaksson, Maxie G. Jollie and Daniel Bayha; undergraduate students, Yamilex Rosado, Ananya Talikoti, Vanessa Ly, Preslyn Philips, Avery Roberts, Jordan Poe, Sydney Tickle and Mikiel Ewida, for their help in implementing some of the activities.

I share the credit of my work with Mary Beth Dallas, Wei Shen, Mari Ines Reyes, Catherine Doyle, Anna Dye, David Deppong and Lorenzo Concia for their technical assistance, helpful ideas and encouragement.

I am indebted to my many colleagues at MARI who supported me, Doreen Mgonja, Deogratius Mark, Joel Erasto, Margareth Lupembe and Christina Kidulile.

This study was supported by grants from the Bill & Melinda Gates Foundation to Mikocheni Agricultural Research Institute and North Carolina State University.

TABLE OF CONTENT

DECLARATION ..... **ii**

DEDICATION ..... **iii**

ACKNOWLEDGEMENTS ..... **iv**

TABLE OF CONTENT ..... **v**

LIST OF TABLES ..... **xii**

LIST OF FIGURES ..... **xiii**

LIST OF APPENDICES ..... **xvi**

ACRONYMS AND ABBREVIATIONS ..... **xvii**

ABSTRACT ..... **xxi**

CHAPTER ONE **1**

INTRODUCTION **1**

1.1 General introduction 1

1.2 Problem statement and justification 3

1.3 Research questions 5

1.4 Objectives 5

1.4.1 General Objective 5

1.4.2 Specific objectives 5

1.5 Null hypotheses 5

CHAPTER TWO 6

LITERATURE REVIEW 6

2.1 Geminivirus diseases in important crops 6

2.1.1 Cassava 7

2.1.2 Tomato 8

2.2 Disease control strategies 8

2.2.1 Phytosanitation 9

2.2.2 Use of resistant planting materials 10

2.2.3 Quarantine regulations 11

2.3 Geminiviruses 11

2.3.1 Background 11

2.3.2 Classification and transmission 12

2.3.3 Geminivirus genome organization 15

2.3.4 Functions of viral genes 16

2.4 Begomovirus replication 18

2.4.1 Rolling circle/recombination-based replication 19

2.4.2 Recombination-dependent replication 20

2.5 Begomovirus transcription 20

2.6 Begomovirus movement 21

2.7 DNA molecules associated with Begomoviruses 21

2.7.1 Satellites 21

2.7.2 Sequences enhancing geminivirus symptoms (SEGS-1 and SEGS-2) 23

2.8 Virus-host interactions 25

2.8.1 Requirements for a successful viral infection 25

2.8.2 Virus infection cycle 26

2.8.3 Virus defenses 26

2.8.4 Virus impact on host factors 28

2.8.4.1 Reprogramming plant cell cycle controls 28

2.8.4.2 The endocycle and viral DNA replication 29

2.8.4.3 Disruption of plant signaling pathways 30

2.9 Analysis of plant virus - host interactions 31

2.9.1 Model host vs natural host systems 31

2.9.2 Arabidopsis thaliana as a model plant 32

2.9.3 Tomato as a model plant 32

2.10 Translation of information from model systems to crops 34

CHAPTER THREE 35

SUITABILITY OF TOMATO AS A MODEL FOR STUDYING GEMINIVIRUS-HOST INTERACTION 35

3.1 Introduction 35

3.2 Materials and methods 38

3.2.1 Plant growth conditions and inoculation protocols 38

3.2.2 Plant inspection and data collection 41

3.2.3 DNA extraction and virus detection 43

3.2.4 DNA ploidy levels 44

3.2.5 Statistical analysis 44

3.3 Results 45

3.3.1 Symptom expression 45

3.3.2 Virus titer 53

3.3.3 Plant height 54

3.3.4 Yield 55

3.3.5 DNA ploidy.....57

3.4 Discussion.....60

CHAPTER FOUR.....64

CHARACTERIZE THE MECHANISM OF GEMINIVIRUS RESISTANCE IN ARABIDOPSIS 64

4.1 Introduction 65

4.2 Materials and methods.....67

4.2.1 Plant growth.....67

4.2.2 Plasmid construction.....67

4.2.3 CaLCuV VIGS of Arabidopsis accessions 68

4.2.4 Microprojectile bombardment of individual leaves with CaLCuV 68

4.2.5 CaLCuV, BCTV and TYLCV agroinoculation and detection.....69

4.2.6 TRV VIGS 69

4.2.7 TRV quantification 70

4.2.8 Genotyping and QTL mapping.....70

4.3 Results 71

4.3.1 Diverse responses to the VIGS vector 71

4.3.2 Lack of viral DNA accumulation in Pla-1 74

4.3.3 Pla-1 is also immune to a Curtovirus....76

4.3.4 Pla-1 shows resistance to TYLCV infection....78

4.3.5 Pla-1 is susceptible to RNA viruses....79

4.3.6 Immunity maps to chromosome 1....81

4.4 Discussion....84

CHAPTER FIVE....90

CHARACTERIZATION OF CMB INFECTION IN ARABIDOPSIS IN THE PRESENCE AND ABSENCE OF SEGS-1....90

5.1 Introduction....90

5.2 Materials and methods....93

5.2.1 Construction of SEGS-1 clones for bombardment....93

5.2.2 Construction of the SEGS-1 clones for Agrobacterium transformation....93

5.2.3 Plant Material and Growth Conditions....94

5.2.4 *Arabidopsis* Transformation....94

5.2.5 Plant inoculation, sample collection and DNA isolation....94

5.2.6 *Nicotiana tabacum* protoplast assays....96

5.2.7 In situ hybridization and immunohistochemistry....96

5.2.8 Analysis of SEGS-1 episomes....97

5.2.9 Analysis of SEGS-1 episomes 98

5.3 Results 100

5.3.1 SEGS-1 enhances ACMV symptoms in Arabidopsis 100

5.3.2 SEGS-1 transgene enhances CMD symptoms in Arabidopsis 106

5.3.3 SEGS-1 breaks Arabidopsis Pla-1 resistance to Cabbage leaf curl virus 109

5.4 Discussion 111

CHAPTER SIX 115

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS 115

6.1 General discussion 115

6.2 Conclusions 119

6.3 Recommendations 120

REFERENCES 122

APPENDICES 169

LIST OF TABLES

Table 3.1: Infectious viral clones used to inoculate 'Florida Lanai' plants by agroinoculation or biolistics. 40

Table 3.2: List of primers used for PCR amplification of viruses in this study. 42

Table 3.3: Comparison between infected and healthy tomato plants for the change in height at different days after inoculation. 48

Table 3.4: Effect of TYLCV, ToMoV and BCTV on yield. 56

Table 3.5: One-way analysis of variance (ANOVA) for means of virus titer (copy number) for TYLCV, ToMoV and BCTV. 56

Table 3.6: Difference between means and significance of pairwise comparison (LSD) for means of virus copy number for TYLCV, ToMoV and BCTV at different days post inoculation. Differences indicated * are significant at the $\alpha < 0.05$ level and ** are significant at the $\alpha < 0.01$ level. 57

Table 5.1: List of primers used for PCR amplification in this study 98

Table 5.2: Infectious viral and SEGS-1 clones used to inoculate plants by agroinoculation or biolistics. 99

Table 5.3: Transformation efficiencies of floral dip Arabidopsis Sei-0 transformed with SEGS-1 clones by Agrobacterium mediated floral dip method. 106

LIST OF FIGURES

Figure 2.1: Genomic organisation of begomoviruses and their associated satellite DNAs. 17

Figure 2.2: Sequence organization of SEGS-1 and SEGS-2, showing a linear map of cloned sequences with GC-rich regions and flanking repeated sequences (green)..... 25

Figure 3.1: Comparison at 45 day-old A: Florida Lanai and B: Micro-Tom tomato varieties..... 45

Figure 3.2: Symptoms observed on Florida Lanai plants mock- and agro-inoculated with TYLCV, ToMoV and BCTV. 46

Figure 3.3: Florida Lanai recovering from ToMoV infection. A: Infection at 14 dpi, B: Infection at 28 dpi, C: Impact of recovery on yield (i) ToMoV, (ii) TYLCV and (iii) BCTV 50

Figure 3.4: Symptoms on inoculated Florida Lanai by biolistics using A: ToMoV and B: TGMV. Bottom row: Florida Lanai infected by whitefly transmission using C: TYLCV and D: ToMoV, showing severe and very mild symptoms respectively. 52

Figure 3.5: Change in plant height for Florida-Lanai tomato plants infected with TYLCV, ToMoV, BCTV and mock at different days post inoculation. Vertical bars represent the standard error (SE) of the means. N=10 for all treatments. 53

Figure 3.6: Histogram of the relative fluorescence intensity of nuclei isolated from leaves of Lanai plants either mock-inoculated or inoculated with ToMoV,

BCTV or TYLCV. The bars represent ploidy percentages for each treatment. Values indicated by * are statistically significant ($P < 0.05$). .58

Figure 3.7: Changes in viral load over time for Florida Lanai infected with A: TYLVC, B: ToMoV and C: BCTV. Vertical bars represent the standard error (SE) of the means. N=7 for all treatments 59

Figure 4.1: Response of different Arabidopsis accessions to the CaLCuVA:CH-42 virus-induced gene silencing (VIGS) vector (a–f) or wild-type CaLCuV (g and h).....72

Figure 4.2: Pla-1 is immune to wild-type CaLCuV. 75

Figure 4.3: Pla-1 is resistant to agroinoculation with CaLCuV, BCTV and TYLCV 77

Figure 4.4: Pla-1 shows reduced virus-induced gene silencing (VIGS) and susceptibility to TRV:AtPDS.....80

Figure 4.5: Quantitative trait locus (QTL) associated with CaLCuV symptoms are located on chromosome 1 of Pla-1. 83

Figure 5.1: SEGS-1 enhances ACMV symptoms in the susceptible Arabidopsis accession Sei-0. 101

Figure 5.2: SEGS-1 enhances ACMV DNA accumulation in the susceptible Arabidopsis accession Sei-0. 104

Figure 5.3: SEGS-1 enhances ACMV DNA-A replication in tobacco protoplasts. 105

Figure 5.4: A SEGS-1 transgene enhances ACMV infection in Arabidopsis SEI-0 plants.....108

Figure 5.5: Exogenous SEGS-1 breaks endogenous resistance in the Arabidopsis accession Pla-1. 110

LIST OF APPENDICES

Appendix I: Tables.....169

Appendix II: Plates.....208

ACRONYMS AND ABBREVIATIONS

ACMBFV *African cassava mosaic Burkina Faso virus*

ACMV *African cassava mosaic virus*

ANOVA Analysis of Variance

BCTV *Beet curly top virus*

CBSD *Cassava brown streak disease*

CMBs Cassava Mosaic Begomoviruses

CMD Cassava mosaic disease

CMGs Cassava mosaic geminiviruses

CMMGV *Cassava mosaic Madagascar virus*

CP Coat protein

CR Common region

CTAB Cetyl trimethyl ammonium bromide

DNA Deoxyribonucleic acid

dNTPs Deoxynucleotides

DPI Days of post-inoculation

EACMCV *East African cassava mosaic Cameroon virus*

EACMKV *East African cassava mosaic Kenya virus*

EACMMV *East African cassava mosaic Malawi virus*

EACMV East *African cassava mosaic virus*

EACMV-UG East *African cassava mosaic virus* Uganda variant

EACMZV East *African cassava mosaic Zanzibar virus*

FAOSTAT Food and Agriculture Organization Statistics

HR Hypersensitive response

ICMV *Indian cassava mosaic virus*

ICTV International Committee on Taxonomy of Viruses

IR Intergenic region

JA Jasmonic Acid

NSP Nuclear shuttle protein

NW New World

ORF Open reading frame

Ori Origin of replication

OW Old World

PCR Polymerase chain reaction

QTL	Quantitative trait loci
RCA	Rolling circle amplification
RCR	Rolling circle replication
RDR	Recombination-dependent replication
REn	Replication enhancer protein
Rep	Replication associated protein
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
RNAi	RNA interference
RT-PCR	Reverse transcription polymerase chain reaction
SACMV	South *African cassava mosaic virus*
siRNA	Small interfering RNA
SLCMV	*Sri-Lankan cassava mosaic virus*
ssDNA	Single-stranded DNA
TGS	Transcriptional gene silencing
TrAP	Transcriptional activation protein
TYLCSV	*Tomato yellow leaf curl Sardinia virus*

TYLCV *Tomato yellow leaf curl*

UCBSV *Ugandan Cassava brown streak virus*

ABSTRACT

Geminiviruses are a group of single-stranded DNA viruses that cause devastating diseases in a wide variety of crops world wide. The family *Geminiviridae* consists of nine genera, many of which have been shown to be transmitted by insect vectors. In tropical and subtropical areas of the world, geminiviruses affect cassava and tomato, which are important food and cash crops for small holder farmers. To design management strategies, information about geminivirus-host interactions and endogenous host resistance is required. The use of model host systems can facilitate acquisition of key information in a timely and cost-effective manner. In the first aim, 'Florida Lanai', a small fast-growing tomato variety, was broadly characterized to establish its suitability as a model system for studying geminiviruses. Infection by three begomoviruses (*Tomato yellow leaf curl virus*, TYLCV; *Tomato mottle virus*, ToMoV; *Tomato golden mosaic virus*, TGMV) and a curtovirus (*Beet curly top virus*, BCTV) was examined. Agroinoculation of TYLCV, ToMoV and BCTV, mechanical inoculation of ToMoV or TGMV using a microsprayer, and whitefly transmission of TYLCV or ToMoV resulted in 100% infection efficiency and rapid disease progress reflected by strong disease symptoms and viral DNA accumulation. Infection had measurable impacts on plant height, flowering and fruit number. Florida Lanai's small size is suitable for limited laboratory space, and its short life cycle allows completion of experiments in a short time. These properties established Florida Lanai as a suitable model host for studying geminiviruses infecting tomato. In the second aim, a virus-induced gene silencing (VIGS) vector derived from the begomovirus, *Cabbage leaf curl virus* (CaLCuV), was used to assess natural variation in virus–host interactions in 2003 *Arabidopsis thaliana* accessions. The screen identified the Pla-1 accession as the only ecotype to show strong resistance to CaLCuV. A major resistance QTL was mapped onto Chromosome 1 using Pla-1 x Col-0 crosses. The resistance is recessive, quantitative and broad-based. Identification of this QTL provides a platform for future research identifying and deploying the corresponding resistance gene in crop plants. In the third aim, SEGS-1, a sequence from the cassava genome that enhances geminivirus symptoms, was characterized in wild-type *Arabidopsis* Sei-0 plants, plants carrying a SEGS-1 transgene, and *Nicotiana tabacum* suspension cells. SEGS-1 increased symptoms development, viral DNA levels and the number of infected cells in both wild-type and transgenic *Arabidopsis* co-inoculated with *African cassava mosaic virus* (ACMV). SEGS-1 also broke host resistance to CaLCuV in co-inoculation experiments of Pla-1 accession, similar to previous results when a resistant cassava cultivar was co-inoculated with a cassava begomovirus and SEGS-1. However, unlike cassava, no SEGS-1 episomes were detected in infected *Arabidopsis* plants. *Nicotiana tabacum* cells also accumulated higher levels of ACMV DNA-A when co-inoculated with SEGS-1. Together, these results demonstrated that SEGS-1 can function with a heterologous host and begomovirus to increase disease and break resistance. Moreover, SEGS-1 can function in a genomic context, indicating that SEGS-1 episomes are not required for disease

enhancement. This information sheds light on the role of SEGS-1 in virus infection and will be useful while formulating management strategies.

CHAPTER ONE

INTRODUCTION

1.1 General introduction

Geminiviruses are plant pathogens that cause economically important diseases in tropical and subtropical regions of the world. They are responsible for many important diseases in staple crops, vegetables, fruits, ornamentals and pastures. Across Africa, geminiviruses are associated with serious losses in cassava (Ndunguru *et al*, 2016; Legg & Fauquet, 2004), tomato (Rajabu *et al*., 2018; Moriones & Navas-Castillo, 2000; Picó *et al*., 1996), common beans (Kim *et al*., 1978) and maize (Shepherd *et al*., 2010).

In sub-Saharan Africa, cassava is one of the most important food and cash crops. The geminiviruses, Cassava mosaic begomoviruses (CMBs), cause a wide range of symptoms and yield losses in cassava. Fargette *et al*. (1988) estimated yield losses varying from 20 to 95% in farmers' fields.

Tomato is also a major vegetable crop in sub-Saharan Africa and is grown in practically every country. Tomato is important as a commercial and a dietary vegetable. Tomato is a good source of vitamins A and C and contains lycopene, a powerful antioxidant (Hedges, & Lister, 2005). Geminiviruses such as Tomato yellow leaf curl (TYLCV) cause estimated yield losses of up to 100% (Rajabu *et al*., 2018; Moriones & Navas-Castillo, 2000). Despite tremendous efforts for intervention, viral diseases are difficult to control or eradicate. Tomato genome has been fully sequenced, and it contains 35,000 genes arranged on 12 chromosomes. The genes cover characteristics such as pathogen resistance, nutritional content and taste. Sequences of these genes and their arrangement in the cromosomes have been described (Tomato Genome Consortium, 2012). Available information makes molecular studies in tomato more informative and reliable.

Since Cassava mosaic disease (CMD) was first reported (Warburg, 1894), management initiatives to mitigate the effect of the disease have been continuous. Focus has mostly been on phytosanitation and breeding for resistance against the viruses. The first and most widely used source of resistance to CMBs was derived from the Ceara rubber tree, *Manihot glaziovii* Müll. Arg. (Jennings, 1976). More resistance work has been done using molecular breeding (Lokko *et al*., 2005; Ribeiro *et al*., 2012) and genetic engineering (Zhang *et al*., 2005; Vanderschuren *et al*., 2007, 2009). Despite the efforts, CMBs are still a persistent problem and continue to invade new areas, causing losses and threatening food security and income.

Fondong *et al*. (2000) reported that the symptoms and impact on yield depend on whether a cassava plant is infected with a single CMB or co-infected by multiple viruses that interact synergistically to cause more severe disease. Another factor determining the amount of disease expression and yield loss is cultivar susceptibility. Cassava cultivars exhibit different levels of resistance to CMB infection. Yield loss is a result of the physiological changes in cassava following infection by CMBs and is more pronounced in susceptible cultivars. Satellite molecules such as Betasatellites and Alphasatellites, which have also been implicated in causing more severe infections, are often associated with geminiviruses (Briddon *et al*., 2003; Briddon & Stanley, 2006).

In recent years, severe CMD and yield losses in cassava have been associated with presence of two DNA molecules designated as sequences enhancing geminivirus symptoms (SEGS-1 and SEGS-2) (Ndunguru *et al*., 2016). In controlled inoculation experiments, both SEGS-1 and SEGS-2 enhance CMD symptoms, and SEGS-1 breaks CMD2-mediated resistance in cassava landraces (Ndunguru *et al*. 2016). The cassava genome contains sequences related to SEGS-1 and SEGS-2. Due to the universal occurrence of SEGS-related sequences in the cassava genome, they pose a new threat to cassava production, and research is necessary to understand how the SEGS interact with CMBs to enhance symptoms and break resistance.

1.2 Problem statement and justification

Food security is a top priority of every society in sub-Saharan Africa Unfortunately, sustainability of food security is threatened by viral diseases. Geminiviruses evolve rapidly through mutation, genetic drift and recombination, resulting in new viruses or strains that can be more virulent than the parental viruses (Rojas *et al*., 2005). The ever-increasing diversity of geminiviruses have allowed them to adopt to new hosts and spread to new geographical locations (Rocha *et al*., 2013; Monci *et al* 2002). These properties make geminivirus diseases difficult to control.

Management of plant viruses is of vital importance to reduce the damage leading to yield losses (Sastry & Zitter, 2014). Disease management strategies need extensive knowledge of virus infection, transmission, spread and host interactions to be able to select the best control measures (Rodrigues *et al*., 2009). Model system in plant biology is a research plant that is used to understand particular biological phenomena about other plants. Studying viruses in their host plant can be difficult because of the complexity of the pathosystems and the limited availability of research tools for many crops. Some complex pathosystems also contain genetic alterations or mutations with a potential to interfere with the quality of experimental results.

Studying viruses can be simplified if a tractable host system is available. Hence, developing plant models to study geminivirus-host interactions may aid in the design of virus management strategies

Tomato is susceptible to a wide range of viral diseases, many of which are associated with significant agronomic losses (Hansen *et al*., 2010; Inoue-Nagata *et al*., 2016). Of the 322 begomoviruses recognized by the International Committee on Taxonomy of Viruses, more than a third infect tomato, underscoring the importance of having a suitable tomato variety for virus studies.

Tomato has long been the preferred system for studying plant-pathogen interactions involving plants from the Solanaceae family (Arie *et al*., 2007), but there is considerable physiological and genetic variation among tomato varieties that affects their suitability for laboratory studies. A good model plant should be easy to grow and maintain, have a short generation time and a well annotated genome, and allow the use of different tools such as various inoculation methods. Model plants are also usually inexpensive to study and readily accessible (Meissner *et al*., 1997; Matsukura *et al.*, 2008).

Cassava, a major factor in food security across sub-Saharan Africa, is susceptible to losses due viral diseases. Two most important of these diseases are African cassava mosaic disease (CMD) and Cassava brown streak disease (CBSD). CMD is caused by cassava mosaic begomoviruses (CMBs) in the *Geminiviridae* family. CBSD is caused by cassava brown streak viruses (CBSVs), single-stranded RNA viruses in the genus Ipomovirus of the family *Potyviridae* (Monger *et al*., 2001; Hillocks and Jennings, 2003). In farmers' fields, it is common to find both CMBs and CBSVs co-infecting a cassava plant, but there is little information about whether the SEGS alter co-infections.

Recently, the severity of CMD symptoms and yield loses have been associated with SEGS-1 and SEGS-2 (Ndunguru *et al*., 2016), but it is not known how the SEGS impact CMD resistance and symptoms. There is no information regarding SEGS interactions with CMBs or the cassava genome. For example, the mechanism employed by SEGS-1 to break the host resistance in the CMD2 resistant TME 3 is unknown. Furthermore, it is still not clear whether the SEGS interact directly with CMBs or indirectly by interfering with or activating host factors that impact disease processes. The cassava genome contains multiple sequences related to SEGS-1, including one sequence that is 99% identical to full-length SEGS-1.

A first step toward addressing these questions is to characterize how the SEGS impact the host at a global level during CMB infection. Given that all cassava germplasms tested to date contain sequences related to the SEGS in their genomes, it is not possible to fully separate interactions due to endogenous and exogenously introduced SEGS.

Arabidopsis plants do not possess SEGS-like sequences in their genomes and provide a clear advantage by eliminating confounding factors due to the genomic copies of SEGS in the cassava genome. Other advantages of *Arabidopsis* include a fast generation time, a well annotated genome, and the availability of mutants in many pathways. To address the knowledge gaps regarding SEGS and CMBs interactions, The study used *Arabidopsis* plants and tobacco protoplasts to characterize how SEGS-1 increase viral disease severity and therefore provide insights on how to better manage CMD.

1.3 Research questions

How do we study complex pathosystems using model plants to generate reliable information relevant to the natural host?

1.4 Objectives

1.4.1 General Objective

The general objective of this study was to generate information on geminivirus-plant interactions

1.4.2 Specific objectives

i. To asses suitability of tomato as a model for studying geminivirus-host interaction
ii. To characterize the mechanism of geminivirus resistance in *Arabidopsis*
iii. To characterize cassava mosaic begomoviruses infection in Arabidopsis plants in the presence and absence of SEGS-1

1.5 Null hypotheses

i. Model plants cannot be used to characterize geminivirus infection
ii. Arabidopsis does not have geminivirus resistance genes
iii. SEGS-1 has no impact on Geminivirus infection in Arabidopsis

CHAPTER TWO

LITERATURE REVIEW

2.1 Geminivirus diseases in important crops

Geminiviruses infect a wide variety of plants, including crops, forage, ornamentals and weeds, and cause a huge economical loss to agriculture and horticulture worldwide. Geminiviruses are transmitted mainly from one infected plant to a healthy plant through insect vectors. Weeds and native plants serve as reservoirs or as alternative hosts for geminiviruses (Ooi *et al*., 1997; Jovel *et al*., 2004; Frischmuth *et al*., 1997). Reservoir plants, which are widespread and highly adaptable, intensify the threat of spread and reoccurrence of geminivirus diseases in cultivated plants.

Geminiviruses cause serious losses in diverse crop species including those important for food security in the developing world. For example, cassava mosaic diseases in sub-Saharan Africa which caused the severe CMD pandemic in the 1990s (Gibson, 1996; Bosque-Perez, 2000). In tomato geminiviruses cause *Tomato yellow leaf curl virus* disease, *Tomato mottle virus* disease and *Beet curl top virus* disease which cause up to 100% in yield loss (Rajabu *et al*., 2018). Geminiviruses also cause Maize streak virus disease a significant maize disease in countries in Eastern and Southern Africa causing estimated 20 to 100% yield loss (Thottappilly *et al*., 1993). Other economically important viral diseases caused by geminiviruses include; *Bean golden mosaic* causing 10 to 100% yield loss of beans (Blair *et al*., 1995; Faria & Maxwell, 1999) and Yellow mosaic disease causing 10 to 90% decrease in grain legumes (Malathi *et al*., 2005).

Cassava and tomato are considered to be two of the most economically important food and cash crops in sub-Saharan Africa. Studying geminivirus in tomato and cassava is significantly important to generate information needed to formulate sustainable management strategies and to inform about other crops.

2.1.1 Cassava

Cassava (*Manihot esculenta* Crantz) is one of the world's most important food crops (Nassar & Ortiz, 2010; Legg *et al*., 2011). It is cultivated in Asia, Africa and Latin America for its storage roots, which are sources of carbohydrate for more than 800 million people (FAO, 2016). Cassava is drought tolerant and thrives and produce stable yield on poor soils in which other crops fail. The broad agro-ecological adaptability of cassava and its ability to produce reasonable yields in poor conditions makes it the basis for food security and an important source of dietary energy at the household level for smallholder farmers in sub-Saharan Africa. In some areas, cassava leaves are also used as green vegetables to supplement important protein lacking in cassava roots. Yeoh and Chew (1976) reported that based on dry leaf weight, cassava protein content ranges from 29 to 39%, with leaf protein production ranging from 242 to 953 Kg/ha. Additionally, cassava leaves are an important source of some essential amino acids (Yeoh & Chew, 1976).

World production of cassava is estimated at 277 million tons on 23 million ha (FAOSTAT, 2016). Africa produces 157 million tons on 17 million ha of land. World cassava productivity is estimated at 13 tons/ha while that of Africa is 10.2 tons/ha (FAOSTAT, 2016). Cassava leaf production in 2016 was 7.8 tons, all of which came from Africa (FAOSTAT, 2016). Cassava is also an important economical crop that generates vital income for smallholder farmers. It is estimated that about 10% of all cassava roots are used in trade (FAOSTAT, 2016). Trade involves selling cassava roots as food and for industrial use. Industrial use of cassava is mainly in the production of commodities such as starch for textile industries, binding agents, ethanol, paper and flavouring agents for Asian cooking. Given its use as an economical source of industrial raw materials, cassava has the potential to contribute significantly to improved livelihoods leading to a transformation of rural African economies and adding severely lacking foreign exchange income to national economies.

2.1.2 Tomato

Tomato (*Solanum lycopersicon*) is in the family *Solanacaea* and the genus *Solanum*. It is herbaceous plant with hundreds of varieties that differ in size and generation time. Tomato is one of the most important vegetable crops in the world. The fruit is rich in lycopene, a strong antioxidant that has beneficial health effects. It is also an important source of vitamins A and C. Tomato is consumed in diverse ways, including raw, as an ingredient in many dishes and sauces, and in drinks. While it is botanically a fruit, it is considered a vegetable for culinary purposes. Present world tomato production is about 177 million tons fresh fruit produced on 4.8 million hectares. Africa produces an estimated 19.8 million tones on 1.3 million ha of land. Productivity of tomato in Africa is very low (17.2 tones/ha) as compared to the world (40.8 tones/ha) (FAOSTAT, 2016). Tomato is susceptible to a wide range of viral diseases, many of which cause significant agronomic losses. The amount of loss can vary greatly from minimal to total (100%), depending on plant variety, virus strain, plant age at infection time and presence of other diseases (Rajabu *et al*., 2018; Hansen *et al*., 2010; Inoue-Nagata *et al*., 2016). Tomato yellow leaf curl disease is caused by begomoviruses and has spread worldwide to become one of the most important viral diseases of tomato (Lefeuvre *et al*., 2010).

2.2 Disease control strategies

The *Geminiviridae* was listed as a family in ICTV for the first time in 1993 (Mayo & Martelli, 1993; Pringle, 1993). Due to high rates of mutation, recombination and reassortment, the number of species has increased tremendously since then (Rojas *et al*., 2005; Inoue-Nagata *et al*., 2006). Geminivirus has been the subject of considerable research, which has intensified even more with the occurrences of new viruses and more virulent strains spreading to new geographical locations. An example is the emergence of *East African cassava mosaic virus*-Uganda (EACMV-UG) and the severe CMD pandemic that spread wide in eastern and central Africa in the 1990s (Legg *et al*., 1999) and the movement of TYLCV from the Mediterranean area to the New World. Several strategies described below have been developed and deployed to control CMD.

2.2.1 Phytosanitation

Phytosanitation involves the removal or destruction of infected plant material that is a source of inoculum for further spread of the disease. Three main features of phytosanitation for geminivirus control are (i) crop hygiene (ii) disease-free planting material, (iii) and removal of diseased plants. The phytosanitation approach is aimed at preventing or delaying virus infection to reduce damage and provide sufficient time to produce a crop before the plant is overwhelmed by the disease.

Crop hygiene involves removal of all diseased cassava or other host plants within and immediately around sites of new plants. This is a conventional means of limiting pests and diseases from re-occurring from previous crops to decrease the risk of carryover of pathogens to new plants. However, diagnosis and detection procedures based only on symptom observation are often unreliable (Zrachya *et al*., 2007). Late virus infection may not result into observable symptoms at the time of harvest, and some infected cultivars are asymptomatic and not identified as diseased. In addition, this strategy is limited by the fact that many viruses have reservoir hosts that act as foci of infection for spread into crops (Bos, 1981; Thresh, 1981). Little is known about alternative hosts of many important geminiviruses, making it impossible to identify and destroy them.

Disease-free planting material is necessary because cuttings are sources for spreading geminiviruses if they are obtained from infected plants. Ensuring that farmers have access to disease-free planting material of good quality is one of the most important elements for a successful agricultural production and development (Fajardo *et al*., 2010). Procedures for obtaining geminivirus-free planting materials can be through careful visual selection from source plants maintained in a vector-free environment. Due to latent infection, it is important for the selected plants to go through established procedures for virus indexing, such as regular testing for viral DNA using polymerase chain reaction (PCR). The generation of disease-free planting materials from infected plants via tissue culture technology can also be used to produce planting materials (Walkey *et al*., 1987; Wang & Hu, 1980).

Rogueing is the removal of diseased plants in a field. This practice requires knowledge of the disease and associated symptoms, and plants must display symptoms for rogueing to be successful. Rogueing is widely applicable and has been used in attempts to control or contain diseases of diverse crops in both temperate and tropical regions (Thresh, 1988), but it is only effective when symptoms are conspicuous, and the symptomatic plants are removed early. Hence, rogueing can have a significant impact for susceptible cultivars but is less effective for resistant or tolerant cultivars.

2.2.2 Use of resistant planting materials

Plant disease resistance is a genetic trait that varies between cultivars, species or genus. Resistance to a given disease is a stable characteristic based on the genetic composition of the plant. Naturally occurring resistance in crops and their wild relatives is exploited by breeders and researchers to introduce or concentrate desirable resistance genes for crop improvement.

Farmers have practiced breeding since the introduction of modern crops. Crop breading has been aimed at adding traits of agricultural importance to crops such as seed/fruit size, yield, storability, taste, color and disease resistance. Conventional breeding for geminivirus resistance has been done in many crops including common bean (Blair & Morales, 2008), Cassava (Okogbenin *et al*., 2007), tomato (Vidavsky & Czosnek, 1998) and maize (Welz *et al*., 1998). Genetically engineered disease resistance is more complex and needs considerable knowledge and facilities to identify, clone and characterize resistance genes. Equally, knowledge and facilities are also needed to introduce and evaluate transgenic resistance genes in plants.

CMD2 a major Geminivirus resistance loci from Nigerian landrace, TMEB3 (Akano *et al.,* 2002) has been introduced in cassava to mediate resistance to CMD, resulting into high yielding cassava varieties that are resistant or tolerant to Geminiviruses (Okogbenin *et al.,* 2012, Rabbi *et al.,* 2014). Such resistance however has been reported to break in some varieties when introduced to farmers field (Ndunguru et al., 2016). Resistance

breaking has been attributed to many factors including emergene of new more virulent virus strains and the presence of sequences in cassava genome enhancing geminivirus symptoms (SEGS) which have shown to break CMD2 resistance in TME 3 cassava variety (Ndunguru *et al.*, 2016).

In maize, resistance to *Maize streak virus* (MSV) has been introduce through breading or genetic engineering resulting into plants with different levels of resistance to MSV. Some plants acquired mediam resistance manifested as reduced symptom severity and low virus titers, while others especially those produced through genetic engineering displayed stronger resistance (*Shepherd et al., 2007;* Vanderschuren et al., 2007).Recently, there have also been efforts to use gene editing technology such as Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) to confer resistance to geminiviruses (Ali *et al*., 2015; Green & Hu, 2017; Zaidi *et al*., 2016). CRISPR - Cas9 is an RNA-guided, gene-editing platform that uses an endonuclease (Cas9) and synthetic guide RNAs to introduce double strand breaks at specific locations in DNA. The use of CRISPR for engineering resistance to geminiviruses in plants is still a developing technology.

2.2.3 Quarantine regulations

To control the spread of diseases from one geographical location to another, countries, states or regions have laws and regulations to restrict the movement of plant materials or other objects carrying disease vectors from infected to disease-free locations.

2.3 Geminiviruses

2.3.1 Background

Geminiviruses comprise a large family of plant viruses with circular single-stranded (ss) DNA genomes (2500–5200 nucleotides in size) that are packaged into double-icosahedral virions (Hanley-Bowdoin *et al*., 2013). They are transmitted by insect vectors to both monocotyledonous and dicotyledonous plants. Geminivirus genomes

consist of either one (monopartite) DNA or two (bipartite) similarly sized DNA-A and DNA-B components (Zerbini *et al*., 2017; Gutierrez, 1999; Hanley-Bowdoin, 2000; Jeske, 2009; Brown *et al.*, 2012; Hanley-Bowdoin *et al*., 2013). Given that their genomes have limited coding capacity, geminiviruses depend heavily on host cellular machineries and interact with a wide range of plant proteins and processes during infection (Hanley-Bowdoin *et al*., 2013)

2.3.2 Classification and transmission

Geminiviruses are classified by the International Committee on Taxonomy of Viruses (ICTV) into nine genera; Becurtovirus, Begomovirus, Curtovirus, Eragrovirus, Mastrevirus, Topocuvirus, Turncurtovirus, Capulavirus and Grablovirus based on genome organization, host range and insect vectors (Zerbini *et al*., 2017).

The Mastrevirus genus includes viruses with single genome components of 2.7 kb that are transmitted by various leafhopper species to monocotyledonous or dicotyledonous plants in a non-propagative, persistent and circulative manner (Stanley *et al*., 2005; Muhire *et al*., 2013). The type member is *Maize streak virus* (MSV). Mastreviruses are normally not transmissible by mechanical inoculation. Mastreviruses have four open reading frames (ORFs). V1 and V2 on the virion-sense strand encode the coat protein gene (CP) that forms the virion and acts as the nuclear shuttle protein (NSP) and the movement protein (MP) for cell-to-cell movement. C1 and C2 on the complementary-sense strand encode Rep (by transcript splicing) and RepA (ORF C1). The Rep protein initiates rolling circle replication and plays a role in the recruitment of host replication factors to the origin of replication. The RepA protein binds to the plant homologue (RBR) of retinoblastoma protein to regulate cell-cycle progression (Kammann *et al*., 1991; Zerbin *et al*., 2017).

The Begomovirus genus currently consists of 322 species of which *Bean golden mosaic virus* (BGMV) is the type member. Begomoviruses have either monopartite (DNA-A genome) or bipartite genomes (DNA-A and DNA-B) (Brown *et al*., 2012; Zerbini *et al*.,

2017) that are transmitted by whiteflies in a persistent, circulative, non-propagative manner to dicotyledonous hosts (Lazarowitz, 1992; Stanley *et al*., 2005). Many begomoviruses are associated with DNA satellites. Begomoviruses have been divided into two major groups based on their genome organization, geographical distribution and genetic diversity. Old World (OW) begomoviruses occur in Africa, Asia, Europe and Australia, while New World (NW) begomoviruses occur in the Americas. All NW begomoviruses have bipartite genomes whereas OW begomoviruses have either monopartite or bipartite genomes (Zhang & Ling, 2011; Zhou, 2013). The DNA-A component of NW begomoviruses is strictly dependent on the DNA-B component for systemic infection, while many DNA-A components of OW bipartite begomoviruses can move and infect systemically in the absence of their DNA-B components (Rojas *et al*., 2005). DNA-A has six open reading frames (ORFs), two in the virion sense (AV1 and AV2) and four in the complementary sense (AC1, AC2 and AC3 and AC4). DNA-B has two ORFs, the virion-sense BV1 and complementary-sense BC1 (Nawaz-ul-Rehman & Fauquet, 2009). (Begomovirus genomes are described in more detail in the next section.)

The Topocuvirus genus contains a single member species, *Tomato pseudo-curly top virus* (TPCTV), which has one genomic component of about 3 kb. It is transmitted by treehoppers to dicotyledonous plants. Topocuviruses have been reported only in the northern hemisphere and are thought to be a recombinant between begomoviruses and mastreviruses (Rojas *et al*., 2005; Briddon *et al*., 1996). Topocurtoviruses have six ORFs; V1 and V2 in the virion sense and C1, C2, C3 and C4 in the complementary sense (Zerbin *et al*., 2017).

The Curtovirus genus contains three species, including *Beet curly top virus* (BCTV) as the type member and an important pathogen in North America and the Middle East (Chen *et al*., 2010). They are monopartite viruses that are transmitted by leafhoppers to dicotyledonous plants in a persistent, circulative, non-propagative manner (Stanley *et al*., 2005; Chen *et al*., 2010). The genome has seven ORFs, three in the sense strand (V1 encoding CP, V2 encoding MP and V3 encoding the V3 protein) and four in the complimentary sense (C1 encoding Rep, C2 encoding the C2 protein, C3 encoding Ren

and C4 encoding the C4 protein) (Hanley-Bowdoin *et al*., 2013). Curtoviruses have been found in association with defective-interfering DNAs, which in some instances reduce symptom severity (Varsani *et al*., 2014).

The Becurtovirus genus consist of viruses with monopartite genomes coding for 5 proteins (three overlapping ORFs, V1, V2 and V3, in the virion sense and two ORFs, C1 and C2 in the complementary sense. V1, V2 and V3 are analogous of their curtovirus counterparts with similar functions but the C1 and C2 are more related to mastreviruses ORFs occupying the same position in in the genome. Becurtoviruses lack C3 and C4 ORFs (Yazdi *et al*., 2008). Becurtoviruses are transmitted by leafhoppers to dicotyledonous plants (Heydarnejad *et al*., 2007; Varsani *et al*., 2014). The type member is *Beet curly top Iran virus* (BCTIV).

The Eragrovirus genus has only one species, *Eragrostis curvula streak virus* (ECSV). It has a monopartite genome coding for 4 proteins (two each in the virion and complementary sense) and is transmitted by an unknown vector (Varsani *et al*., 2009). Eragroviruses have an unspliced Rep and a unique genome organization resembling different geminivirus genera. The locations of theV1 and V2 ORFs correspond to the positions of the CP and MP genes found in other monopartite geminiviruses. The positions of C1 and C2 ORFs correspond to the same positions as Rep and C2 genes in begomoviruses, curtoviruses and topocuviruses (Varsani *et al.*, 2009).

The Turncurtovirus genus consists of monopartite viruses closely resembling curtoviruses but are phylogenetically distinct. They encode 6 rather than the 7 proteins of Curtovirus (V1 and V2 in the virion sense and C1, C2, C3 and C4 in the complementary sense (Briddon *et al*., 2010; Varsani *et al*., 2014). This arrangement is different from that of curtoviruses and mastreviruses in having two vs. three virion-sense genes and four vs. two complementary-sense genes, respectively. *Turnip curly top virus* (TCTV) is the only species in the genus.

In addition to the seven established genera described above, two new genera, Capulavirus and Grablovirus, have been approved by ICTV. The Capulavirus genus includes four species, and its type member virus is *Euphorbia caput-medusae latent virus*. Members of the genus may be transmitted by aphids (Roumagnac *et al*., 2015). The genomes contain 7 ORFs with four in the virion sense (V1, V2, V3 and V4) and three in the complementary sense (C1, C2 and C3)

The Grablovirus genus consists of closely related viruses discovered infecting grapevines in Canada, South Korea and the USA. The type member and the only virus in this genus is *Grapevine red blotch virus* (GRBV), which has a 3.2-kb monopartite genome that is larger than those of other monopartite geminiviruses, which range from 2.7 to 3.0 kb (Krenz *et al*., 2012; Varsani *et al*., 2017). The natural vector is likely to be the three-cornered alfalfa treehopper (*Spissistilus festinus* Say) (Bahder *et al*., 2016). Its genomic organization is unique, with virion and complementary sense ORFs that have no homologues in members of the other geminivirus genera. The genome has three ORFs in the virion sense (V1, V2, and V3) and three in the complementary sense (C1, C2, and C3) (Krenz *et al*., 2012).

2.3.3 Geminivirus genome organization

Bipartite Geminiviruses have two small, circular ssDNA components, DNA-A and DNA-B (Morris *et al*., 1990; Stanley *et al*., 2005; Bull *et al*., 2006). DNA-A and DNA-B are similar in size but differ in their sequences except for the 5’ intergenic region or common region (about 200 to 250 nt), which is present in both components (Zhou, 2013). The DNA-A component includes five or six open reading frames (ORFs), with two (AV1 and AV2 for OW viruses) on the virion-sense strand and four (AC1, AC2, AC3 and AC4) on the complementary-sense strand (Fig. 2.1). DNA-B, which depends on DNA-A for replication, has two ORFs (BV1 and BC1) on the virion and complementary strands.

2.3.4 Functions of viral genes

AV1 encodes the coat protein responsible for encapsidation of progeny virions and whitefly transmission (Unseld *et al*., 2004). AV2 is a pre-coat protein gene (PCP) involved in viral movement (Lazarowitz, 1992; Jeske, 2009) and most likely functions to counteract host gene silencing (Zrachya *et al*., 2007; Sharma & Ikegami, 2010). AC1 encodes the replication-associated protein (Rep) that plays an essential role in the viral rolling circle replication (RCR) and modulation of gene expression (Etessami *et al*., 1991; Arguello-Astorga *et al*., 2004; Nash *et al*., 2011). In RCR, Rep interacts with host DNA replication factors forming a functional replication complex at the viral origin of DNA replication. Rep recognizes a specific sequence in the double-stranded form of the viral origin (Fontes *et al*., 1992, Arguello-Astorga *et al*., 2004). Rep also cleaves the virion-sense strand at

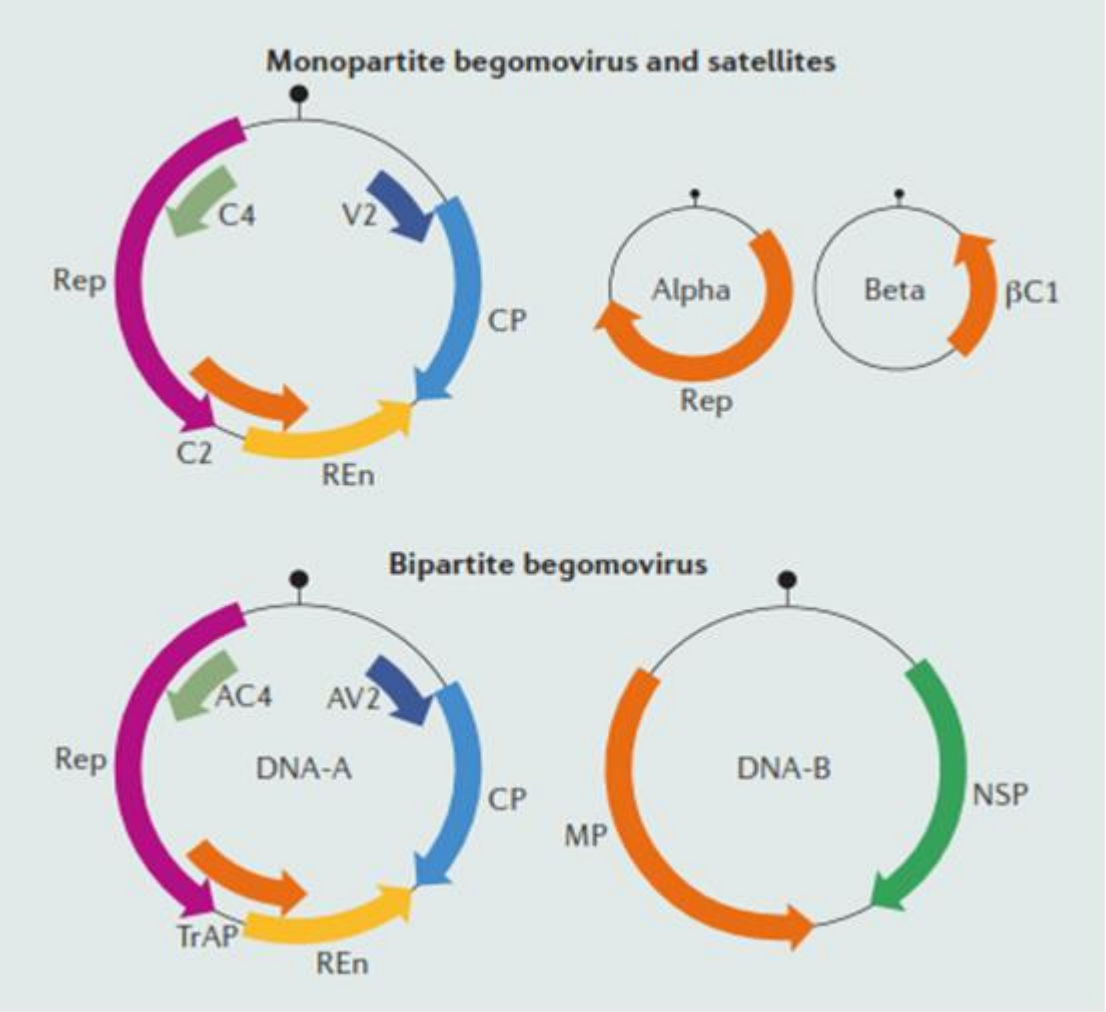


Figure 2.1: Genomic organisation of begomoviruses and their associated satellite DNAs. Monopartite genomes and bipartite DNA A components both contain six ORFs, AVI and AV2 in the sence strand and AC1 – AC4 in the complimentary strand. DNA-B of bipartite begomoviruses has two ORFs; BV1 encoding MP and BC1 encoding NSP. Alphasatellites and betasatellites encode Rep protein and βC1, respectively (Adapted from Hanley-Bowdoin et al., 2013).

Specific site in a conserved hairpin to generate a free 3'-OH that acts as a primer for initiation of RCR. (Heyraud-Nitschke *et al*., 1995; Orozco & Hanley-Bowdoin, 1996). Rep acts as a DNA helicase to unwind viral DNA during leading strand replication (Clerot, & Bernardi, 2006; Singh *et al*., 2008). Rep reprograms plant cell cycle controls through its interactions with the host RBR protein to induce the synthesis of the plant

replication machinery required for viral amplification (Ascencio-Ibanez *et al*., 2008; Hanley-Bowdoin *et al*., 2013). Rep also interferes with the plant DNA methylation machinery and suppresses transcriptional gene silencing (Rodriguez-Negrete *et al*., 2013). Rep interacts with many host factors and is likely to impact a variety of other host functions (for review see Hanley-Bowdoin *et al*., 2013). AC2 encodes the transcriptional activator protein (TrAP) that transactivates expression of the AV1 coat protein gene and the BV1 movement protein gene (Sunter & Bisaro, 1991) and suppresses transcriptional gene silencing (TGS) (Buchmann *et al*., 2009). AC3 encodes the replication enhancer protein (Ren) that increases viral DNA accumulation and interacts with Rep, RBR and PCNA, a host replication protein (Settlage *et al*., 1996, 2005). AC4 encodes an RNA anti-silencing suppressor protein, which is a determinant of symptom expression (Vanitharani *et al*., 2004; Sunitha *et al*., 2013).

BV1 encodes a nuclear shuttle protein (NSP). BV1 localizes to the cell nucleus where it binds to newly formed viral ssDNA and transports it into the cytoplasm (Carvalho *et al*., 2008; Teng, *et al*., 2010). BC1 encodes the movement protein (MP), which facilitates cell-to-cell transport of viral DNA through plasmodesmata to neighboring plant cells (Lucas, 2006). Hence, begomoviruses move within and between cells of host plants by a co-operative action of the two proteins encoded by the DNA-B component (Hehnle *et al*., 2004).

2.4 Begomovirus replication

Because of their small genomes, geminiviruses do not encode their own DNA polymerases and, instead, use host machinery to replicate their genomes. However, most plant cells exit the cell cycle early in development and undergo differentiation. As a consequence, most plant cells do not contain the replicative enzymes necessary for viral DNA synthesis. Geminiviruses have evolved proteins to re-activate S phase in terminally differentiated plant cells and induce the expression of host DNA polymerases. The key player for this strategy for begomoviruses is the Rep protein (Hanley-Bowdoin

et al., 2013), which binds to the host RBR protein to induce cell cycle re-entry and the synthesis of host replication machinery (Liu *et al*., 1999).

2.4.1 Rolling circle/recombination-based replication

Geminiviruses, which have circular, single-stranded DNA (ssDNA) genomes, replicate their genomes in the nucleus using a combination of rolling circle replication (RCR) and recombination dependent replication (RDR). To start the replication cycle, ssDNA is converted to double-stranded DNA (dsDNA). Then, the virus amplifies the dsDNA via the RCR and RDR. Late in replication, ssDNAs are removed from the replication pool by packaging into virions

The virion first enters a plant cell during feeding by the insect vector. The capsid is then disassembled by an unknown mechanism to release viral ssDNA. The virion-sense ssDNA strand is used as a template for synthesis of the complimentary strand and the generation of the viral double-stranded replicative form (RF). It is thought the conversion of ssDNA to dsDNA is catalyzed entirely by host DNA polymerases and associated factors. The RF is used as a template for further viral replication or transcription. Both processes occur in the plant nucleus.

Rolling circle replication is initiated by the virus-encoded, initiator protein Rep, which nicks the virion strand of the RF at a specific site within the origin of replication. Rep has both site-specific nicking and ligase activities for initiation and termination of rolling circle replication. Nicking of dsDNA is followed by covalent crosslinking of the Rep protein to 5' phosphate of the nicked strand while the 3' hydroxyl end is released to serve as primer for DNA synthesis. The nicked strand is displaced as a ssDNA by the DNA helicase activity of Rep and circularized by the ligase activity of Rep (Hehnle *et al*., 2004; Preiss & Jeske, 2003; Hanley-Bowdoin *et al*., 1999; Stenger *et al*., 1991). The ssDNA can be converted to dsDNA by host enzymes as described above or packaged into virions.

2.4.2 Recombination-dependent replication

Analyses of replication intermediates of TYLCV, *Tomato golden mosaic virus* (TGMV), Tomato leaf curl virus (ToLCV), Ageratum mosaic virus (AbMV) and *Beet curl top virus* (BCTV) using a 2D gel electrophoresis and electron microscopy revealed a range of additional intermediates in addition to the previously identified RCR intermediates. This observation suggested that a mode of viral replication exists, which is designated as recombination-dependent replication (RDR) (Albert *et al*., 2005; Jeske *et al*., 2001). The RDR model has 3 steps - processing of broken dsDNA, invasion of the homologous duplex by the 3' end of ssDNA, and DNA heteroduplex extension (branch migration). The three steps are detailed in Kreuzer (2000) and Mosig *et al*., (2001). RDR is thought to play crucial roles in homologous recombination, double-strand break repair, restoration of collapsed replication forks, and adaptive mutation (Kogoma, 1997).

2.5 Begomovirus transcription

After viral dsDNA is generated, it is transcribed in the nucleus by host RNA polymerase II to produce viral mRNAs (Hanley-Bowdoin 2000). The mRNA is translocated to the cytosol where it is translated to generate viral proteins. Viral transcription is bidirectional from promoters located in the 5' intergenic region (IR). Begomoviruses also have a promoter upstream of the AC2/C2 ORF.

Begomoviruses encode two proteins involved in the transcription. Rep acts as a transcriptional repressor when it binds to its binding site in the 5' IR, which is located between the TATA box and the transcriptional start site of the Rep gene (Eagle *et al*., 1994; Sunter *et al*., 1993). Begomoviruses also encode a transcriptional activator, TrAP, specified by the AC2/C2 gene. TrAP activates transcription of the CP gene on DNA-A and the MP gene on DNA-B (Sunter & Bisaro, 1991).

2.6 Begomovirus movement

Virus movement involves the spread of the virus genetic material from the initial inoculated cell to other cells throughout plant. Viral movement occurs locally into adjacent cells via the plasmodesmata (cell-to-cell movement) and systemically through the phloem to distal parts of the plant (Hull, 1989; Carrington *et al*., 1996).

Bipartite begomoviruses, such as Cassava mosaic viruses, Tomato mottle virus, Cabbage leaf curl virus and Tomato golden mosaic virus with genomic components, DNA-A and DNA-B, encode two movement proteins, the nuclear shuttle protein NSP (BV1) and the movement protein MP (BC1), that are required for systemic infection (Rojas *et al*., 2005; Lazarowitz & Beachy 1999). NSP shuttles the viral DNA between the nucleus and cytoplasm, and MP is responsible for cell-to-cell movement by transporting the DNA-NSP complex to the cell periphery and facilitating movement across the cell wall (Rojas *et al*., 2005).

In the monopartite Geminiviruses such as *Tomato yellow leaf curl virus* (TYLCV), the coat protein (CP), V1 and C4 are involved with movement of the virus. The nuclear shuttle function is mediated by the CP, which is localized in the nucleus facilitating import and export of DNA. Thus, CP serves as the functional homolog of bipartite BV1. V1 is localized around the nucleus and at the cell peripheral and C4 is localized to the cell peripheral, are functional homologs of the bipartite BC1 (MP) facilitating cell-to cell movement (Rojas *et al*., 2001; Hanley-Bowdoin *et al*., 2013)

2.7 DNA molecules associated with Begomoviruses

2.7.1 Satellites

One feature that distinguishes plant viruses from animal viruses is their common association with satellite molecules. Based on the type of nucleic acid and encapsidation, satellites can be classified into two broad categories. The first category comprises satellites that encode their own capsid protein referred to as satellite viruses, and the

second category consists of satellites packed by the capsid proteins of their cognate helper viruses referred as satellite RNAs (virusoids) or satellite DNAs (Palukaitis, 2008). Regardless of their types, all satellites share the following features. They are not part of the helper viral genome, are not required for the infection cycle of their helper viruses, and lack general nucleotide sequence homology to their helper viruses (Robinson *et al*., 1999; Mayo et al., 2005; Flores *et al*., 2005). Geminiviruses are known to be helper viruses for a number of distinct DNA satellites. Two DNA satellite groups have been described in association with begomoviruses, i.e. the alpha and beta satellites (Briddon *et al*., 2003; Briddon & Stanley, 2006).

i. Betasatellites

Many OW begomoviruses have monopartite genomes and are commonly found associated with a group of ssDNA satellites known as betasatellites. Betasatellites DNAs are approximately half the size of their helper virus genome (about 1.4 kb) and encode a betaC1 ORF on the complimentary strand. All known betasatellite functions are mediated by the 13 kDa betaC1 protein. The protein plays an important role in the pathogenicity of the helper virus, including symptom induction and suppression of host-mediated transcriptional and post-transcriptional gene silencing. Other functions include involvement in virus movement, increased viral DNA accumulation, binding to DNA/RNA and interaction with the helper virus CP (Saunders *et al.*, 2000; Briddon *et al.*, 2001; Mansoor, *et al*., 2003; Cui *et al.*, 2005; Saeed *et al.*, 2007; Nawaz-ul-Rehman, *et al*., 2009; Muhammad *et al*., 2012). The betaC1 protein also interacts with host factors and pathways such as jasmonic acid responsive genes (Yang *et al*., 2008) and is implicated in suppression of DNA methylation pathways, a key anti-silencing activity (Yang *et al*., 2011). Betasatellites have no sequence homology with their helper viruses except for a nanonucleotide sequence TAATATTAC corresponding to the conserved RCR DNA cleavage site (Briddon *et al*., 2003). Betasatellites depend on their helper viruses for replication, movement and transmission within and between plants. This is achieved when betasatellites are trans-encapsidated by their helper virus coat protein (Briddon *et al*., 2003; Mansoor *et al*., 2006; Briddon & Stanley, 2006).

ii. Alphasatellites

Alphasatellites are ssDNA molecules that are self-replicating, but depend on the helper virus for vector transmission, movement and encapsidation. Alphasatellites have an adenine-rich region (200 nts) and an origin of replication. Their DNAs resemble nanoviruses and contain a nanonucleotide TAGTATTAC motif that occurs in many nanoviruses (Briddon *et al.,* 2004). A begomovirus-associated alphasatellite, originally known as DNA-1, was first described in 1999 for OW monopartite begomoviruses such as *Cotton leaf curl virus* and *Ageratum yellow vein virus* (Saunders & Stanley, 1999; Mansoor *et al*., 1999). Recently, an alphasatellite (Melon chlorotic mosaic alphasatellite) has been found in the NW in association with a bipartite begomovirus *(Melon chlorotic mosaic virus;* MeCMV) (Romay *et al*., 2010). The alphasatellite genome encodes Alpha-Rep, which functions as a replication initiator protein and a suppressor of RNA silencing to overcome host defences and promote viral infection (Nawaz-ul-Rehman *et al*., 2010; Xie *et al*., 2010; Zhou, 2012).

iii. Gammasatellites

Gammasatellites (a.k.a. deltasatellites) are related to betasattelites. They are group of non-coding begomovirus-associated satellite DNAs smaller than 1 kb (~640–750 nucleotides) in size. Their small size and lack of open reading frame distinguish them from alphasatellites and betasatellites. Gammasatellites contain an A-rich region as well as a conserved region ~100 nt in length. The role of gammasatellites in disease development has not yet been reported. Their occurance is widespread, and they have been described in Australia (Dry *et al*., 1997), Puerto Rico and Spain (Rosario *et al*., 2016), Cuba (Fiallo-Olivé *et al*., 2012), Florida (Ng *et al.,* 2011).

2.7.2 Sequences enhancing geminivirus symptoms (SEGS-1 and SEGS-2)

The severe CMD pandemic that devastated cassava plants in most of east and central Africa in the late 1990s and early 2000s was attributed to the emergence of new more

virulent CMB strains, that arose by recombination, synergy between CMB viral species (Legg & Fauquet, 2004), and the appearance of an invasive biotype of *Bemisia tabaci* (Legg *et al*., 2002). Later, two putative circular ssDNA sequences were identified that may have also contributed to the severity of the pandemic. Originally the two DNAs were designated as satDNA-II and satDNA-III (Ndunguru *et al*., 2008). Subsequent studies revealed that sequences related to the satDNAs are present in all cassava genomes tested to date, suggesting that they are likely to be derived from the host genome. Based on this, SatDNA-II and SatDNA-III do not fit the definition of DNA satellites and have been renamed SEGS-1 and SEGS-2 (Sequences Enhancing Geminiviruses Symptoms), respectively (Ndunguru *et al*., 2016).

When SEGS-1 or SEGS-2 was co-inoculated with cassava mosaic viruses in controlled experiments, both SEGS enhanced CMD symptoms, causing severe leaf distortion and yellowing and increased viral DNA accumulation in infected plants (Ndunguru *et al*., 2016). In a cassava landrace carrying the CMD2 resistance locus, co-inoculation of CMBs with SEGS-1 broke resistance resulting in severe CMD symptoms. Episomal forms of both SEGS were detected in CMB-infected cassava but not in healthy cassava. SEGS-2 episomes were also found in virions and whiteflies.

SEGS-1 and SEGS-2 are 1000 and 1200 nt in size, respectively, and are characterized by GC-rich regions and the absence of long open reading frames. SEGS-1 has 7 putative ORFs, while SEGS-2 has 4 potential ORFs (Fig. 2.2, Ndunguru *et al*., 2016). The ORFs may encode functional proteins but their small sizes argue that they do not specify proteins. The SEGS lack the typical geminivirus hairpin structure, characteristic of the origin of replication (Ndunguru *et al*., 2016). SEGS-1 has no homology to geminiviruses or their associated satellites, but the cassava genome contains a sequence that is 99% identical to full-length SEGS-1. The cassava genome also contains three sequences with 84 to 89% identity to SEGS-2 that together encompass all of SEGS-2 except for a 52-bp region, which includes the episomal junction and a 26-bp sequence related to alphasatellite replication origins. These results suggested that SEGS-1 is derived from the cassava genome and facilitates CMB infection as an integrated copy and/or an

episome, while SEGS-2 was originally from the cassava genome but now is encapsidated into virions and transmitted as an episome by whiteflies (Ndunguru *et al*., 2016).

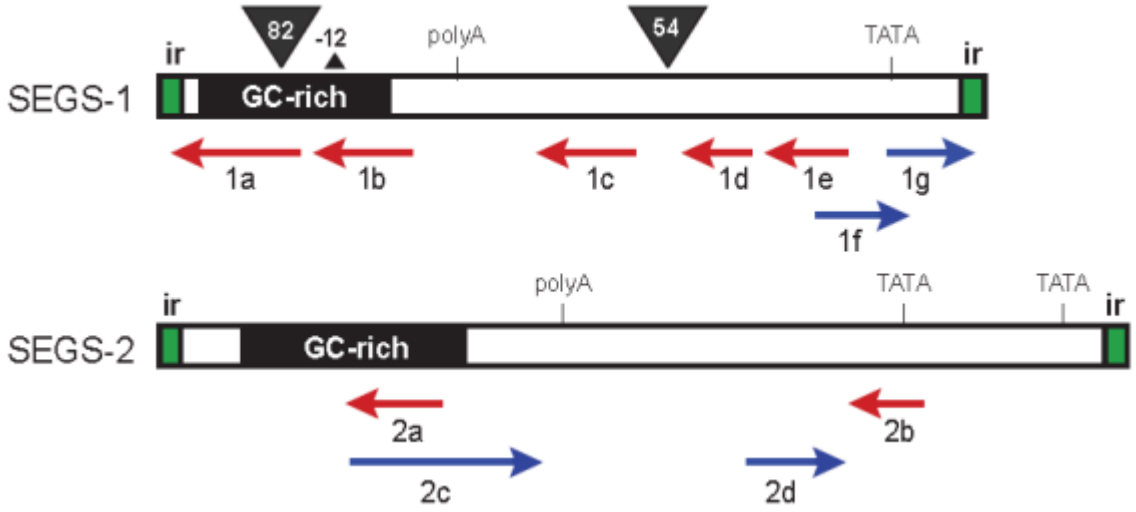


Figure 2.2: Sequence organization of SEGS-1 and SEGS-2, showing a linear map of cloned sequences with GC-rich regions and flanking repeated sequences (green). SEGS-1 has 7 putative ORF and SEGS-2 has 4 putative ORF (2a to 2d) (Hanley-Bowdoin, unpublished)

2.8 Virus-host interactions

2.8.1 Requirements for a successful viral infection

To successfully invade a host and cause infection, plant viruses must accomplish three things: replicate their genomes in host cells, move their genomes throughout the host and suppress host defences. V**irus** cell-to-cell **movement** is facilitated by the **nuclear shuttle protein** and the movement protein facilitates the long-distance movement to other parts of the plant is facilitated by the movement protein (MP). Viruses compete for and interfere with host resources such as the replication factors, and when a substantial

amount of host resources have been used, they disrupt host physiology to cause disease (symptomatic phenotypes) (Pallas & Garcia, 2011). Interference and competition following virus infection can affect a large number of host genes either by inducing or repressing them. These genes can belong to a broad range of plant pathways/cellular processes such as cell cycle and transcriptional control, cell death, signalling and protein turnover pathways, as well as hormonal regulation, macromolecular transport and defence pathways.

2.8.2 Virus infection cycle

Geminivirus-host interactions have been reviewed by Hanley-Bowdoin *et al*. (2013). Infection starts when a whitefly carrying virus feeds on the sap of a healthy leaf and transmits virions to the phloem-associated cells. In the infected plant cells, viral ssDNA is released from the virion and copied to make double-stranded DNA (dsDNA) in a process where plant DNA polymerases use the RNA oligonucleotides to prime complementary strand synthesis. Viral dsDNA assembles into nucleosomes that are transcribed by the host RNA polymerase II to express the Rep protein. Rep initiates viral replication, which occurs through a combination of rolling circle and recombination dependent replication (Egelkrout *et al*., 2001). To move the infection from the nucleus to another cell or through the phloem to a new leaf, two viral proteins are produced and employed; e.g. the movement protein (MP) and the nuclear shuttle protein (NSP).

2.8.3 Virus defenses

Introduction of viruses into host plant cells results in coordinated changes in functions of host components and induction of host defense reactions (Whitham *et al*., 2006). Plant responses include RNA silencing, induced hypersensitivity, systemic acquired resistance and processes such as DNA methylation, which together limit viruses to infected cells and impart resistance to the noninfected tissues (Mandadi & Scholthof, 2013).

Active defenses (hypersensitive resistance) are induced by the pathogen itself. Plants contain a specific R (resistance) gene which recognizes a pathogen, in the other hand the pathogen has a corresponding gene called AVR (avirulent) also usually dominant (Staskawicz *et al.,* 1995). R genes produce specific protein products that confer resistance to a pathogen that produces the corresponding Avr protein. Virus infection initiates the HR response by Avr/R protein interactions that lead to metabolic changes in defense hormone levels, such as salicylic acid (SA), jasmonic acid (JA) and nitric oxide (NO), and the accumulation of reactive oxygen species, such as $O2^-$ and hydrogen peroxide in the infected and noninfected tissues, which together trigger necrotic stress and metabolic alterations to contain virus spread. This can be observed as virus-associated chlorotic lesions or spots, ringspots, and necrotic lesions on leaves, stems and fruits (Moffett, 2009; Mandadi &Scholthof, 2013; Culver & Padmanabhan, 2007).

Systemic acquired resistance (SAR) is a form of induced resistance that is activated throughout a plant after exposure to a virus or other stimuli. SAR induces defense reactions involving both biochemical and cytological changes, and depends on the production of a signal that is translocated to other parts of the plant, where it triggers resistance (Vallad & Goodman, 2004; Gozzo & Faoro, 2013)

Plants also respond to pathogen attack and restrain development of a systemic infection through gene silencing. In plants, gene silencing can occur via transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS). TGS involves stable repression of transcription caused by epigenetic changes, especially promoter DNA methylation resulting in decreased RNA synthesis (Law & Jacobsen, 2010; Vaucheret & Fagard, 2001). Upon geminivirus infection, plants process viral RNA into small interfering RNA (siRNA) and direct its use to other antiviral processes (Grewal & Elgin, 2007; Matzke *et al*., 2009; Ding, 2010). Plants can also establish heterochromatization, which involves changes in the host chromatin structure by histone protein modifications to restrict access to binding proteins (Baulcombe, 2004; Voinnet, 2005).

PTGS or RNA silencing is one of the main plant defense mechanisms against viruses. It involves inactivating viral genes in a cell through the formation of aberrant double stranded viral RNA that is trimmed by the enzyme dicer to form siRNA. The siRNAs are incorporated into the RNA-induced silencing complex (RISC) to induce mRNA degradation (Sijen *et al*., 2001; Bartel, 2004; Hanley-Bowdoin *et al*., 2013). PTGS can also be induced by viruses expressing host genes in a process called virus-induced gene silencing (VIGS) (Baulcombe, 1996). Viruses themselves can be the targets of the PTGS machinery.

2.8.4 Virus impact on host factors

2.8.4.1 Reprogramming plant cell cycle controls

In plant meristems and very young differentiating tissues, a proliferative cell cycle occurs where mitosis is coupled with interphase, during which a cell carries out most of its normal functions. Interphase is divided into three stages G1, S and G2. G1 and G2 stand for first and second gap respectively. G1 is the period after mitosis (M) but before DNA synthesis, during which the cell grows, synthesize proteins and performs other cellular functions in preparation for DNA replication and cell division. S phase follows G1 with the cell actively replicating its DNA. At the completion of S phase, the cell enters G2 and continues to carry out normal functions and growth in advance of mitosis. Many plant cells also undergo the endocycle in which DNA replication is uncoupled from mitosis, resulting in increased genome ploidy. When plant cells differentiate, they exit the cell cycle in G1 or G2, but not during S or M. Fully differentiated cells do not replicate DNA.

Most geminiviruses infect plant cells that have exited the cell cycle and do not express host DNA polymerases (Nagar *et al*., 1995; Hanley-Bowdoin *et al*., 2013). To overcome this barrier, geminiviruses must activate host cell cycle machinery to facilitate efficient virus replication (Hanley-Bowdoin *et al*., 2000). Evidence from gene profiling studies showed that begomovirus infection activates cell cycle associated genes expressed in S

and G2 phase and inhibits genes active in M and G1 phase (Ascencio-Ibanez *et al*., 2008). A key regulator of the plant cell cycle is the retinoblastoma-related (RBR) protein, a plant homolog of the human tumor suppressor RB. RBR regulates the cell cycle, cell maintenance and differentiation in plants. RBR interact with E2F transcription factors to repress transcription of genes encoding host replication proteins (Hanley-Bowdoin *et al*., 2013).

2.8.4.2 The endocycle and viral DNA replication

Meristematic cells proliferate via the mitotic cell cycle to promote growth and development. Outside of the meristem, differentiating cells first go through the mitotic cell cycle to increase cell number followed by some cells transitioning to the endocycle. In the endocycle, cells undergo successive rounds of DNA replication without an intervening mitosis. Some geminiviruses can induce mature host cells to enter the endocycle and replicate both viral and plant chromosomal DNA in a mechanism involving Rep. Not all functions of Rep are known, but Rep is known to indirectly activate the expression of the viral transcriptional activator protein (TrAP), which interacts with host factors to inhibit the plant methyl cycle. Suppression of the methyl cycle reduces viral DNA methylation, facilitating begomovirus replication (Hanley-Bowdoin *et al*., 2013). A study by Ascencio-Ibanez, *et al* (2008) using *Arabidopsis* infected with *Cabbage leaf curl virus* (CaLCuV) showed that the virus interferes with progression of cell cycle by affecting cyclin-dependent kinases (CDK) activated by a regulatory class of proteins called cyclins (CYCs). D-type cyclins (CYCDs) act during G1, and the CYCD3 subclass has been shown to promote the mitotic cycle and prevent the endocycle. In this study, CaLCuV down regulated CYCD3 genes, prompting infected cells to enter the endocycle. Similar results have been reported by Pierce and Rey (2013) working with *Arabidopsis* infected with *South African cassava mosaic virus* (SACMV).

2.8.4.3 Disruption of plant signaling pathways

Upon viral invasion, pathogen-associated molecular patterns and effectors can be detected and recognized by a complex system of plant receptors, that interact with hormonal signaling pathways. Geminiviruses interact with such pathways to induce host processes for viral replication and to interfere with host defenses (Hanley-Bowdoin *et al*., 2013). Key plant receptors in geminivirus infection include some members of the receptor-like kinases (RLKs), a protein kinase family that senses pathogens and triggers defense responses. Over-expression of selected RLKs delays viral symptoms while loss of function increases susceptibility (Morin *et al.,* 1999). Another signaling pathway is the GRIK-SnRK1 protein kinase cascade. SnRK1 is best known for modulating energy status in plants. GRIK activates SnRK1 and only accumulates in young plant cells and geminivirus-infected mature cells. The GRIK-SnRK1 protein kinase cascade is thought to play dual roles in both facilitating and countering geminivirus infection (Thelander *et al*., 2004; Shen *et al*., 2009).

Hormonal signaling pathways: Plant defenses in response to virus infection employ signal transduction networks modulated by the salicylic acid (SA), ethylene (ET) or jasmonic acid (JA) pathways (Clarke *et al*., 2000). Geminivirus infection activates the SA and ET pathways, both of which participate in host defences (Ascencio-Ibanez *et al*., 2008). Plants exhibiting increased SA levels are more resistant to viral infection. JA is a growth inhibitor and also part of the defense response against non-viral pathogens and whiteflies. Genes in the JA pathways are generally suppressed during geminivirus infection (Carvalho & Lazarowitz, 2004, Ascencio-Ibanez *et al.,* 2008). Geminiviruses also interact with cytokines and auxin pathways, which promote cell growth and modulate differentiation. Geminivirus infection activates mechanisms leading to phosphorylation of cytokinins and converts them to their low activity form (Baliji *et al*., 2010). Elevated levels of plant cytokinins can augment plant resistance against virus infection and other pathogens (Pogany *et al*., 2004; Choi *et al*., 2010). Expression of some viral proteins such as Rep, REn, V2 and NSP, can lead to programmed cell death

(apoptosis). Binding of the Rep protein to RBR elicits cell death in mature tissues (Jordan *et al*., 2007). V2 and NSP induce cell death by an unknown mechanism.

2.9 Analysis of plant virus - host interactions

Viruses carry limited amounts of genetic information into plants and depend on their hosts to provide the cellular machinery needed for replication and gene expression. This dependency requires that viral and host components interact at the molecular level. It is at this level that host plants recognize and respond to infecting viruses (Culver, *et al*., 1991). Understanding the mechanisms behind these interactions will provide insight into the development of new techniques for control of plant virus diseases. Development of disease management strategies requires extensive knowledge of virus infection, transmission, spread and effects on host plants to select the best control measures (Rodrigues *et al*., 2009). Studying viruses can be simplified if a tractable host system is available.

2.9.1 Model host vs natural host systems

A model host is an organism that is used for studying a particular biological phenomenon with expectations that the findings will provide insight into infection of other organisms. In contrast, natural hosts represent organisms that a pathogen commonly infects in nature. Model systems usually have been extensively characterized and have many available tools, making studying model organisms more tractable. Many natural hosts are less studied and may contain other factors such as inherent genomic mutations that can confound research. However, care must be taken when generalizing from a model organism to a natural host. Model systems may not be suitable for some questions like identification of the best disease management strategy for a pathosystem.

2.9.2 Arabidopsis thaliana as a model plant

Arabidopsis thaliana (L.) Heynh. Family Name: Brassicaceae (Cruciferae) - Mustard Family, is a short-lived, self-compatible and predominantly inbreeding annual plant that is distributed in temperate regions of Europe, the Far East, and East Africa. It was declared a model plant in 1998 (Meinke *et al*., 1998). Arabidopsis is one of the most widely used model organisms for molecular and genomic studies of higher plants. Arabidopsis plants have several technical advantages including a compact genome of about 130 Mb with a low level of repetitive sequences, small size (6–12-inch height), can be grown in petri dishes, short life cycle (5–6 weeks), large number of seeds per plant (10,000 per plant), easy to transform, large collections of mutants, and the translucent nature of the plant parts that can be used for *in situ* analysis (Saraswathy & Ramalingam, 2011).

2.9.3 Tomato as a model plant

Many tomato varieties have been used as model hosts to analyze virus-host interactions, including Micro-Tom (Compos *et al.*, 2010), Moneymaker (Butterbach *et al*., 2014) and Florida lanai (Nava *et al*., 2013; McKenzie, 2002). Of these tomato cultivars, Micro-Tom and Moneymaker are most widely used (Lima *et al.*, 2004; *Arie et al*., 2007). Micro-Tom, which are 13–20 cm tall, are considered to be the world's smallest tomato (Martí *et al*., 2006; Carvalho *et al*., 2011; Okabe *et al.*, 2011), allowing large numbers of plants to be grown in limited space. Although Florida Lanai reaches a height of 60-90 cm (Augustine *et al*., 1981), it can still be grown in a limited growth chamber space. In contrast, Moneymaker can grow to a height of 200 cm and is heavily branched (http://www.thompson-morgan.com/vegetables/vegetable-seeds/tomato-seeds/tomato-moneymaker/282TM), making it difficult to grow sufficient plants in growth chambers. A major advantage of 'Florida Lanai' over Micro-Tom is that it does not carry recessive mutations, eliminating the risk of confounding effects.

Other characteristics making tomato a good model plant for geminivirus-host interaction includes; successifull tissue culture and in vitro plant regeneration to callus, shoots and roots (Zorzol *et al*., 2007), ability to produce good quality protoplast from different parts of the plant (Tewes *et al*., 1984), easily modified genome (getically transformed) and presence of mutants in many pathways for genetic studies (Zorzol *et al*., 2007;). Presence of developed markers for molecular studies (Zhang *et al*., 2003) and presence of sequenced full genome of tomato which facilitates functional studies of genes (Ranjan *et al*., 2012).

2.9.3.1 Protoplast preparation and transfection

Protoplasts are isolated individual plant cells which have been treated with digesting enzymes to remove their cell walls but possess a plasma membrane and all other cellular components (Cocking, 1972; Davey, 2005). Protoplasts can be prepared from a wide variety of plants tissues including leaves, roots, shoot apices and fruits. Of these, the mesophyll tissue of fully expanded leaves of young plants is most frequently used (Grosset, 1990; Yoo *et al*., 2007). Most plant species can produce protoplasts of good quality, including tobacco (Motoyoshi *et al*., 1973), *Arabidopsis* (Yoo *et al*., 2007), *N. benthamiana* (Rao & Prakash, 1995), tomato (Tewes *et al*., 1984), rice, barley and lettuce (Rao & Prakash, 1995). Most protoplast transient assay systems have been developed for dicots (Yoo *et al*., 2007; Hong *et al*., 2003; Sheen, 2001), but some have also been developed for monocots (Chen *et al*., 2006; Bart *et al*., 2006; Zhang *et al*., 2011; Sheen, 2001; Gronwald & Leonard, 1982).

Transfection involves the introduction of foreign DNA/RNA into the protoplast cells. With their wall removed, protoplasts are able take up foreign DNA/RNA/protein directly from the environment through a number of methods including electroporation, PEG–calcium and microinjection (Mathur & Koncz, 1998). DNA, RNA or reporters can be isolated from protoplasts and quantified to measure replication and/or gene expression. Care must be taken during protoplast isolation and maintenance to ensure that the health and viability is sustained for cell division and regeneration.

2.10 Translation of information from model systems to crops

Model organisms are often used to obtain information relevant to other species that are more difficult to study directly. Information obtained from studying model systems have advanced our understanding of various aspects of plant growth, development and response to pathogens (Meinke *et al*., 1998. Translating information from a model system to crops is a careful process to avoid making mistakes and incorrectly interpreting the information acquired. Results from model organisms can be used to identify factors, gene expression profiles or pathways involved in the studied phenomenon that can guide the identification and testing of similar factors, pathways or gene homologs in crops. More accurate predictions are obtained when the model plant and crop are closely related and more likely to share similar responses.

CHAPTER THREE

SUITABILITY OF TOMATO AS A MODEL FOR STUDYING GEMINIVIRUS-HOST INTERACTION

Abstract

Geminiviruses are devastating single-stranded DNA viruses that infect a wide variety of crops in tropical and subtropical areas of the world. Tomato, which is a host for more than 100 geminiviruses, is one of the most affected crops. Developing plant models to study geminivirus-host interaction is important for the design of virus management strategies. In this study, "Florida Lanai" tomato was broadly characterized using three begomoviruses (*Tomato yellow leaf curl virus*, TYLCV; *Tomato mottle virus*, ToMoV; *Tomato golden mosaic virus*, TGMV) and a curtovirus (Beet curly top virus, BCTV). Infection rates of 100% were achieved by agroinoculation of TYLCV, ToMoV or BCTV. Mechanical inoculation of ToMoV or TGMV using a microsprayer as well as whitefly transmission of TYLCV or ToMoV also resulted in 100% infection frequencies. Symptoms appeared as early as four days post inoculation when agroinoculation or bombardment was used. Symptoms were distinct for each virus and a range of features, including plant height, flower number, fruit number, fruit weight and ploidy, was characterized. Due to its small size, rapid growth, ease of characterization and maintenance, and distinct responses to different geminiviruses, "Florida Lanai" is an excellent choice for comparing geminivirus infection in a common host.

3.1 Introduction

Geminiviruses belong to a large, diverse family of plant infecting viruses (Geminiviridae) that are transmitted by insects and cause economically significant diseases worldwide (Zhang *et al*., 2001; Rojas *et al*., 2005; Hanley-Bowdoin *et al*., 2013). Geminiviruses are among the most economically important pathogens in a variety of crops including vegetables, fruits, root crops, cereals, spices and legumes (Morales &

Anderson, 2001; Mansoor *et al*., 2003; Seal *et al*., 2006). The genomes of geminiviruses consist of either one (monopartite) or two (bipartite) circular, single-stranded DNA molecules, with the components of bipartite viruses known as DNA-A and DNA-B (Zhang *et al*., *2001*; Brown *et al*., 2012; Hanley-Bowdoin *et al*., 2013). Geminiviruses are classified in nine genera according to their genome, host and insect vector (Zerbini *et al*., 2017).

Management of plant viruses is of vital importance to reduce the damage (Sastry and Zitter, 2014), especially in areas where food security is at risk due to high viral diversity and the emergence of more virulent strains (Damsteegt, 1999; Mansoor *et al.,* 2003; Sastry & Zitter, 2014). In 2009, Rodrigues *et al.* (Rodrigues *et al*., 2009) concluded that disease management strategies need extensive knowledge of the virus infection, transmission, spread and their effects on host plants to select the best control measures. Studying viruses can be simplified if a tractable host system is available. The suitability of a host for studying the infection process is determined by its ability to become infected and to allow the virus to replicate and induce typical symptoms (Scholthof *et al.,* 1996).

Geminiviruses have been studied using model plant systems such as *Arabidopsis thaliana* (Muangsan *et al*., 2004; Ascencio-Ibáñez *et al*., 2008; Hanley-Bowdoin *et al*., 2013; Raja *et al*., 2014), *Nicotiana benthamiana* (Goodin *et al*., 2008), *Solanum nigrum* (Urbino *et al*., 2008), and Datura stramonium (Chen *et al*., 2013). These model plants have many advantages including small size, short life cycles, high seed germination rates and ease of genetic analysis (Meissner *et al*., 1997; Meinke *et al*., 1998; Matsukura *et al*., 2008). For example, *Arabidopsis* has one of the smallest genomes, making it useful for genetic manipulation (Bevan & Walsh, 2006). Model plants are also usually inexpensive to study and readily accessible. However, information obtained using model plants may not always accurately reflect viral interactions or processes that occur in a non-model crop or reservoir plants in nature and disease can be the result of specific interactions between a virus and a host (Dawson & Hilf, 1992; Pallas & Garcia, 2011).

Of the 322 begomoviruses recognized by the International Committee on Taxonomy of Viruses, more than a third infect tomato and probably many others can infect solanaceous plants, underscoring the importance of having a suitable tomato variety for virus testing. Tomato (*Solanum lycopersicum* L., Solanaceae) is an herbaceous plant with hundreds of varieties that differ in size and generation time. Tomato has long been the preferred system for studying plant-pathogen interactions involving plants from the Solanaceae family (Arie *et al*., 2007; Meissner *et al.* 1997; Emmanuel & Levy, 2002). Tomato is susceptible to a wide range of viral diseases, many of which are associated with significant agronomic losses (Hanssen *et al*., 2010; Inoue-Nagata *et al*., 2016). As an example, tomato yellow leaf curl disease is caused by begomoviruses and has spread worldwide to become one of the most important viral diseases of tomato (Lefeuvre *et al*., 2010).

There is considerable physiological and genetic variation among tomato varieties that affects their suitability for laboratory studies. Among tomato varieties, Micro-Tom (TGRC accession # LA3911, UC Davis, Department of Plant Sciences, USA), a dwarf tomato cultivar derived from

crossing cv. Florida Basket and Ohio 4013-3 (Scott & Harbaugh, 1989), is widely used in laboratory studies due to its small size (15–20cm in height), rapid life cycle (70–90 days), and because it can be readily and efficiently transformed (Emmanuel & Levy, 2002; Meissner *et al*., 1997, Martí *et al*., 2006; Carvalho *et al*., 2011; Okabe *et al*., 2011; Sun *et al*., 2006). Studies require less time to complete because of its rapid life cycle that can accommodate up to four generations per year. Even though Micro-Tom has been widely adopted, its potential for molecular studies is limited because of its mutant genetic background, which results in brassinosteroid deficiency and deep green rugose leaves induced by the presence of the dwarf (d) and miniature (mnt) recessive genes (Bishop *et al*., 1996; Pnueli *et al*., 1998; Martí *et al*., 2006). The brassinosteroid pathway has been implicated in viral disease and symptom development, and alterations in the pathway may interfere with virus-plant interaction studies in Micro-Tom (Campos *et al*., 2010). Moreover, the gibberellin response is altered in MicroTom (Martí *et al*., 2006)

and further interferes with data interpretation. In addition, Micro-Tom has a mutation in the self-pruning (sp) gene, which controls the regularity of the vegetative-reproductive switch along the compound shoot of tomato. This mutation is responsible for its determinate phenotype (Pnueli *et al*., 1998). Thus, it is important to look for new model systems that are either alternative or complementary to those currently used.

Solanum lycopersicum 'Florida Lanai' is also a small tomato variety that was developed for home gardens (Augustine *et al.*, 1981). It has regular leaves and determinate growth, reaching a height of 60–90cm. Flowers are open pollinated and produce a medium sized fruit (under 450g) maturing approximately 60 days after transplanting or 90 days from seeding. Seed germination rate ranges from 82% to 96%. Even though 'Florida Lanai' plants are small and have a short generation time, they do not carry the recessive genes that compromise the use of Micro-Tom. 'Florida Lanai' has been used previously to characterize a new begomovirus species (Tomato yellow margin leaf curl virus) using biolistics to inoculate infectious clones (Nava *et al*., 2013). It has also been used to study geminivirus-insect interactions (McKenzie, 2002), although there has been no systematic characterization of its suitability as a model system for geminiviruses. In this study, we used three inoculation methods to examine 'Florida Lanai' as a model system for studying diverse geminiviruses that naturally infect tomato.

3.2 Materials and methods

3.2.1 Plant growth conditions and inoculation protocols

Florida Lanai seeds were kindly supplied by J. Scott (University of Florida, USA). The plants were grown in sterile soil from seeds in a walk-in growth chamber at 25°C, 80% humidity and a 16:8 light/dark (LD) cycle. After one week, the seedlings were transplanted into pots and propagated for two more weeks before inoculation. Virus inoculation was done by either Agrobacterium (ABI)-mediated inoculation, low-pressure particle acceleration DNA delivery using a microdrop sprayer (Venganza, Inc.) or whitefly transmission from infected to healthy plant. The infectious clones

corresponding to Beet curly top virus (BCTV), *Tomato yellow leaf curl virus* (TYLCV), *Tomato mottle virus* (ToMoV DNA-A and DNA-B), *Tomato golden mosaic virus* (TGMV DNA-A and DNA-B), *Cabbage leaf curl virus* (CaLCuV DNA-A and DNA-B), are described in Table 3.1. *E. coli* culture clones for TYLCV, ToMoV, TGMV and CaLCuV DNA A and DNA B were prepared in LB broth containing 0.1µg/ml carbenicillin, subsequently grown overnight at 37°C with vigorous shaking. Similarly, their corresponding Agrobacterium clones were prepared in LB broth containing 0.075µg/ml Spectinomycin grown at 30°C. For BCTV, *E. coli* and Agrobacterium clones were prepared in 0.05µg/ml kanamycin LB broth cultured overnight at their respective temperatures. All experiments were repeated three times.

3.2.1.1 Agrobacterium-mediated inoculation

Agrobacterium cultures containing infectious clones in binary vectors were grown in LB broth with their corresponding antibiotics at 30°C overnight. The bacterial cultures were diluted 10-fold with LB media and used to inoculate ten plants for each treatment. For bipartite viruses, equal amounts of cultures corresponding to DNA-A and DNA-B genomes were mixed prior to inoculation. An Agrobacterium strain carrying an empty T-DNA vector was used for mock inoculation. Plants were then returned to the growth chamber. Agroinoculation procedures were described previously by Reyes *et al*. (2013).

3.2.1.2 Biolistics

Plasmid DNA (5µg) carrying infectious clones was coated onto 1µm gold (Au) particle suspensions as described in Cabrera-Ponce *et al*. (1997). The final pellet was resuspended in 65µL of absolute ethanol and used to spray 6 plants (10µL/plant) at 40psi. For the

Table 3.1: Infectious viral clones used to inoculate 'Florida Lanai' plants by agroinoculation or biolistics.

Virus[a]	Plasmid used for biolistics	Plasmid used for agroinoculation	References and comments
BCTV	BCTV in pMON521	BCTV in pMON521	Beet curly top virus (BCTV; strain Logan), a pMON525-based plasmid containing a BCTV DNA containing a partial tandem copy (provided by D. M. Bisaro of Ohio State University, Stenger *et al.*, 1991).
TYLCV	pTYLCV2	pNSB1736	Partial tandem copy of *Tomato yellow leaf curl virus* (TYLCV; Dominican Republic isolate) cloned into pMON721 (Settlage et al., 2005; Reyes *et al*., 2013), from Acc. number AF024715.
ToMoV DNA A	pNSB1906	pNSB1906	Partial tandem copy of *Tomato mottle virus* (ToMoV) DNA-A cloned into pMON721 (Abouzid *et a*l, 1992, Reyes *et al*, 2013)
ToMoV DNA B	pNSB1877	pNSB1877	Partial tandem copy of *Tomato mottle virus* (ToMoV) DNA-B cloned into pMON721 (Abouzid *et al*, 1992, Reyes *et al*, 2013)
TGMV DNA A	pMON1565	pMON337	Partial tandem copy of *Tomato golden mosaic virus* (TGMV) DNA-A (Fontes *et al*., 1994; Orozco and Hanley-Bowdoin, 1996, Elmer *et al*, 1988).
TGMV DNA B	pTG1.4B	pMON393	Partial tandem copy of *Tomato golden mosaic virus* (TGMV) DNA-B cloned in pTG1.4B (Fontes *et al*., 1994; Orozco and Hanley-Bowdoin, 1996, pMON393, first mentioned in Stenger *et al*, 1988).
CaLCuV DNA A	pCpCLCV A.003	pNSB1090	*Cabbage leaf curl virus* (CaLCuV) with a partial tandem copy (Turnage *et al*, 2002; Eagelkrout *et al*, 2002).
CaLCuV DNA B	pCpCLCV B.003	pNSB1091	*Cabbage leaf curl virus* (CaLCuV) with a partial tandem copy (Turnage *et al*, 2002; Eagelkrout *et al*, 2002).

[a] All clones have been designed to contain two viral origins of replication which allow the vector to release a functional viral monomer circularized by Rep and identical to wild-type viral DNA.

Bipartite geminiviruses, 5µg of each viral DNA component were mixed prior to coating the gold particles. The sprayer was positioned 2.5cm from the plant apex. Empty plasmid DNA was used for the mock controls.

3.2.1.3 Whitefly transmission

Experiments were carried out in whitefly proof cages using *Bemisia tabaci* MEAM1 adults from a colony maintained on 'Florida Lanai' tomato at 27°C and a 16:8 LD cycle in an environmental chamber. Approximately 100 adult whiteflies between 2 and 10 days post-eclosion were allowed to acquire virus by caging for 72 hr with a symptomatic 'Florida Lanai' plant infected with either TYLCV or ToMoV. The whiteflies were transferred to new cages containing healthy 'Florida Lanai' plants and allowed to feed continuously. The mock treatment was done by feeding the whiteflies on healthy plants. The plants were inspected for symptoms at 28 days post inoculation (dpi) and leaf samples collected for PCR analysis.

3.2.1.4 Seed transmission

Seeds were harvested from plants showing typical symptoms of TYLCV, ToMoV, BCTV or TGMV. Harvested seeds were washed, dried and sown in new pots. Samples were taken for DNA isolation from one leaflet of the fourth compound leaf (counted from the top of the plant) at 3 and 6 weeks after planting from 6 plants per treatment. Equal amounts of DNA from 6 plants were pooled for each treatment. For BCTV-infected plants, which do not produce fruit if infected early, healthy plants were inoculated with BCTV after initial fruit-setting. Seeds were harvested and analyzed as described above. All pooled samples were analyzed by conventional PCR using virus-specific primers (Table 3.2).

3.2.2 Plant inspection and data collection

Plants were inspected weekly from1dpi to record disease symptoms and plant height. Disease symptoms were recorded by photography using a digital camera (Panasonic

Lumix DMC-FZ28). Plant height (cm) was measured from the base to the tip of the main shoot for each plant (Olaniyi *et al*., 2010). The measurements were recorded as height increase by subtracting initial height of a plant at the time of inoculation from the height measured at the time of data recording. Data were also recorded on yield parameters (number of flowers, number of fruit and fresh fruit weight). The number of flowers was recorded 60 days after planting. The number of fruits and the fresh fruit weight was recorded at harvest (95 days from planting).

Table 3.2: List of primers used for PCR amplification of viruses in this study.

Primer name	Sequence (5′ → 3′)	Virus species	Expected size (nt)
BCTV15-for[a]	CGTTACTGTGACGAAGCATTTG	BCTV	283
BCTV15-rev[a]	CTCCTTCCCTCCATATCCAGTA	BCTV	
TYLCV15-for[b]	CCTCTGGCTGTGTTCTGTTATC	TYLCV	257
TYLCV15-rev[b]	GCAATCTTCGTCACCCTCTAC	TYLCV	
ToMoV pNSB1[c]	GTCCAATACTCTCTCGTCCAATC	ToMoV	239
ToMoV pNSB2[c]	CAGCGGCCTTGTTAATTCTTG	ToMoV	
Sal-Nco[d]	CGACAAAGACGGAGATACTCT	TGMV	397
AL1 RT[d]	GCCTAGTGAACGAGCCCACA	TGMV	
CaLCuV1990-F[e]	ACATACATCAGAGTCGCAAGAG	CaLCuV	223
CaLCuV1990-R[d]	ACTGCCCGGATTCACAATAA	CaLCuV	

[a] Primer designed using GenBank accession nos. NC_001412, M24597, AY134867, EU586260 and JN817383

[b] Primer designed using GenBank accession nos. AM409201, EU085423, AB192965, KC852149 and KJ879950

[c] Primer designed using GenBank accession nos. EF028241, L14460, EU709520 and AY965900.

[d] Primer designed using GenBank accession nos. K02029, JF694490 and JF694488.

[e] Primer designed using GeneBank accession nos. U65529 and DQ178612.

3.2.3 DNA extraction and virus detection

Samples were collected from the fourth compound leaf from the top of individual plants and consisted of a single base leaflet. Independent samples were placed in 2-mL cryovials at 14, 17, 21 and 31dpi from 10 plants for each treatment and frozen immediately in liquid nitrogen. DNA was extracted using the CTAB DNA extraction method (Doyle and Doyle, 1987). DNA concentrations and quality were assessed using a Nanodrop (ThermoScientific™). For plants infected with ToMoV, which showed a recovery phenotype, DNA was prepared from the first, second and third compound leaves from the apex.

A convergent primer pair that amplifies a short DNA fragment (≤300bp) was designed for each virus (Table 3.2) by SciTool Integrated DNA Technology (IDT) using sequences from the Genbank. Primers were first tested in conventional PCR to establish optimum annealing temperature and amplification efficiency before being used in quantitative real-time PCR (qPCR). Viral DNA was quantified using a qPCR standard curve generated by amplification of known amounts of plasmid DNA containing viral sequences (Table 3.1) that was 10-fold serially diluted from 10−1 to 10−4 range. QPCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems), at 95°C denaturation for 10 minutess, then 40 cycles of 95°C for 15 s and 58°C for 30 seconds. CT values were determined automatically by the instrument. The specificity of the reaction was determined by melting curves. Concentration of the template DNA in the reaction mix was converted from ng/µL to copy number/µL using the following formula: (C×10−9/MW)×NA where C=template concentration ng/µL, MW=template molecular weight in Daltons and NA=Avogadro's constant, 6.022×1023. MW was obtained by multiplying the number of base pairs of a plasmid by the average molecular mass of one base pair (660g/mol). A base 10 logarithmic graph of copy number versus the threshold cycle (Ct) for the dilution factor was plotted and used as a standard curve to determine the amount of viral DNA (copy number) in each µL of total DNA in a reaction mix.

The qPCR analyses were performed with the MX300P real-time thermocycler (Stratagene, La Jolla, CA) using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA). The amplification reactions were performed in 50µL containing 0.2µM forward and reverse primers, ultrapure water and the optimum amount of DNA template as determined in titration experiments for the respective viruses. Each virus was tested in a separate 96-well plate in which the first row contained the 10-fold serially diluted plasmid DNA for the standard curve.

3.2.4 DNA ploidy levels

To determine DNA ploidy levels of 'Florida Lanai' infected with different geminiviruses, leaf samples were taken from plants showing symptoms of TYLCV, ToMoV and BCTV as well as from mock-inoculated and healthy plants for comparison. Three biological replicas were collected for each treatment. Ploidy levels were determined using an Accuri™ C6 Flow Cytometer (BD Biosciences). Nuclei suspensions were prepared by chopping ca. 200mg of fresh leaf tissue with a sharp razor blade in chopping buffer (3mL Galbraith buffer+10µL β-ME and 2µL RNase A) for 5 min on ice. Buffer preparation and other processes were done according to the BD Accuri™ C6 Flow Cytometer user manual. Data were plotted using internal BD Accuri C6 software, and peak positions and relative ploidy indices determined.

3.2.5 Statistical analysis

Statistical analysis was performed using Microsoft Excel (Office 2013). Analysis was performed using paired, two-tailed Student's t-test and $p < 0.05$ as the statistically significant cutoff. One-way analysis of variance was used to establish differences among group means and the least significant difference (LSD) test was used in pairwise comparison to analyze differences between means.

3.3 Results

Three inoculation protocols mimicking infection in laboratories and in plants natural environment were used: agroinoculation, particle bombardment and whitefly transmission, to inoculate ‘Florida Lanai’ plants with 5 diverse geminiviruses. A characterization of the effects of inoculation with each virus onto ‘Florida Lanai’ was performed. Also, seed transmission was determined for four of the viruses. A comparison between 'Florida Lanai' and Micro-Tom is shown for healthy plants (Fig. 3.1).

Figure 3.1: Comparison at 45 day-old A: Florida Lanai and B: Micro-Tom tomato varieties.

3.3.1 Symptom expression

Agroinoculation was a very efficient method for inoculating ‘Florida Lanai’ with TYLCV, ToMoV and BCTV resulting in 100% infection. Typical symptoms were observed in plants inoculated with these three viruses (Fig. 3.2A–D). Symptoms started to appear as early as 4 dpi for TYLCV and ToMoV and 7 dpi for BCTV. There were no observable symptoms in plants inoculated with TGMV or CaLCuV and no virus was

detected by PCR. The failure of the TGMV to induce symptoms in tomato is well documented (Wyant *et al*., 2012).

When young plants were agroinoculated with TYLCV (28 days after planting), the plants showed chlorotic leaf margins, upward leaf curling, severe leaf size reduction and flower abscission (Fig. 3.2B). When older plants were inoculated with TYLCV (45 days after planting), symptoms were limited to middle and upper leaves and ca. 85% of the floral buds were lost by abscission. Other symptoms included swelling of veins and severe stunting.

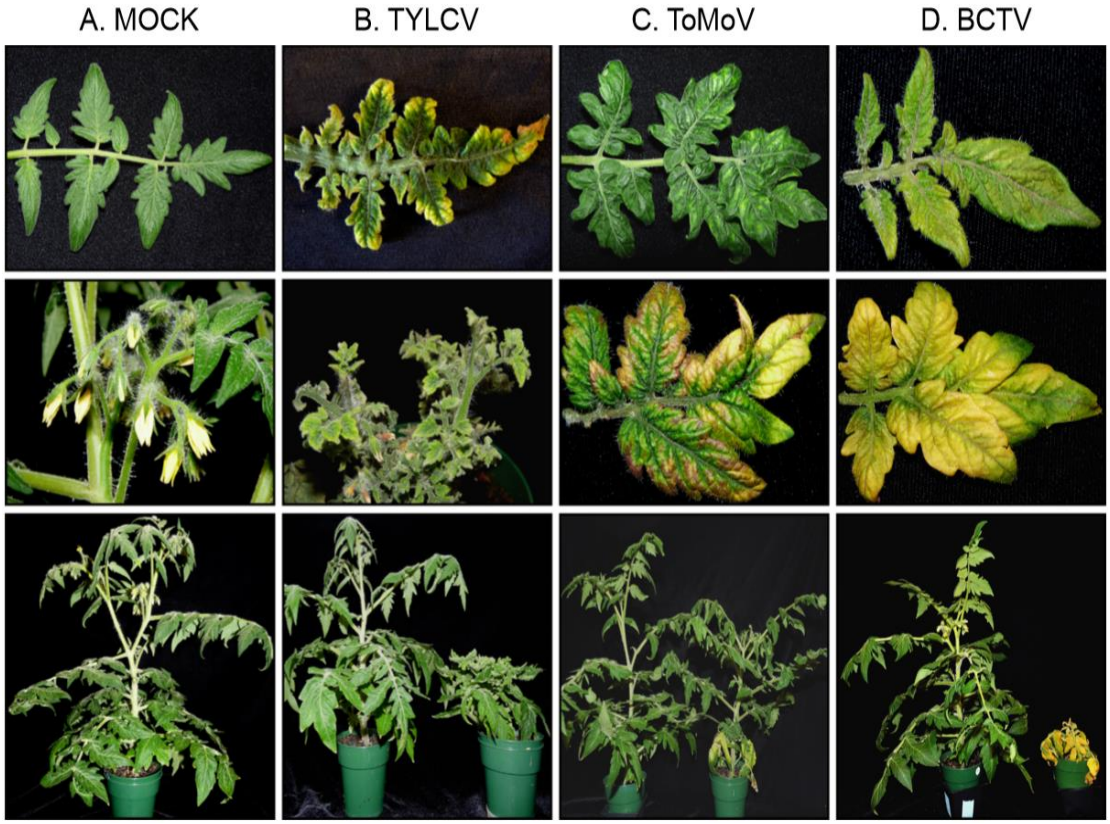


Figure 3.2: Symptoms observed on Florida Lanai plants mock- and agro-inoculated with TYLCV, ToMoV and BCTV.

A: Mock-inoculated plant showing a healthy leaf, healthy flowers and a healthy plant (top to bottom). **B:** TYLCV inoculation showing chlorotic leaf margins, severe leaf size reduction, flower abscission and severe height reduction. **C:** ToMoV inoculation displaying bright yellow mottling in upper leaves, severe yellowing of lower leaves and medium plant height reduction. **D:** BCTV inoculation with general yellowing with mixed shades of green at early stages of infection, deep yellowing of the whole plant and very severe stunting at late stages of infection.

Plants agroinoculated with BCTV developed a general yellowing mixed with green at early stages of infection that progressed to deep yellow at advanced stages (Fig. 3.2D). Leaves were stunted, thicker and crisp with swollen veins. BCTV-infected plants generally exhibited severe stunting (Table 3.3 and Fig. 3.5). Approximately 25% of the plants infected at an early growth stage (28 days after planting) exhibited root decay and were dead by 45 dpi, while the remaining plants did not recover or produce flowers. Plants infected later (45 days after planting) produced a few flowers, which did not open and dropped before fruit set.

Table 3.3: Comparison between infected and healthy tomato plants for the change in height at different days after inoculation.

	Mean (cm)[a]	P-value[b]	% of height reduction
Mock			
7 dpi	2.65±0.66		
14 dp1	5.27±1.28		
21 dpi	7.68±1.56		
28 dpi	8.58±1.31		
35 dpi	11.1±1.26		
TYLCV			
7 dpi	1.05±0.38	≤0.001	60.4
14 dpi	2.09±0.54	≤0.001	60.6
21 dpi	2.52±0.60	≤0.001	67.2
28 dpi	2.99±0.62	≤0.001	65.2
35 dpi	4.31±0.65	≤0.001	61.0
ToMoV			
7 dpi	1.79±0.63	0.008	32.5
14 dpi	3.94±1.04	0.02	25.8
21 dpi	6.15±1.64	0.05	19.9
28 dpi	8.00±0.99	0.28	6.76
35 dpi	10.4±1.42	0.27	6.15
BCTV			
7 dpi	1.93±0.64	0.008	28.3
14 dp1	2.07±0.72	0.002	62.1
21 dpi	2.16±0.73	≤0.001	72.5
28 dpi	2.36±0.87	≤0.001	72.8
35 dpi	2.88±0.15	≤0.001	73.3

[a] Mean±S.D, n=10

[b] Significance level ($P \leq 0.05$)

Plants agroinoculated with ToMoV typically developed a bright yellow chlorotic mottling on younger leaves and severe yellowing, leaf deformation and upward curling on lower leaves (Fig. 3.2C). Compared to plants infected with TYLCV or BCTV, ToMoV-infected plants showed only moderate stunting, less flower abscission and a smaller reduction in fruit (Fig. 3.2, 3.3, and Table 3.3). During ToMoV infection, the yellow chlorotic symptoms observed from 5 to 14dpi changed to a recovery phenotype in which new leaf growth was symptomless and the plant grew faster producing many flowers and fruit (Fig. 3.3 and Table 3.3). ToMoV DNA was detected by PCR in leaves showing the recovery phenotype.

Particle bombardment led to the infection of two of the five viruses used in this study. Virus symptoms were observed in 100% of the 'Florida Lanai' plants inoculated with ToMoV or TGMV by bombardment. No infected plants were observed in equivalent experiments using plasmids corresponding to TYLCV, BCTV or CaLCuV. Plants bombarded with ToMoV developed symptoms indistinguishable from those in agroinoculation experiments (Fig. 3.4A). Bombardment of TGMV DNA resulted in bright yellow coloration along veins (Fig. 3.4B). In comparison, TGMV inoculated plants (*N. benthamiana*) exhibited chlorotic mottling, leaf curling or spiral distortion, which was not observed in Florida Lanai.

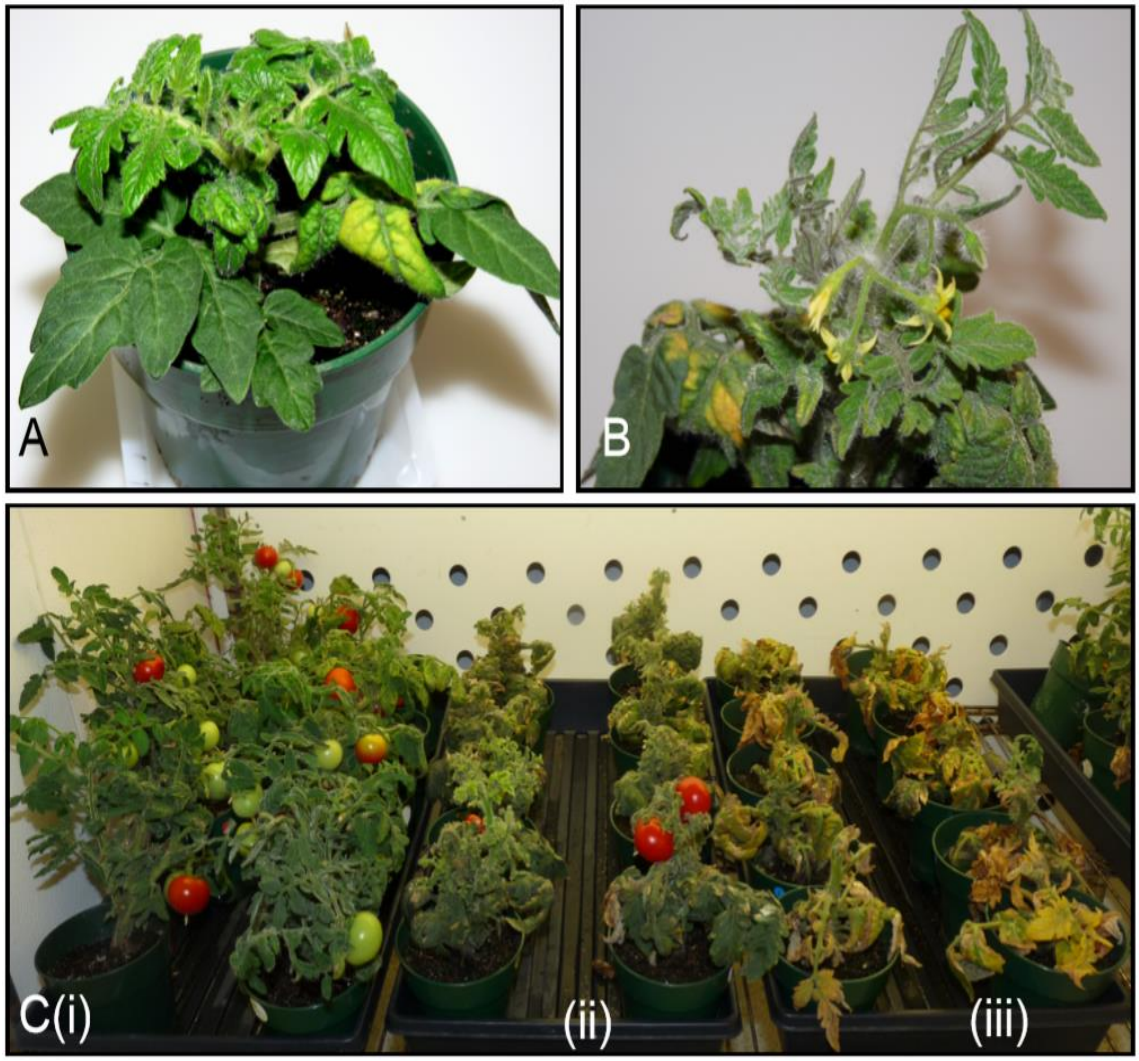


Figure 3.3: Florida Lanai recovering from ToMoV infection. A: Infection at 14 dpi, B: Infection at 28 dpi, C: Impact of recovery on yield (i) ToMoV, (ii) TYLCV and (iii) BCTV

TYLCV and ToMoV were tested in whitefly transmission assays. Based on symptoms and PCR analysis, TYLCV was successively transmitted by viruliferous whiteflies from a TYLCV-infected source plant to a healthy Florida Lanai. By 30 days after introduction of viruliferous whiteflies, the target plants exhibited chlorotic leaf margins, upward curling of leaves, reduced leaf size and other symptoms characteristic of TYLCV infection described above (Fig. 3.4C). Whitefly transmission of ToMoV resulted in a very mild mottling on leaves (Fig. 3.4D). Efficience of transmission by whiteflies of the two viruses was 100%.

A recent study (Kil *et al*., 2016) reported that geminiviruses can be transmitted through seed collected from TYLCV-infected plants. We produced seed from fruit collected from plants infected with TYLCV, ToMoV, BCTV or TGMV. After washing carefully with water, the seeds were planted and F_1 and F_2 progeny plants were examined for symptoms and viral DNA. None of the plants developed symptoms, and PCR assays did not detect viral DNA in any of the plants. These results showed that the geminiviruses we tested are not transmitted through 'Florida Lanai' seed.

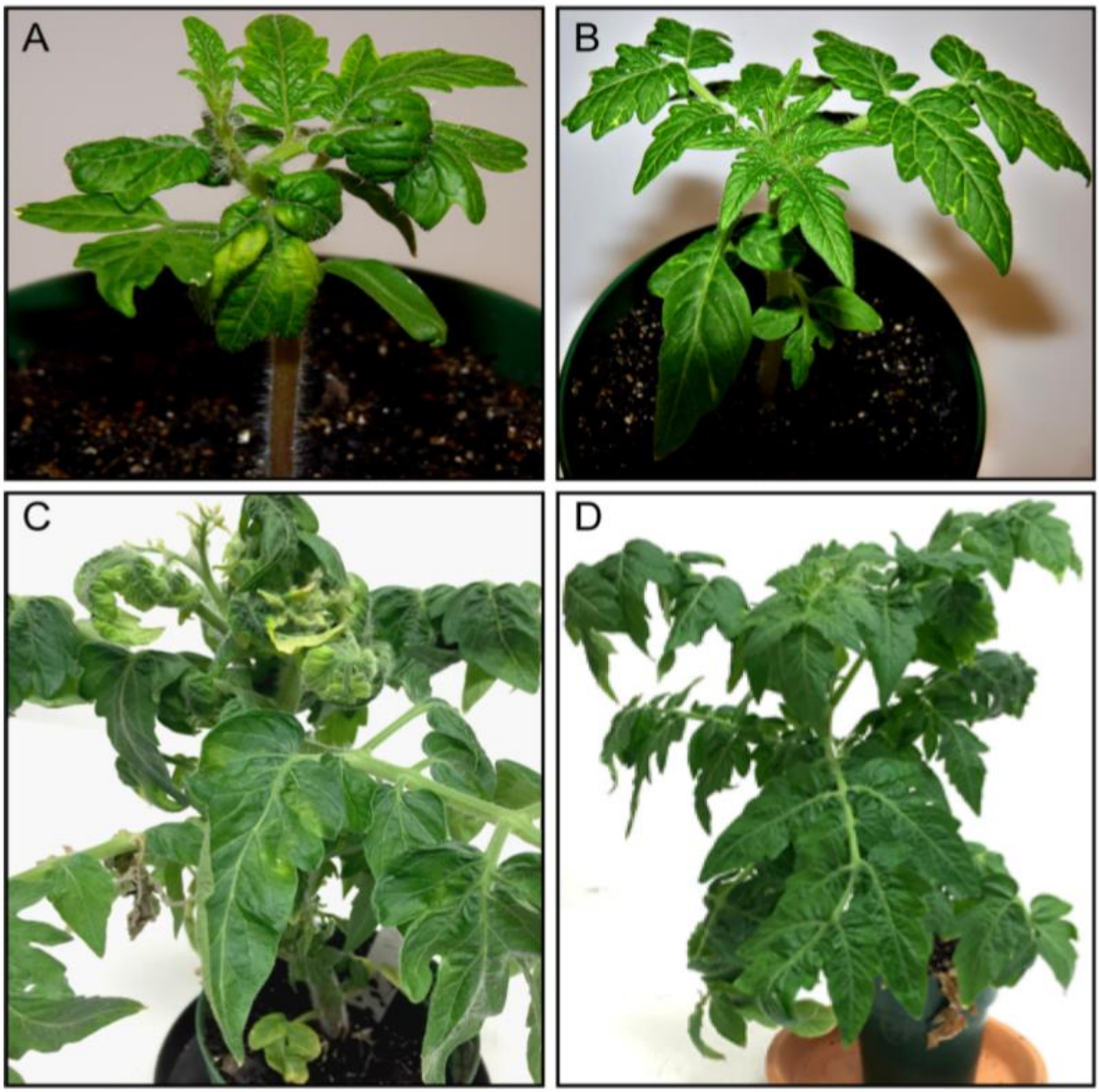


Figure 3.4: Symptoms on inoculated Florida Lanai by biolistics using A: ToMoV and B: TGMV. Bottom row: Florida Lanai infected by whitefly transmission using C: TYLCV and D: ToMoV, showing severe and very mild symptoms respectively.

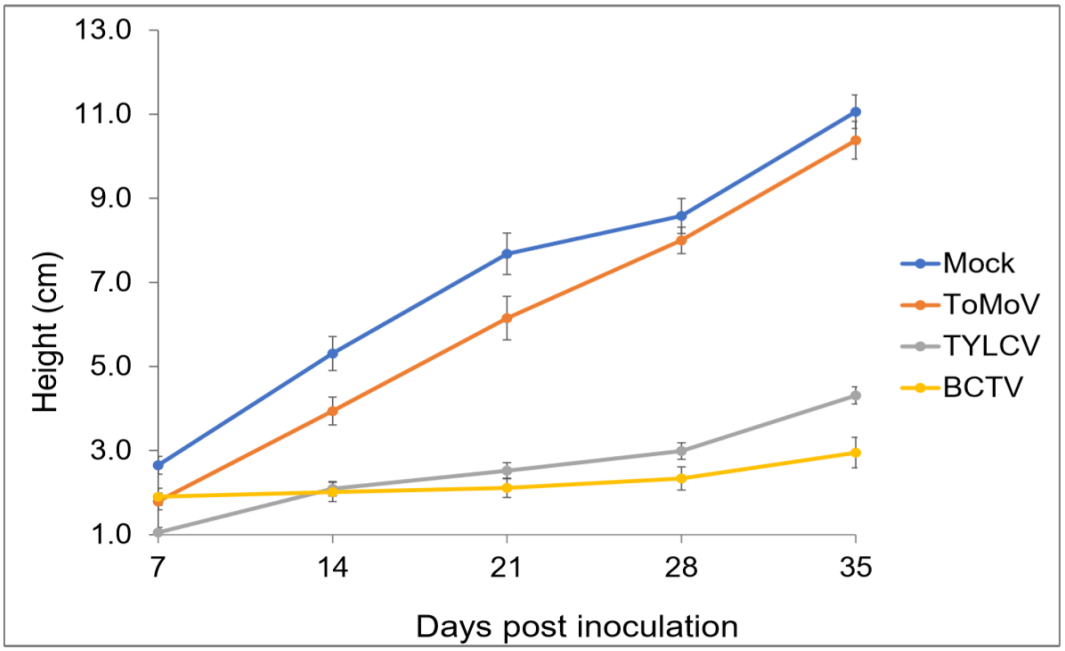


Figure 3.5: Change in plant height for Florida-Lanai tomato plants infected with TYLCV, ToMoV, BCTV and mock at different days post inoculation. Vertical bars represent the standard error (SE) of the means. N=10 for all treatments.

3.3.2 Virus titer

Analyses of virus titer by conventional PCR or qPCR used total DNA extracted from leaves of 'Florida Lanai' plants. Primer pairs (Table 3.2) were optimized to amplify viral DNA at an annealing temperature of 58°C. The TYLCV, ToMoV and BCTV standard curves for qPCR were linear in the range of 50 (1:10 dilution) to 5 x 10^{-6} ng (1:1 x 10^{6} dilution) per reaction ($r^2 > 0.99$). We used 5ng/reaction of total DNA for qPCR analysis of unknown viral DNA titers. This amount (5ng) can be easily measured using a spectrophotometer.

Analysis of variance (ANOVA) showed that virus levels changed over time in all treatments (Fig. 3.7). There was a significant change up to 31 dpi in the means of viral load in plants infected with TYLCV ($F_{3, 24}=5.30$, $p<0.05$), ToMoV ($F_{3,24}=7.28$, $p<0.05$) or BCTV ($F_{3,24}=3.08$, $p<0.05$) (Table 3.5). Mean separation by a LSD test (Table 3.6) showed that virus titer in plants infected with TYLCV increased significantly ($\alpha=0.01$) at 10, 17 and 24 dpi and then decreased at 31 dpi to a level similar to 10dpi. Viral DNA increased in ToMoV-infected plants over a shorter window of time between 10 to 17 dpi ($\alpha=0.05$) and then declined (31 dpi, $\alpha=0.01$) consistent with the recovery phenotype. BCTV infected plants showed a continuous increase in virus titer from 10 to 31 dpi, with a significant increase at 31 dpi ($\alpha=0.01$). This correlates with observed continuous increase in symptom severity over time.

3.3.3 Plant height

Plants infected with TYLCV, ToMoV or BCTV were shorter than the mock-inoculated controls (Table 3.3 and Fig. 3.5). The reduction in height was highly significant ($P<0.05$) for plants infected with BCTV or TYLCV at all sampling times. In contrast, ToMoV infection resulted in a significant height reduction during the initial stages of infection (7 and 14dpi). During the later stages (21 and 28dpi), ToMoV-infected plants underwent recovery and the heights of infected and mock-inoculated plants were not statistically different. The establishment of BCTV infection was initially delayed (Table 3.3), but it ultimately caused the most severe disease symptoms. BCTV caused the largest reduction in the mean plant height (73.3%) at 35 dpi followed by TYLCV (67.2%) at 21 dpi. ToMoV had the smallest effect on Lanai growth. It recorded only 32.5% reduction in plant height at the initial stage of infection (7 dpi) before the plants recovered (Table 3.3).

3.3.4 Yield

Plants infected with TYLCV, BCTV or ToMoV showed reduced yields (Table 3.4). Reductions were most pronounced for TYLCV and BCTV, which were reduced for mean flower number, fruit number and fruit weight (g) per plant by 69.3, 93.5, and 95.3% respectively for TYLCV and 87.8, 100 and 100% respectively for BCTV. In contrast, ToMoV reduced the yield metrics by 8.5, 27.4 and 29.8%, respectively. The reductions were significant ($P < 0.05$) for numbers of flowers and fruit and fruit weight for plants infected with TYLCV and BCTV. The reductions in number of fruit and fruit weight were also significant for ToMoV-infected plants, but the reduction in number of flowers was not. From these results, it appears that TYLCV reduces the number of flowers and the proportion of flowers resulting in fruit due to excessive abscission, while ToMoV does not change the number of flowers produced by plants but increases flower abscission and causes a smaller reduction in fruit size. BCTV impairs the ability of plants to produce viable flowers and had a greater effect on yield than TYLCV. Plants infected early (21 days old) with BCTV produced very few flowers and none of them set fruit (Table 3.4). When older plants (at flowering, 45 days old) were infected with BCTV they formed flower buds that failed to open and eventually died. Generally, all plants including the mock-inoculated controls produced many more flowers that set and produced fruit.

Table 3.4: Effect of TYLCV, ToMoV and BCTV on yield.

	Mean[a]	P-value[b]
Mock		
Mean flower number per plant	18.9±4.15	
Mean fruits number per plant	6.20±1.62	
Mean fruit weight per plant (g)	61.3±14.4	
TYLCV		
Mean flower number per plant	5.80±2.57	≤0.001
Mean fruits number per plant	0.40±0.70	≤0.001
Mean fruit weight per plant (g)	2.91±8.63	≤0.001
ToMoV		
Mean flower number per plant	17.3±5.71	0.48
Mean fruits number per plant	4.50±1.90	0.045
Mean fruit weight per plant (g)	43.0±22.2	0.045
BCTV		
Mean flower number per plant	2.30±0.2.21	≤0.001
Mean fruits number per plant	0±0.00	≤0.001
Mean fruit weight per plant (g)	0±0.00	≤0.001

[a] Mean±S.D, n=10

[b] Significance level ($P \leq 0.05$).

Table 3.5: One-way analysis of variance (ANOVA) for means of virus titer (copy number) for TYLCV, ToMoV and BCTV.

	Source of Variation	SS	df	MS	F	P-value	F crit
TYLCV	Between Groups	8.05E+15	3	2.68E+15	5.29	0.00602	3.01
	Within Groups	1.21E+16	24	5.07E+14			
	Total	2.02E+16	27				
ToMoV	Between Groups	3.66E+16	3	1.22E+16	7.27	0.00123	3.01
	Within Groups	4.03E+16	24	1.68E+15			
	Total	7.69E+16	27				
BCTV	Between Groups	2.11E+14	3	7.03E+13	3.08	0.04649	3.01
	Within Groups	5.48E+14	24	2.28E+13			
	Total	7.59E+14	27				

Table 3.6: Difference between means and significance of pairwise comparison (LSD) for means of virus copy number for TYLCV, ToMoV and BCTV at different days post inoculation. Differences indicated * are significant at the α < 0.05 level and ** are significant at the α < 0.01 level.

		10dpi	17dpi	24dpi	31dpi
TYLCV	10dpi	0	4.99E+7**	2.42E+7 ns	2.06E+7 ns
	17dpi		0	4.75E+8**	5.20E+8**
	24dpi			0	4.48E+7**
	31dpi				0
ToMoV	10dpi	0	5.66E+7*	3.36E+6 ns	4.52E+7 ns
	17dpi		0	6.00E+7*	1.02E+8**
	24dpi			0	4.18E+7 ns
	31dpi				0
BCTV	10dpi	0	2.94E+6 ns	4.99E+6 ns	7.48E+6**
	17dpi		0	2.05E+6 ns	4.54E+6 ns
	24dpi			0	2.50E+6 ns
	31dpi				00

Dpi = days post inoculation.
ns = not significant.

3.3.5 DNA ploidy

Geminivirus infection modifies plant cell cycle controls to support replication of both viral DNA and plant chromosomes leading to increase in genome ploidy (Ascencio-Ibáñez *et al*., 2008). Flow cytometry analysis of leaf nuclei of Lanai plants infected with TYLCV, ToMoV or BCTV and uninfected leaf controls showed four peaks corresponding to nuclei with 2C, 4C, 8C and 16C ploidy (Fig. 3.6). Virus infection changed the distribution of the peaks. A reduction in cells with lower ploidy (2C) and

enrichment in cells with higher ploidy (4C, 8C and 16C) was observed during infection, with BCTV-infected plants displaying the largest changes in ploidy. The differences were found to be statistically significant for 4C and 16C for ToMoV and TYLCV infected plants, as well as for 16C for BCTV infected plants (Fig. 3.6).

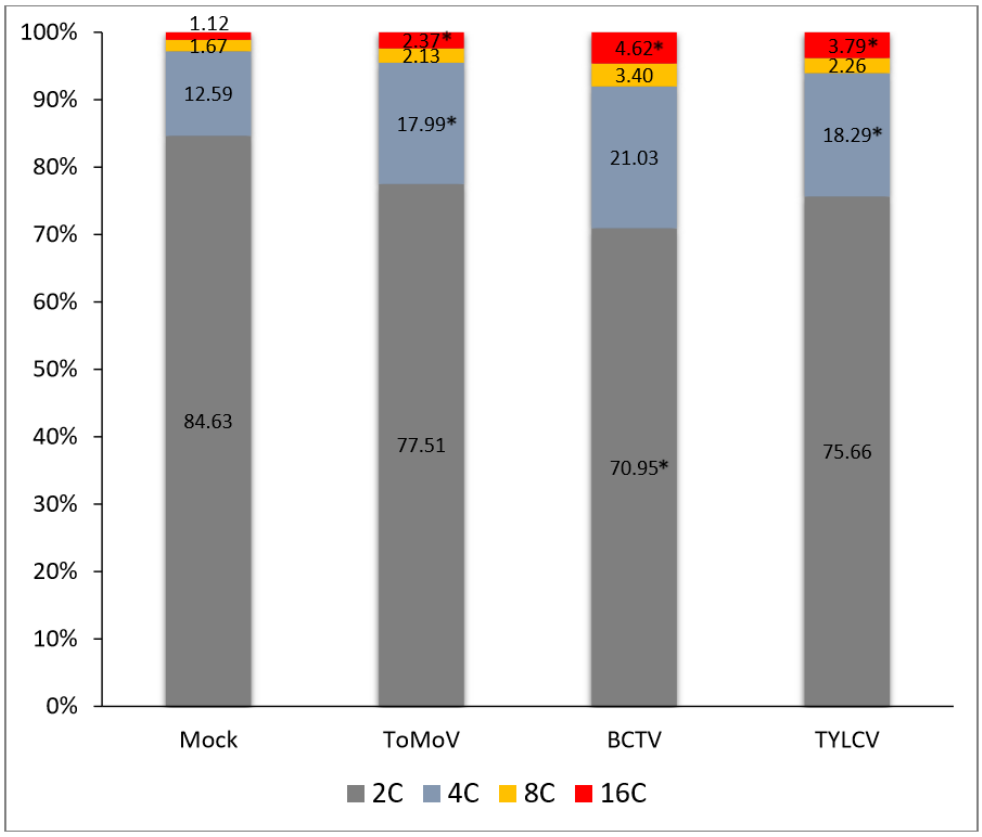


Figure 3.6: Histogram of the relative fluorescence intensity of nuclei isolated from leaves of Lanai plants either mock-inoculated or inoculated with ToMoV, BCTV or TYLCV. The bars represent ploidy percentages for each treatment. Values indicated by * are statistically significant ($P < 0.05$).

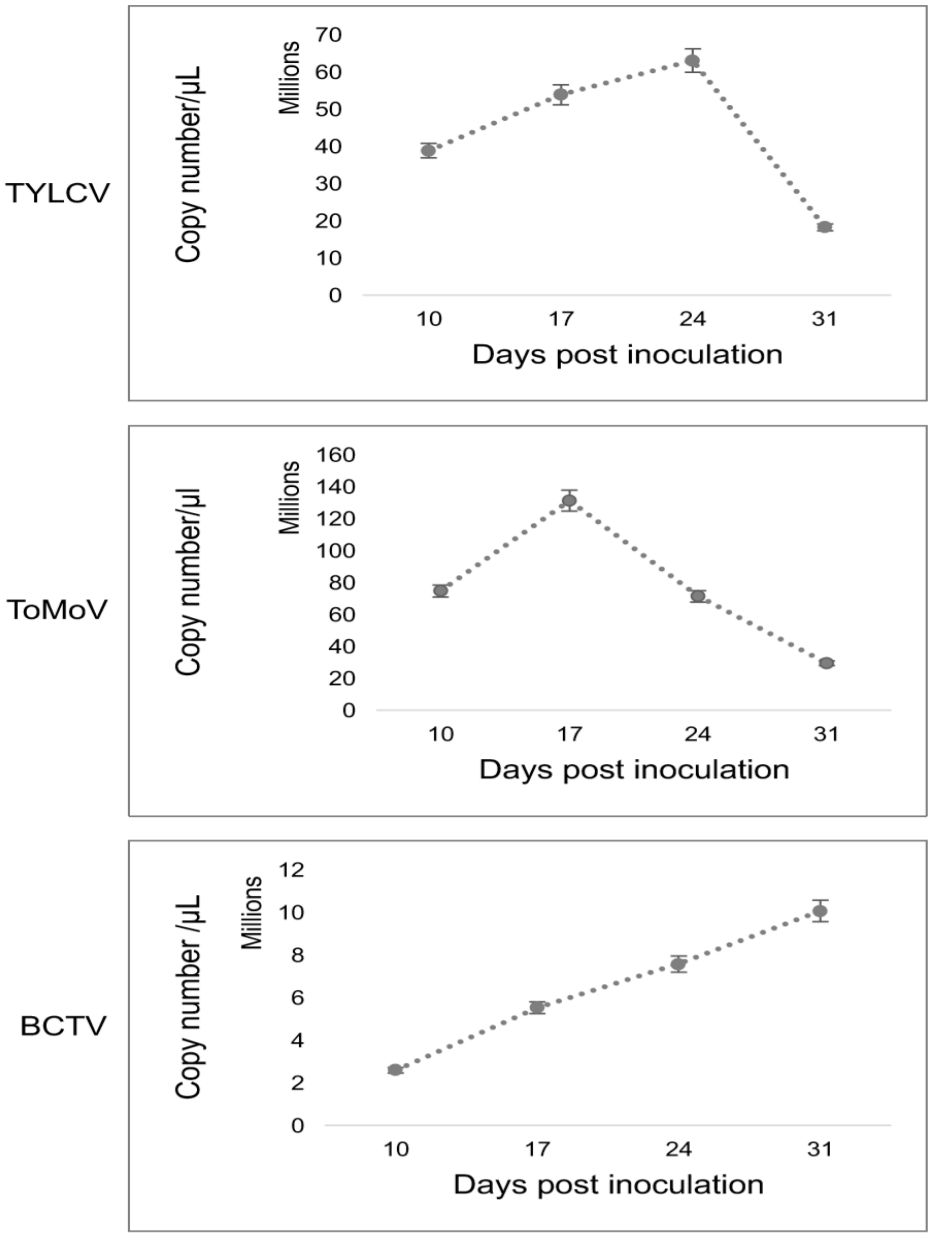


Figure 3.7: Changes in viral load over time for Florida Lanai infected with A: TYLVC, B: ToMoV and C: BCTV. Vertical bars represent the standard error (SE) of the means. N=7 for all treatments

3.4 Discussion

Other studies have highlighted the facility of virus transmission and ability to allow rapid replication as the most important characteristics of a good model plant (Gergerich & Dolja, 2006; MacLean *et al*., 2011). TYLCV is an Old World monopartite begomovirus. ToMoV and TGMV are New World bipartite begomoviruses. Two of these viruses were identified and isolated from tomato, whereas TGMV was identified in tomato but propagated in and isolated from *N. benthamiana* (Cohen & Nitzany, 1966; Matyis *et al*., 1975; Costa, 1976; Buck & Coutts, 1985; Bisaro *et al*., 1982; Abouzid *et al*., 1992; Crespi *et al*., 1995). CaLCuV is a bipartite begomovirus in the Squash leaf curl clade from the New World (Nawaz-ul-Rehman *et al*., 2009). CaLCuV was not cloned from or considered to be a pathogen of tomato. BCTV, which has a single-component genome, is a curtovirus with a broad host range that includes tomato (Bennett, 1971; Chen *et al*., 2010). 'Florida Lanai' plants were readily infected (100% success rate) by TYLCV, ToMoV and BCTV using agroinoculation, regardless of plant growth stage. ToMoV and TGMV were transmitted mechanically by a microdrop-sprayer, while ToMoV and TYLCV were transmitted by whiteflies (whitefly transmission of TGMV was not tested). Together, these results established that 'Florida Lanai' is a versatile model for studying geminivirus infection in tomato. The ability to infect the variety using more than one method of inoculation provides important alternatives when facilities or expertise to carry out other methods are lacking. The inability to inoculate TYLCV and BCTV by bombardment most likely reflects that they are largely phloem limited. With a few exceptions, phloem-limited viruses are not mechanically transmitted (Schneider, 1973; Esau, 1977, Rojas *et al*., 2001; Wyant, *et al*., 2012; Miozzi *et al*., 2014). Although recent reports showed geminivirus seed transmission (Kil *et al*., 2016), no seed transmission was detected for the viruses infecting Florida Lanai. Seeds were washed extensively prior to planting to minimize any potential contamination of the seed coat from surrounding fruit tissue, which may contain virus.

Another observation is the ability of TGMV to infect Florida Lanai. Tomato is thought to be a non-host for TGMV even though the virus was originally found in tomato but maintained and cloned from *N. benthamiana* (Stenger *et al*., 1992; Matyis *et al*., 1975). Florida Lanai was readily infected with TGMV by biolistics with a100% success rate. A previous study (Wyant *et al*., 2012) inoculated three tomato cultivars, including var. Moneymaker, with 25% efficiency.

The observation that 'Florida Lanai' plants displayed typical disease symptoms as early as 4 dpi, is an important characteristic of a good model plant. The short incubation period of the pathogen depends not only on the infectious agent but also on host susceptibility and ability to express symptoms (Dmitry & Van den Ackerveken, 2013). 'Florida Lanai' plants developed viral symptoms quickly, producing typical and distinct symptoms for different geminiviruses and enabling a systematic evaluation of the impact of different viruses in a common host. Walkey (1991) stated that good indicator plants respond to viral infections consistently and distinctively. These are important requirements for a model plant, especially when making a disease diagnosis, fulfilling Koch's postulates or characterizing virus-host interactions. Quantifiable effects of virus infection on symptoms, leaf deformation, plant height, flower number, fruit number, fruit weight, effect on roots, and DNA ploidy were detected.

We used flow cytometry to examine the effect of virus infection on plant ploidy. TYLCV, ToMoV or BCTV infection increased the number of cells with higher ploidy levels (4C, 8C and 16C) and reduced the number of cells with lower ploidy levels (2C). These results confirm previous reports of increases in ploidy in mature leaves during geminivirus infection (Ascencio-Ibáñez *et al*., 2008). The earlier study detected ploidy changes in CaLCuV which is not confined to the phloem. Thus, it was surprising to detect significant ploidy changes for BCTV and TYLCV, both of which have been reported to be phloem-limited in tomato (Schneider, 1973; Esau, 1977, Rojas *et al*., 2001; Miozzi *et al*., 2014), and it will be interesting to characterize further the interactions of these two viruses with 'Florida Lanai'.

The patterns of virus accumulation in 'Florida Lanai' plants infected with TYLCV, ToMoV and BCTV provide more evidence of its suitability as a model system. The patterns related clearly with the severity of symptoms exhibited by the plants, fitting the general concept that higher virus titer leads to more plant damage (Ponz and Bruening, 1986). The kinetics of virus accumulation for TYLCV and BCTV followed general virus infection patterns (Rom *et al*., 1993) and corresponded well with the development and maintenance of severe symptoms throughout the time course of infection. In contrast, ToMoV plants showed significant rise in viral load early in infection followed by a rapid decrease. This decline was associated with the disappearance of symptoms. Reduced virus accumulation and a recovery phenotype are thought to be the consequence of host defenses overcoming the virus (Covey *et al*., 1997; Ratcliff *et al*., 1999; Zhou et al., 2008; Ma *et al*., 2015). One of the main factors in the success of infection is the ability of a given virus to suppress plant silencing pathways (Qu & Morris, 2005; Hanley-Bowdoin *et al*., 2013; Pumplin & Voinnet, 2013). Our results suggested that TYLCV and BCTV have stronger silencing suppressing activities than ToMoV. The use of a common plant host provides an excellent system for studying these differences because it eliminates any effects due to potential differences between host silencing factors across plant species and varieties (Nie & Molen, 2015).

The Florida Lanai tomato variety is an excellent model system for studying and comparing tomato infecting geminivirus-host interactions. Using this model system, researchers can obtain reliable results quickly even when space is limited. 'Florida Lanai' plants are readily infected by different viruses, delivered using different methods, to produce distinct measurable symptoms. More than 60 geminivirus species infect tomato (Inoue-Nagata *et al*., 2016), and we tested only four here. Hence, there is a need for further studies to determine if more geminiviruses can infect Florida Lanai. We recommend Florida Lanai as an excellent tomato variety for use as a model system for agroinoculation studies of TYLCV, ToMoV and BCTV, for mechanical bombardment of ToMoV and TGMV, and for whitefly transmission for TYLCV and ToMoV.

Researchers may find it useful to use Florida Lanai in virus transmission studies, disease epidemiology studies and when investigating various physiological phenomena.

CHAPTER FOUR

CHARACTERIZE THE MECHANISM OF GEMINIVIRUS RESISTANCE IN ARABIDOPSIS

Abstract

Geminiviruses are DNA viruses that cause severe crop losses in different parts of the world, and there is a need for genetic sources of resistance to help combat them. Arabidopsis has been used as a source for virus-resistant genes that derive from alterations in essential host factors. We used a virus-induced gene silencing (VIGS) vector derived from the geminivirus *Cabbage leaf curl virus* (CaLCuV) to assess natural variation in virus–host interactions in 190 Arabidopsis accessions. Silencing of *CH-42*, encoding a protein needed to make chlorophyll, was used as a visible marker to discriminate asymptomatic accessions from those showing resistance. There was a wide range in symptom severity and extent of silencing in different accessions, but two correlations could be made. Lines with severe symptoms uniformly lacked extensive VIGS, and lines that showed attenuated symptoms over time (recovery) showed a concomitant increase in the extent of VIGS. One accession, Pla-1, lacked both symptoms and silencing, and was immune to wild-type infectious clones corresponding to CaLCuV or *Beet curly top virus* (BCTV), which are classified in different genera in the Geminiviridae. It also showed resistance to the agronomically important *Tomato yellow leaf curl virus* (TYLCV). Quantitative trait locus mapping of a Pla-1 X Col-0 F_2 population was used to detect a major peak on chromosome 1, which is designated *gip-1* (*geminivirus immunity Pla-1-1*). The recessive nature of resistance to CaLCuV and the lack of obvious candidate genes near the *gip-1* locus suggest that a novel resistance gene(s) confers immunity.

4.1 Introduction

The Geminiviridae is a large family of circular, single-stranded DNA (ssDNA) plant viruses named for their twinned particles (Hanley-Bowdoin *et al*., 2013). They are classified into different genera depending on their insect vector, genome structure and host range (Hanley-Bowdoin *et al*., 2013; Varsani *et al*., 2014). As a group, geminiviruses infect a broad range of crop plants primarily in tropical and subtropical regions of the world (Moffat, 1999). Their incidence and severity have increased over the past 20 years (Mansoor *et al*., 2006; Navas-Castillo *et al*., 2011), and some resistance strategies used to control them are no longer effective. Breakdown of resistance has been associated with novel disease agents, including ssDNA alphasatellites and betasatellites as well as host-derived sequences enhancing geminivirus symptoms (Nawaz-ul-Rehman & Fauquet, 2009; Ndunguru *et al*., 2016), which are of concern because of their recent emergence and unknown etiology.

Two resistance genes that provide resistance to *Tomato yellow leaf curl virus* (TYLCV), a monopartite member of the Begomovirus genus, were recently identified in tomato (Verlaan *et al*., 2013). Variations in the first gene, Ty-1/Ty-3, which encodes a gamma-type RNA-dependent RNA polymerase (RDR), were found to be responsible for resistance in two different lines of tomato (Verlaan *et al*., 2013). Resistance most likely involves augmentation of host gene silencing pathways and has been associated with increased methylation of viral DNA (Butterbach *et al*., 2014). The second resistance gene, ty-5, encodes an altered version of Pelota (Pelo), which functions in ribosome recycling following translation (Lapidot *et al*., 2015). This recessive resistance is important because it identified an essential host factor and because it will be difficult for the virus to overcome. Identification of both of these genes has facilitated breeding for geminivirus resistance. However, neither resistance gene prevents the accumulation of viral DNA during infection, which can lead to the development of viral mutations that eventually overcome resistance (Arguello-Astorga *et al*., 2007; Richter *et al*., 2016). In addition, there is a risk that Ty-1/Ty-3 resistance will be overcome because geminiviruses encode anti-silencing proteins (Raja *et al*., 2010) and occur in mixed

infections with pathogenic RNA viruses, such as *Cassava brown streak virus* (Mbanzibwa *et al*., 2009), which also interfere with silencing. Geminivirus-associated satellites frequently target host gene silencing pathways (Nawaz-ul-Rehman & Fauquet, 2009; Hanley-Bowdoin *et al*., 2013). There is a need for additional sources of resistance to augment the existing repertoire and enhance the possibility of creating durable resistance.

Arabidopsis thaliana has served as a model plant for studying virus–host interactions, and numerous genes impacting infection have been identified using mutagenized populations or by screening naturally occurring accessions (Ouibrahim *et al*., 2014). Because *Arabidopsis* is self-pollinating, the thousands of accessions collected from around the world function as inbred lines that have adapted to a wide range of environments (Consortium, 2016). These accessions can be used for genome-wide association studies (GWAS) in addition to quantitative trait locus (QTL) mapping and provide powerful tools for uncovering the genetic basis of defective virus–host interactions that can lead to resistance (Pagny *et al*., 2012). For example, the potyvirus resistance gene, eIF4(iso)E, was first identified in an *Arabidopsis* mutant (Lellis *et al*., 2002), and then found to correspond to the broad spectrum potyvirus resistance allele (pvr) that is widely used for breeding (Ruffel *et al*., 2002; Kang *et al*., 2005). The product of this recessive resistance gene participates in translation initiation and has been called the 'weak link' of potyvirus infection (Robaglia & Caranta, 2006). Three resistance genes that limit Tobacco etch virus to inoculated leaves were identified in a mutagenized population of Ler-1 after the trait was first uncovered in naturally occurring populations (Chisholm *et al*., 2000). Recently, the Cvi-0 accession was used to identify a variant form of a phosphoglycerate kinase gene that confers potyvirus resistance (Ouibrahim *et al*., 2014) and provided new insights into the involvement of metabolic enzymes in virus–host interactions.

Several years ago, a screen of *Arabidopsis* accessions was initiated to better understand geminivirus–plant interactions. We were interested in using *Arabidopsis* to study virus-induced gene silencing (VIGS). VIGS takes advantage of a major plant defense

pathway, post-transcriptional gene silencing (PTGS), which results in degradation of the aberrant RNA associated with viral infection (Waterhouse *et al*., 2001). When a host gene fragment is inserted into the virus, mRNA from the host gene is also degraded. Previously (Turnage *et al*., 2002), we showed that Col-0 plants inoculated with a VIGS vector derived from the geminivirus *Cabbage leaf curl virus* (CaLCuV) carrying a fragment of the CH-42 gene (At4G18480; a.k.a. Chlorina-42, CHLI-1), which encodes magnesium chelatase subunit I, developed yellow-white areas due to chlorophyll loss. However, viral symptoms were too severe for its use as a VIGS vector. We screened 190 accessions to identify lines with attenuated symptoms and accidentally identified one line, Pla-1, that showed durable and complete resistance to CaLCuV even when wild-type virus was used for infection. Because of the potential benefits of a natural source of immunity to geminiviruses, we chose to focus on Pla-1. This paper reports results of the VIGS screen and initial characterization of Pla-1 immunity.

4.2 Materials and methods

4.2.1 Plant growth

Seeds were stratified to encourage subsequent germination at 4°C for 3 days on moist autoclaved Metro-Mix 360 soil. For the VIGS screen, plants were grown at 22/20°C during an 8-h light/16-h dark photoperiod. For TYLCV agroinoculation, plants were grown in continuous light at 22°C. All other experiments used plants grown at 20°C under an 8-h light/16-h dark photoperiod with 50% humidity at a light intensity of 140 µmol/m^2sec.

4.2.2 Plasmid construction

All plasmids for geminivirus inoculation carried duplicated 5′ intergenic regions for replicational release in plant cells (Elmer *et al*., 1988). Plasmids carrying the wild-type CaLCuV A DNA (pCPCbLCVA.003), the CaLCuV A DNA VIGS vector (CaLCuVA:CH-42 with a 362-bp fragment of CH-42 in antisense orientation in place of

the coat protein gene, pMTCaLCuVA.008), the CaLCuV A DNA LUC vector (CaLCuVA:LUC with a 623-bp fragment of LUC, pNMCaLCuVA.LUC) and wild-type CaLCuV B DNA (pCPCbLCVB.002) have been described (Turnage *et al*., 2002; Muangsan *et al*., 2004). The replication-deficient CaLCuV A mutant (pCaLCuVA: FSAL1mut) carrying a frameshift mutation in AL1 was created by digesting pCPCbLCVA.003 with NcoI, repairing the cleaved ends and religating it. CaLCuV-containing Agrobacterium plasmids have been described (Egelkrout *et al*., 2002). The BCTV-Logan (Stenger *et al*., 1991) plasmid was provided by D.M. Bisaro of The Ohio State University. TRV plasmids were obtained from the *Arabidopsis* Biological Resource Center (ABRC, Ohio State University). The plasmid carrying TYLCV-IL[DO] (GenBank accession number AF024715) has been described (Reyes *et al*., 2013).

4.2.3 CaLCuV VIGS of Arabidopsis accessions

Of the 190 accessions screened, 173 came from ABRC and were bulked at Paradigm Genetics and 17 were from the collection of 96 natural accessions (Nordborg *et al*., 2005) obtained from ABRC as CS22660. Seedlings at the seven-eight leaf stage were co-bombarded with equal amounts of CaLCuVA:CH-42 or CaLCuVA:LUC and CaLCuV B, as previously described (Turnage *et al.,* 2002). Twenty seedlings per accession were bombarded and 16 accessions were screened simultaneously along with Col-0 controls. Four people independently evaluated symptoms, and silencing and consensus scores (Table A.1) were reached by group discussion.

4.2.4 Microprojectile bombardment of individual leaves with CaLCuV

Equal amounts (2.5 μg) of the wild-type or replication-deficient CaLCuV A and CaLCuV B DNAs were precipitated onto 1-μm gold microprojectiles (Santos *et al*., 2008) and co-inoculated three times into three adjacent mature rosette leaves using a DNA microsprayer (Venganza) at 30 psi. The experiment was repeated twice and included CaLCuV B-inoculated controls.

4.2.5 CaLCuV, BCTV and TYLCV agroinoculation and detection

Five-week-old seedlings were inoculated with an equal mixture of Agrobacterium carrying CaLCuV A and B plasmids, the BCTV or the TYLCV containing plasmid (Ascencio-Ibanez *et al*., 2008). Agrobacterium with an empty vector served as control. Agrobacterium cultures were grown overnight at 30°C until saturation. Plants were inoculated by pricking the area surrounding the shoot apex 10 times and depositing a drop of the culture using a 1-mL syringe with a 27.5-gage needle. Plants were then covered for 24 h. Leaves 6 and 7 (with leaf 1 being the youngest) were pooled at 25 or 35 dpi for genomic DNA extraction using the DNeasy Plant Mini Kit (Qiagen) or a Plant/Fungi DNA isolation kit (Norgen). CaLCuV DNA was detected by PCR using divergent primers CaLCuVAdivPCR-For 5′- CTCTAGGAACATCTGGGCTTCTA and CaLCuVAdivPCR-Rev 5′- CCTTATAATTGCGAGACGCTCT. BCTV DNA was detected using primers BCTV15-for 5′-CGTTACTGTGACGAAGCATTTG and BCTV15-rev 5′-CTCCTTCCCTCCATATCCAGTA. All assays were run in triplicate and included no DNA and target DNA controls. TYLCV was detected by DNA blot hybridization using 100 ng of genomic DNA digested with *SacI*. Blotted membranes were hybridized to an [α-^{32}P]-dATP labeled 1619-bp TYLCV Cla-1 fragment and exposed to CL-X Posure film.

4.2.6 TRV VIGS

Arabidopsis plants at the 12–14 leaf stage were used for TRV VIGS. pTRV1 and pTRV2-AtPDS were introduced into Agrobacterium tumefaciens strain GV3101::pMP90 by electroporation and equal amounts were mixed together before infiltration. Agroinfiltration of six plants per accession (Col-0 and Pla-1) was performed as described previously (Burch-Smith *et al*., 2006).

4.2.7 TRV quantification

Three PDS-silenced leaves were pooled per plant. Total RNA was isolated using an RNeasy Plant Kit (Qiagen, Crawley, UK). First-strand cDNA synthesis was performed using a SuperScript III kit (Invitrogen, Paisley, UK) and the Tobravirus-specific primer 305 5′-GGGCGTAATAACGCTTACG.

Primers and probes derived from the 3′ ORF in TRV RNA1 were used as described (Holeva *et al*., 2006). The 5′ reporter dye was FAM and the 3′ dye was TAMRA (Applied Biosystems). Real-time PCR was performed using the Mx3000P qPCR System (Stratagene). Each assay was performed in triplicate and included either no cDNA template or pTRV1 controls. TRV1 in 10-fold dilutions (10 ng to 100 fg) were run in triplicate as standards for quantification. Crossing threshold (Ct) values were calculated by MxPro QPCR Software (Agilent). TRV1 copy number was calculated using the following formula: (g of TRV1 DNA/(size of TRV1 DNA in bp)/molecular weight of 1 bp) * Avogadro's constant.

4.2.8 Genotyping and QTL mapping

To identify markers, the Pla-1 genome was sequenced using Illumina Sequencing by Synthesis technology at NCSU's Kannapolis campus. DNA was isolated from Pla-1 ecotypeID 7301 using a Qiagen DNeasy kit. About 15 million reads with an average length of 33 bp were aligned to the TAIR 9 version of Col-0 using Bowtie22.1.0 (Langmead *et al*., 2009). SNPs were called using SAMTools (Li *et al*., 2009) and 96 SNPs were used by Illumina (San Diego, CA, USA) to design oligonucleotides for the GoldenGate Genotyping assay with VeraCode Technology.

A total of 440 5-week-old F_2 plants, plus Col-0 and Pla-1 controls, were scored for wild-type CaLCuV infection (Fig. A.6). Three young leaves from each plant were used for DNA extraction (Stepanova *et al*., 2011). DNA was quantified using PicoGreen® (Life

Technologies), and 15 μL^{-1} at 50-100 ng μL^{-1} was sent to the Genomics Core at Case Western Reserve School of Medicine for processing and calling SNPs.

Quantitative trait locus analyses were performed using R/qtl [R version 3.2.2 (2015-08-14); Broman and Sen, 2009] with extended Haley–Knott regression. LOD thresholds were determined by performing 1000 permutations to estimate the 0.05 significance level.

4.3 Results

4.3.1 Diverse responses to the VIGS vector

Screen of *Arabidopsis* accessions was conducted to assess natural variation in response to a geminivirus VIGS vector. The original motivation was to find lines with reduced symptoms, but we were also interested in resistance. Preliminary experiments showed that VIGS in Col-0 was more extensive under short-day conditions (8 h light/16 h dark), which were used for the screen. To standardize results, Col-0 was included in each experiment as an internal control.

Initially, the CaLCuV vector with or without a non-homologous Luciferase fragment was used simply to assess symptoms. When it was realized that the extent of VIGS also varied among accessions, CaLCuV carrying a CH-42 fragment was used, and both symptoms and the extent of silencing were scored. Results from the first experiments are included because each of the 26 accessions tested showed symptoms (Fig. A.1; Table A.1), indicating that they were not resistant to CaLCuV.

A total of 166 accessions were bombarded with CaLCuV:CH-42 to assess both symptoms and silencing. Each accession was placed into one of four classes based on the extent of silencing and symptom severity (Fig. 4.1; Table A.1): Class A, most of the accessions with significant symptoms and silencing, and those with a weak VIGS response; Class B, accessions with low symptoms and high silencing – that would be a suitable host for VIGS; Class C, accessions with very low silencing but severe

symptoms – interesting because of a lack of VIGS; and Class D, accessions with very low symptoms and very low silencing – candidates for resistance. Fig. 4.1 shows examples of accessions from each of the classes, and individual photos of the phenotypic response of each accession, grouped by experiment, are shown in Fig. A.1. Col-0 consistently received high scores for both symptoms and the extent of silencing in each of the experiments (Table A.1; Fig. A.1).

Class A had 144 members and included all accessions not placed in the other classes.

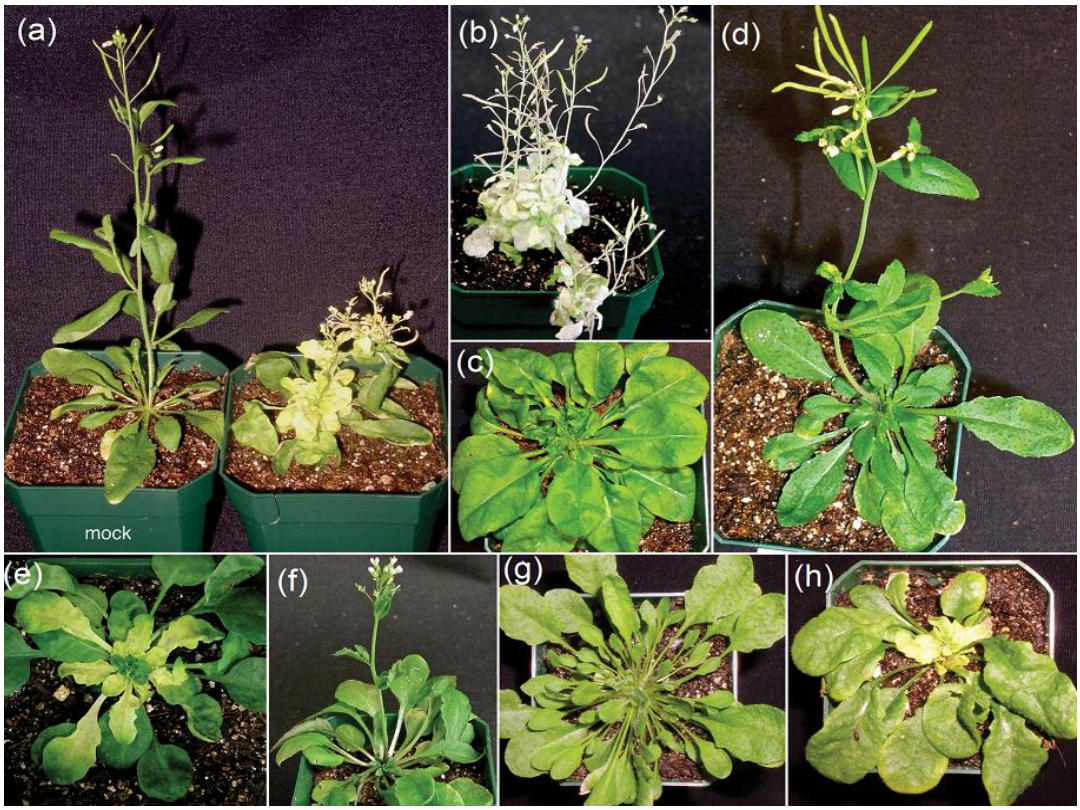


Figure 4.1: Response of different Arabidopsis accessions to the CaLCuVA:CH-42 virus-induced gene silencing (VIGS) vector (a–f) or wild-type CaLCuV (g and h).

(a) Hi-0, a Class A accession, at 29 dpi. Hi-0 on the left was not inoculated.

(b) Kil-0, a Class B accession, at 54 dpi.

(c) Gr-1, a Class C accession, at 25 dpi.

(d) Di-0, a Class D accession, at 27 dpi.

(e) Li-2:1, a Class D accession that shows recovery at 29 dpi (f) and 60 dpi.

(g) Pla-1, which never showed silencing or symptoms, at 28 dpi agroinoculated with wild-type CaLCuV.

(h) Col-0 inoculated with wild-type CaLCuV at 28 dpi.

These accessions had moderate to severe symptoms (138 accessions) or, if mild symptoms, limited silencing (six accessions). Only 20 of these lines had severe symptoms, but strikingly none of them showed an extensive VIGS response (Table A.1; Fig A.1). Class C had three accessions that also showed severe symptoms, but VIGS in these lines was greatly reduced or in some cases absent.

Class B contained 10 accessions with extensive silencing but minimal symptoms. Several accessions stood out for their robust silencing response and attenuated symptoms throughout development. The best VIGS responses were found in Kil-0 (Fig. 4.1b), Le-0, Ka-0 and Gu-1, followed by Ra-0 and Sf-2 (Fig. A.1). Some accessions, in Class A because of their phenotype at ~25 dpi, showed attenuated symptoms at later time points, similar to recovery in wild-type virus infections, and are marked with an asterisk in Table A.1. Fig. A.2 shows three representative accessions that had stunted inflorescences at 26 dpi. At 45–60 days, symptoms attenuated, leaf sizes increased, and inflorescences developed normally, and the plants showed increased silencing.

Class D had six members that showed resistance to the VIGS vector but would need to be retested with wild-type CaLCuV to assess resistance. The bottom row of Fig. 4.1

shows two exceptional examples of Class D resistance. In the first example, plants such as Li-2:1 showed an early VIGS response (Fig. 4.1e) but then appeared to recover to become symptomless (Fig. 4.1f). All five of the Li-2:1 plants showed the same pattern (Fig. A.1). Another accession, PNA-17, also showed a transient VIGS response except that silenced areas were light green rather than yellow (Fig. A.1). These were the only accessions that lost the VIGS phenotype.

The second example, the Pla-1 accession (ecotypeID 7301) from Playa de Aro in Spain, never showed symptoms or silencing. Moreover, no signs of infection were observed when Pla-1 plants were agroinoculated with wild-type CaLCuV, which contains the viral coat protein gene and produces much more severe symptoms than CaLCuVA:CH-42 (Fig. 4.1g).

4.3.2 Lack of viral DNA accumulation in Pla-1

Successful infection by geminiviruses requires viral DNA replication and gene expression as well as nuclear, cellular and long-distance movement (Morra and Petty, 2000; Trejo-Saavedra *et al.,* 2009). To identify the stage where infection was blocked, we inoculated individual leaves of Pla-1 and Col-0 (Fig. 4.2a). A single leaf was inoculated by microprojectile bombardment with wild-type CaLCuV, and the process repeated until three mature leaves from the same plant were inoculated. Symptoms appeared in young, developing leaves of Col-0 at 13 dpi. Leaf curling, stunted development and mild chlorosis were present at 17 dpi and pronounced chlorosis at 22 dpi. In contrast, Pla-1 did not develop symptoms.

We tested for viral DNA accumulation using polymerase chain reaction (PCR) primers that were divergent in pCPCbLCVA.003, a plasmid containing partial tandem copies of wild-type A DNA with duplicated 5′ intergenic regions. The primers were expected to produce a 936-bp product from replicated viral DNA in infected tissues and a ~4.6-kb product from the input plasmid. A replication-deficient mutant containing a frameshift mutation in AL1, which is essential for viral

DNA replication (Elmer *et al*., 1988; Sunter *et a*l., 1990), was tested to distinguish between homologous recombination between the duplicated 5′ intergenic regions of input DNA and low levels of authentic viral DNA replication. The AL1 mutant did not replicate (Fig. A.3) in protoplast assays (Methods A.1).

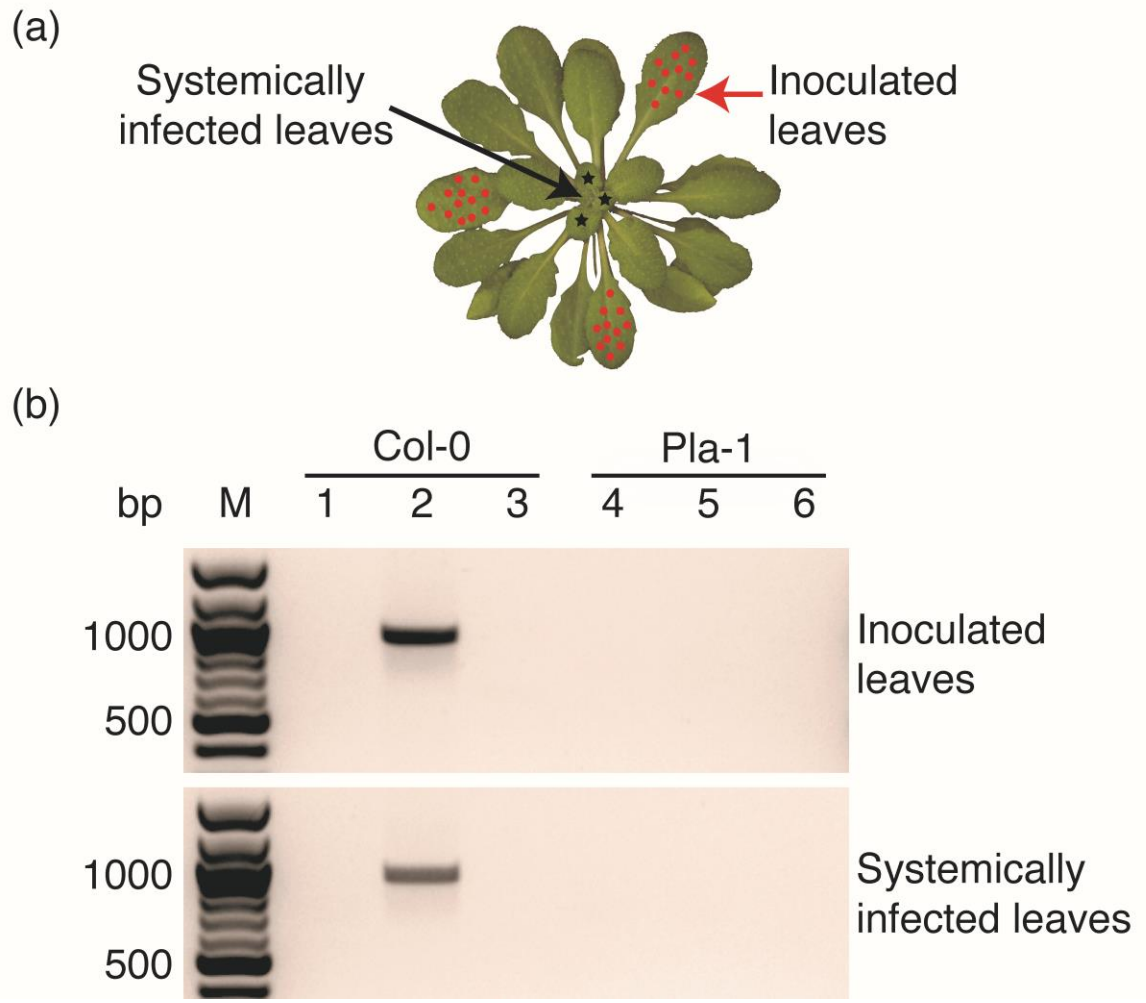


Figure 4.2: Pla-1 is immune to wild-type CaLCuV.

(a) An *Arabidopsis* plant illustrating the three leaves that were inoculated separately (red dots) and leaves in new growth that were tested for systemic infection (black stars). Photo modified from Charles Andres (licensed under CC BY 2.0).

(b) Polymerase chain reaction (PCR) detection of viral DNA in inoculated (upper panel) and systemically infected leaves (lower panel) of Col-0 and Pla-1. Lanes 1 and 4 show mock-inoculated plants, lanes 2 and 5 show plants inoculated with wild-type CaLCuV, and lanes 3 and 6 show plants inoculated with the CaLCuV replication-deficient mutant. Lane M shows DNA size markers. The expected PCR product is 936 bp.

Viral DNA was detected in inoculated leaves of Col-0 at 9 dpi and in systemically infected leaves at 13 dpi. Fig. 4.2b shows viral DNA accumulation at 22 dpi. No viral DNA was detected in Col-0 inoculated with the AL1 mutant, suggesting that viral DNA replication in planta was required for detection by our PCR primers. In Pla-1, none of the DNAs, including wild-type, was detected (Fig. 4.2b). These results show that at least part of the Pla-1 resistance impacts infection at or before viral DNA is replicated and establish that Pla-1 is immune to CaLCuV.

4.3.3 Pla-1 is also immune to a Curtovirus

To test whether Pla-1 resistance extends to other geminiviruses, we challenged Pla-1 plants with Beet curly top virus (BCTV), a member of the Curtovirus genus. Because BCTV is phloem-limited and not efficiently inoculated by bombardment (Briddon *et al*., 1989; Stenger *et al*., 1990), we agroinoculated both CaLCuV and BCTV. Pla-1 inoculated with BCTV (Fig. 4.3a) did not show symptoms. It has been previously reported that another accession, Cen-0, was resistant to BCTV (Park *et al*., 2002). We confirmed that Cen-0 had no symptoms from BCTV but it was susceptible to CaLCuV and displayed similar symptoms as Col-0 – severe chlorosis and leaf deformation (Fig. 4.3a). Symptoms in BCTV-infected Col-0 included leaf curling and deformation plus anthocyanin accumulation, but chlorosis was not apparent.

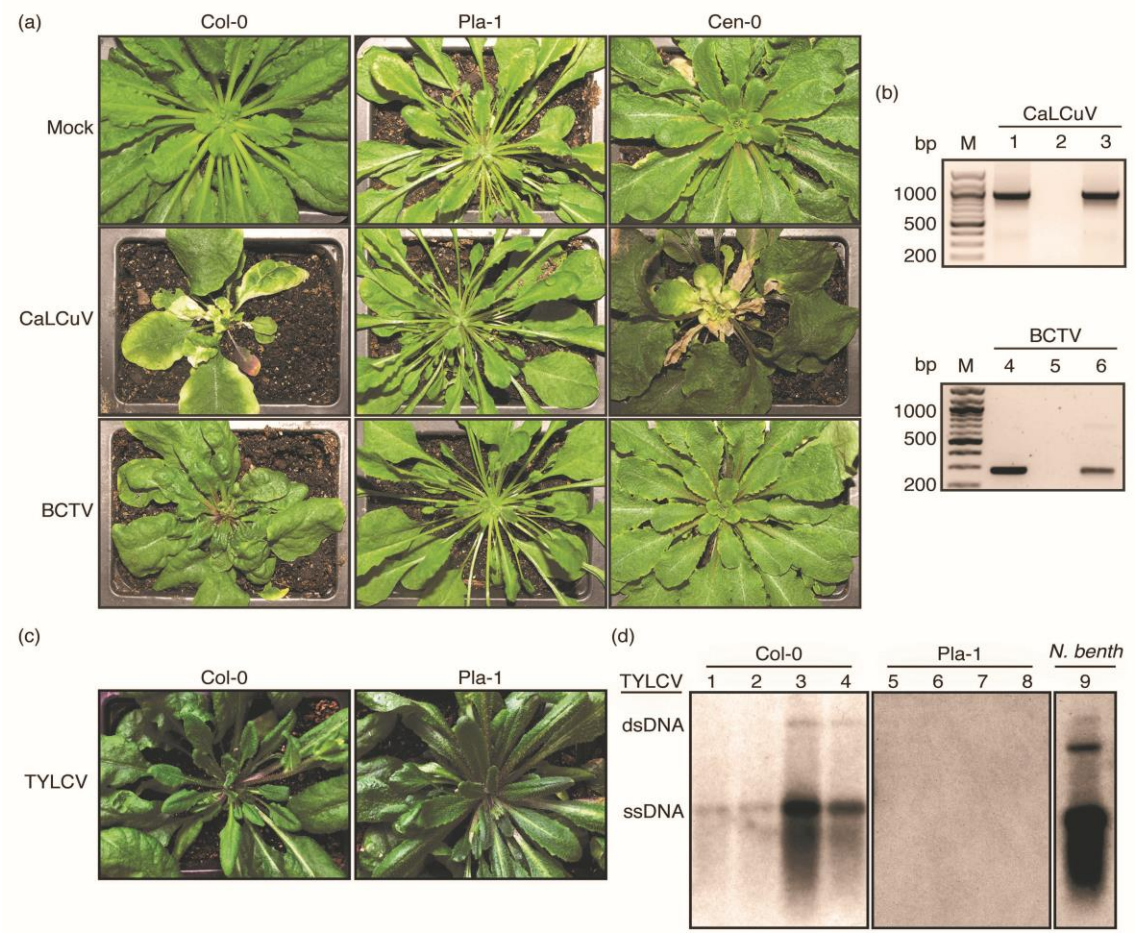


Figure 4.3: Pla-1 is resistant to agroinoculation with CaLCuV, BCTV and TYLCV

(a) Symptom development at 25 dpi in Col-0, Pla-1 and Cen-0 plants agroinoculated with CaLCuV or BCTV.

(b) Polymerase chain reaction (PCR) detection of viral DNA in Col-0 (lanes 1, 4), Pla-1 (lanes 2, 5) or Cen-0 (lanes 3, 6) plants inoculated with CaLCuV (lanes 1–3) or BCTV (lanes 4–6). Lane M shows DNA size markers. The expected PCR product size for CaLCuV is 936 bp and for BCTV is 283 bp.

(c) Symptom development at 35 dpi in Col-0 and Pla-1 plants agroinoculated with TYLCV.

(d) DNA blot hybridization of total DNA from TYLCV inoculated Col-0 (lanes 1–4), Pla-1 (lanes 5–8) and the positive control *Nicotiana benthamiana* (lane 9) at 35 dpi. The

blot was probed with P^{32}-labeled TYLCV DNA and imaged on film. Lanes 1–4 contain DNA from Col-0 plants showing the mildest (lanes 1 and 2) and most severe (lanes 3 and 4) symptoms out of eight inoculated plants. dsDNA, double-stranded DNA. ssDNA, single-stranded DNA.

To test for immunity to BCTV, we used PCR (Fig. 4.3b). Col-0 inoculated with CaLCuV and BCTV showed bands of the predicted sizes indicating that viral DNA was present in systemically infected leaves. However, neither CaLCuV nor BCTV produced detectable viral DNA in Pla-1. CaLCuV DNA accumulated in Cen-0 at levels comparable to Col-0, consistent with the symptoms. Despite the lack of symptoms in Cen-0, BCTV DNA was detected in systemic growth, confirming previous results that Cen-0 is tolerant to BCTV (Park *et al*., 2002). These results demonstrate that Pla-1 has broad-based immunity to geminiviruses and that Cen-0 tolerance is distinct from Pla-1 immunity.

4.3.4 Pla-1 shows resistance to TYLCV infection

Recently, Col-0 was shown to be susceptible to both the severe, Israel strain (IL), and the mild strains of TYLCV (Cañizares *et al*., 2014). Because of the severe crop losses caused by TYLCV globally (Diaz-Pendon *et al*., 2010), testing for susceptibility in Pla-1 was decided. Eight Col-0 and seven Pla-1 plants with a variant of the Israel strain that came from the Dominican Republic (TYLCV-IL[DO]) was agroinoculated. Eight *Nicotiana benthamiana* plants, a known host for TYLCV, were also agroinoculated as a positive control for infection. TYLCV symptoms were clearly present in *N. benthamiana* by 10 dpi but were not apparent in Col-0 or Pla-1. Mild symptoms appeared in Col-0 at 21 dpi, while Pla-1 did not show symptoms, even at 35 dpi when the plants were used for DNA extraction (Fig. 4.3c). DNA:DNA blot hybridization of genomic DNA from the infected plants with a TYLCV probe showed that viral DNA was present in systemically infected leaves of both *N. benthamiana* and each of the eight inoculated Col-0 plants. In contrast, there was no hybridization with DNA from any of the seven inoculated Pla-1 plants (Fig. 4.3d). Together with the lack of symptoms, we conclude that Pla-1 is resistant to TYLCV.

4.3.5 Pla-1 is susceptible to RNA viruses

To determine if Pla-1 immunity was specific to DNA viruses, Pla-1 was tested for infection with the RNA tobravirus Tobacco rattle virus (TRV). Because TRV infection is asymptomatic (Burch-Smith *et al*., 2006), and also interest was in the VIGS response of Pla-1, we used TRV carrying a visible marker for silencing, a fragment of Phytoene Desaturase (AtPDS; At4G14210; Fig. 4.4). PDS is needed for carotenoid biosynthesis, which protects chlorophyll from photobleaching. Although extensive AtPDS silencing was observed in Col-0, the extent of VIGS was reduced in Pla-1, and only five out of six plants showed silencing (Fig. 4.4a). A second experiment showed that new growth in TRV:AtPDS-inoculated Pla-1 was green at later time points while the equivalent leaves in Col-0 plants remained white (Fig. A.4).

To determine whether the reduced TRV:AtPDS VIGS in Pla-1 was due to a defective silencing response or reduced TRV susceptibility, viral RNA accumulation was analyzed using TaqMan®. pTRV1 contains the viral RDR but lacks the AtPDS silencing fragment. The copy number of TRV1, which was normalized to total RNA (μg), was significantly higher in Col-0 plants (69 × 106) compared with Pla-1 plants (26 × 106; $P < 0.05$; Fig. 4.4b). Therefore, Pla-1 shows some resistance to the TRV:AtPDS VIGS vector in comparison to Col-0. Because the reduced silencing could be a direct effect of reduced TRV:AtPDS levels, concluding whether the silencing response was altered in Pla-1 was not possible.

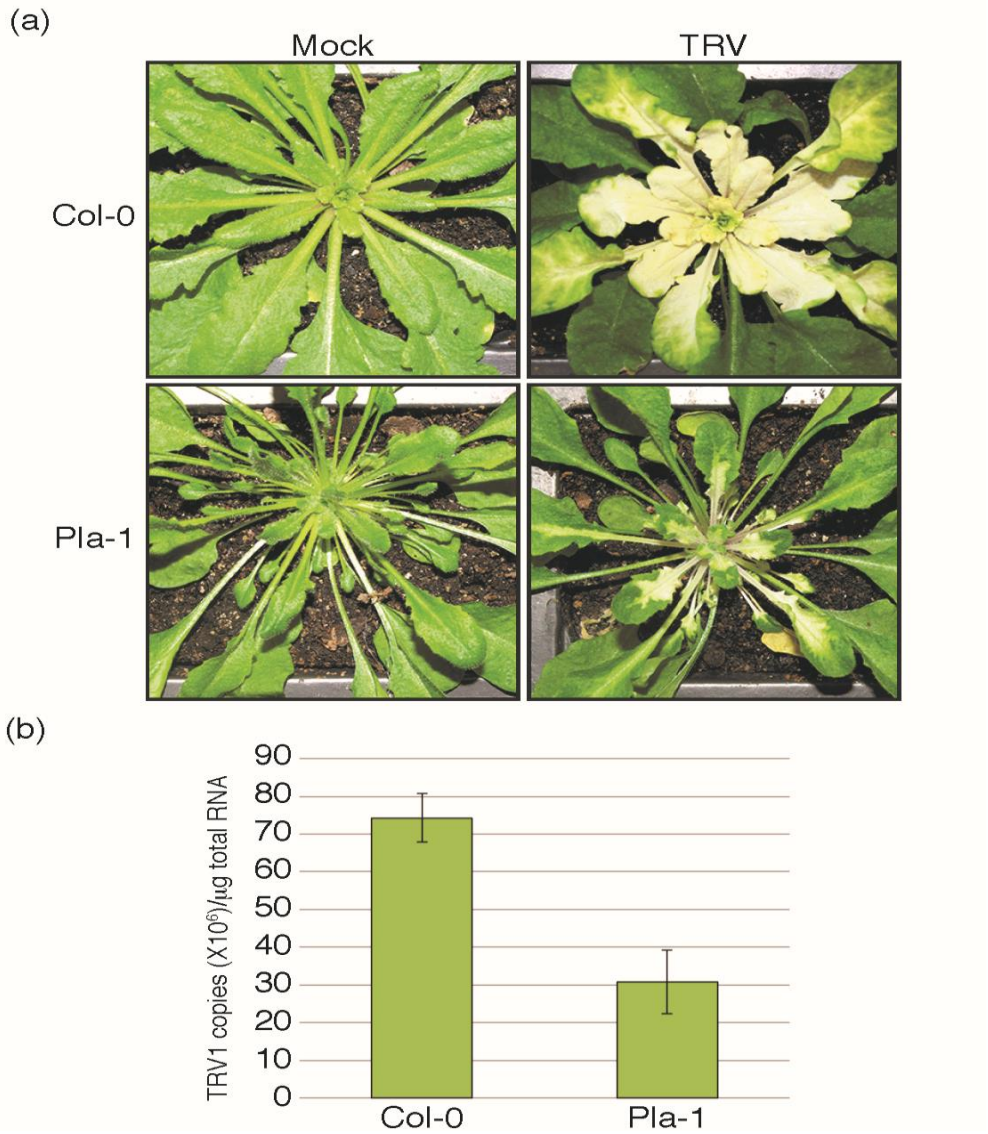


Figure 4.4: Pla-1 shows reduced virus-induced gene silencing (VIGS) and susceptibility to TRV:AtPDS.

(a) Response of Col-0 and Pla-1 to TRV:AtPDS inoculation at 21 dpi.

(b) Histogram of TRV1 copy number per lg of total RNA for Col-0 and Pla-1 TRV:AtPDS-inoculated plants. Error bars show standard error.

Because Pla-1 showed reduced susceptibility to TRV:AtPDS, Pla-1 plants were challenged with another RNA virus, the potyvirus Turnip mosaic virus (TuMV; Methods A.2). All eight TuMV-inoculated Pla-1 plants showed severe symptoms at 18 dpi, indicating susceptibility (Fig. A.5).

4.3.6 Immunity maps to chromosome 1

To determine if Pla-1 immunity corresponded to previously identified resistance genes, we initiated QTL mapping with progeny from a Pla-1 × Col-0 cross. In the first set of experiments, 83 $F_{2:3}$ families were agroinoculated with wild-type CaLCuV and scored for symptoms at 21 dpi in three replicates (Methods A.3). Symptoms were scored on a scale of 1–5 (Fig. A.6), with 1 being no symptoms and 5 being severe chlorosis, stunting and growth arrest. A total of 20 simple sequence length polymorphism (SSLP) markers polymorphic for Pla-1 and Col-0 (Table A.2; Fig. A.7c) were used to construct a QTL map using R/qtl (Broman and Sen, 2009). Plots for the three replicates showed a major peak on chromosome 1 near nga280 (Fig. A.7d) with logarithm of odds (LOD) scores of about 5.5. A LOD threshold of 3.5 was calculated from 1000 permutations at 0.05 confidence, indicating that the peaks were significant. The consistency of results from different members of the $F_{2:3}$ families in three replicates showed that resistance was heritable and suggested that fine-mapping should concentrate on chromosome 1.

To identify single nucleotide polymorphisms (SNPs) for chromosome 1, the Pla-1 genome was sequenced to a depth of 3 ×. Although coverage was low, many SNPs were supported by at least 5 reads. A total of 77 SNPs near nga280 were chosen from Pla-1 sequence and supplemented with 19 previously identified SNPs (Platt *et al.*, 2010) to provide complete coverage of the genome. Illumina's Golden Gate technology was used to identify and call SNPs, and oligonucleotides for the 96 putative SNPs passed Illumina's proprietary criteria for inclusion in the assay. Unfortunately, more than 50% of the SNPs failed to produce useful data, largely due to incomplete data for the Pla-1 genome. Sequences and positions of the 42 successful SNPs are provided in Table A.3.

A total of 440 F_2 progeny were agroinoculated with CaLCuV, scored for symptoms at 28 dpi and used for the second QTL map. Of the 440 plants, 121 plants (27.5%) showed no symptoms (symptom score 1; Fig 4.5a). The ratio of resistant (121) to susceptible (319) plants was similar to the expected ratio (1:3) for recessive resistance with a chi square value of 1.47 ($P = 0.23$ that expected and observed were from different populations). These results were similar to the $F_{2:3}$ families (Fig A.7b) and indicate that immunity is recessive.

The positions of the 42 SNPs used to genotype 440 plants are shown in Fig 4.5b. R/qtl was used to create a QTL map using extended Haley–Knott regression (Feenstra *et al*., 2006). The results show a major QTL, designated gip-1 for geminivirus immunity, Pla-1, at 42.6 centiMorgan (cM) in chromosome 1 with a LOD score of 34 (Fig 4.5b). Following 1000 permutations, the LOD score for 0.05 significance was 2.85. Similar results were obtained using standard interval mapping (Lander and Botstein, 1989) and the original Haley–Knott regression method. These results located a major QTL on the left side of the centromere that contrasts with results from $F_{2:3}$ families, which showed a peak to the right of the centromere. This can be explained by the paucity of SSLP markers in the center of chromosome 1, which likely skewed results from the $F_{2:3}$ families.

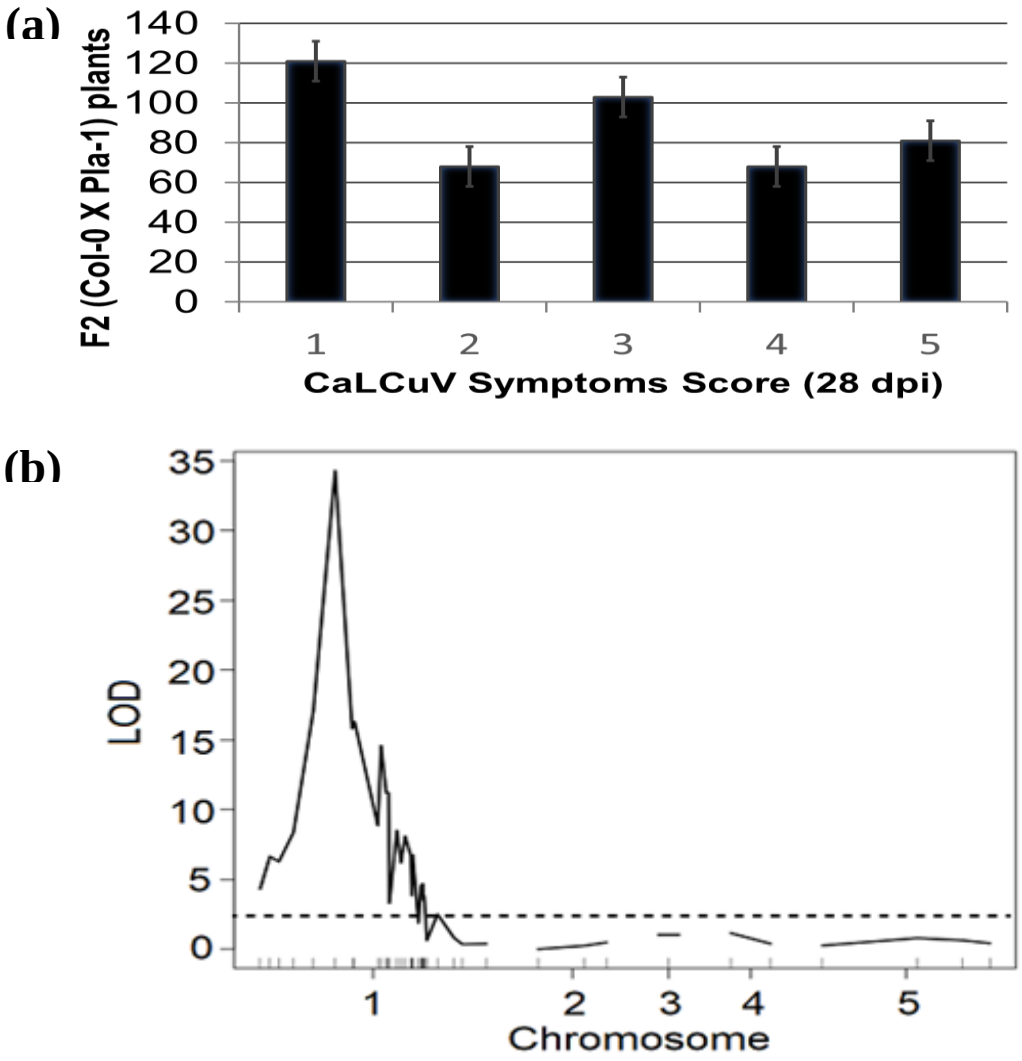


Figure 4.5: Quantitative trait locus (QTL) associated with CaLCuV symptoms are located on chromosome 1 of Pla-1.

(a) 28-dpi symptom responses of the 440 F_2 (Pla-1 x Col-0) plants used for mapping to CaLCuV.

(b) QTL map of symptom responses in the CaLCuV-inoculated F_2 population. Positions of the single nucleotide polymorphism (SNP) markers along the chromosomes are shown as vertical black lines on the x-axis. The black dashed line marks the logarithm of odds (LOD) significance threshold ($P < 0.05$).

Both analyses found a major peak on chromosome 1. In addition, both studies showed that QTLs on other chromosomes do not have a strong impact on the production of CaLCuV symptoms. From these results, we conclude that Pla-1 immunity is distinct from the RDR-like Ty-1/Ty-3 or the Pelota-like ty-5 genes previously identified in tomato, which are located in chromosomes 2 and 4, respectively (Verlaan *et al.*, 2013; Lapidot *et al*., 2015). Therefore, the gene(s) responsible for Pla-1 immunity are likely to be novel. Candidate genes near gip-1, whose sequences are altered compared with the geminivirus-susceptible accession Col-0 and the closely related susceptible accession Pla-0, are listed in Table A.4. This list includes 582 SNPs unique to Pla-1 located in the open reading frames of 161 genes (Methods A.4).

4.4 Discussion

This work began as an effort to identify a suitable host for geminivirus-mediated VIGS in Arabidopsis, but ultimately focused on resistance. After screening 190 Arabidopsis accessions, Pla-1 was identified as the only accession immune to CaLCuV, a whitefly-transmitted bipartite member of Squash leaf clade in the Begomovirus genus of Geminiviridae. Pla-1 was also resistant to BCTV, a leafhopper-transmitted monopartite geminivirus in the Curtovirus genus, and to the agronomically important TYLCV, a whitefly-transmitted monopartite member of the Old World branch of the Begomovirus genus, thereby establishing the broad-based nature of the immunity.

Pla-1 was inoculated with wild-type CaLCuV by microprojectile bombardment into three leaves of the same plant (Fig 4.2) or by agroinoculation (Fig 4.3). In both cases, no viral DNA was recovered in inoculated or systemically infected leaves. In addition, no viral DNA was detected when Pla-1 was inoculated with BCTV. Furthermore, TYLCV could not be detected in any of the inoculated Pla-1 plants. This kind of broad-based immunity is desirable in plant breeding programs because the lack of viral DNA accumulation reduces the chances of viral variants with the potential to break resistance.

Recessive resistance, especially when it is to more than one genus of virus, suggests that a host protein(s) essential for infection is altered or unavailable to the virus (Pagny *et al*., 2012; Ouibrahim *et al*., 2014; Lapidot *et al*., 2015). DNA viruses exploit the host's capacity for protein and nucleic acid synthesis, nuclear trafficking, and cell-to-cell and long-distance movement (Ascencio-Ibanez *et al*., 2008; Hanley-Bowdoin *et al*., 2013). Although viral DNA was not detected in inoculated leaves, the assay may not have been sensitive enough to detect very low-level DNA replication. We included an AL1 frameshift mutant because episomes were detected when a similar construct for *Tomato golden mosaic virus* was transfected into Escherichia coli (Lopez-Ochoa *et al*., 2006). These episomes likely formed by recombination across the duplicated 5′ intergenic regions of input plasmid, but it is not known whether episomes can be formed simply by homologous recombination in plant cells. Therefore, the lower limits of detection in our assay is not known and occurrence of very low levels of viral DNA replication cannot be ruled out. However, when wild-type CaLCuV A is bombarded into mature leaves in the absence of B DNA, which is essential for movement, viral DNA is easily detected. Therefore, at least part of the Pla-1 immunity must involve host processes that target early events in the viral life cycle.

The VIGS screen of *Arabidopsis* accessions resulted in a variety of responses, but two correlations were found – lines that showed reduced symptoms over time (recovery) also showed increased VIGS, and lines that showed the most severe symptoms lacked extensive silencing (Table A.1). Fig A.2 shows the extensive VIGS associated with recovery in some of the accessions at later time points. One explanation for the increased VIGS response is that transcriptional gene silencing (TGS), which methylates viral DNA, stops transcription of viral genes that inhibit silencing. Geminiviruses encode multifunctional anti-silencing proteins that target TGS as well as PTGS (Wang *et al*., 2005; Glick *et al*., 2008; Rodriguez-Negrete *et al*., 2009). Recovery from geminivirus infection has been correlated with increased methylation of viral DNA (Raja *et al*., 2008), but viral DNA is not entirely eliminated in new growth and not all DNAs are methylated (Paprotka *et al*., 2011). If only a few DNAs are transcriptionally active,

anti-silencing protein levels may be reduced compared with early stages of infection. Any transcription of the CH-42 insert could be amplified by RDR6 and cause extensive silencing in new growth.

Viral anti-silencing activity may be especially strong against both TGS and PTGS in other accessions, such as those in Class C that show severe symptoms and very limited PTGS. Previous studies have identified RDR6, SGS3, DCL4 and Hen1 as necessary for VIGS from CaLCuV in *Arabidopsis* (Blevins *et al*., 2006). Plants mutant for RDR6 and SGS3, which encodes a known target of geminivirus anti-silencing proteins (Glick *et al*., 2008), also show more severe symptoms and reduced silencing (Muangsan *et al.*, 2004). The strong correlation between severe symptoms and reduced VIGS suggests that the relative strength of the host gene silencing defense response determines symptom severity in this virus host pathosystem.

Several accessions showed minimal symptoms and extensive silencing. Accessions in Class B, especially Gu-1, Kil-0, Le-0, Sf-2, Mz-0 and Ra-0, could be suitable hosts for VIGS as a functional genomics tool, depending on the goals of the research (Flores *et al*., 2015). We used relatively cool (20–22°C) growth conditions. Because the extent of VIGS increases with temperature (Chellappan *et al*., 2005; Wang *et al*., 2006; Tuttle *et al*., 2008), increased silencing might be obtained for some of these accessions if grown at higher temperatures.

The response of plants in Class D, which showed reduced VIGS as well as reduced symptoms, could reflect mutations in genes for essential host factors, such as Pelo. In ty-5, which has an alternative Pelo allele, TYLCV viral DNA levels are greatly reduced and infection is asymptomatic (Lapidot *et al.*, 2015). However, the lines in Class D need to be screened with wild-type CaLCuV before they are considered to be resistant.

Only one out of 190 accessions (Pla-1) showed a complete lack of symptoms and silencing when inoculated with the VIGS vector, suggesting that VIGS is a sensitive method for distinguishing between resistance and immunity. *Arabidopsis* is susceptible

to a variety of viruses and several screens for resistant accessions have been reported (Leisner and Howell, 1992; Martín Martín *et al*., 1997; Park *et al*., 2002; Rajakaruna and Khandekar, 2007; Ouibrahim *et al*., 2014). However, the only report of virus immunity in *Arabidopsis* described a Col-0 mutant already resistant to tobamoviruses that was subjected to further mutagenesis (Yamanaka *et al*., 2002). Further testing will be needed to determine whether one or more genes comprise the Pla-1 immunity to CaLCuV and whether this accession is unique in showing broad-based immunity to geminiviruses.

The only other study to test the response of *Arabidopsis* accessions to a VIGS vector, which used the same TRV:AtPDS as in Fig 4.4, found very little variation in symptoms or the extent of silencing (Wang *et al.,* 2006). In contrast, we found wide variation in both symptoms and the extent of silencing. We also found a reduction in the extent of VIGS from TRV:AtPDS in Pla-1 compared with Col-0, but this may have been due to reduced levels of the TRV vector. Although resistant to CaLCuV, BCTV, TYLCV and perhaps to some extent TRV, Pla-1 has been shown to be very susceptible to Cauliflower mosaic virus, a DNA virus that replicates through RNA intermediates (Leisner and Howell, 1992), the potyvirus TuMV (Fig A.5), and the comovirus Turnip ringspot virus (Khandekar *et al*., 2007).

A recessive susceptibility locus, sha3, that impacts long-distance movement of the Potyvirus Plum Pox Virus (PPV) was mapped to a 20-kb region on chromosome 3 by combining GWAS of 147 *Arabidopsis* accessions and traditional QTL mapping (Pagny *et al*., 2012). Six different accessions showed resistance to PPV and were allelic for sha3. Our initial attempts at using GWAS have not yielded clear results for gip-1, perhaps because the wild-type CaLCuV virus was not used or, more likely, because immunity is polygenic and one or more alleles are not prevalent among other accessions.

Accessions related to Pla-1, except for Pla-0, showed a reduction in either symptoms or silencing (Fig A.1). Only Pla-0 has been sequenced (Consortium, 2016), and its genome will be useful for eliminating candidate genes. Pla-2 and Pla-3 both showed mild symptoms. Although Pla-4 had strong symptoms, it was placed in Class C due to the

lack of a significant VIGS response (Table A.1). These VIGS responses demonstrate significantly more viral DNA replication and movement than found in Pla-1. Efforts are currently underway to transfer gip-1 to Col-0 to determine if immunity can be retained in a different background. These efforts are complicated by the longer flowering time for Pla-1 and the need to vernalize F_1 progeny to induce flowering.

Resistance genes in tomato have been identified for TYLCV, which is in the same genus as CaLCuV (Begomovirus) but has a single-component genome and is limited to the phloem during infection. The Ty-1/Ty-3 alleles have alterations in an RDR that shows high sequence similarity to *Arabidopsis* RDR3, RDR4 and RDR5 (Verlaan *et al*., 2013), all of which are located on chromosome 2. The recessive mutation in Pelo, at the ty-5 locus, confers strong resistance to TYLCV, probably by inhibiting or slowing down ribosome recycling and reducing protein synthesis in the infected cells (Lapidot *et al*., 2015). The corresponding gene in *Arabidopsis* is located in chromosome 4 and is distinct from the Pla-1 resistance locus on chromosome 1. The Ty-1 and Ty-3 alleles behave differently: Ty-1 is dominant and is specific for TYLCV; while Ty-3 is semi-dominant and also confers partial resistance to the bipartite *Tomato mottle virus*, and the combination of alleles provides stronger resistance than either allele alone (Ji e*t al*., 2007). The ty-5 allele is recessive and provides strong protection against TYLCV but may be associated with reduced growth in the absence of infection (Lapidot *et al*., 2015). Nevertheless, recessive resistance that involves alterations in essential host factors is hard for the virus to overcome. Identification of the genetic basis for the immunity found in Pla-1, which is also recessive, could provide information about another virus–host interaction that can be targeted for resistance. An advantage of using natural variation is that the genes conferring resistance have been selected for fitness, which is important because these viruses target essential host processes. The possibility of using CRISPR/Cas technologies to precisely modify the corresponding genes in crop plants without the need for traditional plant transformation (Puchta, 2016) may speed the deployment of these genes where they are most needed. Candidate genes for conferring geminivirus immunity in the gip-1 locus are listed in Table A.4. The list includes genes

that encode proteins involved in pathogen and stress responses, transcription, hormonal regulation and development. All of these pathways have been implicated in geminivirus infection (Hanley-Bowdoin *et al*., 2013) and their disruption has the potential to interfere with the geminivirus infection process.

Tagging CaLCuV with a marker for VIGS was useful in uncovering dynamic aspects of geminivirus–host interactions that would have been difficult to track in a wild-type virus infection. It was especially useful in identifying CaLCuV immunity in Pla-1. A single peak was identified by QTL mapping of CaLCuV-infected plants, but it still may consist of more than one gene. Still to be determined is whether the CaLCuV immunity in Pla-1 comprises a unique combination of genes that are also present in other accessions or if it includes a rare allele. It also needs to be established whether gip-1 participates in resistance to BCTV and/or TYLCV. Nevertheless, the high LOD score of gip-1, the block of viral DNA accumulation from two distinct geminiviruses in Pla-1 and the resistance shown against TYLCV all suggest that further analyses will be valuable. Efforts are currently underway to identify the molecular basis of Pla-1 immunity.

CHAPTER FIVE

CHARACTERIZATION OF CMB INFECTION IN ARABIDOPSIS IN THE PRESENCE AND ABSENCE OF SEGS-1

Abstract

Cassava is a major crop in Sub-Saharan Africa, where it is grown primarily by small holder farmers. Cassava production is constrained by Cassava mosaic disease (CMD), which is caused by cassava mosaic begomoviruses (CMBs). A previous study showed that SEGS-1 (sequences enhancing geminivirus symptoms), which occurs in the cassava genome and as episomes during viral infection, enhances CMD symptoms and breaks resistance in cassava. We report here that SEGS-1 also increases symptoms, viral DNA levels and the number of infected cells in *Arabidopsis thaliana* co-inoculated with *African cassava mosaic virus* (ACMV) and SEGS-1 sequences. Disease was also enhanced in *Arabidopsis* plants carrying a SEGS-1 transgene inoculated with ACMV alone. Unlike cassava, no SEGS-1 episomal DNA was detected in the transgenic plants during ACMV infection. SEGS-1 also broke host resistance to *Cabbage leaf curl virus* (CaLCuV) in co-inoculation experiments of a resistant *Arabidopsis* accession. Studies using *Nicotiana tabacum* suspension cells showed that SEGS-1 increases viral DNA accumulation in the absence of systemic infection. Together, these results demonstrated that SEGS-1 can function with a heterologous host and virus to increase disease and break resistance. Moreover, SEGS-1 can function in a genomic context, indicating that SEGS-1 episomes are not required for disease enhancement.

5.1 Introduction

Cassava (*Manihot esculenta* Crantz) is a major crop across Africa, providing food and income to over 300 million people. Cassava can be grown on marginal lands and under drought conditions, but its production is severely limited by viral diseases (Okogbenin *et al*., 2013). Cassava mosaic disease (CMD) is one of the most important viral diseases of

cassava, with yield losses ranging from 20 to 95% in sub-Saharan Africa (Thresh *et al*., 1994), contributing to food insecurity and poverty across the region.

Cassava mosaic disease is caused by a complex of DNA viruses collectively designated as cassava mosaic begomoviruses (CMBs). In Africa, eight CMB species are associated with CMD. They include *African cassava mosaic virus* (ACMV), *East African cassava mosaic virus* (EACMV), *East African cassava mosaic Malawi virus* (EACMMV), *East African cassava mosaic Zanzibar virus* (EACMZV), *East African cassava mosaic Kenya virus* (EACMKV), and *South African cassava mosaic virus* (SACMV) (ICTV, 2017). More recently, *Cassava mosaic Madagascar virus* (CMMGV) (Harimalala *et al.*, 2013) and *African cassava mosaic Burkina Faso virus* (ACMBFV) (Tiendrebeogo *et al*., 2012) have been described. There are also several begomovirus strains, including *East African cassava mosaic virus*-Uganda (EACMV-UG), which arose from a recombination event between EACMV and ACMV (Zhou, 2013) and is responsible in part for the severe CMD pandemic in East Africa in the 1990s and 2000s (Gibson, 1996; Otim-Nape *et al.*, 1997).

Begomoviruses constitute the largest genus in the *Geminiviridae*, which is characterized by their double icosahedral particles and small, circular DNA genomes that are packaged into virions as single-stranded DNA (ssDNA) (Zhang *et al*., 2001). Begomovirus genomes also exist as double-stranded DNA (dsDNA) that is involved in viral replication and transcription in infected plants (Hanley-Bowdoin *et al*., 2013). CMB genomes consist of two DNA components designated as DNA-A and DNA-B that together encode 8 viral proteins involved in viral replication and movement throughout the host plant, countering host defenses and whitefly transmission. CMBs are also transmitted through cassava stem cuttings when infected plant material is used for propagation.

Some begomoviruses are associated with DNA satellites that can alter virulence and disease severity (Briddon *et al.*, 2003; Briddon & Stanley, 2006). Begomovirus satellites, which have been classified into three types, also have circular DNAs that are a

quarter or half the size of the viral genomes. Betasatellite and alphasatellite DNAs encode proteins that interfere with host defenses, while gammasatellite DNAs do not contain any open reading frames (ORFs). None of the satellites share homology with host genomes, and all are thought to be transmitted in virions like their helper viruses (Briddon & Stanley, 2006; Zhou, 2013).

A recent report described two small DNA molecules, SEGS-1 and SEGS-2, that were isolated from cassava showing severe, atypical CMD symptoms in Tanzanian fields (Ndunguru *et al.*, 2016). Experiments under controlled laboratory conditions showed that both SEGS-1 and SEGS-2 enhance CMD symptoms when co-inoculated with a CMB onto cassava, resulting in symptoms resembling those observed in the field. SEGS-1, but not SEGS-2, also overcomes endogenous CMD2 resistance. Strikingly, both SEGS-1 and SEGS-2 occur as low copy number episomes in CMD-infected cassava, suggesting that they might behave as satellites. However, the SEGS show no sequence relationship with begomovirus satellites or their helper viruses. Instead, the SEGS are related to sequences in the cassava genome.

The cassava reference genome (Phytozome v6.1) contains a near full-length copy of SEGS-1 (99% identify) and three partial sequences related to SEGS-2 (84-89% identity) as well as many other SEGS-related sequences (Ndunguru *et al*., 2016). Both SEGS are characterized by GC-rich regions and small putative ORFs, but only share 23% overall sequence identity with each other. This distinction is underscored by the observation that SEGS-2 episomes are packaged into virions and occur in whiteflies, suggesting that SEGS-2 is a novel satellite. In contrast, SEGS-1 episomes have only been found in plants and not in virions, consistent with them being derived from the cassava genome and not transmitted by whiteflies. The SEGS-1 genomic sequence has been found in all African and South American cultivars examined to date, including landraces and improved germplasms from breeding programs. Given the widespread and potentially universal presence of SEGS-1 in the cassava genome, it is essential to ask if SEGS-1 activity depends on the formation of an episome or if SEGS-1 can function in a genomic context.

In this study, capacity of genomic and episomal forms of SEGS-1 to enhance viral disease using *Arabidopsis thaliana* as a model plant host was examines. We used *A. thaliana* because it doesn't contain sequences related to SEGS-1 in its genome (Ndunguru *et al.*, 2016).

5.2 Materials and methods

5.2.1 Construction of SEGS-1 clones for bombardment

SEGS-1 clones used in these studies were generated from a pGEM-T Easy plasmid harboring a dimeric copy of SEGS-1 (pGEM-SEGS-1) described in Ndunguru *et al.*, (2016). The dimeric construct was digested with *Eco*RI and cloned into pUC119 at the *Eco*RI site to make pNSB2136 (referred here as S1-2.0, a dimer SEGS-1 in pUC119). The pGEM-SEGS-1 was also digested with *Pst*1/*Bcl*1 to isolate two 500-bp fragments. The fragments were individually cloned into pUC119, which had been digested with *Bam*H1/*Pst*1, to give two half clones - pNSB1827 containing the *Cla*1 site and pNSB1828 containing the *Sal*I site from SEGS-1. pNSB2136 was digested with *Pst*I to give a 1032-bp SEGS-1 monomer fragment, which was re-cloned into pUC119 to form S1-1.0 (SEGS-1 monomer in pUC119). To make S1-1.5a (pNSB1829), pNSB1827 was linearized with *Pst*I and ligated with the SEGS-1 monomer fragment. To make S1-1.5b (pNSB1830), pNSB1828 was digested with *Pst*I and ligated to the SEGS-1 monomer fragment.

5.2.2 Construction of the SEGS-1 clones for Agrobacterium transformation

Agrobacterium for the floral DIP transformation of Arabidopsis were prepared by electroporation of *Agrobacterium tumefaciens* with the SEGS-1 clones in table 5.3. Electroporation protocol according to Shaw (1995) was followed.

5.2.3 Plant Material and Growth Conditions

Arabidopsis thaliana accessions Sei-0 (Lee *et al*., 1994), Col-0 (Turnage *et al*., 2002), and Pla-1 (accession CS28641) were from the *Arabidopsis* Biological Resource Centre (ABRC, Ohio State University). *Arabidopsis* seeds were sown in pots with sterile soil and grown in a walk-in growth chamber at 20°C, 80% humidity and an 8:16 light/dark (LD) cycle. Transplanting was done 14 days after seeding and plants were grown for more three weeks (about 12 leaves per plant) before inoculation. Pla-1 seeds were sown in the same conditions as Sei-0 and Col-0 except that seeds in pots were cold stratified in the dark at 4°C for 3 days before germination in a growth chamber.

5.2.4 *Arabidopsis* Transformation

Transgenic *Arabidopsis* plants were generated using the floral dip procedure according to published protocols (Mara *et al*., 2010) with the following changes. Kanamycin (0.05 mg/mL) was included in LB broth and the selection plates. The aerial part of a plant was immersed in infiltration solution for 1 min. Seeds for antibiotic selection were sterilized in 1.5 mL Eppendorf tubes containing 70% ethanol for two min, followed by treatment with a 10% bleach solution containing 0.02% Triton X-100 for 10 min and washing 3 times with sterile water. Approximately 100 sterile seeds were spread evenly on the selection plates and sealed with aeration tape. The plates were incubated in dark at 4C for 2 days followed by a brief exposure to light for 6 h at room temperature and incubated again for 2 days in dark at the same temperature until the seeds began to geminate. The plates were then placed in constant light at room temperature for full growth of plantlets.

5.2.5 Plant inoculation, sample collection and DNA isolation

Plants were inoculated by biolistic bombardment using a low-pressure particle DNA delivery microsprayer system (Venganza, Inc.). Procedures used for coating gold particles with plasmid DNA and for plant inoculation have been described earlier (Shen

et al., 2014). Plants were inoculated with 1.5 µg per plant of cloned SEGS-1 DNA (S1-1.0, S1-1.5a, S1-1.5b and S1-2.0) and with 0.75 µg of each viral replicon plasmid corresponding to ACMV (Accession Numbers: AF112352 and AF112353) or CaLCuV (Accession Numbers: NC_003866 and NC_003887.1).

Wild-type Sei-0 plants were inoculated in six treatments: Mock (DNA-B alone), ACMV (DNA-A and DNA-B), ACMV + S1-1.0, ACMV + S1-1.5a, ACMV + S1-1.5b and ACMV + S1-2.0. Each treatment consisted of 10 plants and the experiment was repeated 3 times.

The transgenic Sei-0 experiments used 3 plant genotypes (wild-type Sei-0, transgenic Sei-0 carrying a forward SEGS-1 transgene (S1-1.0F) and transgenic Sei-0 carrying a reverse transgene (S1-1.0R) that were inoculated with either mock or ACMV making a total of 6 treatments. Each treatment consisted of 6 plants. The experiment was repeated 3 times.

Pla-1 plants were inoculated with CaLCuV in presence and absence of the SEGS-1 clones. Treatments involved Mock (CaLCuV DNA-B alone), CaLCuV (CaLCuV DNA-A and DNA-B), CaLCuV + S1-1.0, CaLCuV + S1-1.5a, CaLCuV + S1-1.5b and CaLCuV + S1-2.0. *Arabidopsis* Col-0 was used a positive control for CaLCuV infection. Ten plants were inoculated per treatment. The experiment was repeated 3 times.

Plants were inspected at 10, 17 and 24 dpi for symptom appearance (recorded by photography) and symptom severity based on the 1-5 symptom severity scale (scale: 1 = no symptoms to 5 = very severe). Samples for viral DNA analysis were collected in 1.5 ml eppendorf tubes from 2nd youngest leaf (about 1 cm in length) in the rosette center, frozen in liquid nitrogen and stored at −80ºC until DNA extraction. Total DNA was isolated using the CTAB protocol (Doyle *et al*., 1990) and treated with RNase A (0.1 µg/µL) according to the manufacturer instructions (Thermo Scientific™). DNA concentrations were quantified using a NanoDrop ND-1000 (NanoDrop Technologies) and a portion adjusted to 25 ng/µL by diluting with DNAse free water. Viral DNA

accumulation was monitored by semi-quantitative PCR using virus-specific DNA-A primers (Table 5.1). For conventional PCR, a 50 µL PCR reaction containing 1× PCR buffer, 0.2 mM dNTPs, 0.2 µM of each forward and reverse primers, 0.05 U of Taq DNA polymerase (NEB), 100 ng of DNA and nuclease free water to volume was used. PCR cycling conditions were, initial denaturation at 95°C for 5 mins, followed by 30 cycles of denaturation at 94°C for 30 second, annealing temperature in Table 5.1 for 30 second, extension at 72°C for 45 sec. per 1 kb PCR product and final extension was performed at 72°C for 7 mins. Amplified DNA was analyzed in 1% agarose gel electrophoresis and visualized under gel documentation system.

5.2.6 *Nicotiana tabacum* protoplast assays

Protoplasts were prepared from *N. tabacum* NT-1 cells, electroporated with ACMV DNA-A in presence or absence of the SEGS-1 clones, and cultured as described previously (Fontes *et al*., 1994). The transfections included 1.5 µg of ACMV DNA-A and 10 µg of a SEGS-1 clones in the following treatments: Mock (no virus), ACMV DNA-A, ACMV DNA-A + S1-1.0, ACMV DNA-A + S1-1.5a, ACMV DNA-A + S1-1.5b and ACMV DNA-A + S1-2.0. Total DNA was purified 48 h post transfection, and 30 µg was digested with *Dpn*I and linearized with *Bsu*361. Viral DNA accumulation was analyzed by DNA gel blotting using a ^{32}P-labeled ACMV DNA-A probe. Blots were scanned by using a PhosphorImager and quantified by using IQMacV1.2 software (Storm; Amersham, Inc.).

5.2.7 In situ hybridization and immunohistochemistry

For *in situ* hybridization or immunohistochemistry assays, leaf 4 relative to the center of the rosette was harvested, fixed using paraformaldehyde, and embedded int 5% agarose gel in 1xPBS buffer (Shen and Hanley-Bowdoin, 2014). The leaf was sectioned into 100-µm sections using a Leica VT1000S vibratome (Leica Microsystems).

For *in situ* hybridization, digoxigenin-labeled probe corresponding to 415 bp of the ACMV AC1 gene was generated using a PCR DIG Probe Synthesis kit (Roche Diagnostics) and the primer pair ACMV 400F and ACMV 400R (Table 5.1). PCR was performed in 50 µL reaction mix containing 10 ng of cloned ACMV-A DNA (Table 5.2) according to manufacturer's instructions. An identical, unlabeled positive control was also amplified. Labeled and unlabeled PCR products were analyzed on 1% agarose gels to determine labeling success. Before use, the probe was denatured at 100ºC for 5 min and cooled on ice for 5 min.

Immunohistochemistry used a rabbit antiserum against CaLCuV Rep, a biotinylated anti-rabbit IgG secondary antibody and horse radish peroxidase conjugated to streptavidin. Preparation procedures, source of the antibodies and immunohistochemistry assay steps have been described earlier (Shen and Hanley-Bowdoin, 2006; Ascencio-Ibanez *et al*., 2008).

5.2.8 Analysis of SEGS-1 episomes

Wild-type Sei-0 plants and Sei-0 carrying monomeric SEGS-1 transgene were inoculated to produce the treatements, Mock and ACMV, as described earlier except that 6 plants were inoculated per treatment. Leaf samples were collected at 10, 17, 24 and 32 dpi from 2rd and 4th youngest leaves for DNA isolation. The leaves were pooled to give one sample per treatment. The experiment was repeated 3 times.

To detect SEGS-1 episomes, total DNA was isolated and diluted as described above. The DNA (50 µg) was used as template for rolling circle amplification (RCA) using an Illustra™ TempliPhi™ DNA Amplification Kit. (Sigma-Aldrich) as per manufacture instruction. The RCA product was diluted 10-fold with DNase-free water and 1 µL was as template used in a 50-µL PCR reaction containing the primer pair, S2-4F and S2-2R (Table 5.1) using previously established conditions (Ndunguru *et al*., 2016).

5.2.9 Analysis of SEGS-1 episomes

Wild-type Sei-0 plants and Sei-0 carrying monomeric SEGS-1 transgene were inoculated to produce the treatments, Mock and ACMV, as described earlier except that 6 plants were inoculated per treatment. Leaf samples were collected at 10, 17, 24 and 32 dpi from 2rd and 4th youngest leaves for DNA isolation. The leaves were pooled to give one sample per treatment. The experiment was repeated 3 times.

Table 5.1: List of primers used for PCR amplification in this study

	Primer name	Sequence (5′ → 3′)	Virus/ particle	Type	Expected size (nt)	Annealing temp. (°C)
1	CaLuV A dirPCR For [a]	CTCTAGGAACATCTGGGCTTCTA				
2	CaLuV A dirPCR Rev[a]	CCTTATAATTGCGAGACGCTCT	CaLCuV	Divergent	890	53
3	ACMV divLF[b]	GACAAGATCCACTCTCCTACGC				
4	ACMV divLR[b]	CACATTGCGCACTAGCAACGACTT	ACMV	Divergent	1397	53
5	ACMV 400F[b]	CTCAGATGTCAAGTCCTATC				
6	ACMV 400R[b]	ATTGTGTGGGCCTAAAG	ACMV	Convergent	415	47
7	S2-4F[c*]	GGGTAGCCTCTAATCCTTCA				
8	S2-2R[c*]	CAGTTGAACTGCTGAACTGC	SEGS-1	Divergent		57
9	SII hp1F[c**]	TACGCAGCAGCCATCATGGACATC				
10	S2 2R[c**]	CAGTTGAACTGCTGAACTGC	SEGS-1	Divergent		57

[a]Primer designed using GeneBank accession nos. U65529, DQ178610 and DQ178612

[b]Primer designed using GenBank accession nos. AF112352, AY211884, AF366902, HE979768 and KJ887906

[d]Primer designed using a complete sequence of SEGS-1 amplified from cassava genome

*Divergent primer designed to amplify a SEGS-1 episomes

**Divergent primer designed to amplify a SEGS-1 transcript

To detect SEGS-1 episomes, total DNA was isolated and diluted as described above. The DNA (50 µg) was used as template for rolling cicle amplification (RCA) using an Illustra™ TempliPhi™ DNA Amplification Kit. (Sigma-Aldrich) as per manufacture instruction. The RCA product was diluted 10-fold with Dnase-free water and 1 µL used as template in a 50-µL PCR reaction containing the primer pair, S2-4F and S2-2R (Table 5.1) using previously established conditions (Ndunguru *et al*., 2016).

Table 5.2: Infectious viral and SEGS-1 clones used to inoculate plants by agroinoculation or biolistics.

Virus/SEGS-1	Plasmid used for biolistics	Plasmid used for agroinoculation	References and comments
ACMV DNA A	pILTAB409		A partial tandem copy of *African cassava mosaic virus* (ACMV) DNA-A replicon (GenBank Acc. No. AF112352) kindly provided by Claude Fauquet, University of Missouri
ACMV DNA B	pILTAB411		A partial tandem copy of *African cassava mosaic virus* (ACMV) DNA-B replicon (GenBank Acc. No. AF112353) kindly provided by Claude Fauquet, University of Missouri
SEGS-1 monomer (S1-1.0)		pNSB2000	A monomer copy of Sequences Enhancing Geminivirus Symptoms (SEGS-1) in pUC119 to make S1-1.0 and in pMON721 to make pNSB2000
SEGS-1 1.5 mer (S1-1.5a)	pNSB1829	pNSB1903	A partial tandem copy of Sequences Enhancing Geminivirus Symptoms (SEGS-1) in pUC119 to make pNSB1829 and in pMON721 to make pNSB1903
SEGS-1 1.5 mer (S1-1.5b)	pNSB1830	pNSB1902	A partial tandem copy of Sequences Enhancing Geminivirus Symptoms (SEGS-1) in pUC119 to make pNSB1830 and in pMON721 to make pNSB1902
SEGS-1 dimer (S1-2.0)	pNSB2136		A dimeric copy of Sequences Enhancing Geminivirus Symptoms (SEGS-1) in pUC119 to make pNSB2136
CaLCuV DNA A	pCpCLCV A.003	pNSB1090	*Cabbage leaf curl virus* (CaLCuV) with a partial tandem copy (Turnage *et al*, 2002; Eagelkrout *et al*, 2002).
CaLCuV DNA B	pCpCLCV B.003	pNSB1091	*Cabbage leaf curl virus* (CaLCuV) with a partial tandem copy (Turnage *et al*, 2002; Eagelkrout *et al*, 2002).

5.3 Results

5.3.1 SEGS-1 enhances ACMV symptoms in Arabidopsis

It difficult to determine how SEGS-1 enhances begomovirus disease and overcomes resistance in cassava because of the presence of a full-length SEGS-1 sequence and other related sequences in the cassava genome. To overcome this constraint, we asked if SEGS-1 impacts ACMV infection in *Arabidopsis thaliana*, which does not have SEGS-1 related sequences in its genome. For these studies, we bombarded the *Arabidopsis* hypersusceptible accession, Sei-0 (Lee *et al*., 1994), with partial tandem dimers corresponding to ACMV DNA-A and DNA-B in the presence and absence of SEGS-1. We tested several SEGS-1 clones (Fig 5.1A) that were generated from the SEGS-1 clone originally amplified from CMB-infected cassava using a betasatellite universal primer (Ndunguru *et al*., 2016). The S1-2.0 construct contains two full tandem copies of SEGS-1, while S1-1.0 has a single copy. The partial tandem dimers, S1-1.5a and S1-1.5b, have different halves of SEGS-1 duplicated. We used the hypersusceptible *Arabidopsis* accession, Sei-0, and which can be infected by ACMV via bombardment of partial tandem dimers of DNA-A and DNA-B.

Arabidopsis plants infected with ACMV developed symptoms that included leaf curling and deformation, leaf size reduction and flowering inhibition (Fig 5.1B, C and D). However, in the presence of any of the SEGS-1 clones, symptoms appeared earlier and progressed faster compared to plants inoculated with ACMV alone. Based on a symptom severity scale of 1-5 (scale: 1 = no symptoms to 5 = severe, as described in Fig A.6), plants inoculated with ACMV alone had a numerically smaller mean disease severity score compared to plants inoculated with a combination of ACMV and any of the SEGS-1 clones (Fig 5.1D and Table A.5A). Analysis of variance (ANOVA) on the disease severity scores yielded significant variation among treatments at 17, 24 and 31 dpi (Table A.5B). A paired-samples t-test conducted to determine the difference between treatment means showed a significant difference ($p < 0.05$) between the means of plants infected with ACMV alone and those co-inoculated with ACMV and any of the SEGS-1

clones for all the sampling times (Table A.5C). The 17 dpi timepoint was the only sampling time where we found significant difference for the mean of S1-1.0 and S1-2.0 among the co-infected plants.

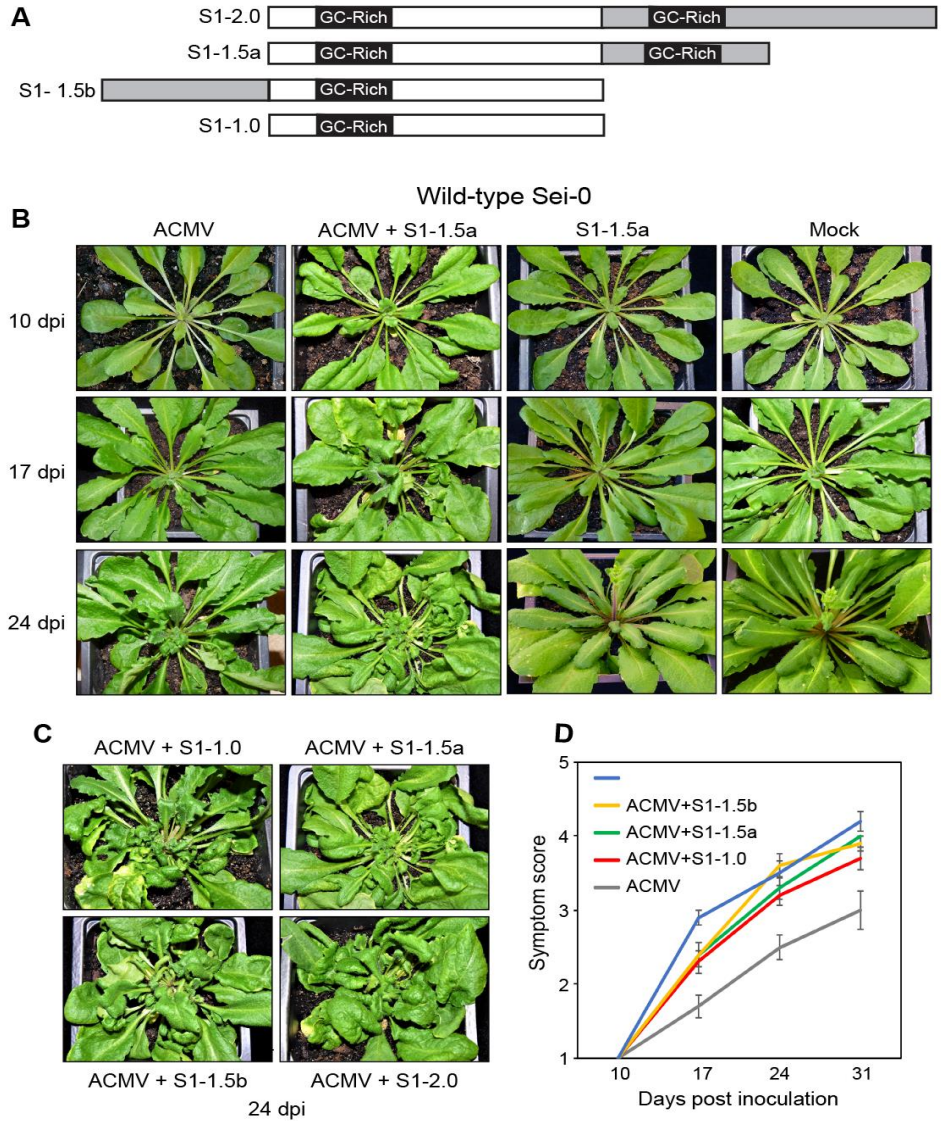


Figure 5.1: SEGS-1 enhances ACMV symptoms in the susceptible Arabidopsis accession Sei-0.

A: SEGS-1 clones used for inoculation (S1-1.0, S1-1.5a, S1-1.5b and S1-2.0). **B:** Time course (10, 17 and 24 dpi) of symptom development in presence or absence of SEGS-1. An ACMV-infected plant shows mild symptoms compared to the severe symptoms

developed by plants co-inoculated with ACMV and the SEGS-1 clone, S1-1.5a. The Mock plant was inoculated with ACMV DNA-B only. **C:** Severe symptom developed on *Arabidopsis* Sei-0 co-inoculated with ACMV and any of the SEGS-1 clones at 24 dpi. **D:** Time course symptom severity trend at four time points (10, 17, 24 and 31 dpi). Compared to ACMV inoculation alone, co-inoculation of ACMV and any of the SEGS-1 clones resulted into earlier symptoms appearance, faster disease progression and higher disease severity score. Bars in symptom severity curve indicate standard error (SE) values of 10 plants/treatment.

End point PCR analysis of total DNA samples revealed that higher levels of viral DNA accumulated in infected plants co-inoculated with ACMV and SEGS-1 than with ACMV alone (Fig 5.2A). Viral DNA was readily detected at 10 dpi in plants co-inoculated with ACMV and SEGS-1, while only trace of amounts of viral DNA were seen at 17 dpi in plants inoculated with ACMV alone (Fig 5.2A). The PCR signals were greater in plants co-inoculated with ACMV and SEGS-1 that ACMV alone at all time points. Plants inoculated with SEGS-1 alone were indistinguishable from mock inoculated plants (Fig 5.1B) and contained no detectable SEGS-1 DNA (Fig 5.2A) at all timepoints with ACMV alone. Mock plants were inoculated with ACMV DNA-B and did not have infected cells.

We then used in situ hybridization to ask if the enhanced symptoms and elevated ACMV DNA levels in the presence of SEGS-1 reflect changes in the pattern of infection. Sections from infected leaves were hybridized with an ACMV A probe labeled with digoxigenon that specifically cross reacts with viral DNA and an anti-digoxigenon detection system that stains virus-positive nuclei with a black precipitate. More virus-positive cells were detected in plants co-inoculated with ACMV and SEGS-1 versus ACMV alone. In all treatments, the infected cells were associated with the vascular bundle and adjacent cells (Fig 5.2B). No staining was observed in sections from the mock inoculated controls, demonstrating the specificity of the *in situ* assay. These results suggested that SEGS-1 enables ACMV to infect more cells around the vascular bundle but not to invade mesophyll and epidermal cells.

The increase in viral DNA accumulation in presence of SEGS-1 is likely due in part the ability of ACMV to establish infection in more cells. Protoplast analysis established wheather the elevated viral DNA levels also reflect an effect of SEGS-1 on viral DNA replication. Co-transfecting an ACMV DNA-A replicon with an empty vector or plasmids carrying SEGS-1 sequences into protoplasts prepared from tobacco (*Nicotiana tabacum*) suspension cells was done and monitored viral DNA accumulation 48 h post transfection on DNA gel blots. Each treatment was performed in triplicate. Higher levels of double-stranded ACMV-A replicative form were detected in the treatments containing the SEGS-1 plasmids (Fig 5.3A, cf. lanes 4-6 with 7-18). The increases were statistically significant ($P<0.05$) except in co-transfection with S1-1.0 (the monomer) (Fig 5.3B). Because cell-to-cell movement does not occur in the protoplast system, the increase levels suggest that SEGS-1 may also enhance viral replication.

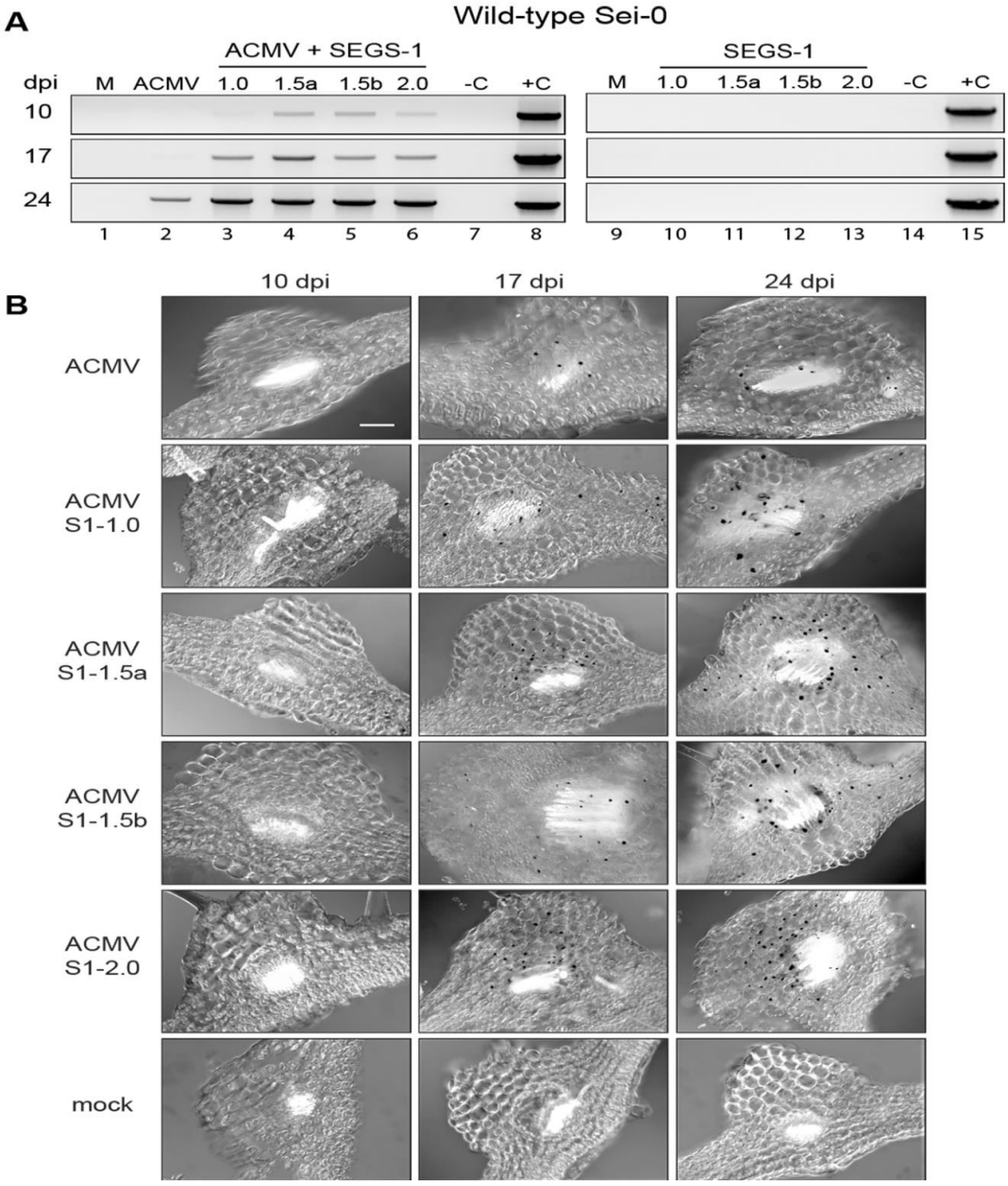


Figure 5.2: SEGS-1 enhances ACMV DNA accumulation in the susceptible Arabidopsis accession Sei-0.

A: Time course of viral genome accumulation in mock (M) (lane 1), ACMV alone (lane 2), or ACMV and one of the SEGS-1 clones, S1-1.0 (lane 3), S1-1.5a (lane 4), S1-1.5b (lane 5) or S1-2.0 (lane 6). A negative no template control and a cloned positive DNA control are indicated by -C and +C (lanes 7 and 6 respectively). **B:** Microscopy images

taken at 24 dpi of in situ hybridization using a PCR DIG-labeled probe showing accumulation of more virus-infected cells in plants co-inoculated with ACMV and one of the SEGS-1 clones than in plants inoculated SEGS-1 enhances viral DNA accumulation in tobacco protoplast cells

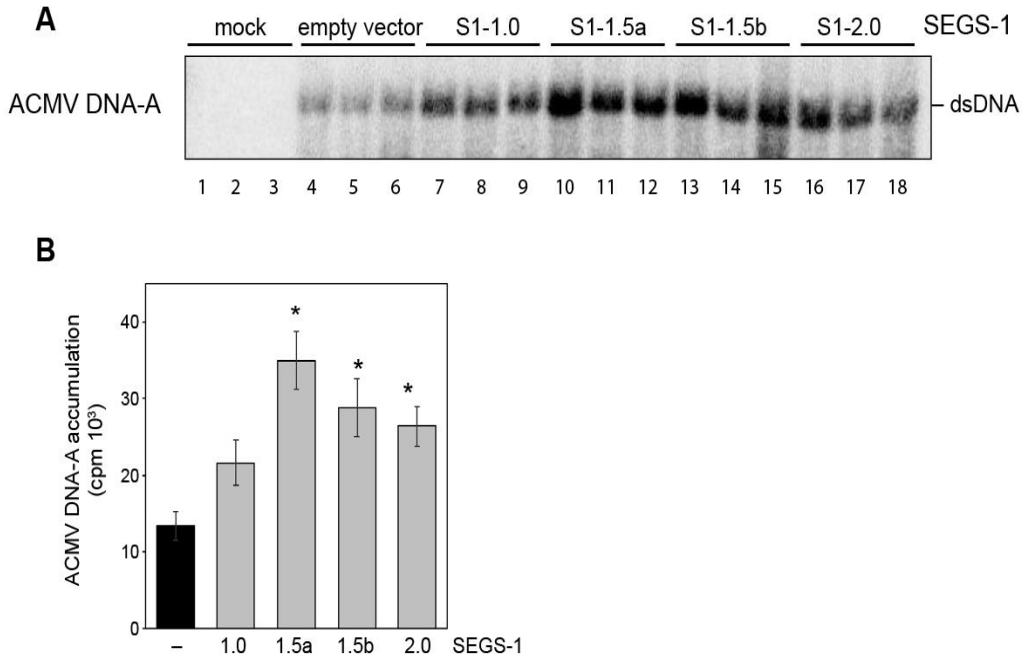


Figure 5.3: SEGS-1 enhances ACMV DNA-A replication in tobacco protoplasts.

A: DNA gel blot showing viral dsDNA accumulation in tobacco protoplast transfected with and ACMV DNA-A replicon in presence or absence of SEGS-1. Analysis of nascent DNA was performed using a radioactive probe [α-32P]dCTP. The transfections are mock (no virus, lanes 1-3), DNA-A + empty vector pUC119 (lanes 4-6), DNA-A + S1-1.0 (lanes 7-9), DNA-A + S1-1.5a (lanes 10-12), DNA-A + S1-1.5a (lanes 13-15) and DNA-A + S1-2.0 (lanes 16-18). **B:** Histogram of the mean relative amount of ACMV DNA-A from the different transfection treatments quantitated with a GelQuant software (Biochem Lab Solutions).

Table 5.3: Transformation efficiencies of floral dip Arabidopsis Sei-0 transformed with SEGS-1 clones by Agrobacterium mediated floral dip method.

T1 population was grown in kanamycin selection plate and number of surviving plantlets with green phenotype and those with bleached phenotype counted. Surviving green plants were then subjected to DNA isolation and PCR detection of the SEGS-1 transgene. Transformation efficiency was calculated as the percentage of the number of PCR positive transgenic plants divided by the total screened seeds.

Construct for transformation	Total no. of seeds screened	No. of survived plants	No. of PCR +ve for transgene	Efficiency (%)
pNSB2000-F	10400	15	13	0.13
pNSB2000-R	7200	12	11	0.15
pNSB1903-R	5000	8	7	0.14
pNSB1903-F	2700	6	6	0.22
Total	25300	41	37	0.15

pNSB2000-F and pNSB1903-F contains SEGS-1 in a reverse orientation

pNSB2000-R and pNSB1903-R contains SEGS-1 in a forward orientation

5.3.2 SEGS-1 transgene enhances CMD symptoms in Arabidopsis

To ask if SEGS-1 can enhance ACMV infection when it is integrated into the *Arabidopsis* genome as it is in cassava, transgenic *Arabidopsis* Sei-0 lines with SEGS-1 integrated into the genome was produced. Four SEGS-1 constructs were used for floral dip transformation that corresponded to the forward and reverse T-DNA orientations of the monomer (S1-1.0F pNSB2000F; S1-1.0R pNSB2000R) and a partial dimer (S1-1.5bF pNSB1903F; S1-1.5bR pNSB1903R) (Table 5.2). Kanamycin resistance screening and PCR analysis of T_1 transformants revealed that the average efficiency rate of transformation was 0.15% (Table 5.2). On kanamycin selection plates, transgenic plants appeared green, healthy and produced strong roots (kanamycin resistant, KanR), whereas the non-transgenic plants were bleached, stunted and failed to produce strong roots (kanamycin sensitive, KanS). A χ^2 test performed on data from T_2 plants indicated that ca. 70% of the transgenic lines contained a single T-DNA insertion with a segregation ratio of 3:1 (KanR:KanS). Only plants segregating according to the 3:1 mendelian ratio (regarded as a single locus insertion) were selected for screening to obtain homozygotes. Homozygous T_3 lines were recovered from about 60% of the

selected T_2 plants. Plants carrying a SEGS-1 transgene plants appeared phenotypically normal, indicating that the SEGS-1 sequences by themselves do not impact *Arabidopsis*, consistent with the observation that inoculation of SEGS-1 DNA by itself has no effect on plants.

Homozygous T_3 Sei-0 plants with SEGS-1 were tested in infection assays with ACMV and compared to infection in wild-type plants to determine whether SEGS-1 is active as a transgene. When ACMV was inoculated alone, the transgenic plants showed earlier onset of CMD symptoms (as early as 10 dpi) and faster disease progression compared to wild-type plants (Fig 5.4A and B). ANOVA on the disease severity scores showed significant variation ($P<0.05$) among treatments means (Table A.6B). A paired-sample t-test showed significant difference ($p < 0.05$) between the means of infected transgenic plants and wild-type Sei-0 plants infected with ACMV at all sampling times except at 10 dpi (Table A.6C). The results were independent of the orientation of SEGS-1 in the T-DNA.

The transgenic SEGS-1 plants also contained higher levels of viral DNA, as detected by end point PCR (Fig 5.4C). *In situ* hybridization revealed more cells were infected with ACMV in the SEGS-1 transgenic plants than wild-type Sei-0 (Fig 5.4B). A few infected cells were seen in the vascular bundles in 10 dpi. In SEGS-1 transgenic plants, the number of infected cells increased rapidly over time (17 and 24 dpi) and some infected cells were found outside the vascular tissue (Fig 5.4B).

SEGS-1 episomes have been reported in CMB-infected cassava plants that may have derived from the cassava genomic copy of SEGS-1 (Ndunguru *et al*., 2016). Hence, we asked if SEGS-1 forms episomes during ACMV infection of the transgenic plants. Total DNA isolated from healthy and infected transgenic Sei-0 plants carrying the SEGS-1 monomer transgene (S1-1.0F) was subjected to rolling circle amplification (RCA) followed by 40 cycles of PCR amplification using divergent primers for SEGS-1 episomes (Table 5.1). Except for the positive plasmid control (S1-1.5a), no PCR product

was observed in three bioreplicates, indicating that SEGS-1 episomes do not occur in transgenic *Arabidopsis*.

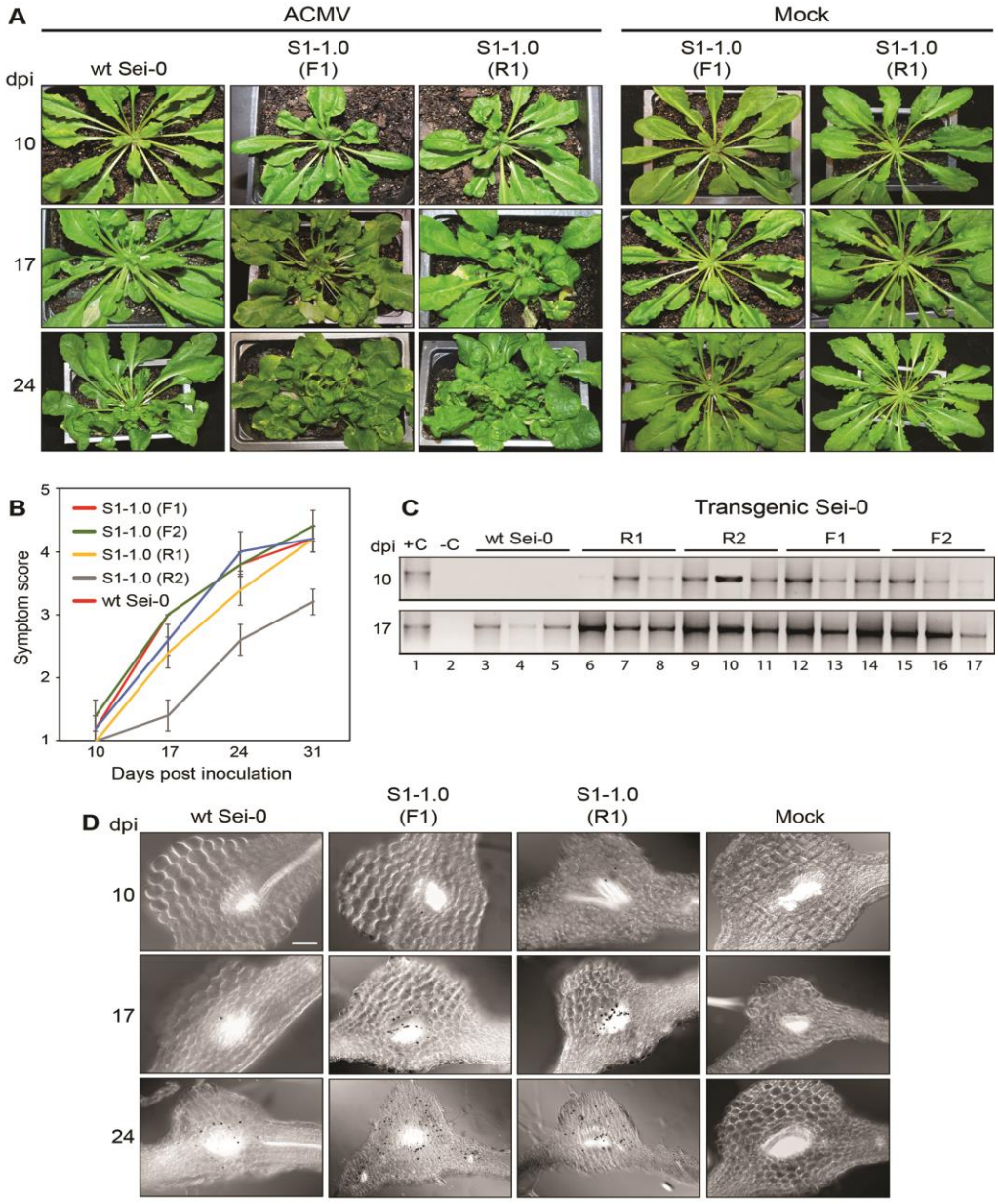


Figure 5.4: A SEGS-1 transgene enhances ACMV infection in Arabidopsis SEI-0 plants.

A: Time course (10, 17 and 24 dpi) of symptom expression on wild-type Sei-0, Sei-0 with a forward (S1-1.0F1) and a reverse (S1-1.0R1) SEGS-1 monomer transgene after inoculation with ACMV and mock treatment. Wild-type plants inoculated with ACMV showed no symptoms at 10 dpi and mild symptom at 24 dpi. Transgenic Sei-0 plants showed disease symptoms as early as 10 dpi that progressed to severe symptoms by 17 dpi. Mock-inoculated plants did not show symptoms. **B:** Time course of symptom severity of transgenic and wild-type plants inoculated with ACMV at 10, 17, 24 and 31 dpi. Transgenic plants exhibited early symptom appearance, fast disease progression and higher disease severity score as compared to wild type Sei-0 plants. Bars indicate standard error (SE) values for 6 plants. **C:** Comparison of the relative amount of virus accumulation in infected wild-type and the transgenic plants (plants with a SEGS-1 reverse transgene; R1 (lanes 6-8) and R2 (lanes 9-11) or with SEGS-1 forward transgene; F1 (lanes 12-14) and F2 (lanes 15-17)) at 10 and 17 dpi. All the transgenic plants had higher virus accumulation at 10 and 17 dpi compared to wild type (lanes 3-5), which did not have detectable virus at 10 dpi and low levels at 17 dpi. **D:** Microscopy images of leaf sections of wild-type and transgenic plants at 10, 17 and 24 dpi from *in situ* hybridization assays using a DIG-labeled ACMV-A probe showing accumulation of more virus-infected cells in transgenic than wild-type plants. Mock plants inoculated with ACMV DNA-B did not have infected cells.

5.3.3 SEGS-1 breaks Arabidopsis Pla-1 resistance to Cabbage leaf curl virus

SEGS-1 can overcome CMD2 resistance to CMBs in cassava (Ndunguru *et al*., 2016). To find out if SEGS-1 can also break resistance in *Arabidopsis*, Pla-1 accession that is immune to infection by *Cabbage Leaf Curl Virus* (CaLCuV), a New World begomovirus that is well adapted to *Arabidopsis* (Reyes *et al*., 2017) was used. Pla-1 plants inoculated with CaLCuV alone did not develop symptoms at any sampling time (7, 14, 21 and 28 dpi) (Fig 5.5A). However, all of the plants co-inoculated with CaLCuV and a SEGS-1 clone showed disease symptoms (Fig 5.5A). At 21 dpi, the co-inoculated plants exhibited severe symptoms of leaf curl, leaf distortion and stunting. The diseased Pla-1 plants did not produce flowers or recover from infection. Interestingly, the infected Pla-1 plants did not show strong chlorosis typically associated with CaLCuV infection of Col-0 plants (Fig 5.5C).

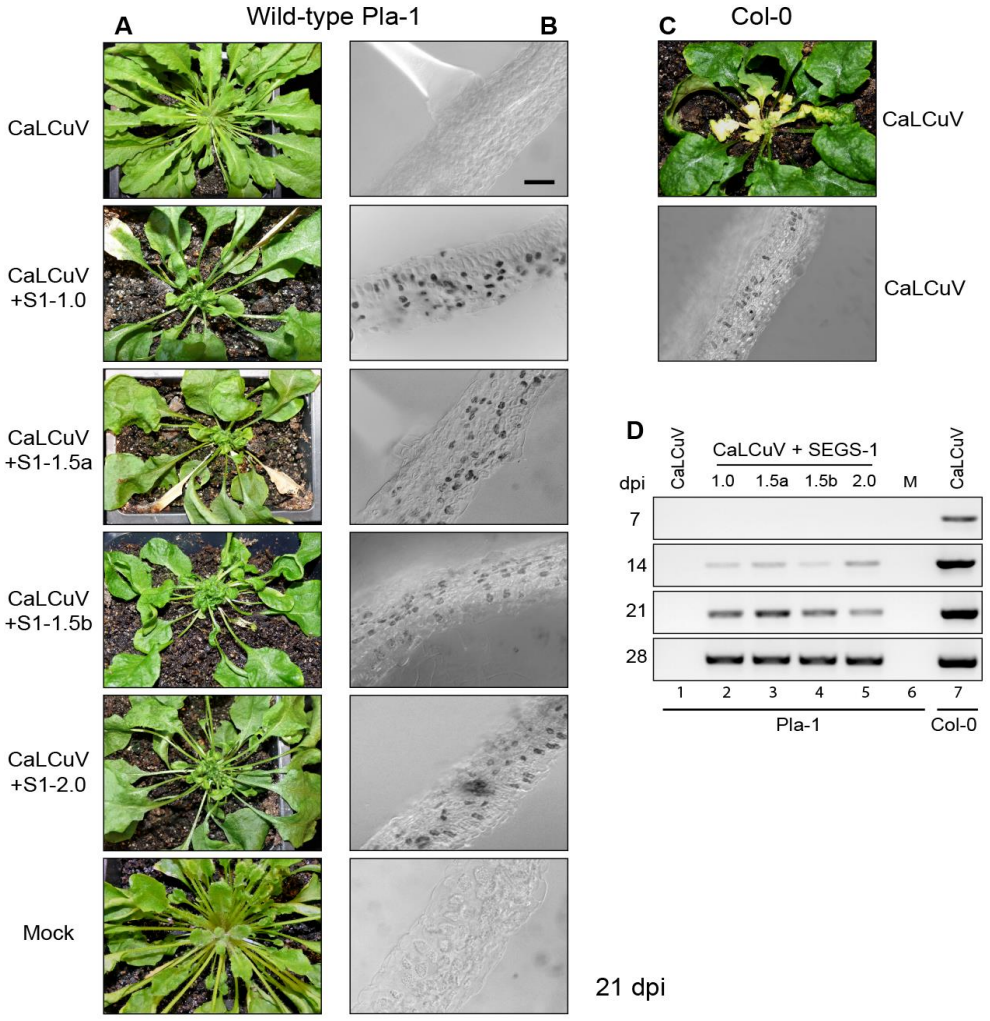


Figure 5.5: Exogenous SEGS-1 breaks endogenous resistance in the *Arabidopsis* accession Pla-1.

A; Symptom expression in Pla-1 plants infected with CaLCuV in presence and absence of SEGS-1 clones. Pla-1 plants inoculated with CaLCuV alone did not show symptoms at all times. All plants inoculated with a combination of CaLCuV and any of the SEGS-1 clones showed symptoms. Symptoms progression was fast, and plants showed severe symptoms at 21 dpi. Mock plants were inoculated with CaLCuV DNA-B and did not show symptoms. **B;** Detection of CaLCuV-infected cells in Pla-1 leaf sections at 21 dpi by in immunohistochemistry. Mock and CaLCuV infected Pla-1 did not contain virus-positive cells. Pla-1 co-infected with CaLCuV and any of the SEGS-1 clones contained many virus-infected cells. **C:** CaLCuV susceptible Col-0 showing diseases symptoms

and virus-infected cells after inoculation with CaLCuV alone. **D;** PCR time course of relative virus DNA accumulation in Pla-1 inoculated with CaLCuV in presence and absence of SEGS-1 clones. No virus was detected in plants inoculated with CaLCuV alone at all sampling times (lane 1). Co-infection of CaLCuV and any of the SEGS-1 clones resulted into a progressive accumulation of the virus from 14 to 28 dpi (lanes 2-5). The Col-0 susceptible accession was used as a positive control (lane 7).

PCR analysis detected viral DNA in all of the co-inoculated plants as early as 14 dpi, but not in Pla-1 plants inoculated only with CaLCuV over the course of the experiment (Fig. 5.5D). Immunohistochemistry using an antibody against CaLCuV Rep detected throughout the leaves of ACMV-infected plants in the presence of SEGS-1 (Fig 5.5B). The Rep staining patterns were similar to those observed in the susceptible Col-0 plants infected with CaLCuV (Fig 5.5C). No disease symptoms, viral DNA, or infected cells were seen in mock-inoculated Pla-1 plantss (Fig 5.5A and B).

5.4 Discussion

Sub-viral molecules associated with begomoviruses may be determinants of virus impact on their host plants. They may result in increased pathogenicity, overcome plant resistance, regulate virus movement and increase virus host range (Cui *et al*., 2004; Kon *et al*., 2007; Saunders *et al*., 2004; Saunders and Stanley, 1999; Venkataravanappa *et al*., 2011). Like satellite DNAs, SEGS-1 has no sequence similarity to begomoviruses and, instead, is 99% identical to a sequence in the cassava genome (Ndunguru *et al*., 2016). Hence, SEGS-1 does not fit the canonical definition of a satellite DNA. Using plant model systems such as *Arabidopsis*, *N. benthamiana* and *N. tabacum* protoplasts, we characterized the interaction between SEGS-1, a begomovirus and the host plant in the absence of potentially confounding SEGS-1 sequences in the cassava genome.

Four clones corresponding to monomeric (S1-1.0), partially dimeric (S1-1.5a, S1-1.5b) and dimeric (S1-2.0) SEGS-1 were analyzed functionally in *Arabidopsis* using bombardment to co-inoculate them with ACMV or CaLCuV. All of the SEGS-1 clones enhanced disease symptoms, increased viral DNA accumulation and resulted in more infected cells, indicating the exact configuration of the cloned sequences did not impact

SEGS-1 function. Similar results were observed in cassava and *N. benthamiana* for a SEG-1 dimer co-inoculated with a CMB (Ndunguru *et al*., 2016). Together, these results establish that SEGS-1 is active in different hosts and with diverse begomoviruses. Hence, the interactions and pathways necessary for SEGS-1 activity are conserved across begomovirus pathosystems and are likely to be fundamental to the infection process.

SEGS-1 is also functional as transgene in the *Arabidopsis* genome, with all infected plants showing disease enhancement. SEGS-1 enhancement activity is more uneven when it is co-bombarded with ACMV. This difference may reflect that a transgene is uniformly distributed while mechanical microspray inoculation of SEGS-1 DNA is more difficult to control and attain uniform delivery (Inoue-Nagata *et al*., 2007). The presence of the SEGS-1 transgene in every cell of an *Arabidopsis* resembles the situation in cassava, in which every cell also has genomic copy of SEGS-1. However, unlike cassava, we found no evidence of SEGS-1 episomes in *Arabidopsis* during infection, indicating that SEGS-1 activity is mediated by the genomic copy. This observation is striking because SEGS-1 enhancement is not a universal feature of CMB infection in cassava, and it is not known if the cassava genomic copy is functional. One possible explanation for this difference is that the genomic copy of SEGS-1 is located in a repressive chromatin environment in cassava while it is in open, more active chromatin in *Arabidopsis*. This idea is consistent with the observation that transgenes tend to insert into more accessible, euchromatic regions. Moreover, the cassava genome contains 17 SEGS-1 related sequences that collectively may be perceived as repetitive DNA and be silenced.

An earlier study showed that CaLCuV does not cause symptoms or accumulate in inoculated or systemic leaves of *Arabidopsis* Pla-1 plants (Reyes *et al*., 2017). In contrast, CaLCuV can readily establish infection Pla-1 plants when it is co-inoculated with SEGS-1. SEGS-1 was also reported to break CMD2 resistance to CMBs in cassava (Ndunguru *et al*., 2016). The resistance genes have not yet been identified in either host, and it is not known if the underlying mechanisms of resistance breaking are similar in

the two pathosystems. However, it will be more straight forward to address resistance breaking in *Arabidopsis* due to the lack of genomic copies of SEGS-1 and the availability of genetic tools that have not been developed for cassava. Recently, it was found that Pla-1 plants with a SEGS-1 transgene are no longer resistant to CaLCuV infection (unpublished results). Such plants can be used to downstream targets of SEGS-1 and provide insight into the pathways involved in the resistance breaking.

Although it is not yet known how SEGS-1 functions to enhance disease and break resistance, results from this study provide insight into potential mechanisms. *In situ* hybridization studies indicated that SEGS-1 increases the number of virus-positive cells in vascular tissue during infection. To mount a systemic infection, a plant virus must be able to move into and through the vascular system of its host (Harries and Ding, 2011; Horns and Jeske, 1991; Lucas and Wolf, 1999). The fact that ACMV-infected cells increase in vascular tissue in presence of SEGS-1 suggested that it might facilitate cell-to-cell movement leading to more infected cells. Given that ACMV-positive cells are confined to vascular tissue even in the presence of SEGS-1 suggested it does not enable the virus to invade other leaf cell populations. SEGS-1 also increases ACMV-A accumulation in transient assays using tobacco suspension cells that support viral DNA replication and gene expression under semi-synchronous conditions independent of cell-to-cell and systemic movement (Brough *et al*., 1992; Motoyoshi, 2018). This result is consistent with SEGS-1 increasing the rate of viral DNA synthesis by modulating the activity or expression of viral and/or host replication factors. Alternatively, SEGS-1 might suppress DNA methylation or other host defenses that inhibits or reduce viral DNA accumulation.

It is important to point out that SEGS-1 product that impacts begomovirus disease processes is not known. We could not detect a SEGS-1 transcript in transgenic *Arabidopsis* and hypothesize that SEGS-1 might function via a small RNA. This idea is supported by the observation that SEGS-1 lack any significant open reading frames and unlikely function via protein.

SEGS-1 enhances begomovirus disease symptoms and virus accumulation, changes the dynamics of disease progression to cause early onset of symptoms, and compromises plant defenses in diverse begomovirus pathosystems. The fact that SEGS-1 functions from a transgene in the *Arabidopsis* genome raises the possibility that the SEGS-1 genomic copy in cassava is also active Thus, SEGS-1 represents a major threat to cassava because all known cultivars contain a genomic SEGS-1. Hence, it is essential to determine how SEGS-1 functions and under what conditions the genomic copy might be activated in cassava. The studies in *Arabidopsis* represent a key step in this direction and in the development of cassava varieties with lasting resistance to begomoviruses and SEGS-1.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

Diseases caused by geminiviruses cause huge losses in food and cash crops, ornamentals and pasture production. Emerging new and more virulent viral species and strains and their associated sub-viral molecules are causing pandemics that threaten food security and economic independence of the most need people across Africa and Asia. Because of their economic relevance, studying these viruses and their host interactions is an important step in designing effective control strategies. However, elucidating these interactions and their underlying molecular mechanisms in the natural host/crop remains challenging because the host pathosystems is generally complex and less amenable for experimentation. This thesis focused on how to study complex pathosystems using simple model plants to generate reliable information relevant to the natural host.

As described in Chapter III, tomato is one of the most desirable model plants for virus-host interaction studies. The limitations of various tomato systems have been documented extensively (Arie *et al*., 2007; Meissner *et al*. 1997; Emmanuel & Levy, 2002). Genetic mutation in some varieties like Micro-Tom have major constraints that could alter or interfere with virus-plant interaction studies (Martí *et al*., 2006). Characterization of Florida Lanai (Augustine, *et al*., 1981), which does not carry known mutations is an important advance towards establishing a more reliable model system for tomato. Because of its ease of infection using different methods (Agroinoculation, mechanical microdrop-sprayer and whitefly transmission), Florida Lanai is among the most accessible model plants for geminivirus studies in Sub-Saharan Africa labs, where facilities or the experience to carry out other methods are often lacking. Inadequate funding also limits choices in technologies in developing countries (Vose & Cervellini, 1983; Chambers & Ghildyal, 1984; Ribaut *et al*., 2010).

Florida Lanai can be used to study diverse geminiviruses including monopartite and bipartite viruses, begomoviruses and curtoviruses, and Old and New World viruses, underscoring the versatility as the system for comparing virus-host interaction in a common host. Other characteristics of Florida Lanai described here and in other reports include transmission efficiency, fast disease progress, development of clear disease symptoms, high virus accumulation and measurable impacts on plant height, flowering, fruit number and size (Dmitry & Van den Ackerveken, 2013; Rom *et al*., 1993; Inoute-Nagata *et al*., 2016). For some geminiviruses, it may be possible to use Florida Lanai as common host rather than using a variety of natural hosts. The common host will simplify research, saving time and improving reliability and comparability of results across different viruses.

Chapter IV focuses on characterizing the mechanism of geminivirus immunity in *Arabidopsis*. VIGS vector derived from the *Cabbage leaf curl virus* (CaLCuV) was used to screen more than 200 *Arabidopsis* accession and to identify *Arabidopsis* accession (Pla-1) that displays strong resistance to two geminiviruses genera, e.g. begomovirus and curtovirus. Identification of genetic resistant to viruses in plants is a key first step in tackling the problems associated with viral diseases. *Arabidopsis* has been used extensively to study and identify resistance genes in response to interaction with a variety of pathogens (Speulman *et al*., 1998). Studies in *Arabidopsis* is facilitated by the well annotated genome with most genes and gene functions identified.

Analysis of viral DNA accumulation established that the Pla-1 accession is immune to both CaLCuV and BCTV infection. The study identified a quantitative trait locus (QTL) involved in the resistance, an important step toward identifying and characterizing the resistance gene(s). Identification of this QTL will provide information about geminivirus-host interactions and disease resistance and may provide new insights into controlling geminivirus disease in crop plants. Information from QTL mapping can also be used for marker-assisted selection of disease resistance (Vinod, 2009).

The geminivirus resistance identified in this study is recessive, suggesting that it is not mediated by one or more R genes, which are dominant and function in a gene-for-gene manner (Flor, 1971; Moffett, 2009; Padmanabhan & Dinesh-Kumar, 2014). Recessive resistance is based on the molecular interactions between viruses and their hosts and is conferred by a recessive gene mutation that encodes a host factor critical for viral infection. Most recessive resistance genes isolated to date are eukaryotic translation initiation factors eIF4E, eIF4G, and their isoforms (Kang *et al*., 2005; Piron *et al*., 2010; Hashimoto *et al*., 2016). Recessive resistance is generally very durable (Parlevliet, 2002), suggesting that the Pla-1 resistance can be translated to crops like cassava to confer broad-based, stable resistance to CMBs.

In chapter V, the *Arabidopsis, N. benthamiana* and *N. tabacum* model systems were used to characterize the impact of SEGS-1 on CMB infection. Generating information regarding SEGS-1 interaction with geminiviruses and host plants is vitally important to understand how SEGS-1 enhances disease and breaks resistance and to guide the formulation of sustainable control strategies that consider SEGS-1. Given the novelty and recent identification of SEGS-1 and SEGS-2, they were considered in the development of current control strategies and may now compromise existing CMD management programs. This possibility is underscored by the observation that some CMD resistance varieties from breeding programs have developed severe CMD symptoms in East African fields (Ndunguru *et al*., 2016).

Four SEGS-1 clones were constructed and shown to enhance disease in plants and protoplasts when co-inoculated with a begomovirus. Functional clones are important tools in plant pathology for characterizing viruses at the molecular and biological levels. The clones also create a ready source and a genetically uniform material for future applications like screening for resistant cultivars (Brewer, *et al* 2017).

SEGS-1 is active in wild-type *Arabidopsis* in co-inoculation experiments with a CMB. A SEGS-1 transgene is also active in *Arabidopsis* inoculated with only a CMB. Thus, It

can be concluded that the pathway(s) necessary for SEGS-1 activity are conserved between *Arabidopsis* and cassava. However, the *Arabidopsis* genome does not contain endogenous sequences related to SEGS-1, making it an excellent host system for functional analysis of SEGS-1 in the absence of endogenous SEGS-1 interference. This is a major problem in the natural cassava host, which contains multiple copies sequences related to of SEGS-1 and SEGS-2 in its genome (Ndunguru *et al*., 2016)

This study established that SEGS-1 is associated with early symptom development, enhanced disease symptoms, fast disease progression and enhanced accumulation of virus in geminivirus infected plants. These functions are similar to those of geminivirus-associated satellite DNAs, which are reported to increase pathogenicity, overcome resistance, regulate virus movement and increase virus host range geminivirus pathosystems (Venkataravanappa *et al*., 2011; Saunders *et al*., 2004; Cui*et al*., 2004; Saeed *et al* 2007).

In situ hybridization studies detected increased number of virus-positive cells in plants co-inoculated with ACMV and SEGS-1 compared to plants infected with ACMV alone, indicating that SEGS-1 might specify a small protein that interacts with a host or viral protein to facilitate or interfere with virus replication, systemic spread, host defenses. However, SEGS-1 does not include a major open reading frame, suggesting that it might not function through protein interactions. Another possibility is that SEGS-1 encodes a small RNA that influences expression of a viral or host protein.

Studies in *N. tabacum* suspension cells confirmed further the impact of SEGS-1 on ACMV infection. Suspension cells co-transfected with ACMV DNA-A and a SEGS-1 clone supported high levels of accumulation of ACMV DNA-A than protoplasts transfected with ACMV DNA-A alone. This result strongly suggested that SEGS-1 promotes virus accumulation in the absence of systemic movement. SEGS-1 may interact with the virus Rep or REn proteins or host replication factors to facilitate production of more viral DNA. Alternatively, SEGS-1 might suppress host DNA

methylation pathways, which contribute host defenses by methylating viral DNA and interfering with viral replication (Raja *et al*., 2008; Baulcombe, 2004). However, we did not detect anti-silencing activity for SEGS-1 in TGS assays, indicating that SEGS-1 is unlikely to alter DNA methylation.

SEGS-1 episomes were not detected in CMB infected *Arabidopsis* plants or SEGS-1 replication in tobacco protoplasts transfected with SEGS-1 alone or with ACMV DNA-A and SEGS-1. These results demonstrated that episome formation is not required for SEGS-1 activity and raise the possibility that the genomic copy of SEGS-1 is functional in cassava. These results also showed that SEGS-1 does not replicate in protoplasts, distinguishing it from geminivirus satellites, which replicate autonomously or are trans-replicated by their helper viruses (Mansoor *et al*., 2003; Sounders *et al*., 2002). We also failed to detect anti-silencing activity for SEGS-1 in TGS or PTGS assays, as has been described for geminivirus satellite proteins (Zhou, 2013; Vanitharani *et al*., 2004), further underscoring that SEGS-1 is distinct from geminivirus satellites.

Similar to CMD2 resistance breaking in cassava (Ndunguru *et al*., 2016), SEGS-1 overcomes *Arabidopsis* Pla-1 resistance to CaLCuV infection. Future studies must address the mechanisms underlying resistance breaking and determine whether similar or different mechanisms overcome Pla-1 resistance in Arabidopsis versus CMD2 resistance in cassava. An important first step will be the identification of the host genes that mediate Pla-1 and CMD2 resistance.

6.2 Conclusions

The research presented here demonstrated how to use model systems to study different aspects of virus-host interactions and their associated sub-genomic DNAs. Tomato cultivar, Florida Lanai was established an excellent model system for geminivirus research, especially for tomato viruses like TYLCV, ToMoV, TGMV and BCTV. Florida Lanai plants are small and can grow and produce many seeds in a small space.

Florida Lanai plants were readily infected by diverse geminiviruses, producing characteristic symptoms and measurable outcomes of virus-host interactions. Florida Lanai is especially well suited for comparisons of different viruses in a common host.

The *Arabidopsis* Pla-1 accession is resistant to viruses in two geminivirus genera, e.g. the begomovirus CaLCuV and the curtovirus BCTV. A major QTL for viral resistance was mapped using F_2 plants of a Pla-1 X Col-0 cross. Analysis indicated that Pla-1 resistance is recessive and may be polygenic.

Four model systems (*Arabidopsis* Sei-0, *Arabidopsis* Pla-1 and *N. benthamiana* plants and *N. tabacum* protoplasts) were used to characterize the impact of SEGS-1 on geminivirus infection. SEGS-1 enhances viral symptoms and viral DNA accumulation, increases the number of virus-infected cells, and increases the rate of infection. *Arabidopsis* Sei-0 plants with a SEGS-1 transgene also show enhanced disease but do not contain SEGS-1 episomes. The failure of SEGS-1 to replicate in tobacco protoplasts or to suppress host silencing pathways distinguish it from geminivirus satellites.

6.3 Recommendations

- The information generated in this study should be used to facilitate designing of management strategies of vital importance to reduce crop damage
- Florida Lanai is recommended as an excellent tomato variety for use as a model system for geminiviruss infecting tomato.
- Researchers will find it useful to use Florida Lanai in virus transmission studies, disease epidemiology studies and when investigating various physiological phenomena
- Pla-1 accession immune to both CaLCuV and BCTV infection and the Quantitative Trait Locus (QTL) involved in the CaLCuV resistance identified in this study, will provide information about geminivirus-host interactions and

disease resistance and will provide new insights into controlling geminivirus disease in crop plants

- Information from QTL mapping can also be used for marker-assisted selection of disease resistance
- For sustainability, it is recommended that furture virus management stratergies do address the presence and impact of Sequence enhancing Geminivirus Symptoms (SEGS)
- Future studies should address the mechanisms underlying resistance breaking and determine whether similar or different mechanisms overcome Pla-1 resistance in Arabidopsis versus CMD2 resistance in cassava

REFERENCES

Abouzid, A. M., Polston, J .A., & Hiebert, E., (1992). The nucleotide sequence of *tomato mottle virus*, a new geminivirus isolated from tomatoes in Florida. *J Gen Virol. 73*, 3225-3229.

Akano, A., Dixon, A., Mba, C., Barrera, E. & Fregene, M., (2002). Genetic mapping of a dominant gene conferring resistance to cassava mosaic disease. *Theor. Appl. Genet. 105*(4), 521-525.

Alberter, B., Rezaian, M.A., & Jeske, H., (2005). Replicative intermediates of Tomato leaf curl virus and its satellite DNAs. *Virol. 331*, 441-448.

Ali, Z., Abulfaraj, A., Idris, A., Ali, S., Tashkandi, M., & Mahfouz, M.M., (2015). CRISPR/Cas9-mediated viral interference in plants. *Genome bio. 16*, 238.

Arguello-Astorga, G., Ascencio-Ibanez, J.T., Dallas, M.B., Orozco, B.M., Hanley-& Bowdoin, L., (2007) High-frequency reversion of geminivirus replication protein mutants during infection. *J. virol. 81*, 11005-11015.

Arguello-Astorga, G., Lopez-Ochoa, L., Kong, L. J., Orozco, B. M., Settlage, S. B., & Hanley-Bowdoin, L., (2004). A novel motif in geminivirus replication proteins interacts with the plant retinoblastoma-related protein. *J. Virol. 78*, 4817–4826.

Arie, T., Takahashi, H., Kodama, M., & Teraoka, T., (2007). Tomato as a model plant for plant-pathogen interactions. Plant Biotech. 24, 135-147.

Ascencio-Ibáñez, J. T., Sozzani, R., Lee, T.J., Chu, T.M., Wolfinger, R.D., Cella, R., & Hanley-Bowdoin, L., (2008). Global analysis of Arabidopsis gene expression uncovers a complex array of changes impacting pathogen

response and cell cycle during geminivirus infection. *Plant Physiol. 148*, 436–454.

Augustine, J. JHarbaugh, B.K.& Crill, J.P. (1981*). Florida Lanai: A Dwarf Tomato for the Patio.* Gainesville [Fla.]: University of Florida,

Bahder, B.W., Zalom, F.G., Jayanth, M. & Sudarshana, M.R., (2016). Phylogeny of geminivirus coat protein sequences and digital PCR aid in identifying Spissistilus festinus as a vector of Grapevine red blotch-associated virus. *Phytopath. 106,* 1223-1230.

Baliji, S., Lacatus, G., & Sunter, G., (2010). The Interaction between Geminivirus Pathogenicity Proteins and Adenosine Kinase Leads to Increased Expression of Primary Cytokinin Responsive Genes. *Virol. 402*, 238-247.

Bart, R., Chern, M., Park, C.J., Bartley, L., Ronald, P.C., (2006). A novel system for gene silencing using siRNAs in rice leaf and stem-derived protoplasts. *Plant methods, 2,* 13.

Bartel, D.P., (2004). MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell 116*, 281-297.

Baulcombe, D.C., (1996). Mechanisms of pathogen-derived resistance to viruses in transgenic plants. *Plant cell, 8,* 1833.

Baulcombe, D.C., (2004). RNA silencing in plants. *Nature, 431*, 356-363.

Bennett, C. W. (1971). *The Curly Top Disease of Sugarbeet and Other Plants*. St. Paul, MN: American Phytopathological Society.

Bevan, M., Walsh, S., (2006). The Arabidopsis genome: A foundation for plant research. *Genome Research, 15*, 1632–1642.

Bisaro, D. M., Hamilton, W. D. O., Coutts, R. H. A., & Buck, K. W., (1982). Molecular cloning and characterization of the two DNA components of *tomato golden mosaic virus*. *Nucleic Acids Res 10*(16), 4913-4922.

Bishop, G.J., Harrison, K., & Jones, J.D.G., (1996). The tomato Dwarf gene isolated by heterologous transposon tagging encodes the first member of a new cytochrome P450 family. *Plant Cell, 8*, 959-969.

Blair, M.W., & Morales, F.J., (2008). Geminivirus resistance breeding in common bean. CAB reviews: perspectives in agriculture, veterinary science, *nutrition and natural resources, 89,* 1-14.

Blevins, T., Rajeswaran, R., Shivaprasad, P.V., Beknazariants, D., Si-Ammour, A., Park, H.S., … & Pooggin, M.M. (2006) Four plant Dicers mediate viral small RNA biogenesis and DNA virus induced silencing. *Nucleic Acids Res, 34*, 6233-6246.

Bos, L., (1981). Wild plants in the ecology of virus diseases. In: Plant Diseases and Vectors: Ecology and Epidemiology. Maramorosch, K. and Harris, K.F. (Eds.), pp.1-33. New York : Academic Press.

Bosque-Perez, N.A., (2000). Eight decades of maize streak virus research. *Virus Res. 71*, 107–121.

Briddon, R.W., Bedford, I.D., Tsai, J.H., & Markham, P.G., (1996). Analysis of the nucleotide sequence of the treehopper-transmitted geminivirus, tomato pseudo-curly top virus, suggests a recombinant origin. *Virol. 219*, 387-394.

Briddon, R.W., Bull, S.E., Amin, I., Idris, A.M., Mansoor, S., Bedford, I.D., … & Markham, P.G., (2003). Diversity of DNA beta, a satellite molecule associated with some monopartite begomoviruses. *Virol. 312*, 106-121.

Briddon, R.W., Bull, S.E., Amin, I., Mansoor, S., Bedford, I.D., Rishi, N., … & Markham, P.G., (2004). Diversity of DNA 1; a satellite-like molecule associated with monopartite begomovirus-DNA β complexes. *Virol. 324*, 462-474.

Briddon, R.W., Heydarnejad, J., Khosrowfar, F., Massumi, H., Martin, D.P., & Varsani, A., (2010). Turnip curly top virus, a highly divergent geminivirus infecting turnip in Iran. *Virus research, 152*, 169-175.

Briddon, R.W., Mansoor, S., Bedford, I.D., Pinner, M.S., Saunders, K., Stanley, J., … & Markham, P.G., (2001). Identification of DNA components required for induction of cotton leaf curl disease. *Virol. 285*, 234-243.

Briddon, R.W., & Stanley, J., (2006). Sub-viral agents associated with plant single stranded DNA viruses. *Virol. 344*, 198-210.

Briddon, R.W., Watts, J., Markham, P.G. & Stanley, J. (1989). The coat protein of beet curly top virus is essential for infectivity. *Virology, 172*, 628-633.

Broman, K.W. & Sen, S. (2009). *A guide to QTL mapping with R/qtl. In Statistics for biology and health*, . Dordrecht: Springer.

Brough, C.L., Gardiner, W.E., Inamdar, N.M., Zhang, X.Y., Ehrlich, M., & Bisaro, D.M., (1992a). DNA methylation inhibits propagation of *tomato golden mosaic virus* DNA in transfected protoplasts. *Plant Mol Biol, 18*, 703-712.

Brown, J.K., Fauquet, C.M., Briddon, R.W., Zerbini, M., Moriones, E., & Navas-Castillo, J., (2012). Geminiviridae. In Virus Taxonomy: Ninth Report of the International Committee on Taxonomy of Viruses, ed. AMQ King, MJ Adams, E.B., Carstens, E.J., Lefkowitz, pp. 351–73. London: Elsevier.

Buchmann, R.C., Asad, S., Wolf, J.N., Mohannath, G., & Bisaro, D.M., (2009). Geminivirus AL2 and L2 proteins suppress transcriptional gene silencing and cause genome-wide reductions in cytosine methylation. *J. Virol. 83*, 5005-5013.

Buck, K.W., & Coutts, R.H.A., (1985). *Tomato golden mosaic virus*. AAB Descriptions of Plant Viruses No. 303. Warwick, U.K: Spottiswode Balantyne Printers Ltd.

Bull, S. E., Briddon, R. W., Sserubombwe, W. S., Ngugi, K., Markham, P. G. & Stanley, J., (2006). Genetic diversity and phylogeography of cassava mosaic viruses in Kenya. J. *Gen Virol., 87*, 3053–3065.

Burch-Smith, T.M., Schiff, M., Liu, Y. & Dinesh-Kumar, S.P., (2006). Efficient virus-induced gene silencing in Arabidopsis. *Plant physiology*, *142*, 21-27.

Butterbach, P., Verlaan, M.G., Dullemans, A., Lohuis, D., Visser, R.G., Bai, Y., & Kormelink, R., (2014). *Tomato yellow leaf curl virus* resistance by Ty-1 involves increased cytosine methylation of viral genomes and is compromised by cucumber mosaic virus infection. *Proc Natl. Acad. Sci. U S A 111*, 12942-12947.

Cabrera-Ponce, J.L., López, L., Assad-García, N., Medina-Arevalo, C., Bailey, A.M., Herrera-Estrella, L., (1997). An efficient particle bombardment system for the genetic transformation of asparagus (Asparagus officinalis L.). *Plant Cell Rep. 16*, 255-260.

Campos, M.L., Carvalho, R.F., Benedito, V.A., & Peres, L.E.P., (2010). Small and remarkable: the Micro-Tom model system as a tool to discover novel hormonal functions and interactions. *Plant Signal. Behav. 5*, 267-270.

Cañizares, M.C., Rosas-Díaz, T., Rodríguez-Negrete, E., Hogenhout, S.A., Bedford, I.D., Bejarano, E.R., … & Moriones, E., (2014). Arabidopsis thaliana, an experimental host for tomato yellow leaf curl disease-associated begomoviruses by agroinoculation and whitefly transmission. *Plant Pathol, 64*, 265-271.

Caplan, J., Padmanabhan, M., & Dinesh-Kumar, S.P., (2008). Plant NB-LRR immune receptors: from recognition to transcriptional reprogramming. *Cell host microb. 3,* 126-135.

Carrington, J.C., Kasschau, K.D., Mahajan, S.K., & Schaad, M.C., (1996). Cell-to-cell and long-distance transport of viruses in plants. *Plant Cell, 8*, 1669.

Carvalho, C.M., Fontenelle, M.R., Florentino,L.H., Santos, A.A., Zerbini, F.M., & Fontes, E.P.B., (2008). A novel nucleocytoplasmic traffic GTPase identified as a functional target of the bipartite geminivirus nuclear shuttle protein. *Plant J. 55,* 869–880.

Carvalho, M F., &Lazarowitz, S.G., (2004). Interaction of the movement protein NSP and the Arabidopsis acetyltransferase AtNSI is necessary for Cabbage leaf curl geminivirus infection and pathogenicity. *J. Virol. 78,* 11161-11171.

Carvalho, R.F., Campos, M.L., Pino, L.E., Crestana, S.L., Zsögön, A., Lima, J.E., Benedito, V.A., & Peres, L.E.P., (2011). Convergence of developmental mutants into a single tomato model system: 'Micro-Tom' as an effective toolkit for plant development research. *Plant Methods 7,* 18.

Chambers, R., & Ghildyal, B.P., (1984). *Agricultural Research for Resource-Poor Farmers: The Farmer-First-and-Last Model, Ford Foundation Discussion Paper no 16,* New Delhi: Ford Foundation

Chellappan, P., Vanitharani, R., Ogbe, F. & Fauquet, C.M., (2005). Effect of temperature on geminivirus-induced RNA silencing in plants. *Plant physiol. 138*, 1828-1841.

Chen, G., Pan, H., Xie, W., Wang, S., Wu, Q., Fang, Y., Shi, X., & Zhang, Y., (2013). Virus infection of a weed increases vector attraction to and vector fitness on the weed Sci. *Rep. 3*, 2253.

Chen, L.F., Brannigan, K., Clark, R., & Gilbertson, R.L., (2010). Characterization of Curtoviruses Associated with Curly Top Disease of Tomato in California and Monitoring for These Viruses in Beet Leafhoppers. *Plant Dis. 94*, 99-108.

Chen, S., Tao, L., Zeng, L., VEGA-SANCHEZ, M.E., Umemura, K., & Wang, G.L., (2006). A highly efficient transient protoplast system for analyzing defense gene expression and protein–protein interactions in rice. Mol. *plant pathol. 7*, 417-427.

Chisholm, S.T., Mahajan, S.K., Whitham, S.A., Yamamoto, M.L. & Carrington, J.C., (2000). Cloning of the Arabidopsis RTM1 gene, which controls restriction of long-distance movement of tobacco etch virus. Proc. Natl. *Acad. Sci. U S A, 97*, 489-494.

Choi, J., Huh, S.U., Kojima, M., Sakakibara, H., Paek, K.H., & Hwang, I., (2010). The cytokinin-activated transcription factor ARR2 promotes plant immunity via TGA3/NPR1-dependent salicylic acid signaling in Arabidopsis. *Dev. Cell, 19*, 284-295.

Clarke, J.D., Volko, S.M., Ledford, H., Ausubel, F.M., & Dong, X., (2000). Roles of Salicylic Acid, Jasmonic Acid, and Ethylene in cpr-Induced Resistance in Arabidopsis. *Plant Cell, 12*, 2175–2190.

Clerot, D., & Bernardi, F., (2006). DNA helicase activity is associated with the replication initiator protein rep of tomato yellow leaf curl geminivirus. *J. Virol. 80,* 11322-11330.

Cocking, E.C., (1972). Plant cell protoplasts-isolation and development. Annu. Rev. Plant Biol. 23, 29-50.

Cohen, S., & Nitzany, F.E., (1966). Transmission and host range of the tomato yellow leaf curl virus. *Phytopath. 56,* 1127-1131.

Consortium, T.G. (2016) 1,135 Genomes Reveal the Global Pattern of Polymorphism in Arabidopsis thaliana. *Cell, 166,* 481-491.

Costa, A.S., (1976). Whitefly transmitted plant diseases. *Annu. Rev. Phytopath. 14,* 429-449.

Covey, S.N., Al-Kaff, N.S., Langara, A., & Turner, D.S., (1997). Plants combat infection by gene silencing. *Nature, 385,* 781-782.

Crespi, S., Noris, E., Vaira, A.M., & Accotto, G. P., (1995). Molecular characterization of cloned DNA from *tomato yellow leaf curl virus* isolate from Sicily. *Phytopathol. Mediterr. 34,* 93-99.

Cui, X., Li, G., Wang, D., Hu, D., & Zhou, X., (2005). A begomovirus DNA-encoded protein binds DNA, functions as a suppressor of RNA silencing, and targets the cell nucleus. *J. Virol. 79,* 10764-10775.

Cui, X., Tao, X., Xie, Y., Fauquet, C.M., & Zhou, X., (2004). A DNAβ associated with Tomato yellow leaf curl China virus is required for symptom induction. *Journal of Virology, 78,* 13966-13974.

Culver, J.N. & Padmanabhan, M.S., (2007). Virus-induced disease: altering host physiology one interaction at a time. *Annu. Rev. Phytopathol., 45*, 221-243.

Culver, J.N., Lindbeck, A.G., Desjardins, P.R. & Dawson, W.O., (1991). *Analysis of tobacco mosaic virus-host interactions by directed genome modification*. In Plant Molecular Biology 2 (pp. 23-33). Boston, MA: Springer.

Damsteegt, V., (1999). New and Emerging Plant Viruses. APSnet Features. Online. doi: 10.1094/APSnetFeature-1999-0999.

Davey, M.R., Anthony, P., Power, J.B., & Lowe, K.C., (2005). Plant protoplasts: status and biotechnological perspectives. *Biotech. Advances, 23*, 131-171.

Dawson, W.O., & Hilf, M.E., (1992). Host-range determinants of plant viruses. Annu. Rev. Plant Physiol. *Plant Mol. Biol. 43*, 527-55.

Diaz-Pendon, J.A., Canizares, M.C., Moriones, E., Bejarano, E.R., Czosnek, H. & Navas-Castillo, J., (2010). Tomato yellow leaf curl viruses: menage a trois between the virus complex, the plant and the whitefly vector. Mol. *Plant Pathol. 11*, 441-450.

Ding, S.W., (2010). RNA-based antiviral immunity. Nat. Rev. *Immunol. 10*, 632.

Dmitry, L., Van den Ackerveken. G., (2013). Susceptibility to plant disease: more than a failure of host immunity. *Trends plant sc. 18*, 546-554.

Doyle, J.J. & J.L. Doyle. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull. 19*, 11-15.

Doyle, J.J., Doyle, J.L., & Hortoriun, L.B., (1990). Isolation oe plant dna from fresh tissue.

Dry, I.B., Krake, L.R., Rigden, J.E., & Rezaian, M.A., (1997). A novel subviral agent associated with a geminivirus: the first report of a DNA satellite. Proceedings of the National Academy of Sciences 94, 7088-7093.

Eagle, P.A., Orozco, B.M., & Hanley-Bowdoin, L., (1994). A DNA sequence required for geminivirus replication also mediates transcriptional regulation. *Plant Cell 6*, 1157-1170.

Egelkrout, E.M., Mariconti, L., Settlage, S.B., Cella, R., Robertson, D., & Hanley-Bowdoin, L., (2002). Two E2F elements regulate the proliferating cell nuclear antigen promoter differently during leaf development. *Plant Cell 14*, 3225-3236.

Egelkrout, E.M., Robertson, D., & Hanley-Bowdoin, L., (2001). Proliferating cell nuclear antigen transcription is repressed through an E2F consensus element and activated by geminivirus infection in mature leaves. *Plant Cell 13*, 1437-1452.

Elmer, J.S., Brand, L., Sunter, G., Gardiner, W.E., Bisaro, D.M., & Rogers, S.G., (1988). Genetic analysis of the *tomato golden mosaic virus*. II. The product of the AL1 coding sequence is required for replication. *Nucleic Acids Res. 16*, 7043-7060.

Emmanuel, E., & Levy, A.A., (2002). Tomato mutants as tools for functional genomics. Curr. *Biol. 5*, 112–117.

Esau, K., (1977). Virus-like particles in nuclei of phloem cells in spinach leaves infected with the curly top virus. *J. Ultrastruct. Res. 61*, 78-88.

Etessami, P., Saunders, K., Watts, J., & Stanley, J., (1991). Mutational analysis of complementary-sense genes of *African cassava mosaic virus* DNA A. *J. Gen. Virol. 72,* 1005-1012.

Fajardo, J., Lutaladio, N., Larinde, M., Rosell, C., Roca, W., & Chujoy, E., (2010). *Quality declared planting material: protocols and standards for vegetatively propagated crops,* Rome: FAO.

FAOSTAT, (2016). *Food and Agriculture Organization of the United Nations,* Rome: FAO.

Fargette, D., Fauquet, C., & Thouvenel, J.C., (1988). Yield losses induced by *African cassava mosaic virus* in relation to the mode and the date of infection. *Trop. Pest Manage. 34*, 89-91.

Feenstra, B., Skovgaard, I.M. & Broman, K.W. (2006). Mapping quantitative trait loci by an extension of the Haley-Knott regression method using estimating equations. *Genetics, 173*, 2269-2282.

Fiallo-Olivé, E., Martínez-Zubiaur, Y., Moriones, E. & Navas-Castillo, J., (2012). A novel class of DNA satellites associated with New World begomoviruses. *Virol. 426*, 1-6.

Flor, H.H., (1971). Current status of the gene-for-gene concept. *Annual rev. phytopath. 9,* 275-296.

Flores, M.A., Reyes, M.I., Robertson, D.N. & Kjemtrup, S., (2015). Persistent virus-induced gene silencing in asymptomatic accessions of Arabidopsis. *Methods Mol. Bio. 1284,* 305-322.

Flores, R., Hernandez, C., Martinez de Alba, E., Daros, J.A., & Di Serio, F., (2005). Viroids and viroid host interactions. *Annu. Rev. Phytopathol. 43*, 117-39.

Fondong, V.N., Pita, J.S., Rey, M.E.C., De Kochko, A., Beachy, R.N., & Fauquet, C.M., (2000). Evidence of synergism between *African cassava mosaic virus* and a new double-recombinant geminivirus infecting cassava in Cameroon. *J. Gen. Virol. 81,* 287-297.

Fontes, E. P. B., Luckow, V. A., & Hanley-Bowdoin, L., (1992). A geminivirus replication protein is a sequence-specific DNA binding protein. *Plant Cell 4*, 597–608.

Fontes, E.P., Eagle, P.A., Sipe, P.S., Luckow, V.A., & Hanley-Bowdoin, L., (1994). Interaction between a geminivirus replication protein and origin DNA is essential for viral replication. *J. Biol. Chem. 269*, 8459-8465.

Frischmuth, T., Engel, M., Lauster, S., & Jeske, H., (1997). Nucleotide sequence evidence for the occurrence of three distinct whitefly-transmitted, Sida-infecting bipartite geminiviruses in Central America. *J. Gen. Virol. 78*, 2675-2682.

Gergerich, R.C., & Dolja, V.V., (2006). *Introduction to plant viruses, the invisible foe*. Plant Health Instructor. DOI: 10.1094/PHI-I-2006-0414-01.

Ghanim, M. & Czosnek, H., (2016). *Interactions between the whitefly Bemisia tabaci and begomoviruses: biological and genomic perspectives*. In Management of Insect Pests to Agriculture (pp. 181-200). Springer, Cham.

Gibson, R., (1996). The report of a survey monitoring the spread of the epidemic of *African cassava mosaic virus* from Uganda into western Kenya. Internal report, Natural Resources Institute (NRI), Chatham, GB.

Glick, E., Zrachya, A., Levy, Y., Mett, A., Gidoni, D., Belausov, E., Citovsky, V., & Gafni, Y., (2008). Interaction with host SGS3 is required for suppression of RNA silencing by *tomato yellow leaf curl virus* V2 protein. Proc. Natl. *Acad. Sci. U S A, 105*, 157-161.

Goodin, M.M., Zaitlin, D., Naidu, R.A., & Lommel, S.A., (2008). Nicotiana benthamiana: Its history and future as a model for plant-pathogen interactions. Mol. *Plant-Microbe Interact. 21*, 1015-1026.

Gozzo, F., & Faoro, F., (2013). Systemic acquired resistance (50 years after discovery): moving from the lab to the field. *J. Agric. Food Chem. 61*, 12473-12491.

Green, J.C., & Hu, J.S., (2017). Editing plants for virus resistance using CRISPR-Cas. *Acta Virologica, 61,* 138-142.

Grewal, S.I., & Elgin, S.C., (2007). Transcription and RNA interference in the formation of heterochromatin. *Nature, 447*, 399.

Gronwald, J.W., & Leonard, R.T., (1982). Isolation and transport properties of protoplasts from cortical cells of corn roots. *Plant Physiol. 70*, 1391-1395.

Grosset, J., Marty, I., Chartier, Y., & Meyer, Y., (1990). mRNAs newly synthesized by tobacco mesophyll protoplasts are wound-inducible. *Plant Mol. Bio. 15*, 485-496.

Gutierrez, C., (1999). Geminivirus DNA replication. Cell. *Mol. Life Sci. 56*, 313–329.

Haber, S., Ikegami, M., Bajet, N., & Goodman, R.M., (1981). Evidence for a divided genome in bean golden mosaic virus, a geminivirus. *Nature 289*, 324.

Hanley-Bowdoin, L., Bejarano, E.R., Robertson, D., & Mansoor, S., (2013). Geminiviruses: masters at redirecting and reprogramming plant processes. *Nat. Rev. Microbiol. 11,* 777-788.

Hanley-Bowdoin, L., Settlage, S.B., Orozco, B.M., Nagar, S. & Robertson, D., (1999). Geminiviruses: models for plant DNA replication, transcription, and cell cycle regulation. *Critical Reviews in Plant Sciences, 18*(1), 71-106.

Hanley-Bowdoin, L., Settlage, S.B., Orozco, B.M., Nagar, S., Robertson, D., 2000. Geminiviruses: Models for plant DNA replication, transcription, and cell cycle regulation. *Crit. Rev. Biochem. Mol. Biol. 35*, 105-140.

Hanley-Bowdoin, L., Settlage, S.B., & Robertson, D., (2004). Reprogramming plant gene expression: a prerequisite to geminivirus DNA replication. *Mol. Plant Pathol. 5*, 149-156.

Hanssen, I.M., Lapidot, M., & Thomma, B.P.H.J.,(2010). Emerging viral diseases of tomato crops. Mol. *Plant-Microbe Interact. 23*, 539-548.

Harimalala, M., De Bruyn, A., Hoareau, M., Andrianjaka, A., Ranomenjanahary, S., Reynaud, B., Lefeuvre, P., & Lett, J.M., (2013). Molecular characterization of a new alphasatellite associated with a cassava mosaic geminivirus in Madagascar. *Arch Virol, 158*, 1829-1832.

Harries, P., & Ding, B., (2011). Cellular factors in plant virus movement: at the leading edge of macromolecular trafficking in plants. *Virol. 411*, 237-243.

Hedges, L.J., & Lister, C.E., (2005). Nutritional attributes of salad vegetables. *Crop and Food Research Confidential Report,* (1473).

Hehnle, S., Wege, C. & Jeske, H., (2004). Interaction of DNA with the movement proteins of geminiviruses revisited. *J. virol. 78*, 7698-7706.

Heslop-Harrison, J.S., (2000). Comparative genome organization in plants: from sequence and markers to chromatin and chromosomes. *Plant Cell 12*, 617-635.

Heydarnejad, J., Abhari, E.H., Yazdi, H.R.B., & Massumi, H., (2007). Curly top of cultivated plants and weeds and report of a unique curtovirus from Iran. *J. Phytopathol. 155*, 321-325.

Heyraud-Nitschke, F., Schumacher, S., Laufs, J., Schaefer, S., Schell, J., Gronenborn, B., (1995). Determination of the origin cleavage and joining domain of geminivirus Rep proteins. *Nucleic acids res. 23*, 910-916.

Hillocks, R.J., & Jennings, D.K., (2003). Cassava brown streak disease: a review of present knowledge and research needs. *Int. J. Pest Manage. 49*, 225-234.

Holeva, R., Phillips, M.S., Neilson, R., Brown, D.J., Young, V., Boutsika, K. & Blok, V.C., (2006). Real-time PCR detection and quantification of vector trichodorid nematodes and Tobacco rattle virus. *Mol. Cell. Probes 20*, 203-211.

Hong, Y., Stanley, J., & van Wezel, R., (2003). Novel system for the simultaneous analysis of geminivirus DNA replication and plant interactions in Nicotiana benthamiana. *J. virol. 77*, 13315-13322.

Horns, T., & Jeske, H., (1991). Localization of abutilon mosaic virus (AbMV) DNA within leaf tissue by *in situ* hybridization. *Virology, 181*, 580-588.

Hull, R., (1989). The movement of viruses in plants. *Ann. Rev. Phytopathol. 27*, 213-240.

Inoue-Nagata, A.K., Lima, M.F., & Gilbertson, R.L., (2016). A review of geminivirus diseases in vegetables and other crops in Brazil: current status and approaches for management. *Hort. bras. 34*, 8-18.

Inoue-Nagata, A.K., Martin, D.P., Boiteux, L.S., Giordano, L.D., Bezerra, I.C., de Avila, A.C., (2006). New species emergence via recombination among isolates of the Brazilian tomato-infecting begomovirus complex. *Braz. J. Agric. Res. 41*, 1329-1332.

Inoue-Nagata, A.K., Nagata, T., de Ávila, A.C., & Giordano, L.D.B., (2007). A reliable begomovirus inoculation method for screening Lycopersicon esculentum lines. *Horticultura Brasileira 25*, 447-450.

Jennings, D.L., (1976). Cassava, *Manihot esculenta* (Euphorbiaceae). In:Simmonds,N. (ed.) Evolution of Crop Plants, London; Longman.

Jeske, H., (2009). Geminiviruses. *Curr. Top Microbiol. Immunol. 331*, 185-226.

Jeske, H., Lu¨tgemeier, M., & Preiss, W., (2001). Distinct DNA forms indicate rolling circle and recombination-dependent replication of Abutilon mosaic geminivirus. *EMBO J. 20*, 6158–6167.

Ji, Y., Schuster, D.J. & Scott, J.W.,(2007). Ty-3, a begomovirus resistance locus near the *Tomato yellow leaf curl virus* resistance locus Ty-1 on chromosome 6 of tomato. Mol. *Breeding, 20*, 271-284.

Jordan, C. V., Shen, W., Hanley-Bowdoin, L.K., & Robertson, D.N., (2007). Geminivirus-induced gene silencing of the tobacco retinoblastoma-related

gene results in cell death and altered development. *Plant Mol. Biol. 65,* 163–175.

Jovel, J., Reski, G., Rothenstein, D., Ringel, M., Frischmuth, T., & Jeske, H., (2004). Sida micrantha mosaic is associated with a complex infection of begomoviruses different from Abutilon mosaic virus. *Archives of Virology, 149*, 829-841.

Kammann, M., Schalk, H.J., Matzeit, V., Schaefer, S., Schell, J., & Gronenborn, B., (1991). DNA replication of wheat dwarf virus, a geminivirus, requires two cis-acting signals. *Virol. 184*, 786-790.

Kang, B.C., Yeam, I., Frantz, J.D., Murphy, J.F. & Jahn, M.M., (2005). The pvr1 locus in Capsicum encodes a translation initiation factor eIF4E that interacts with Tobacco etch virus VPg. *Plant J. 42*, 392-405.

Khandekar, S., Rajakaruna, P. & Leisner, S. (2007). Identification and host relations of Turnip ringspot virus, a novel comovirus discovered in Ohio. *Phytopathology 97*, S57.

Kil, E., Kim, S., Lee, Y., Byun, H., Park, J., Seo, H., … & Lee, S., (2016). *Tomato yellow leaf curl virus* (TYLCV-IL): a seed-transmissible geminivirus in tomatoes. *Sci. Rep. 6*, 19013.

Kim, K.S., Shock, T.L., & Goodman, R.M., (1978). Infection of Phaseolus vulgaris by bean golden mosaic virus: Ultrastructural aspects. *Virol. 89*, 22-33.

Kogoma, T., (1997). Stable DNA replication: interplay between DNA replication, homologous recombination, and transcription. *Microbiol. Mol. Biol. R. 61*, 212-238.

Kon, T., Kuwabara, K., Hidayat, S.H., & Ikegami, M., (2007). A begomovirus associated with Ageratum yellow vein disease in Indonesia: evidence for natural recombination between tomato leaf curl Java virus and Ageratum yellow vein virus-[Java]. *Arch Virol 152*, 1147-1157.

Krenz, B., Thompson, J.R., Fuchs, M., & Perry, K.L., (2012). Complete genome sequence of a new circular DNA virus from grapevine. *J. Virol. 86*, 7715.

Kreuzer, K.N., (2000). Recombination-dependent DNA replication in phage T4. *Trends biochem. sci., 25*, 165-173.

Lander, E.S. & Botstein, D., (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics, 121*, 185-199.

Langmead, B., Trapnell, C., Pop, M. & Salzberg, S.L., (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol. 10*, R25.

Lapidot, M., Karniel, U., Gelbart, D., Fogel, D., Evenor, D., Kutsher, Y., … & M., Levin, I., (2015). A Novel Route Controlling Begomovirus Resistance by the Messenger RNA Surveillance Factor Pelota. *PLoS Genet. 11*, e1005538.

Law, J.A., & Jacobsen, S.E., (2010). Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nature Rev. *Genetics 11*, 204.

Lazarowitz, S.G. (1992). Geminiviruses: genome structure and gene function. CRC Crit. Rev. *Plant Sci. 11*, 327-349.

Lazarowitz, S.G., & Beachy, R.N., (1999). Viral movement proteins as probes for intracellular and intercellular trafficking in plants. *Plant Cell 11*, 535-548.

Lee, S., Stenger, D.C., Bisaro, D.M.,& Davies, K.R., (1994). Identification of loci in Arabidopsis that confer resistance to geminivirus infection. *Plant J. 6*, 525-535.

Lefeuvre, P., Martin, D.P., Harkins, G., Lemey, P., Gray, A.J., Meredith, S., … & Heydarnejad., J., (2010). The spread of *tomato yellow leaf curl virus* from the Middle East to the world. *PLoS Pathog. 6*, e1001164.

Legg, J.P., French, R., Rogan, D., Okao-Okuja, G., & Brown, J.K., (2002). A distinct Bemisia tabaci (Gennadius) (Hemíptera: Sternorrhyncha: Aleyrodidae) genotype 25 cluster is associated with the epidemic of severe cassava mosaic virus disease in 26 Uganda. *Mol. Ecol. 11*, 1219-1229.

Legg, J., & Fauquet, C.M., (2004). Cassava Mosaic Geminiviruses in Africa. *Plant Molecular Bio.56*, 585-599.

Legg, J.P., (1999). Emergence spread and strategies for controlling the pandemic of cassava mosaic virus disease in east and central Africa. *Crop Protection, 18*, 627-637.

Legg, J.P., Jeremiah, S.C., Obiero, H.M., Maruthi, M.N., Ndyetabula, I., Okao-Okuja, G., … & Lava Kumar, P., (2011). Comparing the regional epidemiology of the cassava mosaic and cassava brown streak pandemics in Africa. *Virus Res. 159*, 161-170.

Leisner, S.M. & Howell, S.H. (1992). Symptom Variation in Different Arabidopsis thaliana Ecotypes Produced by Cauliflower Mosaic Virus. *Phytopathology, 82*, 1042-1046.

Lellis, A.D., Kasschau, K.D., Whitham, S.A. & Carrington, J.C. (2002). Loss-of-susceptibility mutants of Arabidopsis thaliana reveal an essential role for eIF (iso)4E during potyvirus infection. *Curr Biol, 12*, 1046-1051.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., & Genome Project Data Processing, S. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics, 25*, 2078-2079.

Lima, J.E., Carvalho, R.F., Neto, A.T., Figueira, A., & Peres, L.E.P., (2004). Micro-MsK: a tomato genotype with miniature size, short life cycle, and improved in vitro shoot regeneration *Plant Sci. 167*, 753–757.

Liu, L., Saunders, K., Thomas, C.L., Davies, J.W., & Stanley, J., (1999). Bean yellow dwarf virus RepA, but not Rep, binds to maize retinoblastoma protein, and the virus tolerates mutations in the consensus binding motif. *Virol. 256*, 270-279.

Lokko, Y., Danquah, E.Y., Offei, S.K., Dixon, A.G.O., &Gedil, M.A., (2005). Molecular markers associated with a new source of resistance to the cassava mosaic disease. *Afr. J. Biotechnol. 4*, 873-881.

Lopez-Ochoa, L., Ramirez-Prado, J. & Hanley-Bowdoin, L., (2006). Peptide aptamers that bind to a geminivirus replication protein interfere with viral replication in plant cells. *J. Virol. 80*, 5841-5853.

Lucas, W.J., (2006). Plant viral movement proteins: agents for cell-to-cell trafficking of viral genomes. *Virol. 344*, 169-84.

Lucas, W.J.,& Wolf, S., (1999). Connections between virus movement, macromolecular signaling and assimilate allocation. *Curr. Opin. Cell Biol. 2*, 192-197.

Ma X., Nicole, M.C., Meteignier, L.V., Hong, N., Wang, G., & Moffett, P., (2015). Different roles for RNA silencing and RNA processing components in virus recovery and virus-induced gene silencing in plants. *J. Exp. Bot. 66*, 919-932.

MacLean, A.M., Sugio,A., Kingdom H.N., Grieve, V.M., & Hogenhout, S.A., (2011). Arabidopsis thaliana as a model plant for understanding phytoplasmas interactions with plant and insect hosts. *Annual Rev. Phytopathol. 49*, 175-195.

Mandadi, K.K. & Scholthof, K.B.G., (2013). Plant immune responses against viruses: how does a virus cause disease?. *Plant cell, 25*, 1489-1505.

Mansoor, S., Briddon, R.W., Bull, S.E., Bedford, I.D., Bashir, A., Hussain, M… & Markham, P.G., (2003). Cotton leaf curl mutational analysis of effectors encoded by monopartite begomoviruses and their satellites 237 disease is associated with multiple monopartite begomoviruses supported by single DNA β. *Arch. Virol. 148*, 1969-1986.

Mansoor, S., Briddon, R.W., Zafar, Y., & Stanley, J., (2003). Geminivirus disease complexes: An emerging threat. *Trends Plant Sci. 8*, 128–134.

Mansoor, S., Khan, S.H., Bashir, A., Saeed, M., Zafar, Y., Malik, K.A., … & Markham, P.G., (1999). Identification of a novel circular single-stranded DNA associated with cotton leaf curl disease in Pakistan. *Virol. 259*, 190-199.

Mansoor, S., Zafar, Y. & Briddon, R.W. (2006) Geminivirus disease complexes: the threat is spreading. *Trends Plant Sci. 11*, 209-212.

Mara, C., Grigorova, B., & Liu, Z., (2010). Floral-dip transformation of Arabidopsis thaliana to examine pTSO2:: β-glucuronidase reporter gene expression. *Journal of visualized experiments: JoVE.*

Martí, E., Gisbert, C., Bishop, G.J., Dixon, M.S., & Garcia-Martinez, J.L., (2006). Genetic and physiological characterization of tomato cv. Micro-Tom. *J. Exper. Botany 57,* 2037-2047.

Martin M. A., Poch, H.L.C., Martinez Herrera, D., & Ponz, F., (1999). Resistances to turnip mosaic potyvirus in Arabidopsis thaliana. Mol. *Plant Microbe Interact. 12,* 1016-1021.

Martín Martín, A., Martínez-Herrera, D., Cabrera y Poch, H.L., & Ponz, F., (1997). Variability in the Interactions between Arabidopsis thaliana Ecotypes and Oilseed Rape Mosaic Tobamovirus. *Australian J. Plant Physio., 24,* 275–281.

Mathur, J., & Koncz, C., (1998). PEG-mediated protoplast transformation with naked DNA. *In Arabidopsis Protocols* 267-276).

Matsukura, C., Aoki, K., Fukuda, N., Mizoguchi, T., Asamizu, E., Saito, T., Shibata, D., & Ezura, H., (2008). Comprehensive Resources for Tomato Functional Genomics Based on the Miniature Model Tomato Micro-Tom. Curr. *Genomics 9,* 436-443.

Matyis, J.C., Silva, D.M., Oliveira, A.R.,& Costa, A.S., (1975). Purification and morphology of tomato golden mosaic virus. *Summa Phytopathol. 1,* 267-75.

Matzke, M., Kanno, T., Daxinger, L., Huettel, B., & Matzke, A.J., (2009). RNA-mediated chromatin-based silencing in plants. *Curr. Opin. Cell Biol. 21,* 367-376.

Mayo, M.A., Leibowitz, M.J., Palukaitis, P., Scholthof, K.B.G., Simon, A.E., Stanley, J., &Taliansky, M., (2005). Satellites In: Fauquet, C.M., Mayo, M.A., Maniloff, J., Desselberger, U., Ball, L.A. (Eds.), *VIIIth Report of the International Committee on Taxonomy of Viruses.,* London: Elsevier/Academic Press.

Mayo, M.A., Martelli, G.P., 1993. New families and genera of plant-viruses. Pp. 496-498.

Mbanzibwa, D.R., Tian, Y., Mukasa, S.B., &Valkonen, J.P., (2009). Cassava brown streak virus (Potyviridae) encodes a putative Maf/HAM1 pyrophosphatase implicated in reduction of mutations and a P1 proteinase that suppresses RNA silencing but contains no HC-Pro. *J. Virol. 83,* 6934-6940.

McKenzie, C.L., (2002). Effect of *Tomato Mottle Virus* (ToMoV) on Bemisia tabaci biotype B (Homoptera: Aleyrodidae) oviposition and adult survivorship on healthy tomato. *Fla Entomol. 85,* 367-368.

Meinke, D.W., Cherry, J.M., Dean, C., Rounsley, S.D., & Koornneef, M., (1998). Arabidopsis thaliana: A model plant for genome analysis. *Sci. 282,* 662-682.

Meissner, R., Jacobson, Y., Melamed, S., Levyatuv, S., Shalev, G., Ashri, A., Elkind, Y., & Levy, A., (1997). A new model system for tomato genetics. *Plant J. 12,* 1465–1472.

Miozzi, L., Napoli, C., Sardo, L., & Accotto, G.P., (2014). Transcriptomics of the interaction between the monopartite phloem-limited Geminivirus Tomato yellow leaf curl Sardinia virus and Solanum lycopersicum highlights a role for plant hormones, autophagy and plant immune system fine tuning during infection. *PLoS One 9,* e89951.

Moffat, A., (1999). Geminiviruses emerge as serious crop threat. *Sci. 286,* 1835.

Moffett, P., (2009). Mechanisms of recognition in dominant R gene mediated resistance. *Adv. Virus Res. 75*, 1-33.

Monci, F., Sanchez-Campos, S., Navas-Castillo, J., & Moriones, E., (2002). A natural recombinant between the geminiviruses Tomato yellow leaf curl Sardinia virus and *Tomato yellow leaf curl virus* exhibits a novel pathogenic phenotype and is becoming prevalent in Spanish populations. *Virol. 303*, 317–326.

Monger, W.A., Seal, S., Cotton, S., & Foster, G.D., (2001). Identification of different isolates of cassava brown streak virus and development of diagnostic test. *Plant Pathol. 50*, 768–775.

Morales, F.J., & Anderson, P.K., (2001). The emergence and dissemination of whitefly-transmitted geminiviruses in Latin America. *Arch. Virol. 146*, 415-441.

Morin, S., Ghanim, M., Zeidan, M., Czosnek, H., Verbeek, M., & van den Heuvel, F.J.M., (1999). A GroEL homologue from endosymbiotic bacteria of the whitefly Bemisia tabaci is implicated in the circulative transmission of Tomato yellow leaf curl virus. *Virol. 256*, 75-84.

Moriones, E., & Navas-Castillo, J., (2000). *Tomato yellow leaf curl virus*, an emerging virus complex causing epidemics worldwide. *Virus res. 71*, 123-134.

Morra, M.R., & Petty, I.T., (2000). Tissue specificity of geminivirus infection is genetically determined. *Plant Cell, 12*, 2259-2270.

Morris, B., Coates, L., Lowe, S., Richardson, K., & Eddy, P., (1990). Nucleotide sequence of the infectious cloned DNA components of *African cassava mosaic virus* (Nigerian strain). Nucl. *Acids Res. 18*, 197-198.

Mosig, G., Gewin, J., Luder, A., Colowick, N. & Vo, D., (2001). Two recombination-dependent DNA replication pathways of bacteriophage T4, and their roles in mutagenesis and horizontal gene transfer. Proceedings of the National Academy oSci. 98, 8306-8311.Motoyoshi, F., 2018. Protoplasts in virology, Plant Protoplasts. CRC Press, pp. 119-137.

Motoyoshi, F., Bancroft, J.B., Watts, J.W., & Burgess, J., (1973). The infection of tobacco protoplasts with cowpea chlorotic mottle virus and its RNA. *J. Gen. Virol. 20*, 177-193.

Muangsan, N., Beclin, C., Vaucheret, H., & Robertson, D., (2004). Geminivirus VIGS of endogenous genes requires SGS2/SDE1 and SGS3 and defines a new branch in the genetic pathway for silencing in plants. *Plant J. 38*, 1004-1014.

Muhammad, S.S., Sharma, P., & Ikegami, M., (2012). *Mutational Analysis of Effectors Encoded by Monopartite Begomoviruses and Their Satellites*, New York: Plant Science, DOI: 10.5772/54518.

Muhire, B., Martin, D.P., Brown, J.K., Navas-Castillo, J., Moriones, E., Zerbini, F.M., … & Varsani, A., (2013). A genome-wide pairwise-identity-based proposal for the classification of viruses in the genus Mastrevirus (family Geminiviridae). *Arch. virol. 158*, 1411-1424.

Nagar, S., Pedersen, T.J., Carrick, K.M., Hanley-Bowdoin, L., & Robertson, D.A., (1995). Geminivirus induces expression of a host DNA synthesis protein in terminally differentiated plant cells. *Plant Cell 7*, 705-719.

Nash, T.E., Dallas, M.B., Reyes, M.I., Buhrman, G.K., Ascencio-Ibanez, J.T., & Hanley- Bowdoin, L., (2011). Functional analysis of a novel motif conserved across geminivirus Rep proteins. *J. Virol.85*, 1182-1192.

Nasser, N., & Ortiz, R., (2010). Breeding cassava to feed the poor. *Scientific American, 302*, 78-82.

Nava, A., Londono, A., & Polston, J.E., (2013). Characterization and distribution of tomato yellow margin leaf curl virus, a begomovirus from Venezuela. *Arch. Virol. 158*, 399-406.

Navas-Castillo, J., Fiallo-Olive, E., & Sanchez-Campos, S., (2011). Emerging virus diseases transmitted by whiteflies. *Annu. Rev. Phytopathol. 49*, 219-248.

Nawaz-ul-Rehman, M.S., & Fauquet, C.M., (2009). Evolution of geminiviruses and their satellites. *FEBS Lett, 583,* 1825-1832.

Nawaz-ul-Rehman, M.S., Mansoor, S., Briddon, R.W., & Fauquet, C.M., (2009). Maintenance of an Old World betasatellite by a New World helper begomovirus and possible rapid adaptation of the betasatellite. *J. virol. 83*, 9347-9355.

Nawaz-ul-Rehman, M.S., Nahid, N., Mansoor, S., Briddon, R.W., & Fauquet, C.M., (2010). Post-transcriptional gene silencing suppressor activity of two non-pathogenic alphasatellites associated with a begomovirus. *Virol. 405*, 300–308.

Ndunguru, J., (2004). *Molecular characterization of Cassava mosaic geminiviruses in Tanzania.* Unpublished PhD Thesis, South Africa: University of Pretoria.

Ndunguru, J., De Leon, L., Doyle, C.D., Sseruwagi, P., Plata, G., Legg, J.P., Thompson, G., … & Hanley-Bowdoin, L., (2016). Two Novel DNAs That Enhance Symptoms and Overcome CMD2 Resistance to Cassava Mosaic Disease. *J. Virol. 90*, 4160-4173.

Ndunguru, J., Fofana, B., Legg, J., Challepan, P., Taylor, N., Aveling, T., Thompson, G., C.M., & Fauquet., (2008). Two novel satellite DNAs associated with bipartite cassava mosaic begomoviruses enhancing symptoms and capable of breaking high virus resistance in a cassava landraces. *Ghent University, Ghent*, 141.

Nie, X., & Molen, T.A., (2015). Host recovery and reduced virus level in the upper leaves after Potato virus Y infection occur in tobacco and tomato but not in potato plants. *Viruses, 7*, 680-698.

Nordborg, M., Hu, T.T., Ishino, Y., Jhaveri, J., Toomajian, C., Zheng, H., … & Bergelson, J. (2005). The pattern of polymorphism in Arabidopsis thaliana. *PLoS Biol, 3*, e196.

Okabe Y., Asamizu E., Saito T., Matsukura C., Ariizumi T., Bres C., … & Ezura, H., (2011). Tomato TILLING technology: development of a reverse genetics tool for the efficient isolation of mutants from Micro-Tom mutant libraries. *Plant Cell Physiol. 52*, 1994-2005.

Okogbenin, E., Egesi, C.N., Olasanmi, B., Ogundapo, O., Kahya, S., Hurtado, P., … de Vicente, C., (2012). Molecular marker analysis and validation of resistance to cassava mosaic disease in elite cassava genotypes in Nigeria. *Crop Sci. 52*, 2576-2586.

Okogbenin, E., Porto, M.C.M., Egesi, C., Mba, C., Espinosa, E., Santos, L.G., … & Ekanayake, I., (2007). Marker-assisted introgression of resistance to cassava mosaic disease into Latin American germplasm for the genetic improvement of cassava in Africa. *Crop Sci. 47*, 1895-1904.

Okogbenin, E., Setter, T.L., Ferguson, M., Mutegi, R., Ceballos, H., Olasanmi, B., & Fregene, M., (2013). Phenotypic approaches to drought in cassava. *Front. Physiol. 4*, 93.

Olaniyi, J.O., Akanbi, W.B., Adejumo, T.A., & Akande, O.G., (2010). Growth, fruit yield and nutritional quality of tomato varieties. *African J. Food Sci. 4*, 398-402.

Ooi, K., Ohshita, S., Ishii, I., & Yahara, T., (1997). Molecular phylogeny of geminivirus infecting wild plants in Japan. *J. plant res. 11*, 247-257.

Orozco, B.M., & Hanley-Bowdoin, L., (1996). A DNA structure is required for geminivirus origin function. *J. Virol. 270*, 148-158.

Otim-Nape, G.W., Bua, A., Thresh, J., Baguma, Y., Ogwal, S., Semakula, G., … & Martin, A., (1997). *Cassava mosaic virus disease in Uganda: the current pandemic and approaches to control*. Cassava mosaic virus disease in Uganda: the current pandemic and approaches to control.

Ouibrahim, L., Mazier, M., Estevan, J., Pagny, G., Decroocq, V., Desbiez, C., … & Caranta, C., (2014). Cloning of the Arabidopsis rwm1 gene for resistance to Watermelon mosaic virus points to a new function for natural virus resistance genes. *Plant J*, *79*, 705-716.

Pagny, G., Paulstephenraj, P.S., Poque, S., Sicard, O., Cosson, P., Eyquard, J.P., … & Decroocq, V., 2012. Family-based linkage and association mapping reveals novel genes affecting Plum pox virus infection in Arabidopsis thaliana. *New Phytol. 196*, 873-886.

Pallas, V., & Garcı´a, J.A., (2011). How do plant viruses induce disease? Interactions and interference with host components. *J. Gen. Virol. 92,* 2691-2705.

Palukaitis, P., (2008). Satellite Nucleic Acids and Viruses. *Encyclopedia of Virology* 526-535.

Paprotka, T., Deuschle, K., Metzler, V., & Jeske, H., (2011). Conformation-selective methylation of geminivirus DNA. *J. Virol. 85*, 12001-12012.

Park, S.H., Hur, J., Park, J., Lee, S., Lee, T.K., Chang, M., Davi, K.R., Kim, J., & Lee, S., (2002). Identification of a tolerant locus on Arabidopsis thaliana to hypervirulent beet curly top virus CFH strain. *Mol. Cells, 13*, 252-258.

Parlevliet, J.E., (2002). Durability of resistance against fungal, bacterial and viral pathogens; present situation. *Euphytica 124*, 147-156.

Picó, B., Díez, M.J., & Nuez, F., (1996). Viral diseases causing the greatest economic losses to the tomato crop. II. The *tomato yellow leaf curl virus*—a review. *Scientia Horticulturae, 67,* 151-196.

Pierce, E.J., & Rey, M.E.C., (2013). Assessing global transcriptome changes in response to South *African Cassava mosaic virus* [ZA-99] infection in susceptible *Arabidopsis thaliana*. *PLoS ONE 8*, e67534.

Piron, F., Nicolaï, M., Minoïa, S., Piednoir, E., Moretti, A., Salgues, A., Zamir, D., Caranta, C. & Bendahmane, A., (2010). An induced mutation in tomato eIF4E leads to immunity to two potyviruses. *PloS one 5*, e11313.

Platt, A., Horton, M., Huang, Y.S., Li, Y., Anastasio, A.E., Mulyati, N.W., …& Borevitz, J.O., (2010). The scale of population structure in *Arabidopsis thaliana*. *PLoS Genet, 6*, e1000843.

Pnueli, L., Carmel-Goren, L., Hareven, D., Gutfinger, T., Alvarez, J., Ganal, M., Zamir, D., & Lifschitz, E., (1998). The self-pruning gene of tomato regulates

vegetative to reproductive switching of sympodial meristems and is the ortholog of CEN and TFL1. *Dev. 125*, 1979-1989.

Pogany, M., Koehl, J., Heiser, I., Elstner, E., & Barna, B., (2004). Juvenility of tobacco induced by cytokinin gene introduction decreases susceptibility to Tobacco necrosis virus and confers tolerance to oxidative stress. Physiol. Mol. *Plant Pathol. 65*, 39-47.

Ponz, F., & Bruening, G., (1986). Mechanism of resistance to plant viruses. Annu. Rev. *Phytopathol. 24*, 355-381.

Preiss, W., & Jeske, H., (2003). Multitasking in replication is common among geminiviruses. *J Virol 77*, 2972-2980.

Pringle, C.R., (1993). Virus taxonomy update. *Arch. Virol. 133*, 491-495.

Puchta, H. (2016). Applying CRISPR/Cas for genome engineering in plants: the best is yet to come. *Curr Opin Plant Biol, 36,* 1-8.

Pumplin, N., Voinnet, O., 2013. RNA silencing suppression by plant pathogens: defense, counter-defence and counter-counter-defence. Nature Rev. Microb. 11, 745-760.

Qin, Y., & Petty, I.T., (2001). Genetic analysis of bipartite geminivirus tissue tropism. *Virol. 291*, 311-323.

Qu, F., & Morris, J.T., (2005). Suppressors of RNA silencing encoded by plant viruses and their role in viral infections. *FEBS Lett., 579*, 5958-5964.

Rabbi, I.Y., Hamblin, M.T., Kumar, P.L., Gedil, M.A., Ikpan, A.S., Jannink, J.L. & Kulakow, P.A., 2014. High-resolution mapping of resistance to cassava

mosaic geminiviruses in cassava using genotyping-by-sequencing and its implications for breeding. *Virus research, 186*, 87-96.

Raja, P., Jackel, J.N., Li, S., Heard, I.M., & Bisaro, D.M., (2014). Arabidopsis double-stranded RNA binding protein DRB3 participates in methylation-mediated defense against geminiviruses. *J. Virol. 88*, 2611-2622.

Raja, P., Sanville, B.C., Buchmann, R.C., & Bisaro, D.M., (2008). Viral genome methylation as an epigenetic defense against geminiviruses. *J. Virol. 82*, 8997-9007.

Raja, P., Wolf, J.N., & Bisaro, D.M., (2010). RNA silencing directed against geminiviruses: post-transcriptional and epigenetic components. Biochim. Biophys. *Acta 1799*, 337-351.

Rajabu, C.A., Kennedy, G.G., Ndunguru, J., Ateka, E.M., Tairo, F., Hanley-Bowdoin, L., & Ascencio-Ibáñez, J.T., (2018). Lanai: A small, fast growing tomato variety is an excellent model system for studying geminiviruses. *J.Virol. Methods, 256*, 89-99.

Rajakaruna, P., & Khandekar, S., (2007). Identification and host relations of Turnip ringspot virus, a novel comovirus from Ohio. *Plant Dis, 91*, 1212–1220.

Ranjan, A., Ichihashi, Y., & Sinha, N.R., (2012). The tomato genome: implications for plant breeding, genomics and evolution. *Genome biol. 13*, 167.

Rao, K.S., & Prakash, A.H., (1995). A simple method for the isolation of plant protoplasts. *J. Biosci. 20*, 645-655.

Ratcliff, F. G., MacFarlane, S.A., & Baulcombe, D.C., (1999). Gene silencing without DNA:RNA-mediated cross-protection between viruses. *Plant Cell 11,* 1207-1216.

Reyes, M.I., Flores-Vergara, M.A., Guerra-Peraza, O., Rajabu, C., Desai, J., Hiromoto-Ruiz, Y.H., … & Ascencio-Ibáñez, J.T., (2017). A VIGS screen identifies immunity in the Arabidopsis Pla-1 accession to viruses in two different genera of the Geminiviridae. *Plant J. 92,* 796-807.

Reyes, M.I., Nash, T.E., Dallas, M.M Ascencio-Ibanez, J.T., & Hanley-Bowdoin, L.J., (2013). Peptide aptamers that bind to geminivirus replication proteins confer a resistance phenotype to *tomato yellow leaf curl virus* and *tomato mottle virus* infection in tomato. *J. Virol. 87,* 9691-9706.

Ribaut, J.M., De Vicente, M.C., & Delannay, X., (2010). Molecular breeding in developing countries: challenges and perspectives. Curr. Opin. *Plant Bio. 13,* 213-218.

Ribeiro, P.F., Akromah, R., & Manu-Aduening, J., (2012). Using marker assisted selection to hasten screening of cassava cultivars developed through introgression of cassava mosaic disease (CMD) resistance into cassava landraces in Ghana. *J. Agric. Sci. Techno. 2,* 74-80.

Richter, K.S., Gotz, M., Winter, S., & Jeske, H., (2016). The contribution of translation synthesis polymerases on geminiviral replication. *Virol. 488,* 137-148.

Robaglia, C., & Caranta, C., (2006). Translation initiation factors: a weak link in plant RNA virus infection. *Trends Plant Sci, 11,* 40-45.

Robinson, D.J.; Ryabov, E.V.; Raja, S.K.; Roberts, I.M., & Taliansky, M.E., (1999). Satellite RNA is essential for encapsidation of groundnut rosette umbravirus

RNA by groundnut rosette assistor luteovirus coat protein. Virol. *254*, 105-114.

Rocha, C.S., Castillo-Urquiza, G.P., Lima, A.T., Silva, F.N., Xavier, C.A., Hora-Júnior, B.T., … & Alfenas-Zerbini, P., (2013). Brazilian begomovirus populations are highly recombinant, rapidly evolving, and segregated based on geographical location. *J. Virol. 87*, 5784-5799.

Rodrigues, S.P., Lindsey, G.G., & Fernandes, P.M.B., (2009). Biotechnological approaches for plant viruses resistance: from general to the modern RNA silencing pathway. *Braz. arch. biol. technol. 52*, 795-808.

Rodriguez-Negrete E., Lozano-Duran R., Piedra-Aguilera A., Cruzado L., Bejarano E.R., & Castillo A.G., (2013). Geminivirus Rep protein interferes with the plant DNA methylation machinery and suppresses transcriptional gene silencing. *New Phytol. 199*, 464-475.

Rodriguez-Negrete, E.A., Carrillo-Tripp, J., & Rivera-Bustamante, R.F., (2009). RNA silencing against geminivirus: complementary action of posttranscriptional gene silencing and transcriptional gene silencing in host recovery. *J. Virol. 83*, 1332-1340.

Rojas, M.R., Hagen, C., Lucas, W. J., & Gilbertson, R.L., (2005). Exploiting chinks in the plant's armor: evolution and emergence of geminiviruses. *Annu. Rev. Phytopathol. 43*, 361-394.

Rojas, M.R., Jiang, H., Salati, R., Xoconostle-Cázares, B., Sudarshana, M.R., Lucas, W.J., & Gilbertson, R.L., (2001). Functional analysis of proteins involved in movement of the monopartite begomovirus, *Tomato yellow leaf curl virus*. Virol. *291*, 110-125.

Rom, M., Antignus, Y., Gidoni, D., Pilowsky, M., & Cohen, S., (1993). Accumulation of *tomato yellow leaf curl virus* DNA in tolerant and susceptible tomato lines. *Plant Dis. 77*, 253-257.

Romay, G., Chirinos, D., Geraud-Pouey, F., & Desbiez, C., (2010). Association of a typical alphasatellite with a bipartite New World begomovirus. *Arch. Virol. 155*, 1843–1847.

Rosario, K., Marr, C., Varsani, A., Kraberger, S., Stainton, D., Moriones, E., Polston, J.E., & Breitbart, M., (2016). Begomovirus-associated satellite DNA diversity captured through vector-enabled metagenomic (VEM) surveys using whiteflies (Aleyrodidae). *Viruses, 8*, 36.

Roumagnac, P., Granier, M., Bernardo, P., Deshoux, M., Ferdinand, R., Galzi, S., … & Mesléard, F., (2015). Alfalfa leaf curl virus: An aphid-transmitted geminivirus. *J. virol. 89*, 9683-9688.

Ruffel, S., Dussault, M.H., Palloix, A., Moury, B., Bendahmane, A., Robaglia, C., & Caranta, C., (2002). A natural recessive resistance gene against potato virus Y in pepper corresponds to the eukaryotic initiation factor 4E (eIF4E). *Plant J, 32*, 1067-1075.

Saeed, M., Zafar, Y., Randles, J.W., & Rezaian, M.A., (2007). A monopartite begomovirus-associated DNA-ß satellite substitutes for the DNA B of a bipartite begomovirus to permit systemic infection. *J. Gen. Virol. 88*, 2881-2889.

Santos, A.A., Florentino, L.H., Pires, A.B., & Fontes, E.P. (2008). Geminivirus: biolistic inoculation and molecular diagnosis. *Methods mol. Bio. 451*, 563-579.

Saraswathy, N., & Ramalingam, P., (2011). *Genomes of model organisms. Concepts and Techniques in Genomics and Proteomics*. Netherlands: Woodhead Publishing.

Sastry, K.S., & Zitter, T.A., (2014). Management of Virus and Viroid Diseases of Crops in the Tropics. In: Plant Virus and Viroid Diseases in the Tropics, (Volume 2 pp. 149-480). Epidemiology and Management; Sastry KS, Zitter TA (eds); Netherlands: Springer.

Saunders, K., Bedford, I.D., Briddon, R.W., Markham, P.G., Wong, S.M., & Stanley, J., (2000). A unique virus complex causes *Ageratum* yellow vein disease. Proc. Nat. *Aca. Sci. USA 97*, 6890-6895.

Saunders, K., Norman, A., Gucciardo, S., & Stanley, J., (2004). The DNA β satellite component associated with ageratum yellow vein disease encodes an essential pathogenicity protein (βC1). *Virol. 324*, 37-47.

Saunders, K., Salim, N., Mali, V.R., Malathi, V.G., Briddon, R., Markham, P.G., & Stanley, J., (2002). Characterisation of Sri Lankan cassava mosaic virus and Indian cassava mosaic virus: evidence for acquisition of a DNA B component by a monopartite begomovirus. *Virol. 293*, 63–74.

Saunders, K., & Stanley, J., (1999). A nanovirus-like DNA component associated with yellow vein disease of Ageratum conyzoides: evidence for interfamilial recombination between plant DNA viruses. *Virol. 264*, 142-152.

Schneider, H., (1973). Cytological and histological aberrations in woody plants following infection with viruses, mycoplasmas, rickettsias, and flagellates. Annu. Rev. *Phytopathol. 11*, 119-146.

Scholthof, H.B., Scholthof, K.B., & Jackson, A.O., (1996). Plant virus gene vectors for transient expression of foreign proteins in plants. *Annu. Rev. Phytopathol. 34*, 299-323.

Scott, J.W., & Harbaugh, B.K., (1989). *Micro-Tom: a miniature dwarf tomato*. Gainesville, Fla, Agricultural Experiment Station, Institute of Food and Agricultural Sciences, Florida: University of Florida.

Seal, S.E., van denBosch, F., & Jeger, M.J., (2006)). Factors influencing begomovirus evolution and their increasing global significance: implications for sustainable control. *Crit. Rev. Plant Sci. 25*, 23-46.

Settlage, S. B., See, R. G., & Hanley-Bowdoin, L., (2005). Geminivirus C3 protein: replication enhancement andprotein interactions. *J. Virol. 79*, 9885–9895.

Settlage, S.B., Miller, A.B., & Hanley-Bowdoin, L**.**, (1996). Interactions between geminivirus replication proteins. *J. Virol. 70***,** 6790–6795.

Sharma, P., & Ikegami, M., (2010). Tomato leaf curl Java virus V2 protein is a determinant of virulence, hypersensitive response and suppression of posttranscriptional gene silencing. *Virol. 396*, 85-93.

Shaw, C.H., 1995. *Introduction of cloning plasmids into Agrobacterium tumefaciens. In Plant Gene Transfer and Expression Protocols*. (pp. 33-37). Totowa, NJ: Springer.

Sheen, J., (2001). Signal transduction in maize and Arabidopsis mesophyll protoplasts. *Plant Physiol. 127*, 1466-1475.

Shen, W., Dallas, M.B., Goshe, M.B., & Hanley-Bowdoin, L., (2014). SnRK1 phosphorylation of AL2 delays Cabbage leaf curl virus infection in Arabidopsis. *J. Virol. 88,* 10598-10612.

Shen, W., Dallas, M.B., Goshe, M.B., & Hanley-Bowdoin, L., (2014). SnRK1 phosphorylation of AL2 delays cabbage leaf curl virus infection in Arabidopsis. *J. Virol 88,* 10598-10612.

Shen, W., &Bowdoin, L., (2006). Geminivirus infection up-regulates the expression of two Arabidopsis protein kinases related to yeast SNF1- and mammalian AMPK-activating kinases. *Plant Physiol. 142*, 1642-1655.

Shen, W., Reyes, M.I., & Hanley-Bowdoin, L., (2009). Arabidopsis protein kinases GRIK1 and GRIK2 specifically activate SnRK1 by phosphorylating its activation loop. *Plant Physiol. 150*, 996–1005.

Shepherd, D.N., Mangwende, T., Martin, D.P., Bezuidenhout, M., Kloppers, F.J., Carolissen, C.H., …. & Thomson, J.A., (2007). Maize streak virus-resistant transgenic maize: a first for Africa. *Plant Biotechnol.J.5*, 759-767.

Vanderschuren, H., Stupak, M., Fütterer, J., Gruissem, W. & Zhang, P., (2007). Engineering resistance to geminiviruses–review and perspectives. Plant Biotechnol. J. 5, 207-220.

Shepherd, D.N., Martin, D.P., Van der Walt, E., Dent, K., Varsani, A., &Rybicki, E.P., (2010). Maize streak virus: an old and complex 'emerging'pathogen. Mol. *Plant Pathol. 11,* 1-12.

Sijen, T., Fleenor, J., Simmer, F., Thijssen, K.L., Parrish, S., Timmons, L., Plasterk, R.H.A., Fire, A., (2001). On the role of RNA amplification in dsRNA-triggered gene silencing. *Cell 107*, 465–476.

Singh, D.K., Malik, P.S., Choudhury, N.R., & Mukherjee, S.K., (2008). MYMIV replication initiator protein (Rep): Roles at the initiation and elongation steps of MYMIV DNA replication. *Virol. 380,* 75-83.

Speulman, E., Bouchez, D., Holub, E.B., & Beynon, J.L., (1998). Disease resistance gene homologs correlate with disease resistance loci of Arabidopsis thaliana. *Plant J. 14*, 467-474.

Stanley, J., Boulton, M.I., & Davies, J.W., (2005). Geminiviridae. eLS.

Staskawicz, B.J., Ausubel, F.M., Baker, B.J., Ellis, J.G. & Jones, J.D., (1995). Molecular genetics of plant disease resistance. *Sci. 268*, 661-667.

Stenger, D.C., Carbonaro, D., & Duffus, J.E., (1990). Genomic characterization of phenotypic variants of beet curly top virus. *J. Gen. Virol. 71*, 2211-2215.

Stenger, D.C., Davis, K.R., & Bisaro, D.M., (1992). Limited replication of *Tomato golden mosaic virus* DNA in explants of nonhost species. *Mol. Plant-Microbe Interact. 5,* 525-527.

Stenger, D.C., Revington, G.N., Stevenson, M.C., & Bisaro, D.M., (1991). Replicational release of geminivirus genomes from tandemly repeated copies: evidence for rolling-circle replication of a plant viral DNA. Proc. *Natl. Acad. Sci. USA 88,* 8029-8033.

Stenger, D.C., Revington, G.N., Stevenson, M.C., & Bisaro, D.M., (1991). Replicational release of geminivirus genomes from tandemly repeated copies: evidence for rolling-circle replication of a plant viral DNA. Proc. *Natl. Acad. Sci. U S A, 88*, 8029-8033.

Stepanova, A.N., Yun, J., Robles, L.M., Novak, O., He, W., Guo, H., Ljung, K., & Alonso, J.M., (2011). The Arabidopsis YUCCA1 flavin monooxygenase functions in the indole-3-pyruvic acid branch of auxin biosynthesis. *Plant Cell 23*, 3961-3973.

Sun, H.J., Uchii, S., Watanabe, S.,& Ezura, H., (2006). A highly efficient transformation protocol for Micro-Tom, a model cultivar for tomato functional genomics. *Plant Cell Physiol. 47*, 426-431.

Sunitha, S., Shanmugapriya, G., Balamani, V., & Veluthambi, K., (2013). Mungbean yellow mosaic virus (MYMV) AC4 suppresses post-transcriptional gene silencing and an AC4 hairpin RNA gene reduces MYMV DNA accumulation in transgenic tobacco. *Virus Genes 46*, 496-504.

Sunter, G., & Bisaro, D.M., (1991). Transactivation in a geminivirus: AL2 gene product is needed for coat protein expression. *Virol. 180*, 416-419.

Sunter, G., & Bisaro, D.M., (2003). Identification of a minimal sequence required for activation of the *tomato golden mosaic virus* coat protein promoter in protoplasts. *Virol. 305*, 452-462.

Sunter, G., Hartitz, M.D., &Bisaro, D.M., 1993. *Tomato golden mosaic virus* leftward gene expression: autoregulation of geminivirus replication protein. Virol. 195, 275-280.

Sunter, G., Hartitz, M.D., Hormuzdi, S.G., Brough, C.L.. & Bisaro, D.M., (1990). Genetic analysis of *tomato golden mosaic virus*: ORF AL2 is required for coat protein accumulation while ORF AL3 is necessary for efficient DNA replication. *Virol. 179*, 69-77.

Teng, K., Chen, H., Lai, J., Zhang, Z., Fang, Y., Xia, R., Zhou, X., Guo, H., & Xie, Q., (2010). Involvement of C4 Protein of Beet Severe Curly Top Virus (Family *Geminiviridae*) in Virus Movement. *PLoS NE 5*(6), e11280. doi:10.1371/journal.pone.0011280.

Tewes, A., Glund, K., Walther, R., & Reinbothe, H., (1984). High yield isolation and rapid recovery of protoplasts from suspension cultures of tomato (Lycopersicon esculentum). *J. Plant Physiol. 113*, 141-150.

Thelander, M., Olsson, T., Ronne,H., (2004). Snf1-related protein kinase 1 is needed for growth in a normal day–night light cycle. *The EMBO J. 23*, 1900-1910.

Thottappilly, G., Bosque-P´erez, N.A., &Rossel, H.W., (1993). Viruses and virus diseases of maize in tropical Africa. *Plant Pathol. 42*, 494-509.

Tjallingii WF, Gabrys B (1999). *Anomalous stylet punctures of phloem sieve elements by aphids*. Entomol. Exp. appl. Dordrecht: Kluwer Academic Pub.

Thresh, J., Fargette, D., Otim-Nape, G.W., (1994). Effects of African cassava mosaic geminivirus on the yield of cassava. *Tropical Science-London 34*, 26-26.

Thresh, J.M., (1981). *Pests, pathogens, and vegetation: the role of weeds and wild plants in the ecology of crop pests and diseases: the outcome of a meeting arranged.* New York: University of York.

Thresh, J.M., (1988). Eradication as a virus disease control measure. Control of plant diseases: costs and benefits/edited for the British Society for Plant Pathology by BC Clifford and E. Laster.

Tiendrebeogo, F., Lefeuvre, P., Hoareau, M., Harimalala, M.A., De Bruyn, A., Villemot, J., … & Lett, J.M., (2012). Evolution of *African cassava mosaic virus* by

recombination between bipartite and monopartite begomoviruses. *Virol. J. 9*, 67.

Tomato Genome Consortium, (2012) tomato genome sequence provides insights into fleshy fruit evolution. *Nature 485*, 635.

Trejo-Saavedra, D.L., Vielle-Calzada, J.P. & Rivera-Bustamante, R.F., (2009). The infective cycle of Cabbage leaf curl virus (CaLCuV) is affected by CRUMPLED LEAF (CRL) gene in Arabidopsis thaliana. *Virol. J, 6*, 169.

Turnage, M.A., Muangsan, N., Peele, C.G. & Robertson, D., (2002). Geminivirus-based vectors for gene silencing in Arabidopsis. *Plant J. 30*, 107-114.

Tuttle, J.R., Idris, A.M., Brown, J.K., Haigler, C.H. & Robertson, D., (2008). Geminivirus-mediated gene silencing from Cotton leaf crumple virus is enhanced by low temperature in cotton. *Plant physiol. 148*, 41-50.

Unseld, S., Frischmuth, T., Jeske, H., (2004). Short deletions in nuclear targeting sequences of *African cassava mosaic virus* coat protein prevent geminivirus twinned particle formation. *Virol. 318*, 90–101.

Urbino, C., Thébaud, G., Granier, M., Blanc, S., & Peterschmitt, M., (2008). A novel cloning strategy for isolating, genotyping and phenotyping genetic variants of geminiviruses. *Virol. J. 5*, 135-10.

Vallad, G.E., & Goodman, R.M.,(2004). Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop sci. 44*, 1920-1934.

Vanderschuren, H., Akbergenov, R., Pooggin, M. M., Hohn, T., Gruissem, W., & Zhang, P., (2007). Transgenic cassava resistance to *African cassava mosaic*

virus is enhanced by viral DNA-A bidirectional promoter-derived siRNAs. *Plant Mol. Bio. 64,* 549-557.

Vanderschuren, H., Alder, A., Gruissem, W., Zhang, P., (2009)**.** Dose-dependent RNAi-mediated geminivirus resistance in the tropical root crop cassava. *Plant Mol. Bio. 70,* 265-272.

Vanitharani, R., Chellappan, P., Pita, J.S., & Fauquet, C.M., (2004). Differential roles of AC2 and AC4 of cassava geminiviruses in mediating synergism and suppression of posttranscriptional gene silencing. *J. Virol.78*, 9487-9498.

Varsani, A., Navas-Castillo, J., Moriones, E., Hernandez-Zepeda, C., Idris, A., Brown, J.K., Murilo Zerbini, F., Martin, D.P., (2014). Establishment of three new genera in the family Geminiviridae: Becurtovirus, Eragrovirus and Turncurtovirus. *Arch. Virol. 159,* 2193-2203.

Varsani, A., Roumagnac, P., Fuchs, M., Navas-Castillo, J., Moriones, E., Idris, A., … & Martin, D.P., (2017). Capulavirus and Grablovirus: two new genera in the family Geminiviridae. *Arch. Virol. 162,* 1819-1831.

Varsani, A., Shepherd, D.N., Dent, K., Monjane, A.L., Rybicki, E.P., & Martin, D.P., (2009). A highly divergent South African geminivirus species illuminates the ancient evolutionary history of this family. *Virol. J. 6,* 36.

Vaucheret, H., & Fagard, M., (2001). Transcriptional gene silencing in plants: targets, inducers and regulators. *TRENDS Gen. 17,* 29-35.

Venkataravanappa, V., Reddy, C.L., Swaranalatha, P., Jalali, S., Briddon, R.W., & Reddy, M.K., (2011). Diversity and phylogeography of begomovirus-associated beta satellites of okra in India. *Virol. J. 8*, 555.

Verlaan, M.G., Hutton, S.F., Ibrahem, R.M., Kormelink, R., Visser, R.G., Scott, J.W., Edwards, J.D., & Bai, Y., (2013). The *Tomato Yellow Leaf Curl Virus* resistance genes Ty-1 and Ty-3 are allelic and code for DFDGD-class RNA-dependent RNA polymerases. *PLoS Genet 9,* e1003399.

Vidavsky, F., & Czosnek, H., (1998). Tomato breeding lines resistant and tolerant to tomato yellow leaf curl virus issued from Lycopersicon hirsutum. *Phytopath. 88,* 910-914.

Vinod, K.K., (2009). Genetic mapping of quantitative trait loci and marker assisted selection in plantation crops. vitro Techniques in Plantation Crops, pp.111-132.

Voinnet, O., (2005). Induction and suppression of RNA silencing: insights from viral infections. Nat. Rev., *Genet. 6,* 206-221.

Vose, P.B., & Cervellini, A., (1983). Problems of scientific research in developing countries. *IAEA Bulletin 25,* 37-40.

Walkey, D. (1991). Applied plant virology. 2nd edition. Chapman and Hall, London.

Walkey, D.G.A., Webb, M.J.W., Bolland, C.J., & Miller, A., (1987). Production of virus-free garlic (Allium sativum L.) and shallot (A. ascalonicum L.) by meristem-tip culture. *J. hort. sci. 62,* 211-220.

Wang, C., Cai, X., Wang, X.,& Zheng, Z., (2006). Optimization of tobacco rattle virus-induced gene silencing in Arabidopsis. *Funct Plant Biol, 33,* 347-355.

Wang, H., Buckley, K.J., Yang, X., Buchmann, R.C., & Bisaro, D.M., (2005). Adenosine kinase inhibition and suppression of RNA silencing by geminivirus AL2 and L2 proteins. *Journal of virology, 79,* 7410-7418.

Wang, P.J., & Hu, C.Y., (1980). *Regeneration of virus-free plants through in vitro culture. In Adv. Biomed.* Eng. Med. Phys. 18, 61-99. Berlin, Heidelberg: Springer.

Warburg, O., (1894). Die kulturpfl anzen usambaras. *Mitteilungen aus den Deutschen Schutzgebieten 7*, 131.

Waterhouse, P.M., Wang, M.B. & Lough, T., (2001). Gene silencing as an adaptive defence against viruses. *Nature 41*, 834-842.

Welz, H.G., Schechert, A., Pernet, A., Pixley, K.V. & Geiger, H.H., (1998). A gene for resistance to the maize streak virus in the African CIMMYT maize inbred line CML202. *Mol. breeding 4*, 147-154.

Whitham, S.A., Yang, C. & Goodin, M.M., (2006). Global impact: elucidating plant responses to viral infection.Mol. *Plant Microbe Interact. 19*, 1207-1215.

Wyant, P.S., Kober, S., Schwierzok, A., Kocher, C., Schäfer, B., Jeske, H., & Wege, C., (2012). Cloned *tomato golden mosaic virus* back in tomatoes. *Virus Res. 167*, 397-403.

Xie Y., Wu P., Liu P., Gong H., & Zhou X., (2010). Characterization of alphasatellites associated with monopartite begomovirus/betasatellite complexes in Yunnan, China. *Virol. J. 7*, 178.

Yamanaka, T., Imai, T., Satoh, R., Kawashima, A., Takahashi, M., Tomita, K., Kubota, K., Meshi, T., Naito, S. & Ishikawa, M., (2002). Complete inhibition of tobamovirus multiplication by simultaneous mutations in two homologous host genes. *J. Virol. 76*, 2491-2497.

Yang, J.Y., Iwasaki, M., Machida, C., Machida, Y., & Zhou, X., (2008). betaC1, the pathogenicity factor of TYLCCNV, interacts with AS1 to alter leaf development and suppress selective jasmonic acid responses. *Genes Dev. 22*, 2564–2577.

Yang, X., Xie, Y., Raja, P., Li, S., Wolf, J.N., Shen, Q., Bisaro, D.M., & Zhou, X., (2011). Suppression of methylation-mediated transcriptional gene silencing by βC1-SAHH protein interaction during geminivirus-betasatellite infection. *PLoS pathog. 7*, e1002329.

Yazdi, H.B., Heydarnejad, J., & Massumi, H., (2008). Genome characterization and genetic diversity of beet curly top Iran virus: a geminivirus with a novel nonanucleotide. *Virus genes 36*, 539-545.

Yeoh, H.H., & Chew, M.Y., (1976). Protein content and amino acid composition of cassava leaf. *Phytochemistry, 15*, 1597-1599.

Yoo, S.D., Cho, Y.H., & Sheen, J., (2007). Arabidopsis mesophyll protoplasts: a versatile cell system for transient gene expression analysis. Nature protocols 2, 1565.

Zaidi, S.S.E.A., Mansoor, S., Ali, Z., Tashkandi, M., & Mahfouz, M.M., (2016). Engineering plants for geminivirus resistance with CRISPR/Cas9 system. Trends in plant science, 21(4), pp.279-281.

Zerbini, F.M., Briddon, R.W., Idris, A., Martin, D.P., Moriones, E., Navas-Castillo, J., … & Consortium, I.R., (2017). ICTV Virus Taxonomy Profile: Geminiviridae. J. Gen. *Virol. 98*, 131-133.

Zhang, L.P., Lin, G.Y., Nino-Liu, D. & Foolad, M.R., (2003). Mapping QTLs conferring early blight (Alternaria solani) resistance in a Lycopersicon esculentum× L. hirsutum cross by selective genotyping. *Mol. Breeding 12*, 3-19.

Zhang, P., Vanderschuren ,H., Fütterer, J., & Gruissem, W., (2005). Resistance to cassava mosaic disease in transgenic cassava expressing antisense RNAs targeting virus replication genes. *Plant Biotech. J. 3*, 385-397.

Zhang, S.C., & Ling, K.S., (2011). Genetic diversity of sweet potato begomoviruses in the United States and identification of a natural recombinant between sweet potato leaf curl virus and sweet potato leaf curl Georgia virus. *Arch.Virol. 156*, 955-968.

Zhang, W., Olson, N.H., Baker, T.S., Faulkner, L., Agbandje-McKenna, M., Boulton, M.I., Davies, J.W., & McKenna, R., (2001). Structure of the maize streak virus geminate particle. *Virol. 279*, 471-477.

Zhang, Y., Su, J., Duan, S., Ao, Y., Dai, J., Liu, J., Wang, P., Li, Y., Liu, B., Feng, D., & Wang, J., (2011). A highly efficient rice green tissue protoplast system for transient gene expression and studying light/chloroplast-related processes. *Plant methods 7*, 30.

Zhou, M., (2012). RNA silencing suppression activity encoded by plant-infecting DNA satellites from Africa. Second cycle, A1E. Uppsala: SLU, Dept. of Plant Biology and Forest Genetics. Volume 131. Retrieved from; http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-1638.

Zhou, X., (2013). Advances in Understanding Begomovirus satellites. *Annu. Rev. Phytopathol. 51*, 357–81.

Zhou, Y., Ryabov, E., Zhang, X., & Hong, Y.,(2008). Influence of viral genes on the cell-to-cell spread of RNA silencing. *J. Exp. Bot. 59*, 2803-2813.

Zorzoli, R., Pratta, G.R., Rodríguez, G.R. & Picardi, L.A., (2007). Advances in biotechnology: tomato as a plant model system. Funct. *Plant Sci. Biotechnol. 1*, 146-159.

Zrachya, A., Glick, E., Levy, Y., Arazi, T., Citovsky, V., & Gafni, Y., (2007). Suppressor, of RNA silencing encoded by *Tomato yellow leaf curl virus*-Israel. *Virol. 358*, 159-165.

APPENDICES

Appendix I: Tables

Table A.1 Response of 190 Arabidopsis accessions to inoculation with the CaLCuVA:CH-42 VIGS vector or to CaLCuVA:LUC.

Accession*	Class★	#symptomtic/#total	Symptoms¶	Silencing§	dpi of photos	VIGS vector insert	Stock Number♯	Web Fig. No.
An-1	B	2/5	+	+++	22 dpi	CH-42	CS22626	Fig. S1n
Bay-0	A	2/5	+++	N/A	22 dpi	EV	CS22633	Fig. S1n
Bla-14	A	10/20	++	+	31 dpi	CH-42		Fig. S1k, bottom
Bla-2	A	13/20	++	++	31 dpi	CH-42		Fig. S1k, top
Bor-1	A	1/5	++	+++	36 dpi	CH-42	CS22590	Fig. S1n
Br-0	A	2/5	+	++	29 dpi	CH-42	CS28095	Fig. S1n
C24	A	1/5	+++	++	21 dpi	CH-42	CS22620	Fig. S1n
Cen-0	A	11/20	+++	++	29; 25 dpi	CH-42	CS28131	Fig. S1a; Fig. 4
Chi-0	E	N/A	++	N/A	14 dpi	LUC		Fig. S1o
Chi-1	A	10/20	+	++	25 dpi	CH-42		Fig. S1a
Chi-2	A	4/20	+++	+	26 dpi	CH-42		Fig. S1a
Cl-0	E	N/A	+	N/A	14 dpi	LUC		Fig. S1o
Cnt-1	A	9/20	+	++	22 dpi	CH-42		Fig. S1i, top
Co	A	3/12	+	++	28 dpi	CH-42		Fig. S1j, bottom
Co-1	A	6/20	++	+++	25 dpi	CH-42		Fig. S1a
Co-2	A	5/20	++	+	25 dpi	CH-42		Fig. S1a
Co-3	E	N/A	+++	N/A	13 dpi	LUC		Fig. S1o

Co-4	A	6/20	+++	++	25 dpi	CH-42		Fig. S1g, top
Col-0	A	10/20	++	++	29;25;32;29;x;31;14 dpi	CH-42		Fig. S1a,b;S1c;S1g;S1h;S1j;S1k;S1o; Fig. 4
Col-1	A	6/20	++	++	30 dpi	CH-42		Fig. S1k, top
Col-5	A	6/20	++	+	29 dpi	CH-42		Fig. S1h, top
Col-6	A	8/20	++	++	31 dpi	CH-42		Fig. S1k, top
Ct-1	A	4/5	++	+++	28 dpi	CH-42	CS22639	Fig. S1n
Cvi-0	A	13/20	++	++	29 dpi	CH-42		Fig. S1a
Da-0	E	N/A	+++	N/A	12 dpi	LUC		Fig. S1o
Db-0	A	9/20	++	++	25 dpi	CH-42		Fig. S1c, top
Db-1	A	3/20	+++	++	32 dpi	CH-42		Fig. S1g, bottom
Db-2	A	5/20	+++	++	27 dpi	CH-42		Fig. S1a
Di-0	D	6/20	-	+	25 dpi	CH-42		Fig. S1b, bottom
Di-1	A	9/20	++	+++	26 dpi	CH-42		Fig. S1b, top
Di-2	E	N/A	+	N/A	14 dpi	LUC		Fig. S1o
Do-0	A	10/20	++	+++	25 dpi	CH-42		Fig. S1c, top
Dr-0	A	11/20	++	+++	25 dpi	CH-42		Fig. S1c, top
Dra-0	A	7/20	+	++	20 dpi	CH-42		Fig. S1d
Dra-1	E	N/A	++	N/A	14 dpi	LUC		Fig. S1o
Dra-2	A	4/20	+	++	29 dpi	CH-42		Fig. S1i, top
Edi-0	A	4/20	++	++	25 dpi	CH-42		Fig. S1c, top
Ei-2	E	N/A	+++	N/A	14 dpi	LUC		Fig. S1o
Ei-6*	E	N/A	++	N/A	14 dpi	LUC		Fig. S1o
Eil-0	A	15/20	++	++	28 dpi	CH-42		Fig. S1b, top
El-0	A	4/20	+	++	25 dpi	CH-42		Fig. S1c, bottom
Ema-1*	A	9/20	++	++	22 dpi	CH-42		Fig. S1i, top

En-1	A	6/20	+	++	30 dpi	CH-42	Fig. S1f, bottom
En-2	A	7/20	++	++	30 dpi	CH-42	Fig. S1f, top
Ep-0	A	3/20	+	++	25 dpi	CH-42	Fig. S1b, top
Er-0	A	8/20	++	+	20 dpi	CH-42	Fig. S1d
Est-0	A	2/10	-	++	26 dpi	CH-42	Fig. S1g, top
Est-1	A	2/20	+	+	25 dpi	CH-42	Fig. S1c, bottom
Et-0	A	4/20	++	+	26 dpi	CH-42	Fig. S1g, top
Fe-1*	A	2/20	+	++	26 dpi	CH-42	Fig. S1a
Fi-0	A	10/20	+++	++	27 dpi	CH-42	Fig. S1a
Fl-1	A	2/20	++	++	27 dpi	CH-42	Fig. S1b, bottom
Fr-2	A	7/20	+	++	20 dpi	CH-42	Fig. S1d
Fr-3	A	3/20	+++	++	25 dpi	CH-42	Fig. S1g, top
Fr-4	A	5/20	++	++	25 dpi	CH-42	Fig. S1c, top
Fr-5	E	N/A	+++	N/A	13 dpi	LUC	Fig. S1o
Fr-6	E	N/A	++	N/A	14 dpi	LUC	Fig. S1o
Fr-7	E	N/A	+++	N/A	14 dpi	LUC	Fig. S1o
Ga-0	A	8/20	+++	++	27 dpi	CH-42	Fig. S1a
Ga-2	A	3/20, 13/20	+; ++	+; +++	26; 30 dpi	CH-42	Fig. S1b, top; S1f, bottom
Gd-1	A	9/20	++	++	30 dpi	CH-42	Fig. S1f, bottom
Ge-1	A	5/20	+++	+	25 dpi	CH-42	Fig. S1g, top
Ge-2	A	6/20	+++	+	28 dpi	CH-42	Fig. S1f, top
Gie-0*	A	4/20	++	++	20 dpi	CH-42	Fig. S1d
Go-2	A	9/20	++	++	27 dpi	CH-42	Fig. S1a
Gr-1	C	10/20	+++	-	29 dpi	CH-42	Fig. S1b, top
Gr-2	A	4/20	+++	+	29 dpi	CH-42	Fig. S1b, top
Gr-3*	A	8/20	++	++	31 dpi	CH-42	Fig. S1k, top

Gr-4	A		+++	++	28 dpi	CH-42		Fig. S1b, top
Gr-5	E	N/A	+++	N/A	14 dpi	LUC		Fig. S1o
Gr-6*	A	4/20	++	++	34 dpi	CH-42		Fig. S1e, top
Gu-0	A	8/20	++	++	29 dpi	CH-42		Fig. S1h, top
Gu-1	B	3/20 0/20	-	+++	25; 29 dpi	CH-42		Fig. S1b, bottom; S1h, bottom
Gy-0	E	N/A	+++	N/A	14 dpi	LUC		Fig. S1o
Ha-0	E	N/A	++	N/A	13 dpi	LUC		Fig. S1o
Hi-0	A	10/20	++	+++	29 dpi	CH-42		Fig. S1b, top
Hl-2	A	13/20	+++	+	22 dpi	CH-42		Fig. S1i, top
Hl-3	D	1/8	+	+	28 dpi	CH-42		Fig. S1j, top
Hn-0	A	7/20	++	++	25 dpi	CH-42		Fig. S1m
Ji-3	E	N/A	++	N/A	14 dpi	LUC		Fig. S1p
Ka-0	B	16/20	-	+++	20 dpi	CH-42		Fig. S1d
Kas-1*	A	7/20	++	+++	29 dpi	CH-42		Fig. S1i, top
Kb-0*	A	6/20	++	++	22 dpi	CH-42		Fig. S1l, top
Kil-0	B	6/20	+	+++	25 dpi	CH-42		Fig. S1g, top
Kl-1	A	10/20	++	+++	27 dpi	CH-42		Fig. S1a
Kl-3	E	N/A	+	N/A	21 dpi	LUC		Fig. S1p
Kl-4	E	N/A	+	N/A	13 dpi	LUC		Fig. S1p
Kl-5	A	6/20	+	+	25 dpi	CH-42		Fig. S1c, bottom
Kn-0	A	3/20	++	++	25 dpi	CH-42		Fig. S1c, top
Knox-18	A	2/5	++	+++	42 dpi	CH-42	CS22567	Fig. S1n
Kr-0	A	5/20	++	++	25 dpi	CH-42		Fig. S1c, bottom
Kro-0	A	8/20	+	++	25 dpi	CH-42		Fig. S1c, top
La-0	E	N/A	++	N/A	14 dpi	LUC		Fig. S1p
Lc-0	A	13/20	++	++	30 dpi	CH-42		Fig. S1e, bottom

Le-0	B	0/20	-	+++	25 dpi	CH-42		Fig. S1g, bottom
Li-1	A	9/20	++	++	27; 14 dpi	CH-42		Fig. S1j, top; S1p
Li-10	A	6/20	++	+++	25 dpi	CH-42		Fig. S1m
Li-2:1	D	5/20	+	++	29 dpi	CH-42		Fig. S1i, top
Li-3*	A	8/20	++	++	29 dpi	CH-42		Fig. S1i, top
Li-5	A	8/20	++	+	31 dpi	CH-42	CS28131	Fig. S1k, top; Fig. S1p
Li-5	A	6/20, 4/29	++	+++	30; 31; 21 dpi	CH-42		Fig. S1b, top; S1f, bottom; S1p
Li-5:3	A	7/20	++	++	25 dpi	CH-42		Fig. S1m
Li-7	A	0/20	+	++	29 dpi	CH-42		Fig. S1i, top
Limeport	A	8/20	++	+++	31 dpi	CH-42		Fig. S1k, top
Lin	A	12/20	++	++	31 dpi	CH-42		Fig. S1k, top
Lip-0	A	4/20	+	++	28 dpi	CH-42		Fig. S1j, top
Lm-2	E	1/5	+++	N/A	20 dpi	EV		Fig. S1n; Fig. S1q
Lo-2	A	4/20	++	++	28 dpi	CH-42		Fig. S1j, top
Lu-1*	A	5/20	++	+++	29 dpi	CH-42		Fig. S1i, top
Ma-0	A	10/20	++	+	25 dpi	CH-42		Fig. S1m
Mnz-0	A	2/20	++	++	28 dpi	CH-42		Fig. S1j, top
Ms-0	E	2/5	+++	N/A	20 dpi	EV	CS22655	Fig. S1n
Mt-0	A	7/20	++	++	25 dpi	CH-42		Fig. S1m
Mz-0	B	2/5	+	+++	35 dpi	CH-42	CS22636	Fig. S1n
Nc-1	A	6/20	++	+++	25 dpi	CH-42		Fig. S1m
Nie-0	A	3/5	++	++	60 dpi	CH-42	CS28563	Fig. S1q
No-0	A	8/20	++	++	22 dpi	CH-42		Fig. S1k, bottom
Nok-3	A	3/5	+++	+	26 dpi	CH-42	CS28571	Fig. S1n; q
Nol-1	A	5/20	+	++	22 dpi	CH-42		Fig. S1l, bottom
Nw-1	A	7/20	++	++	30 dpi	CH-42		Fig. S1f, bottom

Old-1	A	9/20	++	++	25 dpi	CH-42		Fig. S1j, top
Ove-0	A	4/20	+	+	29 dpi	CH-42		Fig. S1e, bottom
Oy-0	A	3/20	++	++	30 dpi	CH-42		Fig. S1f, top
Oy-1	A	11/20	++	+++	29 dpi	CH-42		Fig. S1i, top
Pa-2	A	5/20	+++	++	34 dpi	CH-42		Fig. S1f, top
Pa-3	A	7/20	+	++	29 dpi	CH-42		Fig. S1h, top
Per-1	A	12/20	++	++	25 dpi	CH-42		Fig. S1a
Pf-0	A	12/20	++	++	30 dpi	CH-42		Fig. S1f, bottom
Pi-0	A	2/20	++	++	20 dpi	CH-42		Fig. S1d
Pi-2	B	6/20	+	+++	26 dpi	CH-42		Fig. S1a
Pla-0	A	17/20	++	++	29 dpi	CH-42	CS1459	Fig. S1a
Pla-1	D	0/20	-	-	27 dpi	CH-42	CS28641	Fig. S1a
Pla-2	A	4/20	+	+	34 dpi	CH-42	CS28640	Fig. S1e
Pla-3	A	7/20	+	++	30 dpi	CH-42	CS28643	Fig. S1e
Pla-4	C	4/20	++	-	30 dpi	CH-42	CS28644	Fig. S1e, top
PNA-17	D	2/5	+	-	19, 28 dpi	CH-42	CS22570	Fig. S1n
Po-0	A	11/20	++	+++	28 dpi	CH-42		Fig. S1a
Po-1	E	N/A	+++	N/A	14 dpi	LUC		Fig. S1p
Pog-0	E	N/A	++	N/A	13 dpi	LUC		Fig. S1p
Pr-0	E	N/A	++	N/A	12 dpi	LUC		Fig. S1p
Pro-0	A	4/5	++	+++	36 dpi	CH-42	CS22649	Fig. S1n
Pt-0	A	4/20, 0/20	+	++	25; 29 dpi	CH-42		Fig. S1b, bottom; S1i, bottom
Ra-0	B	9/20	+	+++	25 dpi	CH-42		Fig. S1c, top
Rak-2	A	5/20	++	+	30 dpi	CH-42		Fig. S1f, bottom
Rd-0	A	4/20	+	++	26 dpi	CH-42		Fig. S1g, bottom
Ri-0	A	6/20	+	+	25 dpi	CH-42		Fig. S1a

Rld-2	A	13/20	++	+++	29; 27 dpi	CH-42		Fig. S1j, bottom
Rou-0	A	5/20	++	++	20 dpi	CH-42		Fig. S1d
RRS-7	A	1/5	++	+++	43 dpi	CH-42	CS22564	Fig. S1n
Rsch-0	E	N/A	++	N/A	13 dpi	LUC		Fig. S1p
Rsch-4	E	N/A	++	N/A	14 dpi	LUC		Fig. S1p
Ru-0	A	12/20	++	+++	29 dpi	CH-42		Fig. S1i, top; S1j, bottom
Santa Clara	A	7/20	+++	++	30 dpi	CH-42		Fig. S1k, top
Sav-0	A	2/20	+	++	25 dpi	CH-42		Fig. S1j, bottom
Se-0	A	4/5	+	++	60 dpi	CH-42	CS22646	Fig. S1q
Sf-2	B	4/20	+	+++	25 dpi	CH-42		Fig. S1m
Sg-1*	A	7/20	++	++	30 dpi	CH-42		Fig. S1k, top
Sg-2*	A	10/20	++	+++	31 dpi	CH-42		Fig. S1k, top
Sha	A	2/5	+	++	28 dpi	CH-42	CS28736	Fig. S1n
St-0	A	4/8	+	++	25 dpi; 60 dpi	CH-42		Fig. S1j, top; Fig. S1q
Stw-0	A	11/20	++	++	25 dpi	CH-42		Fig. S1i, top
Ta-0*	A	10/20	++	++	29 dpi	CH-42		Fig. S1h, top
Ts-1	A	7/20	+++	++	29 dpi	CH-42		Fig. S1h, top
Ts-2	A	5/20	+	++	29 dpi	CH-42		Fig. S1h, bottom
Ts-3	A	8/20	+	++	29 dpi	CH-42		Fig. S1h, top
Ts-7	A	0/20	+	++	27 dpi	CH-42		Fig. S1j, bottom
Tsu-0	A	4/20	++	++	22 dpi	CH-42		Fig. S1l, top
Tsu-1	A	8/20	++	++	22 dpi	CH-42		Fig. S1l, top
Tu-0	A	13/20	++	+++	27 dpi	CH-42		Fig. S1j, top
Ty-0	A	8/20	++	++	26 dpi	CH-42		Fig. S1j, top
Uk-3	C	4/20	++	+	25 dpi	CH-42		Fig. S1g, top
Uk-4	A	6/20	+++	++	30 dpi	CH-42		Fig. S1l, top

Wc-1	A	3/20	++	+	27 dpi	CH -42		Fig. S1j, bottom
Wei-0	A	4/20	++	+++	31 dpi	CH -42		Fig. S1k, bottom
Wil-1	A	10/20	++	++	29 dpi	CH -42		Fig. S1i, top
Wil-2	A	3/20	++	+++	26 dpi	CH -42		Fig. S1i, top
Wil-3	A	8/20	++	++	20 dpi	CH -42		Fig. S1d
Ws	A	6/20	+	++	25 dpi	CH -42		Fig. S1c, bottom
Ws-0	E	1/5	+	N/A	21 dpi	EV	CS28825	Fig. S1n
Ws-1	D	3/20	+	+	30 dpi	CH -42		Fig. S1k, bottom
Ws-2	A	6/20	+	++	30 dpi	CH -42		Fig. S1k, bottom
Ws-3	A	9/20	++	++	29 dpi	CH -42		Fig. S1h, top
Wt-1	B	9/20	+	+++	36 dpi	CH -42		Fig. S1l, top
Wt-2	A	2/20	+++	++	26 dpi	CH -42		Fig. S1g, bottom
Wt-3	A	12/20	+	++	22 dpi	CH -42		Fig. S1l, top
Wt-4	A	8/20	++	++	29 dpi	CH -42		Fig. S1i, bottom
Wt-5	B	2/5	+	+++	29 dpi	CH -42	CS28836	Fig. S1n
X-0	A	8/20	++	+++	31 dpi	CH -42		Fig. S1l, top
XX-0	A	6/20	+	++	22 dpi	CH -42		Fig. S1l, top
XXX-0	A	6/20	++	+++	29 dpi	CH -42		Fig. S1h, top
Zu-1	A	2/20	++	+	29 dpi	CH -42		Fig. S1h, bottom

* Showed attenuated symptoms and increased silencing at later time points

♯ Most accessions were ordered from ARBC in 2000 and bulked up at Paradigm Genetics. Stock numbers are given for accessions ordered more recently.

★ Class
class A: Moderate to severe symptoms; if mild symptoms, limited silencing compared to B
class B: Mild symptoms, silencing extensive

class C: Moderate to severe symptoms, silencing absent or limited to less than 10% of of 1-2 leaves

class D: Mild or no symptoms, reduced or delayed silencing if present

class E: Symptoms present, silencing not evaluated

Symptoms:
- no detectable symptoms
+ mild leaf curling
++ moderate symptoms, abrupt reduction in leaf size (>50%) but inflorescence elongates

+++ severe symptoms and inflorescence is stunted

§ Silencing:
- no detectable yellow areas
+ yellow areas on very few leaves, less than 10% of those leaves
++ yellow/white areas on multiple leaves, more than 10% of some leaves are yellow

+++ large areas are completely yellow/white, multiple contiguous sets of leaves show >90% silencing

Table A.2 SSLP markers for Pla-1 and Col-0.

SSLP marker	Chromosome	cM	Primer Sequence 1	Primer Sequence 2
nga59	1	2.9	TTAATACATTAGCCCAGACCCG	GCATCTGTGTTCACTCGCC
F19P19-75410	1	4.7	CCACGTAGGTCAAGAAGAAGAAG	TGTCTGCTGCGATAGAGAGAG
nga63	1	11.48	ACCCAAGTGATCGCCACC	AACCAAGGCACAGAAGCG
AthS0392	1	44.6	TTTGGAGTTAGACACGGATCTG	GTTGATCGCAGCTTGATAAGC
nga 280	1	83.83	GGCTCCATAAAAAGTGCACC	CTGATCTCACGGACAATAGTGC
ATPase	1	117.86	GTTCACAGAGAGACTCATAAACCA	CTGGGAACGGTTCGATTCGAGC
nga1145	2	9.6	GCACATACCCACAACCAGAA	CCTTCACATCCAAAACCCAC
nga168	2	73.77	GAGGACATGTATAGGAGCCTCG	TCGTCTACTGCACTGCCG
nga162	3	20.56	CTCTGTCACTCTTTTCCTCTGG	CATGCAATTTGCATCTGAGG
ciw11	3	43	CCCCGAGTTGAGGTATT	GAAGAAATTCCTAAAGCATTC
nga6	3	86.41	TGGATTTCTTCCTCTCTTCAC	ATGGAGAAGCTTACACTGATC
ciw5	4	10	GGTTAAAAATTAGGGTTACGA	AGATTTACGTGGAAGCAAT
nga1111	4	29.64	GGGTTCGGTTACAATCGTGT	AGTTCCAGATTGAGCTTTGAGC
ciw7	4	65	AATTTGGAGATTAGCTGGAAT	CCATGTTGATGATAAGCACAA
nga1107	4	104.73	GCGAAAAAACAAAAAAATCCA	CGACGAATCGACAGAATTAGG
CTR1	5	10	CCACTTGTTTCTCTCTCTAG	TATCAACAGAAACGCACCGAG
nga151	5	29.62	GTTTTGGGAAGTTTTGCTGG	CAGTCTAAAAGCGAGAGTATGATG
nga139	5	50.48	AGAGCTACCAGATCCGATGG	GGTTTCGTTTCACTATCCAGG
AthPHYC	5	71.13	CTCAGAGAATTCCCAGAAAAATCT	AAACTCGAGAGTTTTGTCTAGATC
ciw9	5	88	CAGACGTATCAAATGACAAATG	GACTACTGCTCAAACTATTCGG

Table A.3 SNPs for Pla-1 and Col-0

SNP name	Chromosome	Position[a]	Sequence [Col-0/Pla-1 SNP]
AtMSQTsnp11	1	1602137	ACCTCCTTGGATTGCT[C/T]AGGAAGCCCAGACAAT
AtMSQTsnp14	1	2775953	TCTTCATACCCAAAGG[T/A]GGGACCACTTCGGAAC
AtMSQTsnp18	1	3872591	CCTTCTTAGCCCTCTT[C/G]ACATCCACAGGATTCG
AtMSQTsnp30	1	5629166	CAATATAAACCCTGTA[T/C]TATTATTTTATTACTT
AtMSQTsnp41	1	8015459	TAGGATGTGGCTCAAA[C/T]GAGTTTCTAGCCGAAG
10.6	1	10659973	GTTTAGATGAAGCTAA[G/A]ATTGTTGTTACTACTG
12.7	1	12700862	TTTGGGATTATGTACG[G/T]ATGGTTTTAATCCATT
13	1	13000466	CATTTGGGCGTTTCCT[G/A]AACGTTGATTTGGCCG
15.8	1	15798699	GTTATTGCTTTCCCTG[A/G]CATCTCTAGCGTTGTA
16.2	1	16199259	AATGAAGACTATGATC[G/A]ATTTAACAGAGGAAGC
16.8	1	16802822	ATGCATTCTATTTTAT[T/C]ATCTATATATACATTT
17.1	1	17100330	CAGCGGTTATATCATC[T/G]AGTTCTGCTACTCCTG
17.2	1	17201183	CTTTTACTACTGTGAA[T/C]TCTGTTGTGTTGTAGA
17.6	1	17591300	CTCATGAAGCCTCTAT[C/T]TTTATTTTCTTGTACT
18.1	1	18093701	TCAAGAAAAGGAATCG[C/T]CGGAGGTGCTGAGGAT
18.6	1	18600884	ATCACAGAGAGGCAGA[A/G]AAAACATACTTGATCA
19.1	1	19100049	GAATCACAAAGAAAGC[A/G]GAAGCACTGTCCAAAC
19.8	1	19800653	TGCAGAAGAGATACAA[G/A]GAAGCTGCACCAGCAA
19.9	1	19901441	AGGTAACACGTTCACG[G/A]ACATAGTGATAATCAA
20	1	20017703	GGTTACTTGCGGCGGT[G/A]GAAGCAAAGGTGAAAT
20.7	1	20704014	CATATATTTACAAAAA[A/G]ATCATTAAAAGTAATT
21	1	20996708	GCACTAAGTAGCATCA[A/G]AAGATAGTGAAAATTA
21.15	1	21149989	ATGGAATTACGATGTT[T/C]GATGATCAAGGGATCG

21.25	1	21252174	GTAAAGTACTTGCCCA[G/C]ACAGTCACATTTGCTT
21.3	1	21309070	TAGTGAAATACCCTTT[C/G]CCACTTTGTTGCACGA
21.4	1	21396934	TGCAAAAGATCGGTTT[A/T]AAATGAAGTCTTTGTT
21.7	1	21693814	TAACTTGATCTTCAAC[T/C]CCCTGAAATTGCTCGC
21.8	1	21731203	GACTGCTCACAATATC[C/A]TATTTATGTAACTCAT
23	1	22988455	GCTCTGCTACTGGTTT[C/A]AACAATCCCAAAGGTC
25	1	24998851	GGTTGGGGATTTTGCT[A/G]TAACTGGAGGTAGTGG
26	1	26000869	GATCTACTTCAACAAC[C/A]ACCACTACCACGGCGT
29	1	28904537	AAGTGAATTAATAGCT[G/T]ACAAAAATAAAAACAA
AtMSQTsnp128	2	5013938	CTCACATTTTCTTCGG[T/G]ATCATGCTCTTATCAA
AtMSQTsnp145	2	10559838	CGAACAAATGCATGTG[C/T]GGTCACGAGGGTAGAA
AtMSQTsnp159	2	13258047	ATTAAAGGCAAGGTAC[C/T]CTACCTCAGCCATATC
AtMSQTsnp222	3	15869439	CGCTTATTGCCTTGTA[T/G]GAATCTGTCAAACACA
AtMSQTsnp288	4	11984761	GAAGACCAAGAGGAAG[A/G]CCAGCGGGATCCAAGA
AtMSQTsnp306	4	16742066	TGAAAAATAATCAGAA[G/C]AATTTGTAAACATGAG
AtMSQTsnp343	5	3606966	CTTGGTCGTTGGTTTT[T/C]GATCGAGTGAGAAGCG
AtMSQTsnp370	5	15047966	AAAACATTACTAATCG[A/G]ATATACAATAACACAC
AtMSQTsnp394	5	20501104	GAATTGTTGCTCACCG[A/G]ATCAACCTTACTTGAA
AtMSQTsnp406	5	23798622	TTGGACATCTGACATA[A/G]TATTCACTTCTTTCCT

[a]Positions based on TAIR10 *Arabidopsis thaliana* Col-0 genome

Table A.4 Candidate Genes for geminivirus immunity Pla-1-1 (gip-1)

Chromosome 1 Position[a]	SNP Pla-0	Pla-1	Col-0[a]	Locus	Gene Description	Gene expression during CaLCuV infection[b]
8106360	T	C	T	AT1G22910	RNA-binding (RRM/RBD/RNP motifs) family protein	No Significant Change
8185933	T	C	T	AT1G23090	Encodes AST91 mRNA for sulfate transporter (SULTR3)	No Significant Change
8255847	T	G	T	AT1G23250	Caleosin-related family protein	No Significant Change
8312119	G	C	G	AT1G23400	Promotes the splicing of chloroplast group II introns (CAF2)	Upregulated
8322458	G	C	G	AT1G23440	Peptidase C15, pyroglutamyl peptidase I-like protein	No Significant Change
8333421	G	C	G	AT1G23480	encodes a gene similar to cellulose synthase (CSLA03)	No Significant Change
8367727	G	A	T	AT1G23640	OBP32pep protein	No Significant Change
8367881	C	G	C	AT1G23640		
8368069	C	T	C	AT1G23640		
8368153	A	G	A	AT1G23640		
8389401	T	G	T	AT1G23720	Proline-rich extensin-like family protein	No Significant Change
8389411	T	A	G	AT1G23720		
8389434	G	A	G	AT1G23720		
8389443	T	C	T	AT1G23720		
8437919	A	C	A	AT1G23880	NHL domain-containing protein	No Significant Change
8519086	T	C	T	AT1G24070	encodes a gene similar to cellulose synthase (CSLA10)	No Significant Change
8542549	C	A	C	AT1G24147	transmembrane protein	N/A
8542567	G	C	G	AT1G24147		
8563867	G	C	G	AT1G24190	Enhances AtERF7-mediated transcriptional repression (SNL3)	No Significant Change
8581068	A	C	A	AT1G24220	paired amphipathic helix repeat-containing protein	N/A
8589072	G	T	G	AT1G24250	Paired amphipathic helix (PAH2) superfamily protein	N/A
8589311	G	C	G	AT1G24250		
8589318	G	C	G	AT1G242		

				50		
8595969	C	T	C	AT1G24260	Member of the MADs box transcription factor family (SEP3)	No Significant Change
8646483	G	T	G	AT1G24380	no-apical-meristem-associated carboxy-terminal domain protein	N/A
8683812	T	C	T	AT1G24490	Homologue of the Alb3/Oxa1/YidC family. ALBINA 4 (ALB4)	No Significant Change
8707862	A	G	A	AT1G24570	transmembrane protein, putative (DUF707)	N/A
8803643	C	T	C	AT1G25054	UDP-3-O-acyl N-acetylglycosamine deacetylase family protein (LPXC3)	N/A
8808666	A	T	A	AT1G25083	Glutamine amidotransferase type 1 family protein	N/A
8837567	A	C	A	AT1G25220	Catalyzes the first step of tryptophan biosynthesis (ASB1)	N/A
8837694	T	C	T	AT1G25220		
8837769	A	C	A	AT1G25220		
8837808	T	A	T	AT1G25220		
8837979	T	C	T	AT1G25220		
8837987	A	G	A	AT1G25220		
9042024	T	C	T	AT1G26150	PROLINE-RICH EXTENSIN-LIKE RECEPTOR KINASE 10 (PERK10)	Upregulated
9042461	A	G	A	AT1G26150		
9078620	A	G	A	AT1G26240	Proline-rich extensin-like family protein	No Significant Change
9120595	A	C	A	AT1G26360	METHYL ESTERASE 13 (MES13)	No Significant Change
9156629	C	A	C	AT1G26480	14-3-3 protein GF14iota (grf12)	No Significant Change
9209675	C	G	C	AT1G26640	ISOPENTENYL PHOSPHATE KINASE (IPK)	No Significant Change
9209677	G	T	G	AT1G26640		
9209854	T	C	T	AT1G26640		
9213416	G	C	G	AT1G26660	Prefoldin chaperone subunit family protein	No Significant Change
9223040	C	T	C	AT1G26680	transcriptional factor B3 family protein	No Significant Change
9283882	G	A	G	AT1G26799	Plant self-incompatibility protein S1 family	N/A
9303785	T	A	T	AT1G26850	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	Upregulated
9330998	G	A	G	AT1G269	hypothetical protein	N/A

				21		
9379220	G	A	G	AT1G270 20	plant/protein	N/A
9397233	G	T	G	AT1G270 70	PROTEIN TARGETING TO STARCH 2 (PTST2)	No Significant Change
9412954	T	C	T	AT1G271 10	Tetratricopeptide repeat (TPR)-like superfamily protein	N/A
9436070	T	G	T	AT1G271 70	Transmembrane receptors / ATP binding protein	No Significant Change
9436109	C	T	C	AT1G271 70		
9436235	T	C	T	AT1G271 70		
9436292	G	T	G	AT1G271 70		
9436313	G	T	G	AT1G271 70		
9436319	C	T	C	AT1G271 70		
9436325	A	C	A	AT1G271 70		
9436332	C	A	C	AT1G271 70		
9436334	T	G	T	AT1G271 70		
9436369	G	A	G	AT1G271 70		
9436375	A	C	A	AT1G271 70		
9436672	C	T	C	AT1G271 70		
9436686	T	C	T	AT1G271 70		
9436693	C	T	C	AT1G271 70		
9436696	C	T	C	AT1G271 70		
9436799	C	G	C	AT1G271 70		
9436816	C	T	C	AT1G271 70		
9436830	A	G	A	AT1G271 70		
9436839	T	G	T	AT1G271 70		
9436863	G	A	G	AT1G271 70		
9436873	A	G	A	AT1G271 70		
9436882	C	G	C	AT1G271 70		

9437007	G	A	G	AT1G27170	
9437142	A	C	A	AT1G27170	
9437171	G	T	G	AT1G27170	
9437204	T	G	T	AT1G27170	
9437209	T	G	T	AT1G27170	
9437322	T	G	T	AT1G27170	
9437398	T	C	T	AT1G27170	
9437403	A	G	A	AT1G27170	
9437420	G	A	G	AT1G27170	
9437442	G	A	G	AT1G27170	
9437454	A	C	A	AT1G27170	
9437518	C	G	C	AT1G27170	
9437542	G	C	G	AT1G27170	
9437992	G	A	G	AT1G27170	
9437996	G	A	G	AT1G27170	
9438097	C	G	C	AT1G27170	
9438159	T	G	T	AT1G27170	
9438324	C	T	C	AT1G27170	
9438511	C	T	C	AT1G27170	
9438632	C	A	C	AT1G27170	
9438644	A	T	A	AT1G27170	
9438805	T	C	T	AT1G27170	
9438910	C	T	C	AT1G27170	
9438940	T	C	T	AT1G27170	
9441318	C	A	C	AT1G27180	Disease resistance protein (TIR-NBS-LRR class) N/A
9441332	A	C	A	AT1G27180	

9441340	A	C	A	AT1G27180
9441352	C	A	C	AT1G27180
9441358	G	C	G	AT1G27180
9441365	C	A	C	AT1G27180
9441370	A	C	A	AT1G27180
9441999	T	G	T	AT1G27180
9442072	C	A	C	AT1G27180
9442095	G	C	G	AT1G27180
9442101	T	G	T	AT1G27180
9442114	G	T	G	AT1G27180
9442137	T	C	T	AT1G27180
9442313	T	A	T	AT1G27180
9442407	C	T	C	AT1G27180
9442433	G	A	G	AT1G27180
9442436	A	G	A	AT1G27180
9442451	A	T	A	AT1G27180
9442565	T	G	T	AT1G27180
9442568	C	T	C	AT1G27180
9442573	G	T	G	AT1G27180
9442575	G	A	G	AT1G27180
9442591	C	T	C	AT1G27180
9442593	G	A	G	AT1G27180
9442597	G	C	G	AT1G27180
9442605	G	C	G	AT1G27180
9442676	A	C	A	AT1G27180
9442683	A	G	A	AT1G27180

9442689	C	G	C	AT1G27180
9442701	T	G	T	AT1G27180
9442710	C	T	C	AT1G27180
9442711	A	G	A	AT1G27180
9442717	T	C	T	AT1G27180
9442722	C	T	C	AT1G27180
9442821	A	T	A	AT1G27180
9442824	C	G	C	AT1G27180
9442827	C	T	C	AT1G27180
9442840	C	A	C	AT1G27180
9442848	T	C	T	AT1G27180
9442850	T	G	T	AT1G27180
9442905	G	T	G	AT1G27180
9442908	C	T	C	AT1G27180
9442926	G	T	G	AT1G27180
9442934	A	T	A	AT1G27180
9442938	A	C	A	AT1G27180
9442947	C	G	C	AT1G27180
9442950	T	G	T	AT1G27180
9442965	G	A	G	AT1G27180
9442982	G	A	G	AT1G27180
9443055	G	A	G	AT1G27180
9443255	A	C	A	AT1G27180
9443267	G	T	G	AT1G27180
9443276	G	T	G	AT1G27180
9443287	C	T	C	AT1G27180

9443301	T	C	T	AT1G27180
9443308	C	T	C	AT1G27180
9443311	C	T	C	AT1G27180
9443317	A	C	A	AT1G27180
9443345	C	T	C	AT1G27180
9443361	G	A	G	AT1G27180
9443410	T	G	T	AT1G27180
9443414	A	G	A	AT1G27180
9443431	C	T	C	AT1G27180
9443455	G	C	G	AT1G27180
9443605	A	G	A	AT1G27180
9443609	G	A	G	AT1G27180
9443617	G	T	G	AT1G27180
9443630	G	A	G	AT1G27180
9443639	G	A	G	AT1G27180
9443641	C	T	C	AT1G27180
9443668	A	C	A	AT1G27180
9443674	A	G	A	AT1G27180
9443686	T	C	T	AT1G27180
9443689	G	C	G	AT1G27180
9443698	C	A	C	AT1G27180
9443719	A	C	A	AT1G27180
9443739	A	C	A	AT1G27180
9443758	T	C	T	AT1G27180
9443772	G	A	G	AT1G27180
9443790	G	T	G	AT1G27180

9443810	T	C	T	AT1G27180
9443861	G	A	G	AT1G27180
9443873	T	G	T	AT1G27180
9443875	C	T	C	AT1G27180
9443880	C	A	C	AT1G27180
9443891	G	A	G	AT1G27180
9443940	T	A	T	AT1G27180
9443953	G	T	G	AT1G27180
9443962	G	A	G	AT1G27180
9443983	G	A	G	AT1G27180
9443990	G	C	G	AT1G27180
9444088	A	T	A	AT1G27180
9444110	C	T	C	AT1G27180
9444114	T	G	T	AT1G27180
9444117	G	T	G	AT1G27180
9444145	G	C	G	AT1G27180
9444151	A	C	A	AT1G27180
9444155	A	G	A	AT1G27180
9444163	G	A	G	AT1G27180
9444179	T	G	T	AT1G27180
9444247	C	T	C	AT1G27180
9444250	T	A	T	AT1G27180
9444281	C	T	C	AT1G27180
9444466	T	G	T	AT1G27180
9444468	A	G	T	AT1G27180
9444490	G	A	G	AT1G27180

9444499	A	T	A	AT1G27180
9444508	G	T	G	AT1G27180
9444511	A	C	G	AT1G27180
9444597	G	A	G	AT1G27180
9444601	G	A	G	AT1G27180
9444608	C	G	C	AT1G27180
9444631	G	C	G	AT1G27180
9444643	C	T	C	AT1G27180
9444646	A	T	A	AT1G27180
9444655	A	G	A	AT1G27180
9444663	T	G	T	AT1G27180
9444668	C	T	C	AT1G27180
9444702	C	G	C	AT1G27180
9444724	A	C	A	AT1G27180
9444728	G	A	G	AT1G27180
9444734	C	A	C	AT1G27180
9444790	A	C	A	AT1G27180
9444797	T	C	T	AT1G27180
9444809	A	C	A	AT1G27180
9444826	G	A	T	AT1G27180
9444831	A	T	A	AT1G27180
9444847	G	A	G	AT1G27180
9444865	T	C	T	AT1G27180
9444879	A	G	A	AT1G27180
9445116	C	T	C	AT1G27180
9445237	C	A	C	AT1G27180

9445249	A	T	A	AT1G27180		
9445308	A	C	A	AT1G27180		
9445410	T	C	T	AT1G27180		
9445991	A	C	G	AT1G27180		
9640788	G	A	G	AT1G27700	Syntaxin/t-SNARE family protein	No Significant Change
9777541	C	T	C	AT1G28050	B-box type zinc finger protein with CCT domain-containing protein (BBX13)	No Significant Change
9813892	C	G	C	AT1G28120	ubiquitin thioesterase otubain-like protein	No Significant Change
9869727	G	A	G	AT1G28240	strawberry notch protein (DUF616)	No Significant Change
9874300	A	C	A	AT1G28250	transmembrane protein	No Significant Change
9973178	T	A	T	AT1G28400	GATA zinc finger protein	No Significant Change
10010903	C	T	C	AT1G28470	NAC domain containing protein 10 (NCA010)	No Significant Change
10084776	C	T	C	AT1G28700	Nucleotide-diphospho-sugar transferase family protein	N/A
10148743	C	G	T	AT1G29060	Encodes a golgi localized QcSNARE involved in response to salt and osmotic stress	No Significant Change
10217131	G	A	G	AT1G29240	transcription initiation factor TFIID subunit, putative (DUF688)	Downregulated
10225855	G	T	G	AT1G29260	PEROXIN 7 (PEX7)	No Significant Change
10230235	A	T	A	AT1G29270	transcription factor bHLH35-like protein	No Significant Change
10252321	A	C	A	AT1G29310	SecY protein transport family protein	No Significant Change
10256972	C	T	C	AT1G29320	Transducin/WD40 repeat-like superfamily protein	N/A
10268791	C	G	A	AT1G29350	RNA polymerase II degradation factor-like protein (DUF1296)	No Significant Change
10268793	A	T	A	AT1G29350		
10270241	T	C	T	AT1G29350		
10270434	G	T	G	AT1G29350		
10272843	A	T	A	AT1G29350		
10272849	C	G	C	AT1G29350		
10299370	C	T	C	AT1G29410	PHOSPHORIBOSYLANTHRANILATE ISOMERASE 3 (PAI3)	No Significant Change

10312360	G	T	G	AT1G29470	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	Upregulated
10327875	A	C	A	AT1G29540	LOW protein: protein BOBBER-like protein	N/A
10331487	A	C	T	AT1G29550	Eukaryotic initiation factor 4E protein (EIF4E1B)	No Significant Change
10331560	G	A	G	AT1G29550		
10341749	G	A	G	AT1G29590	Eukaryotic translation Initiation Factor 4E3 (eIF4E3)	N/A
10341754	T	A	T	AT1G29590		
10341765	A	T	A	AT1G29590		
10341776	G	T	G	AT1G29590		
10341811	G	A	G	AT1G29590		
10347657	C	T	C	AT1G29620	Cytochrome C oxidase polypeptide VIB family protein	No Significant Change
10348446	C	A	C	AT1G29620		
10381885	C	A	T	AT1G29690	CONSTITUTIVELY ACTIVATED CELL DEATH 1 (CAD1)	Downregulated
10405351	C	T	C	AT1G29730	Leucine-rich repeat transmembrane protein kinase	No Significant Change
10431460	C	A	C	AT1G29790	S-adenosyl-L-methionine-dependent methyltransferases superfamily protein	No Significant Change
10431914	G	T	G	AT1G29790		
10431928	T	A	T	AT1G29790		
10431932	T	C	T	AT1G29790		
10431934	T	C	T	AT1G29790		
10434752	A	C	A	AT1G29800	RING/FYVE/PHD-type zinc finger family protein	N/A
10436538	G	A	G	AT1G29810	Transcriptional coactivator/pterin dehydratase (F1N18.100)	N/A
10436560	C	T	C	AT1G29810		
10436699	A	G	A	AT1G29810		
10436723	G	A	G	AT1G29810		
10436732	A	T	A	AT1G29810		
10436738	T	C	T	AT1G29810		

10436758	T	C	T	AT1G29810		
10436827	T	A	T	AT1G29810		
10436834	G	T	G	AT1G29810		
10436837	G	T	G	AT1G29810		
10436843	C	T	C	AT1G29810		
10436852	T	G	T	AT1G29810		
10436864	G	T	G	AT1G29810		
10436866	G	T	G	AT1G29810		
10436880	T	A	T	AT1G29810		
10436976	T	A	T	AT1G29810		
10458090	T	A	T	AT1G29870	tRNA synthetase class II (G, H, P and S) family protein (CLSD3)	No Significant Change
10459794	G	A	G	AT1G29880	glycyl-tRNA synthetase / glycine-tRNA ligase	No Significant Change
10459851	T	A	T	AT1G29880		
10459856	G	T	G	AT1G29880		
10460100	A	T	A	AT1G29880		
10460118	A	G	A	AT1G29880		
10460126	A	C	A	AT1G29880		
10460178	A	G	A	AT1G29880		
10460211	C	T	C	AT1G29880		
10474870	C	G	C	AT1G29920	CHLOROPHYLL A/B-BINDING PROTEIN 2 (CAB2)	N/A
10494407	G	A	G	AT1G29950	basic helix-loop-helix (bHLH) DNA-binding superfamily protein (SACL3)	No Significant Change
10494407	G	A	G	AT1G29951	Upstream open reading frames (uORFs)	N/A
10494407	G	A	G	AT1G29952	Upstream open reading frames (uORFs)	N/A
10609356	C	T	C	AT1G30170	hypothetical protein (DUF295)	N/A
10716598	C	G	A	AT1G30360	Early-responsive to dehydration stress protein (ERD4)	Upregulated

10722267	C	G	C	AT1G30380	PHOTOSYSTEM I SUBUNIT K (PSAK)	Upregulated
10723169	T	C	T	AT1G30380		
10729370	T	A	G	AT1G30400	glutathione S-conjugate transporting ATPase (AtMRP1) mRNA (ABCC1)	No Significant Change
10751389	A	C	A	AT1G30420	member of MRP subfamily. ATP-BINDING CASSETTE C11 (ABCC11)	N/A
10761830	C	A	C	AT1G30440	Phototropic-responsive NPH3 family protein	No Significant Change
10769189	T	A	T	AT1G30450	member of Cation-chloride co-transporter family (CCC1)	Upregulated
10770133	T	C	T	AT1G30455	cyclin/Brf1-like TBP-binding domain-containing protein	N/A
10791636	G	T	G	AT1G30480	recombination and DNA-damage resistance protein (DRT111)	No Significant Change
10859986	C	G	C	AT1G30630	Coatomer epsilon subunit	No Significant Change
10879438	G	T	G	AT1G30670	basic helix-loop-helix (bHLH) DNA-binding superfamily protein	N/A
10879940	G	A	G	AT1G30670		
10918711	G	A	G	AT1G30760	Encodes a BBE-like enzyme (ATBBE-LIKE 13)	No Significant Change
11039291	C	T	C	AT1G30960	GTP-binding family protein (ERA-2)	No Significant Change
11047346	C	T	C	AT1G30974	Encodes a Plant thionin family protein	N/A
11092176	T	C	T	AT1G31080	F-box family protein	No Significant Change
11092311	T	C	T	AT1G31080		
11092316	A	C	A	AT1G31080		
11094845	A	G	A	AT1G31090	F-box family protein	N/A
11094848	A	T	A	AT1G31090		
11094883	T	A	T	AT1G31090		
11094895	G	A	G	AT1G31090		
11094919	T	A	T	AT1G31090		
11094925	G	A	G	AT1G31090		
11094928	G	C	G	AT1G31090		
11094938	T	A	T	AT1G31090		

11094939	G	A	G	AT1G31090		
11094950	T	C	T	AT1G31090		
11158045	G	T	G	AT1G31220	N10-formyltetrahydrofolate-dependent phosphoribosylglycinamide formyltransferase	No Significant Change
11166954	T	A	C	AT1G31250	Proline-rich family protein	No Significant Change
11166957	A	T	A	AT1G31250		
11176255	C	T	C	AT1G31260	member of Fe(II) transporter isolog family (ZIP10)	No Significant Change
11230548	T	A	T	AT1G31358	MICRORNA404 (MIR404)	N/A
11238337	T	G	T	AT1G31370	Ubiquitin-specific protease family C19-related protein	No Significant Change
11238666	G	A	G	AT1G31370		
11238691	A	T	G	AT1G31370		
11238754	A	T	A	AT1G31370		
11238807	C	T	C	AT1G31370		
11240502	C	G	C	AT1G31380	TRAF-like family protein	No Significant Change
11240508	G	A	G	AT1G31380		
11240681	T	A	T	AT1G31380		
11240722	A	G	A	AT1G31380		
11240984	T	A	T	AT1G31380		
11241019	A	T	A	AT1G31380		
11243233	A	C	A	AT1G31390	TRAF-like family protein	N/A
11243239	T	A	T	AT1G31390		
11243245	C	T	C	AT1G31390		
11243269	C	G	C	AT1G31390		
11243323	T	G	T	AT1G31390		
11243351	G	C,T	G	AT1G31390		
11243373	C	G	C	AT1G31390		

11243386	A	T	A	AT1G31390		
11243507	A	T	A	AT1G31390		
11243603	T	C	T	AT1G31390		
11243636	C	T	C	AT1G31390		
11243875	T	A	T	AT1G31390		
11243968	A	G	C	AT1G31390		
11243973	T	C	T	AT1G31390		
11243988	G	A	G	AT1G31390		
11244004	A	G	A	AT1G31390		
11244011	G	T	G	AT1G31390		
11244062	A	G	A	AT1G31390		
11244097	C	A	T	AT1G31390		
11244101	C	A	C	AT1G31390		
11244181	T	G	C	AT1G31390		
11244186	T	C	T	AT1G31390		
11244278	T	A	G	AT1G31390		
11245386	A	G	C	AT1G31400	TRAF-like family protein	No Significant Change
11245388	C	T	C	AT1G31400		
11245668	G	A	G	AT1G31400		
11245759	C	T	C	AT1G31400		
11256051	C	G	C	AT1G31440	SH3 domain-containing protein (SH3P1)	No Significant Change
11258281	A	C	A	AT1G31440		
11289655	A	G	A	AT1G31540	Disease resistance protein (TIR-NBS-LRR class) family	Downregulated
11290458	T	C	T	AT1G31540		
11291306	A	T	A	AT1G31540		
11291326	G	C	T	AT1G31540		

11291549	C	T	C	AT1G31540		
11292137	A	G	A	AT1G31540		
11292248	G	C	G	AT1G31540		
11292830	C	T	C	AT1G31540		
11292860	G	A	G	AT1G31540		
11292873	A	T	A	AT1G31540		
11292884	A	C	A	AT1G31540		
11293409	C	T	C	AT1G31540		
11312982	T	C	T	AT1G31600	RNA-binding (RRM/RBD/RNP motifs) family protein (TRM9)	No Significant Change
11312991	G	T	G	AT1G31600		
11313036	G	A	G	AT1G31600		
11313092	T	C	T	AT1G31600		
11313099	T	C	T	AT1G31600		
11313105	C	T	C	AT1G31600		
11313112	T	C	T	AT1G31600		
11313123	C	T	C	AT1G31600		
11318937	A	T	A	AT1G31630	AGAMOUS-like 86 (AGL86)	No Significant Change
11319009	C	T	C	AT1G31630		
11319013	C	T	C	AT1G31630		
11339048	T	A	T	AT1G31670	Copper amine oxidase family protein	No Significant Change
11368916	T	A	T	AT1G31740	Encodes a putative β-galactosidase (BGAL15)	N/A
11373075	G	A	G	AT1G31760	SWIB/MDM2 domain superfamily protein (SWIB5)	No Significant Change
11377324	A	C	G	AT1G31770	ATP-binding cassette 14 (ABCG14)	No Significant Change
11412904	T	A	T	AT1G31814	FRIGIDA LIKE 2 (FRL2)	No Significant Change
11458646	C	A	C	AT1G31910	GHMP kinase family protein	No Significant Change
11458917	G	T	G	AT1G31910		

11458921	G	T	G	AT1G31910
11458930	C	A	C	AT1G31910
11458945	T	G	T	AT1G31910
11458981	A	C	A	AT1G31910
11459014	G	C	G	AT1G31910
11459064	T	G	T	AT1G31910
11459096	T	A	T	AT1G31910
11459125	G	T	G	AT1G31910
11459169	T	C	T	AT1G31910
11459218	C	T	C	AT1G31910
11459256	T	C	T	AT1G31910
11459274	A	G	A	AT1G31910
11459280	C	T	C	AT1G31910
11459334	G	T	G	AT1G31910
11459339	A	T	A	AT1G31910
11459361	G	A	G	AT1G31910
11459398	G	C	G	AT1G31910
11459674	A	T	A	AT1G31910
11459808	T	C	T	AT1G31910
11459857	T	G	T	AT1G31910
11459863	T	G	T	AT1G31910
11459871	A	T	A	AT1G31910
11460023	G	A	G	AT1G31910
11460027	T	C	T	AT1G31910
11460060	A	G	A	AT1G31910
11460072	C	T	C	AT1G31910

11460086	T	C	T	AT1G31910
11460130	C	A	C	AT1G31910
11460146	G	A	G	AT1G31910
11460233	C	A	C	AT1G31910
11460260	C	T	C	AT1G31910
11460370	T	C	T	AT1G31910
11460471	A	T	A	AT1G31910
11460529	A	G	A	AT1G31910
11460642	T	A	T	AT1G31910
11460712	G	T	G	AT1G31910
11460783	A	C	A	AT1G31910
11460840	C	T	C	AT1G31910
11460894	G	A	G	AT1G31910
11460969	G	C	G	AT1G31910
11460974	A	G	A	AT1G31910
11461142	G	A	G	AT1G31910
11461170	G	T	G	AT1G31910
11461227	G	T	G	AT1G31910
11461278	A	C	G	AT1G31910
11461292	A	G	A	AT1G31910
11461323	T	C	T	AT1G31910
11461362	C	T	C	AT1G31910
11461376	A	T	A	AT1G31910
11461520	C	T	C	AT1G31910
11461526	A	G	A	AT1G31910
11461535	G	A	G	AT1G31910

11461559	G	T	G	AT1G31910		
11461566	G	A	G	AT1G31910		
11461574	T	C	T	AT1G31910		
11461577	C	T	C	AT1G31910		
11461640	C	T	C	AT1G31910		
11461656	T	C	T	AT1G31910		
11461671	A	C	A	AT1G31910		
11461683	G	A	G	AT1G31910		
11467199	A	T	A	AT1G31930	Encodes XLG3 (extra-large G protein 3) (XLG3)	No Significant Change
11510502	C	T	C	AT1G32010	Myosin heavy chain-like protein	No Significant Change
11510527	G	A	G	AT1G32010		
11510768	A	T	A	AT1G32010		
11511442	G	A	G	AT1G32010		
11515522	G	T	G	AT1G32030	plant-specific B3-DNA-binding domain protein (DUF313)	No Significant Change
11529271	T	G	T	AT1G32050	SCAMP family protein (SCAMP5)	No Significant Change
11539018	A	T	A	AT1G32080	Encodes a plant LrgAB/CidAB protein (LrgB)	Upregulated
11568185	T	G	A	AT1G32150	Encodes a G group bZIP transcription factor family member (AtbZIP68)	No Significant Change
11568189	A	T	G	AT1G32150		
11606271	C	A	T	AT1G32210	Encodes protein involved in suppression of apoptosis (ATDAD1)	No Significant Change
11607179	A	G	A	AT1G32210		
11609045	G	A	G	AT1G32220	NAD(P)-binding Rossmann-fold superfamily protein	Upregulated
11613799	A	C	A	AT1G32230	RADICAL-INDUCED CELL DEATH1 (RCD1)	Downregulated
11627554	T	G	T	AT1G32240	Encodes a member of the KANADI family of putative transcription factors (KAN2)	No Significant Change
11739175	T	A	T	AT1G32460	hypothetical protein	Downregulated
11739387	C	G	C	AT1G32470	Single hybrid motif superfamily protein	Upregulated

11757392	A	T	A	AT1G32510	NAC domain containing protein 11 (NCA011)	N/A
11767981	A	G	A	AT1G32540	Encodes a protein with 3 plant-specific zinc finger domains (LOL1)	Upregulated
11775012	C	A	C	AT1G32560	LATE EMBRYOGENESIS ABUNDANT 4-1 (AtLEA4-1)	No Significant Change
11776862	G	A	G	AT1G32570	hypothetical protein	No Significant Change
11777380	T	C	T	AT1G32570		
11784181	C	T	C	AT1G32580	MULTIPLE ORGANELLAR RNA EDITING FACTOR 5 (MORF5)	No Significant Change
11795485	A	G	A	AT1G32600	F-box associated ubiquitination effector family protein	No Significant Change
11796461	A	T	A	AT1G32610	hydroxyproline-rich glycoprotein family protein	No Significant Change
11797821	T	A	T	AT1G32630	FAM50A-like protein	No Significant Change
11811306	G	A	G	AT1G32660	F-box and associated interaction domains-containing protein	No Significant Change
11821061	A	C	A	AT1G32690	DUF740 family protein	No Significant Change
11836528	C	T	C	AT1G32720	Cytochrome C oxidase polypeptide VIB family protein	N/A
11836547	G	A	G	AT1G32720		
11841603	G	A	G	AT1G32730	electron carrier/iron ion-binding protein	No Significant Change
11869827	T	A	T	AT1G32780	GroES-like zinc-binding dehydrogenase family protein	No Significant Change
11902871	G	T	G	AT1G32850	ubiquitin-specific protease 11 (UBP11)	No Significant Change
11907250	T	C	T	AT1G32860	Glycosyl hydrolase superfamily protein	No Significant Change
11912518	G	A	G	AT1G32870	NAC DOMAIN PROTEIN 13 (NAC13)	Downregulated
11912550	T	C	T	AT1G32870		
11913576	A	G	A	AT1G32870		
11972127	G	A	G	AT1G33055	hypothetical protein	No Significant Change
11972186	T	C	T	AT1G33055		No Significant Change
11978219	A	T	A	AT1G33060	NAC 014	No Significant Change
11988182	G	A	G	AT1G33080	MATE efflux family protein	No Significant Change
11988307	G	A	G	AT1G33080		No Significant Change
12075593	A	T	A	AT1G33290	P-loop containing nucleoside triphosphate hydrolases superfamily	No Significant Change

					protein	
12192624	N	C	N	AT1G33612	Leucine-rich repeat (LRR) family protein	N/A
12222477	G	A	G	AT1G33720	Member of CYP76C	Downregulated
12222941	G	C	G	AT1G33720		
12235446	G	A	G	AT1G33750	Terpenoid cyclases/Protein prenyltransferases superfamily protein (TPS22)	No Significant Change
12259668	C	A	C	AT1G33790	jacalin lectin family protein	No Significant Change
12281253	C	A	C	AT1G33830	P-loop containing nucleoside triphosphate hydrolases superfamily protein	No Significant Change
12304432	C	T	C	AT1G33880	Avirulence induced gene (AIG1) family protein	No Significant Change
12313313	G	A	G	AT1G33900	P-loop containing nucleoside triphosphate hydrolases superfamily protein	No Significant Change
12320072	C	T	C	AT1G33920	Phloem protein 2-A4 (PP2-A4)	No Significant Change
12320250	A	C	A	AT1G33920		
12334535	A	C	A	AT1G33950	Avirulence induced gene (AIG1) family protein	No Significant Change
12334846	T	G	T	AT1G33950		
12334868	C	A	C	AT1G33950		
12335367	T	G	T	AT1G33950		
12335384	T	A	T	AT1G33950		
12335761	C	T	C	AT1G33950		
12335936	T	C	T	AT1G33950		
12336311	A	C	A	AT1G33950		
12337115	G	C	G	AT1G33950		
12337223	C	A	C	AT1G33950		
12353445	A	T	A	AT1G33980	Involved in mRNA surveillance (UPF3)	No Significant Change
12356465	T	C	T	AT1G33990	METHYL ESTERASE 14 (MES14)	Downregulated
12394772	C	G	C	AT1G34050	Ankyrin repeat family protein	N/A
12394853	C	T	C	AT1G340		

				50		
12394930	C	T	C	AT1G340 50		
12394984	C	T	C	AT1G340 50		
12395156	T	A	T	AT1G340 50		
12397939	G	A	G	AT1G340 60	Pyridoxal phosphate (PLP)-dependent transferases superfamily protein (TAR4)	N/A
12399889	G	A	G	AT1G340 65	S-adenosylmethionine carrier 2 (SAMC2)	No Significant Change
12419399	G	C	G	AT1G341 10	Leucine-rich receptor-like protein kinase family protein (RGI5)	No Significant Change
12494096	A	G	A	AT1G342 70	Exostosin family protein	No Significant Change
12494533	G	T	G	AT1G342 70		
12514564	G	T	A	AT1G343 15	Transmembrane protein	N/A
12515823	C	G	C	AT1G343 15		
12558601	C	G	C	AT1G343 90	Auxin response factor 22 (ARF22)	N/A
12566342	G	A	G	AT1G344 00	Hypothetical protein	No Significant Change
12566407	A	C	A	AT1G344 00		
12566493	A	T	A	AT1G344 00		
12566497	A	T	A	AT1G344 00		
12577729	G	C	G	AT1G344 10	Auxin response factor 21 (ARF21)	No Significant Change
12577740	G	A	G	AT1G344 10		
12578186	A	G	A	AT1G344 10		
12578203	C	T	C	AT1G344 10		
12578211	C	T	C	AT1G344 10		
12579520	T	C	T	AT1G344 10		
12582568	G	A	G	AT1G344 18	SHORT OPEN READING FRAME 15 (SORF15)	N/A
12596833	C	A	C	AT1G344 60	B1 type cyclin (CYCB1;5)	No Significant Change
12596993	C	T	C	AT1G344 60		
12598273	G	A	G	AT1G344		

				60		
12598788	G	T	G	AT1G344 60		
12615912	T	C	T	AT1G345 10	Peroxidase superfamily protein	No Significant Change
12616320	C	T	C	AT1G345 10		
12616371	T	C	T	AT1G345 10		
12616407	C	T	C	AT1G345 10		
12616545	G	T	G	AT1G345 10		
12616550	T	C	T	AT1G345 10		
12616558	T	C	T	AT1G345 10		
12616576	A	G	A	AT1G345 10		
12616632	G	A	G	AT1G345 10		
12616687	G	C	G	AT1G345 10		
12616858	G	A	G	AT1G345 10		
12736790	G	T	G	AT1G347 50	Protein phosphatase 2C family protein	Downregulated
12737362	G	T	G	AT1G347 50		
12769731	A	T	A	AT1G347 95	Encodes a Plant thionin family protein	N/A
12769833	T	C	T	AT1G347 95		
12769981	C	T	C	AT1G347 95		
12803836	T	C	T	AT1G348 60	Encodes a Plant thionin family protein	N/A
12807190	T	C	T	AT1G349 30	Encodes a Plant thionin family protein	N/A
12929053	C	A	C	AT1G352 40	Auxin response factor 20 (ARF20)	N/A
12929422	A	T	A	AT1G352 40		
12932946	C	A	G	AT1G352 50	Thioesterase superfamily protein. ACYL-LIPID THIOESTERASE 2 (ALT2)	No Significant Change
12978774	A	G	A	AT1G353 40	ATP-dependent protease La (LON) domain protein	Upregulated
12978844	C	G	C	AT1G353 40		

[a]Based on TAIR10 Arabidopsis thaliana Col-0 genome

[b]Ascencio-Ibanez, J.T., Sozzani, R., Lee, T.J., Chu, T.M., Wolfinger, R.D., Cella, R. and Hanley-Bowdoin, L. (2008) Global analysis of Arabidopsis gene expression uncovers a complex array of changes impacting pathogen response and cell cycle during geminivirus infection. Plant physiology, 148, 436-454.

N/A (Information not available)

Table A.5 Symptom score analysis of wild type Arabidopsis Sei-0 infected with ACMV in presence or absence of SEGS-1. **A:** Mean of symptoms score ± Standard error. **B:** Analysis of variance (ANOVA). **C:** T-test analysis of the means.

A.

Treatment →	ACMV	ACMV+S1-1.0	ACMV+ S1-1.5a	ACMV+S1-1.5b	ACMV+ S1-2.0
10 dpi					
17 dpi	1.7±0.15	2.3±0.15	2.4±0.16	2.4±0.16	2.9±0.10
24 dpi	2.5±0.166	3.2±0.13	3.3±0.15	3.6±0.16	3.5±0.16
31 dpi	3.0±0.26	3.7±0.15	4.0±0.00	3.9±0.10	4.2±0.13

B.

ANOVA

	Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
17 dpi	Between Groups	7.28	4	1.82	8.53125	3.27E-05	2.578739
	Within Groups	9.6	45	0.213333			
	Total	16.88	49				
24 dpi	Between Groups	6.12	4	1.53	5.296154	0.001394	2.578739
	Within Groups	13	45	0.288889			
	Total	19.12	49				
31 dpi	Between Groups	8.52	4	2.13	9.042453	1.9E-05	2.578739
	Within Groups	10.6	45	0.235556			
	Total	19.12	49				

C.

		ACMV	ACMV+S1-1.0	ACMV+S1-1.5a	ACMV+S1-1.5b	ACMV+S1-2.0
17 dpi	ACMV	0	0.048*	0.014*	0.014*	0.001**
	ACMV+mono		0	0.899 ns	0.899 ns	0.048*
	ACMV+1.5a			0	0.899 ns	0.138 ns
	ACMV+1.5b				0	0.138 ns
	ACMV+dimer					0
24 dpi	ACMV	0	0.023*	0.007**	0.001**	0.001**
	ACMV+mono		0	0.899 ns	0.387 ns	0.643 ns

	ACMV+1.5a			0	0.643 ns	0.892 ns
	ACMV+1.5b				0	0.899 ns
	ACMV+dimer					0
31	ACMV	0	0.019*	0.001**	0.001**	0.001**
dpi	ACMV+mono		0	0.625 ns	0.880 ns	0.163 ns
	ACMV+1.5a			0	0.899 ns	0.880 ns
	ACMV+1.5b				0	0.625 ns
	ACMV+dimer					0

Table A.6 Symptom score analysis of SEGS-1 transgenic plants infected with ACMV. A: Mean of symptoms score ± Standard error. B: Analysis of variance (ANOVA). C: T-test analysis of the means.

A.

	10 dpi	*17 dpi*	*24 dpi*	*31 dpi*
S1-1.0R	2±0*	3.4±0.55	3.6±0.55	4.8±0.45
S1-1.0F	2±0.89	3.6±0.55	3.6±0.55	4.6±0.55
Sei-0	1.17±0.41	1.83±0.75	2.8±0.45	3.4±0.55

*Mean±SD

B.

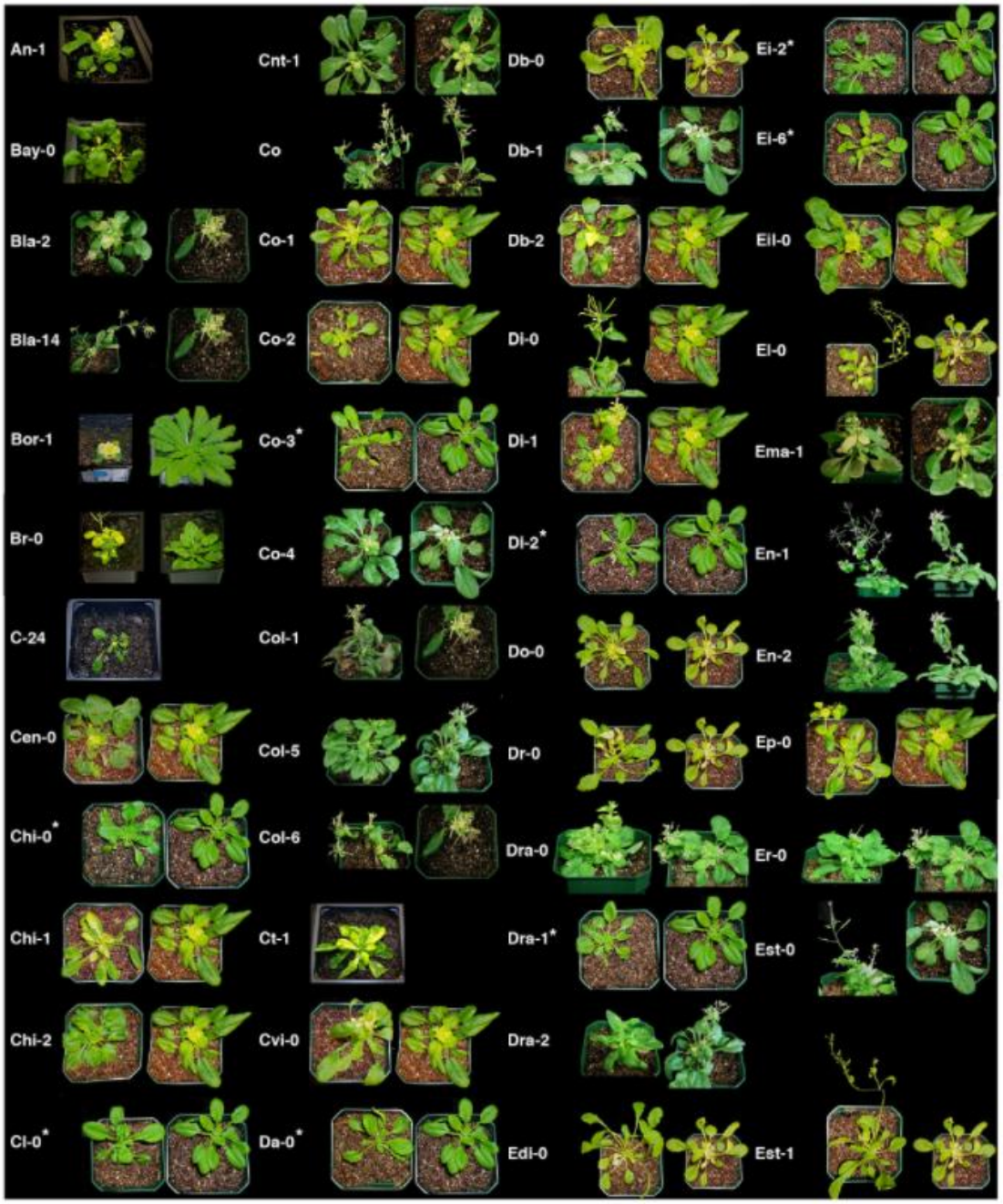
	Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
10 dpi	Between Groups	2.70	2	1.348	3.905	0.045	3.739
	Within Groups	4.83	14	0.345			
	Total	7.53	16				
17 dpi	Between Groups	10.52	2	5.258	13.062	0.001	3.806
	Within Groups	5.23	13	0.403			
	Total	15.75	15				
24 dpi	Between Groups	2.13	2	1.067	4.000	0.047	3.885
	Within Groups	3.20	12	0.267			
	Total	5.33	14				
31 dpi	Between Groups	5.73	2	2.867	10.750	0.002	3.885
	Within Groups	3.20	12	0.267			
	Total	8.93	14				

C.

		Sei-0	S1-1.0R	S1-1.0F
10 dpi	Sei-0	0	0.004**	0.077
	S1-1.0R		0	0.621
	S1-1.0F			0
17 dpi	Sei-0	0	0.003**	0.002**
	S1-1.0R		0	0.580
	S1-1.0F			0
24 dpi	Sei-0	0	0.036*	0.036*
	S1-1.0R		0	1
	S1-1.0F			0
31 dpi	Sei-0	0	0.002**	0.009**
	S1-1.0R		0	0.545
	S1-1.0F			0

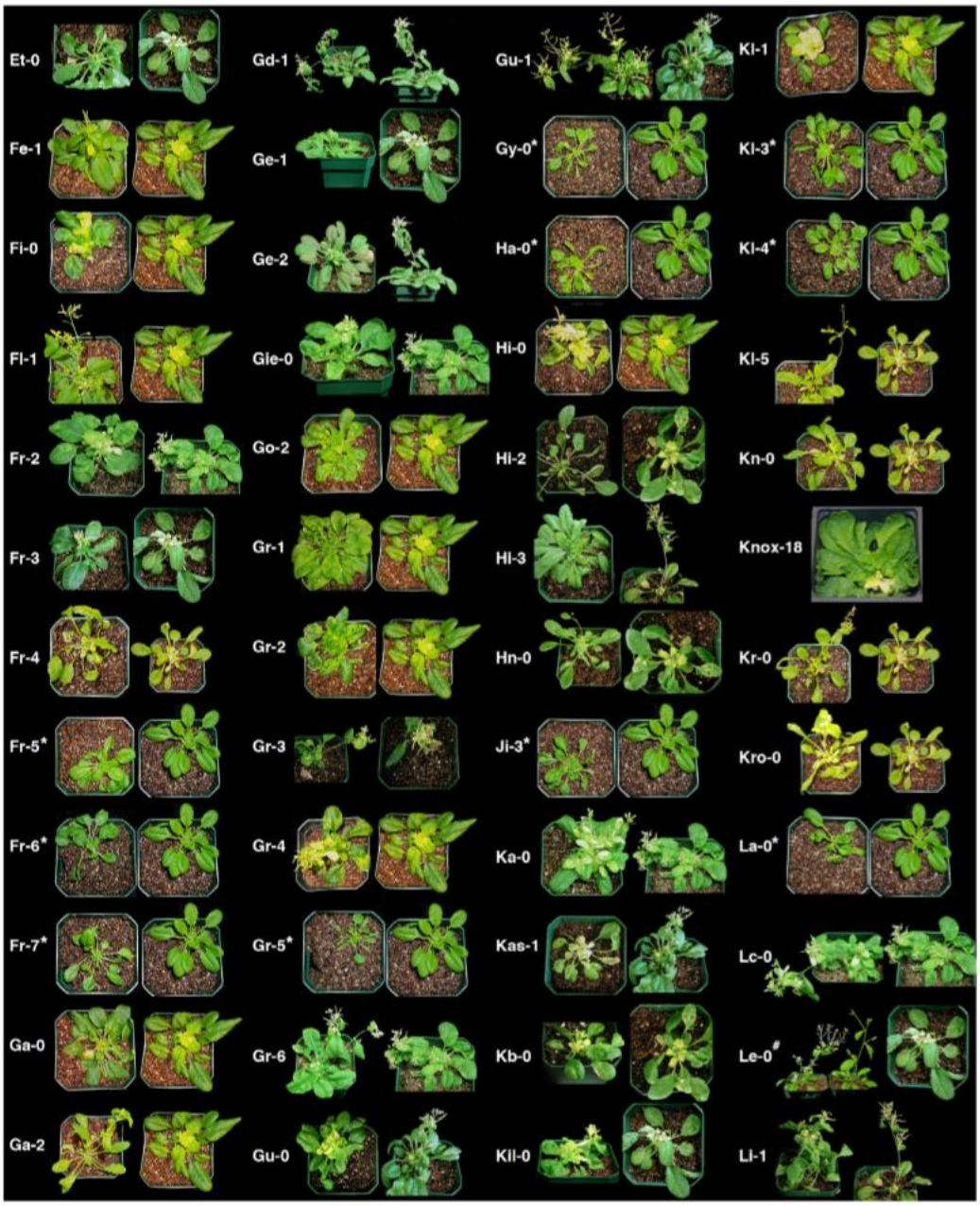
Appendix II: Plates

A.



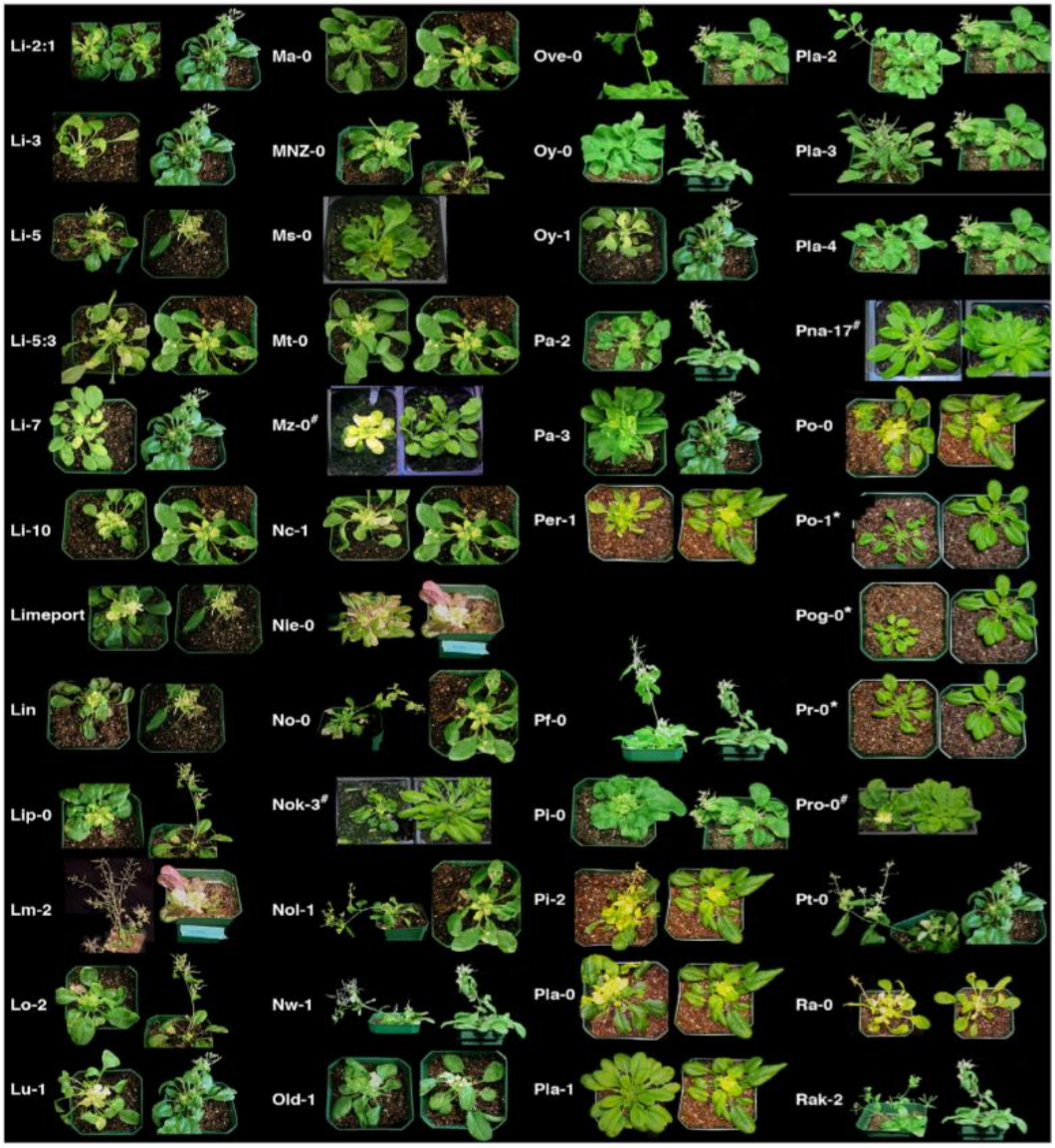
*Both the accession and Col-0 were inoculated with a GFP vector to assess symptoms
** Plant on the right is the same accession planted at the same time but not inoculated

B.


Et-0
Gd-1
Gu-1
Kl-1
Fe-1
Ge-1
Gy-0*
Kl-3*
Fi-0
Ge-2
Ha-0*
Kl-4*
Fl-1
Gie-0
Hi-0
Kl-5
Fr-2
Go-2
Hi-2
Kn-0
Fr-3
Gr-1
Hl-3
Knox-18
Fr-4
Gr-2
Hn-0
Kr-0
Fr-5*
Gr-3
Ji-3*
Kro-0
Fr-6*
Gr-4
Ka-0
La-0*
Fr-7*
Gr-5*
Kas-1
Lc-0
Ga-0
Gr-6
Kb-0
Le-0#
Ga-2
Gu-0
Kil-0
Li-1

*Both the accession and Col-0 were inoculated with a GFP vector to assess symptoms

** Plant on the right is the same accession planted at the same time but not inoculated



*Both the accession and Col-0 were inoculated with a GFP vector to assess symptoms
** Plant on the right is the same accession planted at the same time but not inoculated

D.

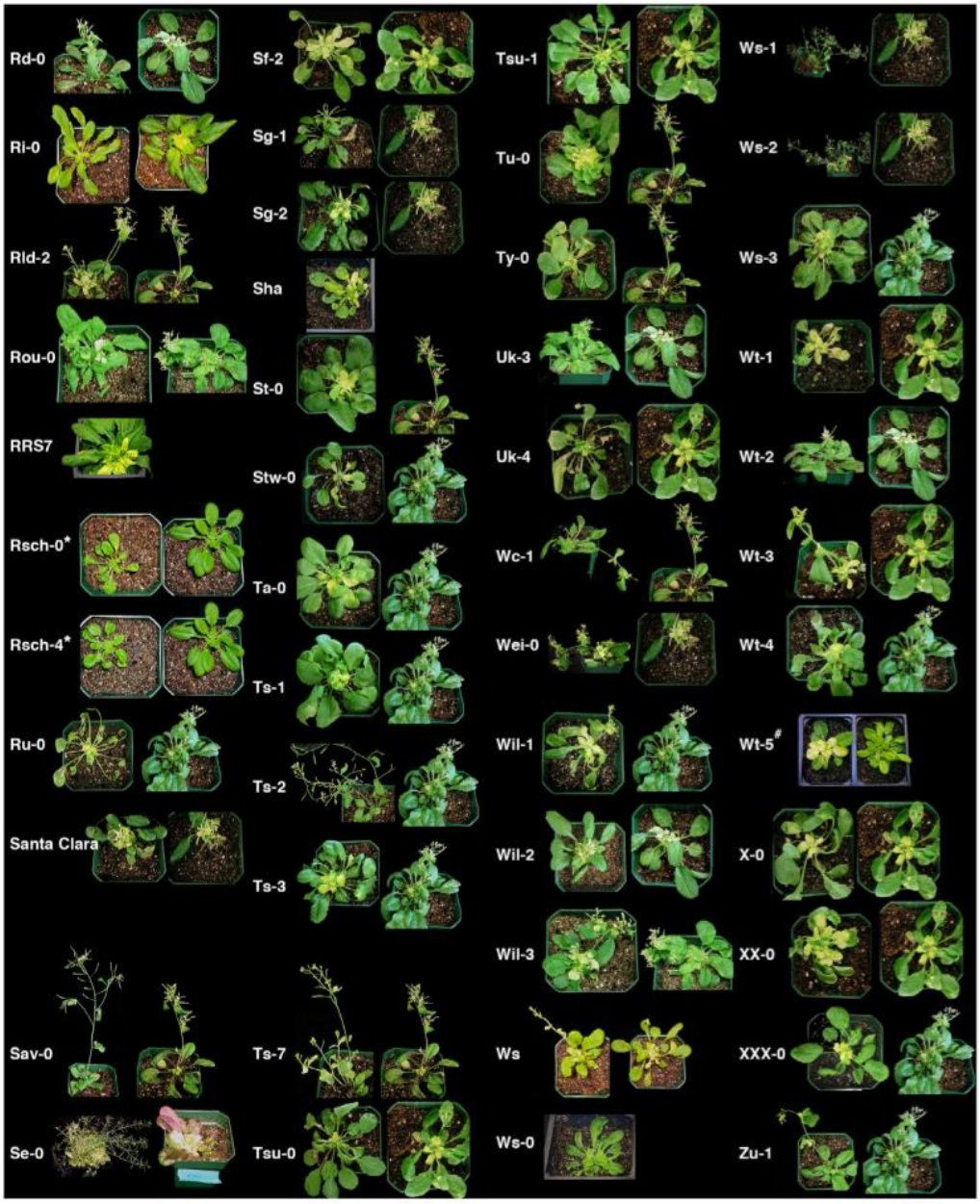


Figure A.1 **(A-D)** Thumbnail images of CH-42 VIGS in different Arabidopsis accessions. Most of the accessions are paired with Col-0 plant (right) inoculated at the same time. Table A.1 additional information. *Both the accession and Col-0 were inoculated with a GFP vector to assess symptoms. **Plant on the right is the same accession planted at the same time but not inoculated.

Figure A.2: Examples of accessions with attenuated symptoms and increased silencing over time. Photos on the left show three accessions at the same time of the screen, 25 dpi, when they were put into Class A due to symptoms and limited silencing. However, photos on the left suggest they belong in Class B at 45–55 dpi. Oy-0 did not show VIGS until 30 dpi.

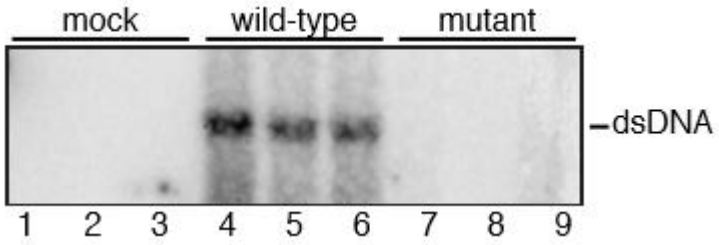


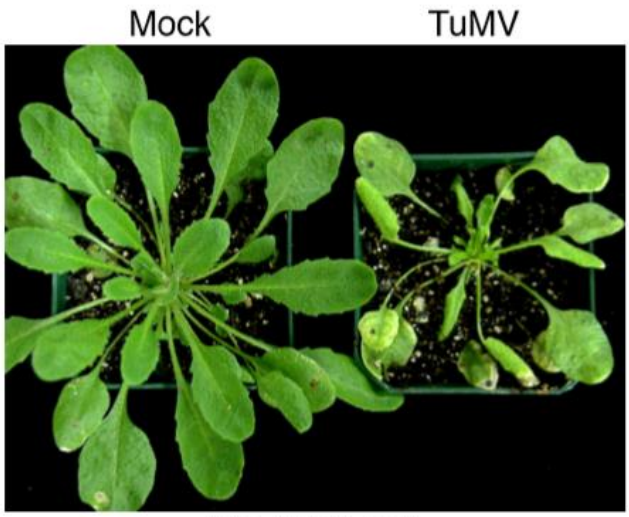
Figure A.3 CaLCuV AL1 frameshift mutation abolishes viral DNA replication in *Nicotiana tabacum* (NT1) protoplasts. Protoplasts were electroporated with no viral DNA (mock) (lanes 1-3), wild-type CaLCuV A DNA (lanes 4 to 6) or the replication deficient CaLCuV A mutant DNA (lanes 7 to 9) in triplicate. Total DNA was extracted at 48-h post transfection and analyzed by DNA gel blotting using a 32Plabeled CaLCuV DNA-A probe. dsDNA, double-stranded DNA.

Figure A.4: New growth in Pla-1 lacks TRV:AtPDS VIGS at later time points compared

to Col-0. The same plants are shown at two different time points, 26 and 31 dpi. The 4

youngest leaves of Pla-1 plants lack visible VIGS at 31 dpi while, in Col-0, they retain

silencing



Pla-1 (18 dpi)

Figure A.5 Pla-1 is susceptible to TuMV. Mock-inoculated (left panel) and TuMVinoculated Pla-1 (right panel) photographed at 18 dpi.

Figure A.6 CaLCuV symptom score key. Photographs of F2 plants agroinoculated with wild-type CaLCuV representing the symptom score on a scale from 1-5. 1, no symptoms; 2, curling of young leaves; 3, reticulated chlorosis in young and older leaves, leaf deformation and stunting; 4, prominent chlorosis and curling of new and older leaves, twisting of older leaves, leaf deformation and stunting; 5, severe chlorosis and stunting, new growth arrest and meristem area death

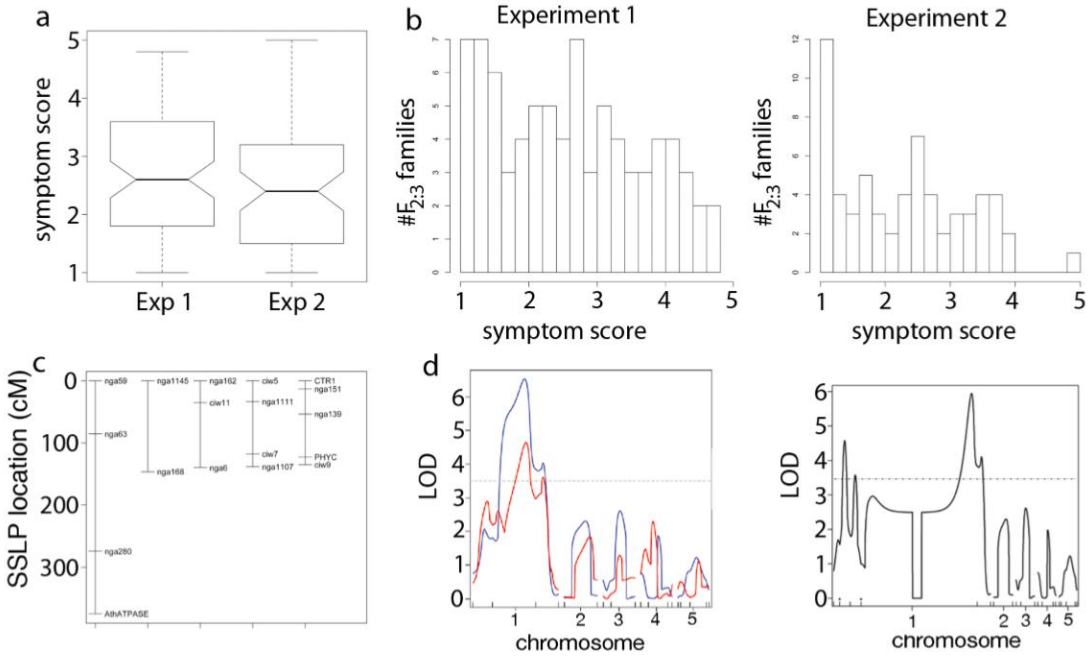


Figure A.7 QTL maps from F2:3 families. 81 families derived from a cross between Pla-1 and Col-0 were scored for symptoms in three separate experiments. A box plot (a) and histogram (b) of the responses in experiments 1 and 2 are shown to compare symptom severity and overall variation. In the histogram, the number of families is shown on the Y axis and symptom level on the X axis. The first experiment scored 14 families as resistant (average score less than 0.5) out of 81 total (chi square 2.61, P = .11) while the second had 16 out of 73 total (chi square .37, P = .54). The relative position of the SSLP markers used for QTL mapping is shown in (c). In (d), QTL maps for the first 2 experiments are shown on the left. In the third experiment (right side), 2 additional SSLP markers were added that flank nga59 and nga63; their positions are

indicated by asterisks on the x axis of the QTL plot. The F19P19-75410 marker is to the left of nga59 and the AtS0392 marker is on the right of nga63.

Supporting Experimental Methods

Method A.1 CaLCuV A DNA replication assay in Nicotiana tabacum (NT1) protoplasts. Protoplasts were prepared from NT1 cells and electroporated with 10 µg of the wild-type CaLCuV A (pCPCbLCVA.003) plasmid (positive control), the replication-deficient CaLCuV A mutant plasmid (pCaLCuVA:FSAL1mut) or no viral DNA (negative control) using a published protocol (Fontes et al., 1994). The transfected cells were analyzed for CaLCuV A DNA replication as previously described (Shen et al., 2014).

Method A.2 TuMV inoculation. The TuMV inoculum consisted of freshly prepared crude sap from TuMV-infected Arabidopsis leaves. To obtain the crude sap, leaves were ground in 50 mM Phosphate Buffer, pH 7.5 (Martin Martin *et al*., 1999). Two to three rosette leaves of five-week-old Pla-1 were dusted with carborundum and rubbed with 5-10 µL of TuMV inoculum. Symptoms were scored, and plants were photographed at 18 dpi.

Method A.3 QTL mapping using F2:3 families. In the first set of mapping experiments, 83 different F2 plants were selfed to create F2:3 families and 5 F3 progeny from each family were agroinoculated with wild-type CaLCuV and scored for symptoms at 21 dpi. DNA from the 5 progeny was pooled and scored for the presence of 20 SSLP markers. Members of the 83 F2:3 families were inoculated in three different experiments and R/qtl was used to analyze the results.

Method A.4 Generation of the geminivirus immunity candidate gene list. Pla-1 was sequenced using the Illumina Hi-Seq platform at the Genomics Science Laboratory at NCSU. To ensure high fidelity sequence, an entire lane was devoted to Pla-1. A .sam file of the aligned sequencing data was created using BWA mem 0.7.15. We converted the

.sam file to sorted .bam using samtools sam to sorted bam 0.1.19.sh. Duplicated reads were removed using samtools rmdup 0.1.19. 167,172,583 reads were properly paired. A Pla-1 SNP list was generated using samtools mpileup 0.1.19 with Col-0 (version TAIR10) as the reference genome. There are 31,245 SNPs between Col-0 and Pla-1. A list of Pla-0 Indels/Addtions/SNPs was downloaded from the 1001 genomes website (http://tools.1001genomes.org/vcfsubset/#select_strains). The Pla-0 list was limited to SNPs and compared to Pla-1 over the region between 8 to 13 megabases on chromosome 1 using Sequel Pro 1.1.2. SNPs duplicated in the two genomes were removed to create a new list of SNPs unique to Pla-1. Out of the 31,245 SNPs, 28,203 SNPs are shared between Pla-1 and Pla-0 and 3042 SNPs are unique Pla-1, which are potential candidate SNPs for geminivirus immunity. A list of the genes found in the same region was downloaded from TAIR (https://gbrowse.arabidopsis.org/cgi-bin/gb2/gbrowse/arabidopsis/) and used to eliminate intergenic SNPs. A list of candidate genes containing one or more Pla-1-specific SNPs was created using Sequel Pro 1.1.2 (Table A.4). Gene descriptions were obtained from TAIR (https://www.arabidopsis.org/tools/bulk/genes/index.jsp).

Supporting References

Fontes, E.P., Eagle, P.A., Sipe, P.S., Luckow, V.A., Hanley-Bowdoin, L., 1994. Interaction between a geminivirus replication protein and origin DNA is essential for viral replication. J. Biol. Chem. 269, 8459–8465.

Martin Martin, A., Cabrera y Poch, H.L., Martinez Herrera, D., Ponz, F., 1999. Resistances to turnip mosaic potyvirus in Arabidopsis thaliana. Mol. Plant Microbe Interact. 12, 1016-1021.

Shen, W., Dallas, M.B., Goshe, M.B., Hanley-Bowdoin, L., 2014. SnRK1 phosphorylation of AL2 delays Cabbage leaf curl virus infection in Arabidopsis. J. Virol. 88, 10598-10612.