

**Molecular and Structural Approaches to the Control of Mixed Viral Infections
in Sweet Potato and an Emerging Virus in Cucurbits**

Clara Ontañón Rojas

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UNIVERSIDAD AUTÓNOMA DE BARCELONA

Facultad de Biociencias

Departamento de Biología Animal, Biología Vegetal y Ecología

Programa de Doctorado en Biología y Biotecnología Vegetal

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Dissertation presented by Clara Ontañón Rojas for the degree of Doctor in Plant
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Dr. Juan José López-Moya

Thesis director

Clara Ontañón Rojas

Ph.D. Candidate

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El éxito y el fracaso, los forja uno paso a paso

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“Ten cuidado con lo que sueñas, a veces se cumple”

SUMMARY

SUMMARY

Viral diseases have a significant impact on agriculture production worldwide. Sweet potato (*Ipomoea batatas*) is one of the highest yielding food crops globally; however, its production is severely constrained by numerous pathogens, particularly viruses. Vegetative propagation and insect-mediated transmission favour the accumulation of multiple viruses and the occurrence of mixed infections. To address an effective control of viral diseases considering the complexity of virus-vector-host interactions requires attention to aspects like the characterization of viral functions, virion structures and virus-virus interactions. The present thesis addresses the characterization of key biological aspects of the sweet potato pathosystem, focusing on two prevalent sweet potato viruses belonging to the genus *Potyvirus*, Sweet potato virus 2 (SPV2) and Sweet potato feathery mottle virus (SPFMV), both transmitted by aphids, and the crinivirus Sweet potato chlorotic stunt virus (SPCSV), transmitted by whiteflies. In addition, other plant pathosystem involving emerging viruses, such as Tomato leaf curl New Delhi virus (ToLCNDV), are analyzed to explore the use of virus-like particles (VLPs) as an experimental platform.

In the first chapter, mutations in SPV2 isolates were characterized and evaluated their possible effects on aphid transmissibility after successive mechanical passages. Additionally, the genome sequence of an SPFMV isolate was completed to and compared with other previously characterized SPFMV isolate. Viral gene products located in the 5' region were analyzed to determine their RNA silencing suppressor (RSS) activity revealing a functional variability.

The second chapter focused on the complex sweet potato virome, characterized by the coexistence of viruses from different families, predominantly *Potyviridae* and *Geminiviridae*. Particular attention was given to mixed infections responsible for the sweet potato virus disease (SPVD), caused by coinfection of SPFMV and the crinivirus SPCSV. Transcriptome analysis revealed significant changes in host gene expression, affecting processes such as photosynthesis, metabolism and plant-pathogen interactions. Gene modules associated with viral infection were revealed by Weighted gene co-expression network analysis (WGCNA).

In the third chapter the use of VLPs as tools to study viral infection dynamics was explored in the alternative pathosystem of the begomovirus ToLCNDV, evaluating the infectivity in zucchini and *N. benthamiana* plants. ToLCNDV VLPs were produced adapting a system based on transient expression of the coat protein together with the self-replicating alphasatellite DNA of AYVV. Western blotting and electron microscopy

(EM) imaging confirmed coat protein overexpression and assembly into VLPs of different morphologies. In addition, a new infectious clone of ToLCNDV DNA-A was generated, and its infectivity and whitefly transmissibility was validated in the presence of DNA-B, providing a platform to identify determinants of transmission.

Overall, the results provide new insights into the molecular determinants involved in virus-plant and virus-vector interactions, improving our understanding of mixed viral infections for the development of effective and durable disease control strategies.

RESUMEN

Las enfermedades virales tienen un impacto significativo en la producción agrícola en todo el mundo. La batata (*Ipomoea batatas*) es una de las especies cultivadas para alimentación de mayor rendimiento a nivel mundial; sin embargo, su producción se ve gravemente limitada por numerosos patógenos, particularmente virus. La propagación vegetativa y la transmisión mediada por insectos favorecen la acumulación de múltiples virus y la ocurrencia de infecciones mixtas. Para lograr un control eficaz de las enfermedades virales, considerando la complejidad de las interacciones virus-vector-huésped, se requiere prestar atención a aspectos como la caracterización de las funciones virales, la estructura de los viriones y las interacciones virus-virus. En la presente tesis se aborda la caracterización de aspectos biológicos clave del patosistema de batata, centrándose en dos virus de batata prevalentes pertenecientes al género *Potyvirus*, el virus 2 de la batata (SPV2) y el virus del moteado plumoso de la batata (SPFMV), ambos transmitidos por pulgones, y en el crinivirus virus del enanismo clorótico de la batata (SPCSV), transmitido por moscas blancas. Además, se analizan otros patosistemas vegetales que involucran virus emergentes, como el virus del enrollado de la hoja del tomate de Nueva Delhi (ToLCNDV), para explorar el uso de partículas similares a virus (VLPs) como plataforma experimental.

En el primer capítulo se caracterizaron las mutaciones en los aislados de SPV2 y se evaluaron sus posibles efectos sobre la transmisibilidad del pulgón después de pases mecánicos sucesivos. Además, se completó la secuencia del genoma de un aislado de SPFMV y se comparó con otros aislados de SPFMV caracterizados previamente. Se analizaron los productos génicos virales ubicados en la región 5' para determinar su actividad como supresores del silenciamiento de ARN (RSS), revelando una variabilidad funcional.

El segundo capítulo se centró en el complejo viroma de la batata, caracterizado por la coexistencia de virus de diferentes familias, predominantemente *Potyviridae* y *Geminiviridae*. Se prestó especial atención a las infecciones mixtas responsables de la enfermedad viral de la batata (SPVD), causada por la coinfección del SPFMV y el crinivirus SPCSV. El análisis del transcriptoma reveló cambios significativos en la expresión de genes del huésped, afectando procesos como la fotosíntesis, el metabolismo y las interacciones planta-patógeno. Los módulos genéticos asociados con la infección viral fueron revelados mediante el análisis de redes de coexpresión génica ponderada (WGCNA).

En el tercer capítulo se exploró el uso de VLPs como herramientas para estudiar la dinámica de la infección viral en el patosistema alternativo del begomovirus ToLCNDV, evaluando la infectividad en plantas de calabacín y *N. benthamiana*. Las VLPs de ToLCNDV se produjeron adaptando un sistema basado en la expresión transitoria de la proteína de la cápside junto con el ADN del alfasatélite autorreplicante del AYVV. La inmunotransferencia Western junto con la microscopía electrónica (ME) confirmaron la sobreexpresión de la proteína de la cápside y su ensamblaje en partículas similares a virus de diferentes morfologías. Además, se generó un nuevo clon infectivo del AND-A del ToLCNDV, y su infectividad y transmisibilidad por mosca blanca se validaron en presencia del ADN-B, proporcionando una nueva plataforma para identificar los determinantes de la transmisión.

En general, los resultados ofrecen nuevas perspectivas sobre los determinantes moleculares implicados en las interacciones virus-planta y virus-vector, mejorando nuestra comprensión de las infecciones virales mixtas para el desarrollo de estrategias de control de enfermedades que sean eficaces y duraderas.

RESUM

Les malalties virals tenen un impacte significatiu en la producció agrícola a tot el món. La batata (*Ipomoea batatas*) és un dels cultius alimentaris de major rendiment a nivell mundial; no obstant això, la seva producció es veu greument limitada per nombrosos patògens, particularment virus. La propagació vegetativa i la transmissió mediada per insectes afavoreixen l'acumulació de múltiples virus i l'aparició d'infeccions mixtes. Per tal d'aconseguir un control eficaç de les malalties virals, considerant la complexitat de les interaccions virus-vector-hoste, cal prestar atenció a aspectes com la caracterització de les funcions virals, les estructures dels virions i les interaccions virus-virus. En aquesta tesi s'aborda la caracterització d'aspectes biològics clau del patosistema de la batata, centrant-se en dos virus de la batata prevalents que pertanyen al gènere *Potyvirus*; el virus 2 de la batata (SPV2) i el virus del clapejat plomós de la batata (SPFMV), tots dos transmesos per pugons, així com el crinivirus virus del nanisme cloròtic de la batata (SPCSV), transmès per mosques blanques. A més, s'analitzen altres patosistemes vegetals que impliquen virus emergents, com el virus de l'enrotllament de la fulla del tomàquet de Nova Delhi (ToLCNDV), per explorar l'ús de partícules similars a virus (VLPs) com a plataforma experimental.

En el primer capítol es van caracteritzar les mutacions en els aïllats de SPV2 i es van avaluar els seus possibles efectes sobre la transmissibilitat per purgons després de passades mecàniques successives. A més a més, es va completar la seqüència del genoma d'un aïllat de SPFMV i es va comparar amb altres aïllats de SPFMV caracteritzats prèviament. Es van analitzar els productes gènics virals ubicats a la regió 5' per determinar la seva activitat com a supressors del silenciament d'ARN (RSS), revelant una variabilitat funcional.

El segon capítol es va centrar en el complex viroma de la batata, caracteritzat per la coexistència de virus de diferents famílies, predominantment Potyviridae i Geminiviridae. Es va prestar especial atenció a les infeccions mixtes responsables de la malaltia viral de la batata (SPVD), causada per la coinfecció de l'SPFMV i el crinivirus SPCSV. L'anàlisi del transcriptoma va revelar canvis significatius en l'expressió de gens de l'hoste, que afectaven processos com la fotosíntesi, el metabolisme i les interaccions planta-patogen. Els mòduls genètics associats amb la infecció viral es van identificar mitjançant l'anàlisi de xarxa de coexpressió gènica ponderada (WGCNA)

Al tercer capítol es va explorar l'ús de VLPs com a eines per estudiar la dinàmica de la infecció viral en el patosistema alternatiu del begomovirus ToLCNDV, avaluant la infectivitat en plantes de carbassó i *N. benthamiana*. Les VLPs de ToLCNDV es van

produir adaptant un sistema basat en l'expressió transitòria de la proteïna de la càpside juntament amb l'ADN de l'alfasatèl·lit autoreplicant d'AYVV. La transferència Western i la microscòpia electrònica (ME) van confirmar la sobreexpressió de la proteïna de la càpside i el seu assemblatge en partícules similars a VLPs de diferents morfologies. A més, es va generar un nou clon infecció d'ADN-A del ToLCNDV, i la seva infectivitat i transmissibilitat per mosca blanca es van validar en presència d'ADN-B, proporcionant una plataforma per identificar els determinants de la transmissió.

En general, els resultats ofereixen noves perspectives sobre els determinants moleculars implicats en les interaccions virus-planta i virus-vector, millorant la nostra comprensió de les infeccions virals mixtes per al desenvolupament d'estratègies de control de malalties eficaces i duradores.

ABBREVIATIONS

Abbreviations and nomenclature of viruses and subviral entities

Abbreviation	Traditional name	Binomial name (ICTV)
ACMV	African cassava mosaic virus	<i>Begomovirus manihotis</i>
ANRSV	Areca palm necrotic ringspot virus	<i>Arepavirus arecae</i>
APV1	Areca palm velavirus 1	<i>Velarivirus arecae</i>
AWMV	Algerian watermelon mosaic virus	<i>Potyvirus algeriaense</i>
AYYV	Ageratum yellow vein virus	<i>Begomovirus ageravenae</i>
BaYMV	Barley yellow mosaic virus	<i>Bymovirus hordeiluteum</i>
BeYDV	Bean yellow dwarf virus	<i>Mastrevirus cicerparvi</i>
BCTV	Beet curly top virus	<i>Curtovirus betae</i>
BPYV	Beet pseudo-yellows	<i>Crinivirus pseudobetae</i>
BVMoV	Bellflower veinal mottle virus	<i>Bevemovirus campanulae</i>
BVY	Blackberry virus Y	<i>Brambyvirus rubi</i>
BYV	Beet yellows virus	<i>Closterovirus flavibetae</i>
CABYV	Cucurbit aphid-borne yellows virus	<i>Polerovirus CABYV</i>
CBSV	Cassava brown streak virus	<i>Ipomovirus brunusmanihotis</i>
CCYV	Cucurbit chlorotic yellows virus	<i>Cucurbit chlorotic yellows virus</i>
CeLV	Celery latent virus	<i>Celavirus apii</i>
CFMMV	Cucumber fruit mottle mosaic virus	<i>obamovirus maculafructi</i>
CGMMV	Cucumber green mottle mosaic virus	<i>Tobamovirus viridimaculae</i>
CLSV	Cucumber leaf spot virus	<i>Aureusvirus cucumis</i>
CIYVV	Clover yellow vein virus	<i>Potyvirus trifolii</i>
CMV	Cucumber mosaic virus	<i>Cucumovirus CMV</i>
CpCDV	Chickpea chlorotic dwarf virus	<i>Mastrevirus cicerparvi</i>
CPMMV	Cowpea mild mottle virus	<i>Carlavirus vignae</i>
CRCSV	Common reed chlorotic stripe virus	<i>Phragmivirus phragmii</i>
CTV	Citrus tristeza virus	<i>Closterovirus tristezae</i>
CuSBV	Cucumber soil-borne virus	<i>Cucumber soil-borne virus</i>
CVYV	Cucumber vein yellowing virus	<i>Ipomovirus cucumisvenae</i>
CYNMV	Chinese yam necrotic Mosaic virus	<i>Macluravirus dioscoreachinense</i>
CYSDV	Cucurbit yellow stunting disorder virus	<i>Crinivirus cucurbitae</i>
EMDV	Eggplant mottled dwarf virus	<i>Alphanucleorhabdovirus melongenae</i>
ENMV	Endive necrotic mosaic virus	<i>Potyvirus chichorii</i>
KGMMV	Kyuri green mottle mosaic virus	<i>Tobamovirus kyuri</i>
LIYV	Lettuce infectious yellows virus	<i>Crinivirus lactucaflavi</i>
MeCMV	Melon chlorotic mosaic virus	<i>Begomovirus cucumismusivi</i>
MNSV	Melon necrotic spot virus	<i>Gammacarmovirus melonis</i>
MVBMV	Melon vein-banding mosaic virus	<i>Melon vein-banding mosaic virus</i>
MWMV	Moroccan watermelon mosaic virus	<i>Potyvirus citrullimoroccense</i>
ONMV	Oat necrotic mottle virus	<i>Tritimovirus avenae</i>
PeWBVYV	Pepper whitefly-borne vein yellows virus	<i>Polerovirus PEWBVYV</i>
PPV	Plum pox virus	<i>Potyvirus plumpoxi</i>
PRSV	Papaya ringspot virus	<i>Potyvirus papayanuli</i>
PVX	Potato virus X	<i>Potexvirus ecspotati</i>
PVY	Potato virus Y	<i>Potyvirus yituberosi</i>
PYVV	Potato yellow vein virus	<i>Crinivirus flavisolani</i>

RGMV	Ryegrass mosaic virus	<i>Rymovirus lolii</i>
RoYMV	Rose yellow mosaic virus	<i>Roymovirus rosae</i>
SPCSV	Sweet potato chlorotic stunt virus	<i>Crinivirus ipomeae</i>
SPFMV	Sweet potato feathery mottle virus	<i>Potyvirus batataplumei</i>
SPLCHnV	Sweet potato leaf curl Henan virus	<i>Begomovirus ipomoeahenanense</i>
SPLCRV	Sweet potato leaf curl Canary virus	<i>Begomovirus ipomoeacanaryense</i>
SPLCSiV	Sweet potato leaf curl Sichuan virus	<i>Begomovirus ipomoeasichuanprimi</i>
SPLCSPV	Sweet potato leaf curl Sao Paulo virus	<i>Begomovirus ipomoeasaopauloense</i>
SPLCV	Sweet potato leaf curl virus	<i>Begomovirus ipomoeae</i>
SPLCD1	Sweet potato leaf curl deltasatellite 1	<i>Deltasatellite ipomoeaprimi</i>
SPLV	Sweet potato latent virus	<i>Potyvirus batatalatentis</i>
SPMCSV	Sweet potato leaf curl South Carolina virus	<i>Begomovirus ipomoeasouthcarolinaense</i>
SPMMV	Sweet potato mild mottle virus	<i>Ipomovirus lenisbatatae</i>
SPMV	Sweet potato mosaic virus	<i>Begomovirus ipomoeamusivi</i>
SPPV	Sweet potato pakakuy virus	<i>Badnavirus occultipomeae</i>
SPSMV-1	Sweet potato symptomless virus 1	<i>Mastrevirus ipomoeae</i>
SPV2	Sweet potato virus 2	<i>Potyvirus duobatatae</i>
SPVC	Sweet potato virus C	<i>Potyvirus cebatatae</i>
SqLCV	Squash leaf curl virus	<i>Begomovirus cucurbitapeponis</i>
SqMV	Squash mosaic virus	<i>Comovirus cucurbitae</i>
TeMV	Telfairia mosaic virus	<i>Potyvirus telfairiae</i>
TEV	Tobacco etch potyvirus	<i>Potyvirus nicotianainsculpentis</i>
TICV	Tomato infectious chlorosis virus	<i>Crinivirus contagichlorosis</i>
TMV	Tobacco mosaic virus	<i>Tobamovirus tabaci</i>
TNV	Tobacco necrosis virus	<i>Tobacco necrosis virus</i>
ToCMoV	Tomato chlorotic mottle virus	<i>Begomovirus solanumpallidivariati</i>
ToCV	Tomato chlorosis virus	<i>Crinivirus tomatichlorosis</i>
ToLCNDV	Tomato leaf curl New Delhi Virus	<i>Begomovirus solanumdelhiense</i>
ToLCV	Tomato leaf curl virus	<i>Begomovirus solanumaustraliaense</i>
ToSRV	Tomato severe rugose virus	<i>Begomovirus solanumseverugosi</i>
TriMV	Triticum mosaic virus	<i>Poacevirus tritici</i>
TuMV	Turnip mosaic virus	<i>Potyvirus rapae</i>
TVMV	Tobacco vein mottling virus	<i>Potyvirus nicotianavenamaculae</i>
TYLCSV	Tomato yellow leaf curl Sardinia virus	<i>Begomovirus solanumflavusardiniaense</i>
TYLCV	Tomato yellow leaf curl virus	<i>Begomovirus coheni</i>
WLMV	Watermelon leaf mottle virus	<i>Potyvirus citrullufolimaculae</i>
WmCSV	Watermelon chlorotic stunt virus	<i>Begomovirus citrulli</i>
WmCSV	Watermelon chlorotic stunt virus	<i>Begomovirus citrulli</i>
WMV	Watermelon mosaic virus	<i>Potyvirus citrulli</i>
ZGMMV	Zucchini green mottle mosaic virus	<i>Tobamovirus cucurbitae</i>
ZYFV	Zucchini yellow fleck virus	<i>Potyvirus pepo</i>
ZYMV	Zucchini yellow mosaic virus	<i>Potyvirus cucurbitaflaviteselati</i>

General abbreviations

35S	Cauliflower mosaic virus 35S promoter
AAP	Acquisition access period
AAP	Abridged anchor primer
ACN	Acetonitrile
AGO	Argonaute
AP	Adapter primer
Asn	Asparagine
Asp	Aspartic acid
AUAP	Abridged universal amplification primer
BP	Biological Process
C	Common
CC	Cellular component
cDNA	Complementary DNA
CLCuD	Cotton leaf curl disease
CP	Coat protein
CPm	Minor coat protein
CR	Common region
Cryo-EM	Cryo-electron microscopy
csDNA	Complementary-strand DNA
DCL	Dicer-like
DEGs	Differentially expressed genes
DI	Defective interfering
DIG	Digoxigenin
DNA	Deoxyribonucleic acid
dpa	Days post agroinfiltration
dpi	Days post inoculation
dsDNA	Double stranded DNA
dsDNA-RT	Reverse-transcribing double stranded DNA
dsRNA	Double stranded RNA
DTT	Dithiothreitol
EA	East African
elf4E	Eukaryotic initiation factor 4E
ETI	Effector-triggered immunity
GARD	Genetic algorithm for recombination detection
GFP	Green fluorescent protein
Glu	Glutamic acid
GO	Gene Ontology
GSPs	Gene-specific primers
h	Hours
ha	hectares
HCPPro	Helper component protease
hDNA	Heterogenous DNA
HF	High fidelity
HR	Hypersensitive response
HRP	Horse radish peroxidase
HSP70	Heat-shock protein 70
IAA	Iodoacetamide
IAP	Inoculation access period
IbRPL2	Ribosomal protein L2 from <i>Ipomoea batatas</i>

ICTV	International Committee on Taxonomy of Viruses
IR	Intergenic region
J	Joule unit of energy
kb	Kilobases
kDa	Kilodaton
KEEG	Kyoto Encyclopedia of Genes and Genomes
LB	Luria-Bertani medium
Leu	Leucine
Lys	Lysine
M	Molar
MEAM	Middle East-Asia Minor 1
MED	Mediterranean
MF	Molecular function
min	Minutes
miRNAs	microRNAs
mL	Mililiter
mm	Milimetres
mM	Milimolar
MP	Movement protein
N ₂	Liquid nitrogen
NIa	Nuclear inclusion protein a
NIb	Nuclear inclusion protein b
NLRs	Nucleotide-binding leucine-rich repeat receptors
nm	Nanometre
NOS	Nopaline synthase terminator for transcriptional termination
NSP	Nuclear shuttle protein
NW	New World
O	Ordinary
OD	Optical density
ORF	Open reading frame
OW	Old World
PAMP	Pathogen associated molecular patterns
PCA	Principal component analysis
PCD	Programmed cell death
PCR	Polymerase chain reaction
Phe	Phenylalanine
PIPO	Pretty interesting potyviral ORF
PISPO	Pretty interesting sweet potato potyviral ORF
PRR	Pattern recognition receptors
PTGS	Posttranscriptional gene silencing
PTI	PAMP-triggered immunity
qRT-PCR	Quantitative reverse transcription-PCR
RACE	Rapid amplification cDNA Ends
RC	Russet crack
RCR	Rolling circle replication
RdRP	RNA-dependent RNA polymerase
REn	Replication enhancer
Rep	Replication protein
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid

RNAi	RNA interference
RNA-seq	RNA sequencing
ROS	Reactive oxygen species
RSS	RNA silencing suppressor
RT	Reverse transcription
RT	Room temperature
SA	Salicylic acid
SDS	Sodium dodecyl sulfate
sec	Seconds
Ser	Serine
siRNA	Small interfering RNA
spp.	Species
SPVD	Sweet potato viral disease
ssDNA	Single stranded DNA
ssRNA	Single stranded RNA
ssRNA-RT	Reverse-transcribing single stranded RNA
TdT	Terminal deoxynucleotidyl transferase
TEM	Transmission electron microscopy
TGS	Transcriptional gene silencing
Thr	Threonine
ToLCD	Tomato leaf curl disease
TrAP	Transcriptional activator protein
TYLCD	Tomato yellow leaf curl disease
Tyr	Tyrosine
UTR	Untranslated region
UV	Ultraviolet
V	Volt
v/v	Volume/volume
Val	Valine
VPg	Genome-linked viral protein
vsiRNA	Viral small interfering RNA
VST	Variance-stabilized transformation
w/v	Weight/volume
WGCNA	Weighted gene co-expression network analysis
YLD	Yellow Leaf Disease
µg	Microgram
µL	Microliter

CONTENTS

1. INTRODUCTION	1
1.1 Viruses.....	1
1.2 Plant viral pathogenesis: viruses, crops and transmission vectors	3
1.2.1 Plant viruses.....	3
1.2.1.1 The family <i>Potyviridae</i>	5
The genus Potyvirus	8
Sweet potato feathery mottle virus (SPFMV)	12
Sweet potato virus 2 (SPV2).....	14
1.2.1.2 The family <i>Closteroviridae</i>	15
The genus Crinivirus	16
Sweet potato chlorotic stunt virus (SPCSV)	17
1.2.1.3 The family <i>Geminiviridae</i>	18
The genus Begomovirus	23
Tomato leaf curl New Delhi Virus (ToLCNDV)	25
1.2.2 Crops	27
1.2.2.1 Impact of plant viruses in sweet potato crops.....	27
Sweet potato	27
Sweet potato viral diseases	28
1.2.2.2 Impact of plant viruses in cucurbit crops	29
Cucurbit crops.....	29
Cucurbits viral diseases.....	29
1.2.2.3 Plant defence responses.....	31
Antiviral RNAi	32
Virus counter defence mechanisms.....	33
1.2.3 Players in virus transmission	34
Aphids	34
Whiteflies	35
1.2.3.1 The vector transmission process.....	37
1.2.3.1.1 Interference with transmission	38
1.2.3.2 Single and mixed infections	40
1.3 Virus like-particles: advances in plant sciences	41
2. OBJECTIVES	45
3. MATERIALS AND METHODS	51
3.1 Biological materials	51
3.1.1 Virus sources	51
3.1.2 Plants	51
3.1.3 Transmission vectors.....	52

3.1.4 Bacterial strains.....	52
3.1.5 Plasmids and cloning vectors	53
3.2 Virus inoculation	55
3.2.1 Mechanical inoculation	55
3.2.2 Insect-mediated inoculation	55
Aphid-mediated inoculation / Aphid mediated non-persistent transmission ...	55
Whitefly-mediated inoculation	56
3.2.3 Agrobacterium-mediated virus inoculation	56
3.3 Nucleic acid manipulation	56
3.3.1 DNA extraction from plant material	56
3.3.2 RNA extraction from plant material and reverse transcription	57
3.3.3 PCR amplification.....	57
3.3.4 Nucleic acid gel electrophoresis	58
3.4 Genome sequencing and sequence analysis.....	58
3.4.1 Genome sequencing of AM-MB1 SPFMV isolate	58
3.4.2 Sequence alignment and phylogenetic analysis	60
3.5 Transient expression of heterologous proteins <i>in planta</i>	60
3.5.1 Assessment of RNA silencing suppressor activity	60
Construct of binary plasmids associated to viral proteins	60
Agrobacterium-mediated co-infiltration assay.....	62
GFP extraction and measurements	62
Protein extraction and immunoprotein techniques (Western Blot analysis) 62	
Statistical analysis.....	63
3.6 Molecular quantification and transcriptomic analysis of viral accumulation.....	63
3.6.1 Viral load quantification	63
3.6.2 Libraries construction and sequencing	64
3.6.3 RNA-seq data processing and analysis	64
Differential gene expression analysis.....	65
Functional annotation and enrichment analysis	66
Weighted gene co-expression network analysis (WGCNA).....	66
3.7 <i>In planta</i> VLPs production and construction of ToLCNDV infectious clone.....	67
3.7.1 Production of AYVV-VLPs in <i>N. benthamiana</i> tissues by agroinfiltration..	67
3.7.2 Production of ToLCDNV-VLPs in <i>N. benthamiana</i> by agroinfiltration	67
3.7.3 Construction of ToLCNDV infectious clone for agroinoculation.....	68
3.8 Virions and Virus-like particles purification	70
3.8.1 Purification of SPV2 virions	70
3.8.2 Purification of ToLCNDV virions	70

3.8.3 Purification of begomovirus VLPs	71
3.8.4 Negative staining and TEM imaging	71
3.8.5 Mass spectrometry: Preparation of gel slices for trypsin digestion	72
4. RESULTS - CHAPTER I	75
Deciphering mechanisms underlying virus transmission and suppression of silencing in sweet potato potyviruses	75
4.1 Exploring the requirements for insect-mediated transmissibility of SPV2	75
4.1.1 Evaluation of SPV2 transmission: Infected host plants and purified virions	75
Sequence analysis and recombination of SPV2 sequences.....	77
4.2 Characterization of different SPFMV isolates and their relevant functions of plant viruses such as silencing suppressor activity	81
4.2.1 Comparative genomic sequencing of two SPFMV isolates	81
4.2.1.1 Complete genome sequencing of AM-MB1 isolate.....	81
4.2.1.2 Comparative analysis of P1 and PISPO region	84
4.2.1.3 Phylogenetic analysis of P1	88
4.2.2 Comparison of RSS activity in gene products of both isolates of SPFMV	89
4.2.2.1 SPFMV proteins conferring local RNA silencing activity	89
4.2.2.2 <i>Cis</i> or <i>trans</i> co-expression of P1 and HCPro does not affect their local RSS capacity	94
4.2.2.3 Effect of viral proteins in local and systemic movement of the silencing signal.....	96
DISCUSSION - CHAPTER I	99
5. RESULTS - CHAPTER II	111
RNA-seq profiling of sweet potato plants under SPFMV single infection and whitefly- mediated SPCSV-SPFMV co-infection.....	111
5.1 Characterization of the sweet potato virome using RNA-seq uncovers diverse plant-infecting viruses with SPFMV predominance	111
5.2 Temporal dynamics of SPFMV accumulation and symptom development in single and mixed infection SPCSV.....	113
5.3 Transcriptomic signatures underpin the SPFMV accumulation enhancement during SPCSV co-infection	117
5.4 From genome mapping to transcription structure: exploratory RNA-seq analysis of SPFMV and SPCSV co-infection.....	118
5.4.1 Exploratory Principal Component Analysis (PCA) of transcriptomic profiles in virus-Infected sweet potato plants	118
5.4.2 Hierarchical clustering identifies time-associated transcriptional response patterns	119
5.4.3 Identification and functional characterization of differentially expressed genes revealed transcriptional changes	120
5.4.3.1 Functional classification and enrichment analysis of DEGs.....	120

5.4.3.2 KEGG pathways influenced analysis.....	121
5.4.4 Weighted gene co-expression network analysis (WGCNA) reveals gene modules associated with viral accumulation	123
6. RESULTS - CHAPTER III	139
<i>In planta</i> platforms of ToLCNDV particles as exploratory tools toward vector transmission mitigation.....	139
6.1 Assessment of ToLCNDV infectivity and virion purification across different host species	139
6.2 <i>In planta</i> production of AYVV and ToLCNDV VLPs using the self-replicating AYVV alphasatellite DNA.....	141
6.3 <i>In planta</i> assembly of ToLCNDV bigeminate VLPs using pCAMBIA:GFP- ToLCNDV	145
6.4 Generation and functional validation of a native-length ToLCNDV DNA-A clone and assessment of its infectivity and transmission.....	148
DISCUSSION - CHAPTER III	153
7. CONCLUSIONS	163
8. ANNEX I	167
9. BIBLIOGRAPHY	181

INTRODUCTION

1. INTRODUCTION

1.1 Viruses

More than a century ago, M. W. Beijerinck identified a novel infectious agent while investigating tobacco mosaic disease. Distinct from bacteria, he originally described it as a “contagious living fluid” (Beijerinck, 1898; Creager 2022). Later, Beijerinck termed this causal agent as Tobacco mosaic virus (acronym TMV), marking the first time a virus was ever identified, currently corresponding to the species *Tobamovirus tabaci* in the family *Virgaviridae*. Viruses are obligate intracellular parasites, meaning they cannot accomplish essential biological processes outside of the host cell. However, they possess a remarkable capacity to hijack the host’s molecular machinery to complete their viral functions and achieving the production of virus progeny.

Being viruses the most genetically diverse group of microorganisms worldwide, their classification can be complex and challenging. Currently, the most widely accepted system includes the categories proposed by the International Committee on Taxonomy of Viruses (ICTV, <https://ictv.global/files/info>) based on the high-level classification introduced by Baltimore in 1971. The Nobel-Prize winning biologist David Baltimore categorized viruses into 7 specific classes according to the nature of nucleic acid incorporated into virions, including positive-sense single-strand RNA viruses (+ssRNA), negative-sense single-strand RNA viruses(-ssRNA), double stranded RNA viruses (dsRNA), reverse-transcribing viruses with positive-strand RNA genomes (ssRNA-RT), reverse-transcribing viruses with double-strand DNA genomes (dsDNA-RT), single-stranded DNA viruses (ssDNA), and double-stranded DNA viruses (dsDNA) (Baltimore 1971; Abrescia *et al.*, 2012; Koonin *et al.*, 2015; Koonin *et al.*, 2020) (Fig. 11A).

Viral genomes are composed of either RNA or DNA, which can be single- or double-stranded, arranged as circular or linear molecules and organized as one or multiple molecular segments. The composition of viromes differ significantly between of prokaryotes and eukaryotes hosts. In both bacteria and archaea, the vast majority of the viruses possess dsDNA genomes, with ssDNA viruses being the second most common group. In contrast, eukaryotes host a highly diverse RNA viruses (particularly of the positive-strand class) and as well as reverse-transcribing elements and retroviruses (Koonin *et al.*, 2015). To date, according to ICTV, over 2,100 virus species have been identified as infecting plants. Currently, all characterized plant viruses possess compact genomes, ranging from approximately 1 to 20 kb. The majority possess RNA as their genetic material, with +ssRNA viruses being the most prevalent, followed by -ssRNA and

Introduction

ssDNA viruses (Koonin *et al.*, 2020; Tatineni and Hein, 2023; Lozano-Durán 2024) (Fig.11B).

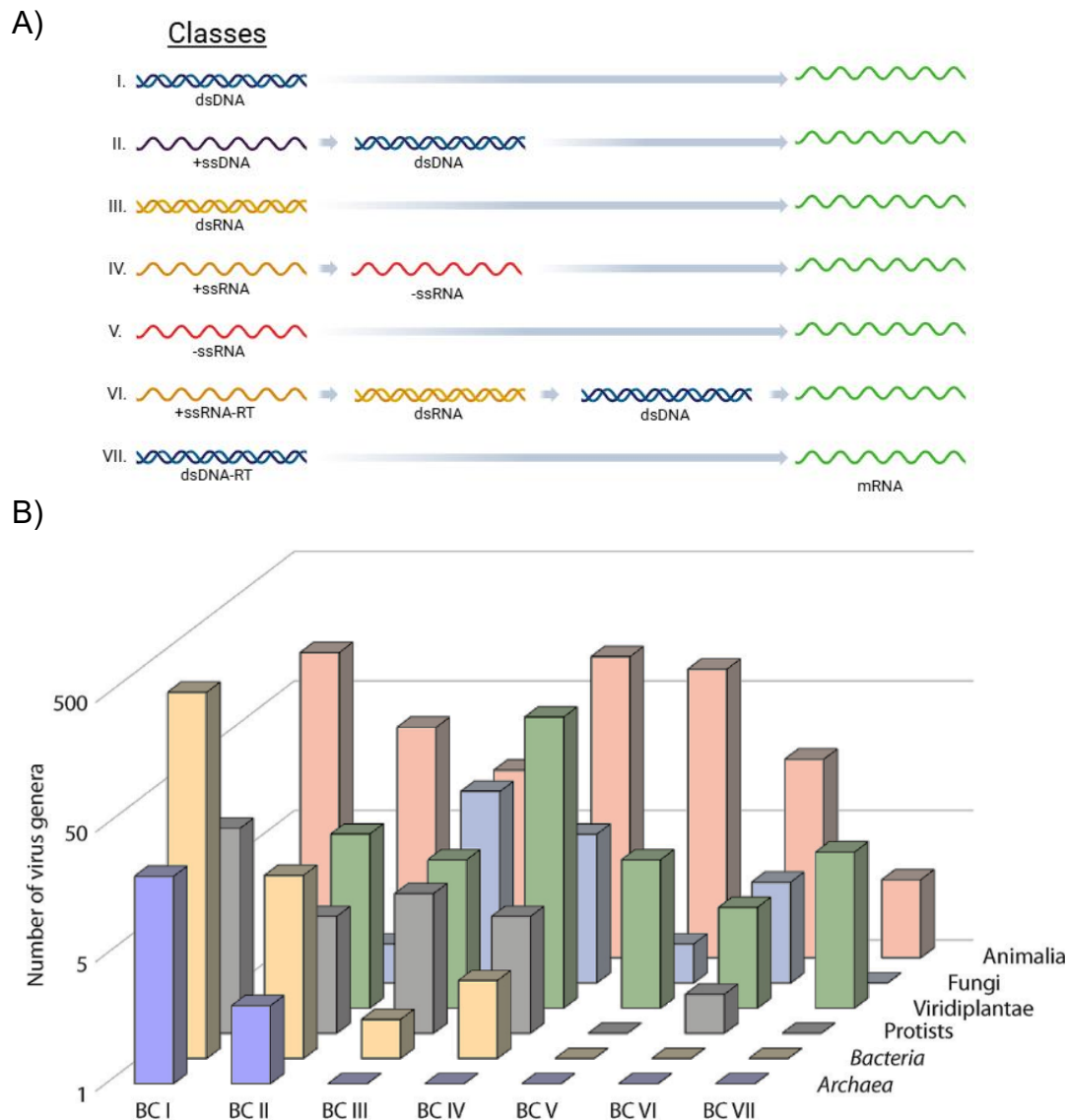


Figure 11. A) Baltimore classification (BC) system of viruses based on their mechanisms of mRNA synthesis. B) Distribution of the seven classes of the BC across the major divisions of prokaryotes and eukaryotes. Panel B adapted from Koonin *et al.* (2020).

In the extracellular environment, viruses are susceptible to different stresses, including physical stress, ultraviolet radiation or radioactivity. To preserve the integrity of their genomic material from the harsh environment, virus encapsulate their nucleic acid within a proteinaceous shell, known as the capsid and mainly composed by one or more coat proteins (CP). Virus particles are distinguishing in “enveloped viruses” or “non-enveloped viruses” depending on they possess a lipid “envelope”. Enveloped viruses are prevalent in animals, in contrast, plant viruses possess almost exclusively non-enveloped viruses,

specifically with icosahedral or helical filamentous or rod-shaped capsid structures (Loutan 2016). Since the helical structural elucidation of TMV (Bernal and Fankuchen, 1941a, b; Franklin 1955; Franklin *et al.*, 1957; Stubbs *et al.*, 1977; Abrescia *et al.*, 2012; Sevvana *et al.*, 2021), over 500 distinct species of filamentous plant viruses have been identified. Many ssRNA plant viruses are encapsidating by a single type of coat protein in a simple helical array, such as members of *Tobamovirus* or *Potyvirus* genera among others; however, plant viruses which have a segmented genome, for example tobnaviruses and hordeiviruses, are packaged in multiple compact helical filaments (Stubbs and Kendall, 2012). Other plant viruses may be enveloped by an icosahedral shell. The genomes of these viruses are packaged completely within an icosahedral capsid, which makes this geometric structure impose spatial constraints that limit the size of the viral genome. In contrast, helical viruses tend to have larger genomes due to the viral nucleic acid coils into a helical configuration and the capsid proteins wind around the external or internal part of the viral genome, forming elongated rod-like structures (Stubbs and Kendall, 2012; Loutan 2016). Additionally, a few plant viruses exhibit a complex structural architecture that does not strictly conform the canonical helical or icosahedral shape. Geminiviruses, in particular, display a sophisticated structure where the viral particles are formed of two icosahedral hemispheres, each missing a pentamer at the interface between them, resulting in a twinned particle (Hipp *et al.*, 2017; Saunders *et al.*, 2020).

1.2 Plant viral pathogenesis: viruses, crops and transmission vectors

1.2.1 Plant viruses

Plant viruses are responsible for nearly half of the reported emerging and re-emerging infectious plant diseases, reducing crop quality and yield, and causing annual losses estimated around >30 billion dollars worldwide (Anderson *et al.*, 2004; Sastry and Zitter, 2014).

Combination of anthropogenic and environmental factors (multiple factors) have contributed to the recent and rapid deterioration of the global plant virus disease landscape. One of the major drivers is the expansion of international trade in plants and plant products, facilitating the introduction of viral pathogens into regions where they were formerly absent. Tendency of multinational seed companies to produce seed crops in warmer regions combined with the faster air and sea transport and weak quarantine and regulatory systems in many developing countries, contribute to boost global virus dissemination pathways (Anderson *et al.*, 2004; Jones 2009; Jones and Naidu, 2019). Another crucial factor compromising crop integrity is the widespread adoption of

Introduction

monoculture practices characterized by high plant density and uniform genetic backgrounds. Crops which have never been grown before as monocultures may exhibit increased vulnerability to novel viral infections (Jones and Naidu, 2019; Tatineni and Hein, 2023). Climate change further exacerbates the situation, making viral disease epidemics more difficult to control. The increase of temperatures, greenhouse gas concentration and meteorological phenomena are affecting directly and indirectly in global agricultural crops, causing alterations in cultivation systems, types of crops grown, and the abundance and distribution of cultivated and weed virus reservoir hosts and virus vectors (Canto *et al.*, 2009; Jones 2009; Jones and Barbetti, 2012; Jones and Naidu, 2019; Jones 2021).

Unlike animals, plants are sessile organisms and not significant transmitters of viruses except for some examples of pollen transmission or seed, and the human-mediated dispersal. Then, most plant viruses rely on biological vectors for their spread and survival, ensuring efficient transmission from plant to plant. Among all known vectors, insects represent by far the most dominant group, transmitting around 65% of described plant viruses (Ng and Falk, 2006; Hogenhout *et al.*, 2008; Fereres 2015; Trebicki 2020). Members of the genus *Begomovirus*, such as African cassava mosaic virus (ACMV) or Tomato yellow leaf curl virus (TYLCV) are transmitted by insect vectors and they are among the most devastating and widespread pathogens in tropical and subtropical food crops, causing substantial yield losses in cassava crops in sub-Saharan Africa and tomato plants in the Indian subcontinent, respectively (Navas-Castillo *et al.*, 2011; Loebenstein and Thottappilly, 2013; Rojas *et al.*, 2018; Jones and Naidu, 2019). Moreover, the polyphagous nature of many insect vectors, combined with plant viruses that are generalists and can infect a wide range of host species, contributes to the frequent occurrence of mixed infections (Syller 2012; Moreno and López-Moya, 2020). Sweet potato viral disease (SPVD) is a good example of the consequences of a mixed virus infection between a potyvirus, Sweet potato feathery mottle virus (SPFMV), and the crinivirus Sweet potato chlorotic stunt virus (SPCSV). In this case, when both viruses encounter in the same plant, result in a synergistic interaction causing losses up to 90% of the crop yield (Karyeija *et al.*, 2000; López-Moya and Moreno, 2020).

Phytoviruses employ different strategies to aid in their dispersal and many of these strategies are complex and often specific to the virus, vector and host plant species, as well as influenced by environmental conditions. Despite their importance, the mechanisms underlying these interactions remain poorly understood, highlighting the need for a more comprehensive understanding of plant virus epidemiology to enable effective management now and in the future (Trebicki *et al.*, 2020). Since the study of

plant viruses began, “plant virus disease epidemiology” has been defined in various ways, including “the complex association between a virus and its host plant resulting in disease, and the factors that influence spread within the host plant population” (Wilson 2014; Jones 2021). Thus, to understand and manage plant virus diseases effectively, it is essential to study virus-host-vector pathosystem within its specific agroecological situation. In the case of vector-borne viruses, numerous intrinsic and extrinsic factors influence the trilateral interactions between host plants, viruses and insect vectors such as the mode of transmission (persistent or non-persistent), the diversity of vector species or host plants, the nature of infections: single or mixed, the ability to insect-borne viruses to manipulate host plant phenotypes and vector behaviour to improve their transmission, among others. In addition to the previous aspects, climate change is increasingly modulating the interactions between pathosystem components, making the study more complex and challenging for researchers. Needless to say, when no vector is involved, the disease triangle become less complex, simplifying epidemiological analysis (Jones and Naidu, 2019).

Traditional control methods including the use of chemical pesticides against insect vectors are facing important regulatory limitations and in parallel becoming less effective for controlling plant virus transmission due to the development of resistance among insect populations. Therefore, new approaches that integrate the understanding of molecular vector-virus interactions and the development of resistant crop varieties can offer robust combined protection (Aranda and Freitas-Astúa, 2017).

1.2.1.1 The family *Potyviridae*

The family *Potyviridae* composes the largest taxonomic group of plant-infecting viruses with RNA genomes, only second after the family *Geminiviridae* (DNA genomes). It includes about 20% of the known plant viruses and is responsible for more than half the viral crop damage in the world (López-Moya and García, 2008). According to the ICTV, the *Potyviridae* family currently contains around 259 definite species grouped in thirteen different genera: *Arepavirus*, *Bevemovirus*, *Brambyrius*, *Bymovirus*, *Celavirus*, *Ipomovirus*, *Macluravirus*, *Phragmivirus*, *Poacevirus*, *Potyvirus*, *Roymovirus*, *Rymovirus*, and *Tritimovirus*, while twenty-eight species remain still unassigned (Table I1) (<https://ictv.global/report/chapter/potyviridae>).

Introduction

Table 11. List of genera within the *Potyviridae* family, indicating their corresponding type member species and natural vector.

Genus	Type member species	Vector
<i>Arepavirus</i>	Areca palm necrotic ringspot virus (ANRSD)	Unknown
<i>Bevemovirus</i>	Bellflower veinal mottle virus (BVMoV)	Unknown
<i>Brambyvirus</i>	Blackberry virus Y (BVY)	Unknown
<i>Bymovirus</i>	Barley yellow mosaic virus (BaYMV)	Plasmodiophorids
<i>Celavirus</i>	Celery latent virus (CeLV)	Unknown
<i>Ipomovirus</i>	Sweet potato mild mottle virus (SPMMV)	Whiteflies
<i>Macluravirus</i>	Chinese yam necrotic Mosaic virus (CYNMV)	Aphids
<i>Phragmivirus</i>	Common reed chlorotic stripe virus (CRCSV)	Unknown
<i>Poacevirus</i>	Triticum mosaic virus (TriMV)	Mites
<i>Potyvirus</i>	Potato virus Y (PVY)	Aphids
<i>Roymovirus</i>	Rose yellow mosaic virus (RoYMV)	Eriophyd mites (lack of experimental confirmation)
<i>Rymovirus</i>	Ryegrass mosaic virus (RGMV)	Eriophyd mites
<i>Tritimovirus</i>	Oat necrotic mottle virus (ONMV)	Eriophyd mites

The genome of potyviruses consists of a monopartite positive-sense single-strand RNA molecule (+ssRNA) ranging from 8.2 to 11.5 kilobases (kb) and encapsidating within 650-950 nm flexible filamentous virus particles. As an exception member, *Bymovirus* genus exclusively possess a bipartite genome being encapsidated separately in two distinct modal lengths of 250-300 nm and 500-600 nm. Morphologically, *Potyviridae* virions share the same structural composition where multiple copies of identical CP subunits (95% of the particle) wind helically surrounding the viral RNA genome (5% of the particle) (Valli *et al.*, 2021; Inoue-Nagata *et al.*, 2022).

The genome of the monopartite potyvirids is based on a +ssRNA, where the 5' end of the genomic RNA presents a terminal protein (VPg) and the 3' end is polyadenylated. The RNA comprises a large Open Reading Frame (ORF) that spans the whole genome and codes for a polyprotein of a significant size (340-370 kDa), flanked by two relatively short 5' and 3' untranslated (UTR) regions, apart from an unusually larger 5'UTR in poaceviruses. Once translated during the infection process, the polyprotein generates a

set of mature and multifunctional proteins after ongoing an autoproteolytic processing cascade (Valli *et al.*, 2021). At least eight different protein products are conserved in *Potyviridae* family members, encompassing from middle to the C-terminus of polyprotein genome, including 8 mature gene products: P3, 6KI, CI, 6K2, VPg, NIa-Pro, NIb and CP. Additionally, the existence of a common short ORF embedded within the P3 cistron codes, through transcriptional slippage, and generates two more protein products termed P3N-PIPO and P3N-ALT (Chung *et al.*, 2008; Valli *et al.*, 2021). Unlike the central and C-terminus regions of the genome, the N-terminal region exhibits greater variability among the genera within the family. P1 is the first protein of the polyprotein and is the least conserved proteinase among the potyvirus (Fig I.2) (Adams *et al.*, 2005b; Valli *et al.*, 2007; Pasin *et al.*, 2022). Around 10% of the recognized potyvirus species lack P1; for instance, arenaviruses, beynoviruses and macluraviruses do not encode a P1 or P1-like protein, whereas other genera, including *Bymovirus* genus, do encode this protein divided in two products, P2-1 and P2-2 (Fig I.2). Another difference in the genome of some ipomoviruses is the absence of the second gene product termed Helper Component protease (HCPPro) and its replacement by an extra P1-like proteinase (P1b), as in the case of Cucumber vein yellowing virus (CVYV) (Fig I.2). Interestingly, in other ipomoviruses such as Cassava brown streak virus (CBSV) an extra gene product similar to host pyrophosphatases denominated Maf1/HAM1-like pyrophosphatase (HAM1) has been found inserted between NIb and CP (Mbanzibwa *et al.*, 2009; Valli *et al.*, 2015) (Fig I.2). Also, another peculiarity found in the bromovirus type virus, Blackberry virus Y (BVY) and the potyvirus Endive necrotic mosaic virus (ENMV), is the presence of an Alkylation B domain within the P1 cistron (Susaimuthu *et al.*, 2008; Pasin *et al.*, 2022). Finally, sweet potato-infecting potyviruses encode for an additional gene product, a pretty interesting sweet potato potyviral ORF (PISPO) embedded in the P1 cistron, giving rise to a frameshift protein, the P1N-PISPO, again produced by the same mechanism as the transframe P3N-PIPO (Rodamilans, *et al.*, 2015; Mingot *et al.*, 2016; Untiveros *et al.*, 2016).

Introduction

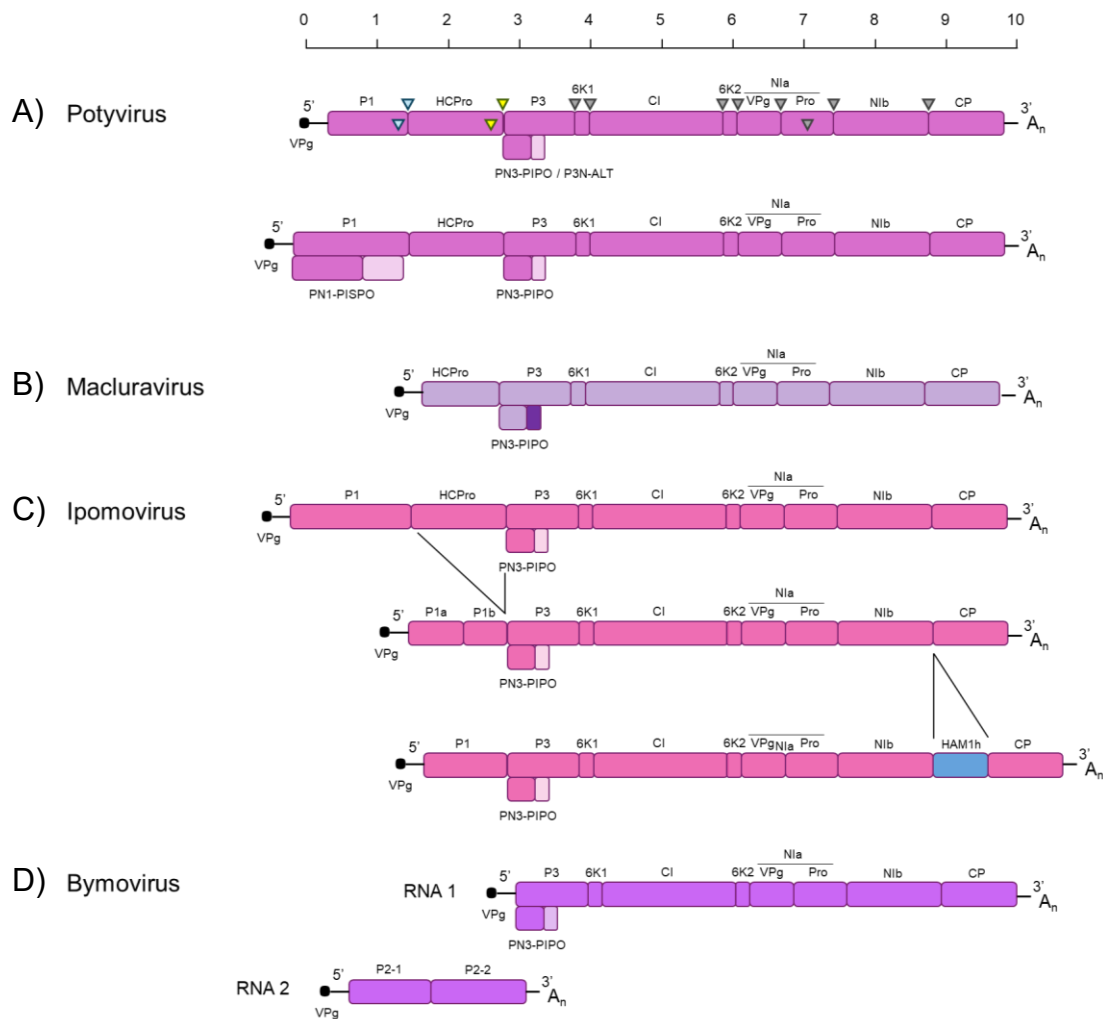


Figure I2. Schematic representation of the genomic organization of selected genera in the *Potyviridae* family (*Potyvirus*, *Macluravirus*, *Ipomovirus*, and *Bymovirus*.) Viral RNA is depicted as a solid line, with VPg linked to the 5' end and a poly(A) tail at the 3' end. Mature proteins are shown as labeled boxes; triangles indicate proteolytic cleavage sites (color-coded by protease in the first example). Products generated by polymerase slippage at conserved motifs (G_2A_6 and related) are shown below other products to indicate their different frame. A) *Potyvirus* genomes: PVY (top) and SPFMV (bottom). B) *Macluravirus*, lacking the P1 cistron. C) *Ipomovirus*: SPMMV, CVYV, and CBSV, with HCPPro present only in SPMMV, while CVYV encode P1a and P1b, and CBSV with an additionally encoded HAM1h between Nib and CP. D) *Bymovirus* (BYMV), with a bipartite +ssRNA genome encoding two polyproteins; RNA2 produces P2-1 and P2-2. Structures of other minority genera (*Arepavirus*, *Bevemovirus*, *Brambyvirus*, *Celavirus* and other cases of individual unassigned viruses can be consulted at <https://ictv.global/report/chapter/potyviridae/potyviridae>).

The genus *Potyvirus*

Potyvirus represents the largest genus within the family *Potyviridae*, currently comprising 215 described species distributed worldwide (<https://ictv.global>). Potyviruses can infect a wide range of plant species across numerous botanical families. Several members rank among the top ten most economically significant plant viruses due to their ability to

cause devastating diseases in crops of high agronomical importance globally (Yang *et al.*, 2021).

Virions contain a +ssRNA molecule of 9.7-11 kb that is encapsidated by several hundreds of CP subunits approximately 2000 CPs per virion. These subunits assemble into flexuous filaments with helical symmetry of around 680-900 nm in length and 11-13 nm in diameter. Thus, the virion composition is predominantly protein-based, with 95% of the particle weight consisting of CP and the rest corresponding to the viral RNA (5%).

Inside the infected cells, the viral RNA of potyviruses is uncoated and translated into a single polyprotein further proteolytically cleaved into up to 10 mature proteins by viral-encoded proteinases. In most of the cases, the resulting functional products include the P1, HCPro, P3, 6K1, CI, 6K2, NIa-VPg, NIa-Pro, NIb and the CP. As already mentioned in the previous section, there are two additional transframe products, P3N-PIPO and P3N-ALT, which are produced from translation of RNA variants generated by a +1 or -1 slippage of the viral polymerase in conserved G₂A₆ nucleotide motifs located inside of P3 genomic region. These partially frameshifted or truncated proteins share the N-terminal region of the P3 cistron (Chung *et al.*, 2008; Revers and García, 2015; Valli *et al.*, 2018). Interestingly, only potyviruses infecting sweet potato plants encode for an additional gene product, the P1N-PISPO. This protein corresponds to the first N-terminal gene product of the polyprotein within the P1-coding region and result from RNA variants with a G₂A₇ in the sequence, generated by polymerase slippage in the G₂A₆ conserved motif (Rodamilans *et al.*, 2015). The functionalities of the individual proteins are outlined below, following their sequential order from the N- to C-terminus:

The P1 protein functions as a serine protease that self-cleaves at its C-terminus, thereby releasing itself from the viral polyprotein (Verchot *et al.*, 1991). Among potyvirus proteins, P1 exhibits the great divergence in both length and amino acid sequence. Moreover, this protein is extremely variable in size, and its N-terminus is hypervariable, intrinsically disordered and dispensable for its proteolytic activity (Valli *et al.*, 2007; Pasin *et al.*, 2014). P1 contributes to viral multiplication, *in trans*, specifically, stimulating the genome amplification (Verchot and Carrington, 1995a, 1995b; Revers and García, 2015). Additionally, this protein has been shown to enhance the pathogenicity of heterologous plant viruses during synergistic interactions (Pruss *et al.*, 1997; Valli *et al.*, 2007). P1 interacts with several potyviral proteins involved in the viral replication process, including HCPro (Merits *et al.*, 1999; Rajamäki *et al.*, 2005), and it has been shown to increase the pathogenicity of heterologous plant viruses during synergistic interactions (Pruss *et al.*, 1997; Valli *et al.*, 2007). There is evidence suggesting that when P1 is positioned

Introduction

upstream of HCPro *in cis*, it can strengthen the suppressor activity of the protein HCPro (Kasschau and Carrington, 1998; Anandalakshmi *et al.*, 1998; Valli *et al.*, 2007). However, more recent findings relate this effect with a P1-mediated enhancement of HCPro translation, independently of the RNA silencing (Tena Fernández *et al.*, 2013; Pasin *et al.*, 2014).

P1N-PISPO transframe product is generated by transcriptional slippage and is embedded within P1 genomic region. This protein is similar in size and partially in sequence to P1, as it consists of the N-terminal region of P1 (P1N) merged to the PISPO sequence. Functional analysis performed by previous members of our group demonstrated that P1N-PISPO is able of suppressing the RNA silencing pathway (Mingot *et al.*, 2016; Untiveros *et al.*, 2016). However, despite these findings, the precise biological role of this protein during viral infection remains unclear, and further studies are required to fully elucidate its function (Rodamilans *et al.*, 2021).

HCPro protein is probably one of the most studied potyviral protein and it can be safely considered a *Potyvirus* core component (Pasin *et al.*, 2022). HCPro is involved in multiple functions including polyprotein processing, virus transmission mediated by aphid vectors and suppression of host antiviral RNA silencing (Syller 2006; Valli *et al.*, 2018). Like P1, this protein autocatalytically cleaves its C-terminus, in this case at the junction between the HCPro C-terminus and P3, thanks to its protease activity (Carrington *et al.*, 1989; Valli *et al.*, 2015). In addition, multitasking HCPro protein has been shown to interact with many viral and host proteins, however because of its functional relevance, the most studied role has been the interaction with the CP protein in the aphid transmission (Revers and García, 2015). As previously mentioned, several studies have shown that HCPro from members of the genus *Potyvirus* generally have RNA silencing suppression (RSS) activity, whereas in other genera, this function relies on other viral proteins (Giner *et al.*, 2010; Tatineni *et al.*, 2012; Young *et al.*, 2012; Mingot *et al.*, 2016; Untiveros *et al.*, 2016). Different mechanisms by which HCPro acts as an RNA-silencing suppressor have been proposed in Valli *et al.*, 2018.

The P3 protein has as-yet undefined functions. Nevertheless, it is required for viral replication (Klein *et al.*, 1994), viral pathogenicity and symptom development (Revers and García, 2015). The presence in all potyvirids of the overlapping P3N-PIPO gene, which plays a direct role in cell-to-cell movement (Cui *et al.*, 2017) links this region with additional functions.

The 6KI peptide has a relevant role in potyviral infection contributing to the regulation of the viral multiplication (Waltermann and Maiss, 2006; Cui and Wang, 2016). Proteins

encoded within the P3-6KI region have been implicated in determining host range and pathogenicity (Valli *et al.*, 2021).

The CI is the largest potyviral protein and forms cylindrical inclusions pinwheel-shaped in the cytoplasm of cells infected with potyviruses. It exhibits ATPase and RNA helicase activities, actively taking part in the process of replication (Fernández *et al.*, 1997). Several studies have shown that CI is able to associate with other viral proteins such as P3N-PIPO while aiding the cell-to-cell viral movement through plasmodesmata (Wei *et al.*, 2010b; Movahed *et al.*, 2017; Wang 2021).

6K2 is a trans-membrane protein which plays a key role in the formation of endoplasmic reticulum-derived vesicles during viral replication (Restrepo-Hartwig and Carrington, 1994; Movahed *et al.*, 2019).

The NIa protein is partially processed resulting in two functionally cistrons, the NIa-VPg and the protease NIa-Pro. The VPg protein is covalently attached to the 5'-end of the genome and interacts with most of the potyviral proteins (Jiang and Laliberté, 2011; Elena and Rodrigo, 2012). NIa-VPg is involved in numerous functions including counteract antiviral RNA silencing, interacting with the eukaryotic initiation factor eIF4E (Cheng and Wang 2017; Saha and Mäkinen 2020), playing roles in replication possibly by priming the RNA synthesis (Valli *et al.*, 2015) and has ATPase activity also when bound to the NIa-Pro domain (Mathur and Savithri, 2012; Valli *et al.*, 2021). The NIa-Pro is the third proteinase responsible for the proteolytic processing of the central and C-terminal regions of the potyviral polyprotein (Adams *et al.*, 2005b). Furthermore, NIa-Pro has been also shown to exhibit non-specific double-stranded DNA degradation activity in host cells, having a regulatory mechanism that modulates host gene expression during the viral infection (Anindya and Savithri, 2004; Revers and García, 2015).

The NIb protein is the RNA-dependent RNA polymerase therefore responsible for potyviral replication (Hong and Hunt, 1996). Together with NIa, its name refers to the forms of crystalline inclusions, mainly found in the nucleus, as predicted by the presence of specific signals in its sequence (Kassanis 1939; Knuhtsen *et al.*, 1974).

The CP is the last product of the viral polyprotein in the C-terminus and is primarily implicated in encapsidating and protecting the RNA genome. Apart from virion assembly and genome protection, CP also plays a role in cell-to-cell movement (Rojas *et al.*, 1997), long-distance movement, and aphid transmission through its interaction with HCPro (Blanc *et al.*, 1997).

Introduction

Potyvirus are naturally spread by aphids in a nonpersistent manner, meaning that vectors can acquire and transmit virus particles in the range of seconds to minutes. Several studies have demonstrated that aphid behaviour during the initiation of early probing, characterized by frequent intracellular puncture made during the first minutes after landing, are correlated with efficient acquisition and lead to transmit potyviruses with high efficiency (Fernández-Calvino *et al.*, 2006). In 1974, and based in the results described at that time, Govier and Kassanis suggested a molecular mechanism by which HCPro and CP participate in the viral transmission process through interactions among them and with the stylet lining surfaces, naming this phenomenon 'bridge hypothesis' (Govier and Kassanis, 1974b; Valli *et al.*, 2018). Two independent domains of the HCPro are required for the bridge hypothesis to occur: a KITC motif at the N-terminal region of HCPro would participate in binding to unknown structure on the aphid stylet facilitating virus transmission (Atreya and Pirone, 1993; Huet *et al.*, 1994) and the PKT domain in the central region participate in the direct interaction between a highly conserved 'DAG' motif within the CP and essential for vector transmission (Atreya *et al.*, 1991, 1995; Huet *et al.*, 1994; Peng *et al.*, 1998, Blanc *et al.*, 1997; Blanc *et al.*, 1998). Predictions based on the bridge hypothesis were confirmed in several studies including the identification of HCPro retention sites in aphid stylets (Moreno *et al.*, 2012; Wang *et al.*, 1999) and the location of HCPro in a protruding tip at one end of the virion (Manoussopoulos *et al.*, 2000; Torrance *et al.*, 2006; Valli *et al.*, 2018).

Sweet potato feathery mottle virus (SPFMV)

SPFMV (genus *Potyvirus*, family *Potyviridae*) is one of the most widespread viruses affecting sweet potato (*Ipomoea batatas*) worldwide (Clark *et al.*, 2012). Its presence was first documented in East Africa during the 1950s (Sheffield, 1957), and has since been reported in nearly all tropical and subtropical regions where the crop is cultivated. The host range of SPFMV is limited to members of *Convolvulaceae* family, including *Ipomoea* spp. and some additional wild plants, which can serve as virus reservoirs when occur around sweet potato crop fields (Tugume *et al.*, 2008). As member of potyviruses, SPFMV can be transmitted by aphids in a nonpersistent manner, including *Myzus persicae*, *Aphis gossypii*, *A. craccivora*, and *Lipaphis erysimi*. Furthermore, it has been reported that the virus can be transmitted mechanically to various *Ipomoea* spp., and also to *Nicotiana benthamiana* and *Chenopodium amaranticolor* (Loebenstein 2015), although these later experimental hosts can be isolate specific. Symptoms in sweet potato plants infected by SPFMV are mild or even non-present; they consist of chlorotic spotting, vein banding, green venial patterns, ringspotting or lack of purple borders, depending on the pigmentation of the sweet potato cultivar (Valverde *et al.*, 2007) (Fig.

13). Nonetheless, when SPFMV is found in con-infection with the crinivirus SPCSV, the symptomatology is aggravated, causing the designated sweet potato virus disease (SPVD) (Karyeija *et al.*, 2000; Gibson and Kreuze, 2015).

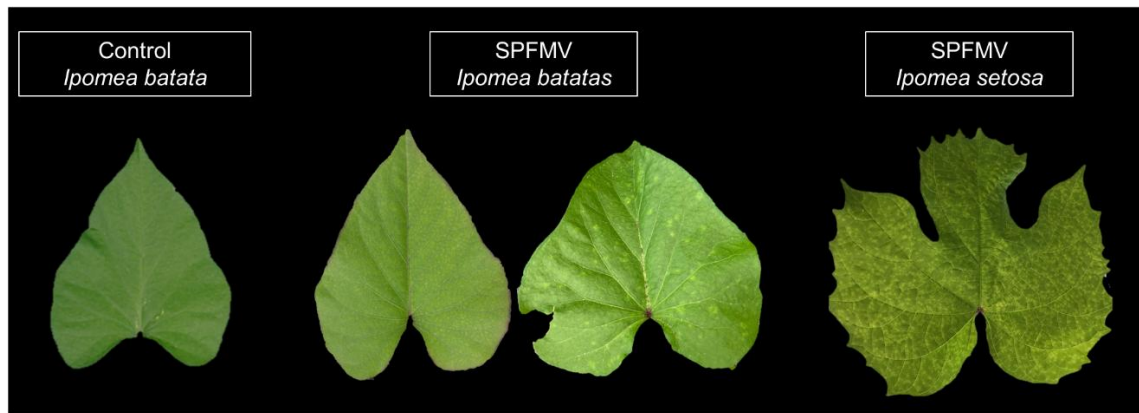


Figure 13. Symptoms of SPFMV infection in leaves of *I. batata* (center) and *I. setosa* (right). A non-infected control leaf is shown for comparison (left).

SPFMV reference genome (NC_001841.1) comprises 10820 nucleotides, excluding the polyadenylated tail at the 3' end, and encodes the canonical potyviral polyprotein: P1, HCPro, P3, 6K1, CI, 6K2, NIa-VPg, NIa-Pro, NIb and CP (Sakai *et al.*, 1997). As previously described, sweet potato-infecting viruses generate two additional ORFs via polymerase slippage at G₂A₆ motifs, resulting in the translation of P1N-PISPO and P3N-PIPO. P1N-PISPO protein was initially predicted through bioinformatic analysis (Clark *et al.*, 2012; Li *et al.*, 2012) and subsequently validated by RNA sequencing data (Rodamilans *et al.*, 2015) for most potyviruses related to SPFMV and infecting sweet potato hosts, with the exception of Sweet potato latent virus (SPLV) (genus *Potyvirus*, family *Potyviridae*) (Wang *et al.*, 2013). Previous members of our research group explored the functional characterization of SPFMV P1N-PISPO, demonstrating that P1N-PISPO can suppress the plant RNA silencing machinery, thereby assuming a role typically attributed to HCPro, which until then had been almost universally regarded as the potyviral RSS protein (Mingot *et al.*, 2016; Untiveros *et al.*, 2016).

The P1 of SPFMV isolates is larger than the equivalent protein in other species within the *Potyvirus* genus. Its C terminal region containing the Proteinase domain is well-conserved among members of the potyvirus genus, however its N-terminus and central regions used to be hypervariable and only shares some homology with other members of the SPFMV lineage and with the P1 of the ipomovirus Sweet potato mild mottle virus (SPMMV) (Untiveros *et al.*, 2010; Li *et al.*, 2012). SPFMV isolates have been initially grouped into four different strains: East African, russet crack (RC), ordinary (O) and common (C), but subsequently the considered C strain members were reclassified as a

Introduction

separate virus specie termed as Sweet potato virus C (SPVC) (Kreuze *et al.*, 2000; Untiveros *et al.*, 2010). Alongside SPFMV, viruses SPV2, SPVG and SPVC are considered as distinct species of the *Potyvirus* genus. These viruses form a separate phylogenetic lineage and share highly homologous sequences, likely resulting from multiple recombination events among them (Untiveros *et al.*, 2008).

Sweet potato virus 2 (SPV2)

Sweet potato virus 2 (SPV2) was first isolated in Taiwan (Rossel and Thottappilly, 1988), and since then, its presence has been confirmed in many sweet potato growing areas such as Nigeria, United States, Australia, Spain or China (Souto *et al.*, 2003; Tairo *et al.*, 2006; Trenado *et al.*, 2007; Ateka *et al.*, 2007; Perez-Egusquiza *et al.*, 2009; Li *et al.*, 2012; Almeyda *et al.*, 2013). Sequence analysis of its 3' genomic region, together with its biological and serological properties, confirmed SPV2 as a distinct specie from SPFMV and SPVG (Tairo *et al.*, 2006). It belongs to the *Potyvirus* genus (family *Potyviridae*) and exhibits the molecular features characteristic of potyviral members; its genome consists of a positive-sense single-strand RNA that encodes the canonical potyviral polyprotein (Ateka *et al.*, 2004; Li *et al.*, 2012; Wei *et al.*, 2024). In addition, Mingot *et al.*, (2016) confirmed the expression of the transframe protein P1N-PISPO in SPV2, similar to what has been observed in other potyvirus infecting- sweet potato (Rodamilans, *et al.*, 2015). In terms of biological properties, the host range of SPV2 has been confirmed for several *Ipomoea* spp (*I. batatas*, *I. coccinea*, *I. nil*, *I. purpurea*, *I. setosa*) and *Nicotiana* spp (*N. benthamiana*, *N. clevelandii*, *N. hesperis*, *N. occidentalis* P1 and *N. occidentalis* ssp. *obliqua*) (Ateka *et al.*, 2004). Symptoms developed by SPV2 infected plant are mild, including mottling, vein clearing and leaf malformation and curling, the latter two being generally observed in *N. benthamiana*. Nonetheless, although SPV2 causes mild or no symptoms in single infections, its impact becomes significant in co-infections with SPCSV, leading to severe yield losses. Its role in SPVD has often been underestimated due to the widespread presence of SPFMV (Untiveros *et al.*, 2008; Tairo *et al.*, 2006). Phylogenetic analyses placed SPV2 within the SPFMV lineage, with around 65% sequence identity, often causing serological cross-reactions (Li *et al.*, 2012). Due to the high sequence similarity, particularly in the CP-terminal region, early studies may have led to misidentification of SPV2 as SPFMV in mixed infection (Souto *et al.*, 2003; Untiveros, *et al.*, 2008).

Similar to other potyviruses, SPV2 was predicted to be transmitted in a non-persistent manner by aphids from plant to plant.

1.2.1.2 The family *Closteroviridae*

The family *Closteroviridae* comprises viruses with the longest RNA genomes among all known plant viruses, ranging from 13 to 19.5 kb in length (Dolja *et al.*, 2006). According to Rubino *et al.*, (2025) the recent inclusion of *Pyrus* virus A in the genus *Velarivirus* increases the number of recognized species in the family to 58. These species are currently classified into seven different genera, *Ampelovirus*, *Bluvavirus*, *Closterovirus*, *Crinivirus*, *Menthavirus*, *Olivavirus*, and *Velarivirus* based on their biological traits, such as semi-persistent mode of transmission by specific vectors, virion length, number of ORFs, organizations and genomic RNAs. Their classification also reflects phylogenetic reconstructions based on conserved protein domains present in members of the family *Closteroviridae*, including the presence of the HSP70 homolog, RNA dependent RNA-polymerase and coat protein (Martelli *et al.*, 2012; Fuchs *et al.*, 2020). The genomes of *Closteroviridae* members may be mono- bi- or tripartite, consisting of a positive-sense single-stranded RNA that accounts for approximately 5-6% of the total particle weight. The 5'-end of the genome is probably capped, and the 3'-end is not polyadenylated and typically lacks tRNA-like structures, but it can contain several hairpin structures (Agranovsky 2016). The 5'-terminal region encompasses overlapping ORFs coding for the replication-associated proteins. Translation of these genes involving +1 ribosomal frameshifting, resulting in production of different mature products. In contrast, the 3'-proximal genes are expressed through a set of 3'-coterminal subgenomic RNAs. A distinctive feature of these RNA viruses is the presence of the gene for a homolog to the cellular heat-shock proteins HSP70 chaperones (termed HSP70h), and the production of duplicated genes or genes fragments, such as major and minor CPs, termed as CP and CPm, respectively (Agranovsky *et al.*, 1995; Agranovsky *et al.*, 2016).

Closteroviridae virions are flexuous, filamentous particles constructed with a helical symmetry, characteristically ranging from 650 nm to 2,200 nm in length and 10-13 nm in diameter (<https://ictv.global/report/chapter/potyviridae/potyviridae>). Two types of CP are the most abundant structural components involved in closteroviral particle assembly. The major CP coats around 95% of the viral genomic RNA, forming the 'main body' of the filamentous particles, whereas the CPm forms a 'tail' that includes the 5'-terminal portions of the genome. In certain viruses as with Beet yellows virus (BYV) and Citrus tristeza virus (CTV), additional proteins (e.g., HSP70h and p60) are incorporated at one extremity of the virion, forming a distinctive structure referred to as 'rattlesnake' or 'heterodimeric' (Martelli and Candresse, 2001; Agranovsky 2021).

Members of *Closteroviridae* family are able to infect a wide range of hosts, including herbaceous, fruits and woody plants (e.g., cucumber, tomato, sweet potato, blackberry,

Introduction

pineapple, sugar beets, citrus, plum, cherry, pistachio, grapevine) and can cause either acute or chronic infections in economically important crops, estimated to millions of dollar losses annually. For instance, Citrus tristeza virus (CTV) is considered the most destructive viral disease affecting citrus plantations worldwide, while Grapevine leafroll associated viruses can markedly impair vine health and significantly reduce grape and wine quality (Martelli 2019). However, the natural and experimental host range of individual species is generally narrow, with notable exceptions found within the genus *Crinivirus*. Disease symptoms may be highly variable according the host species; they can induce mottling, yellowing and reddening of the leaves, stunting or even symptomless infections such as in pineapple cultivars. Few members of the family *Closteroviridae* are transmissible by mechanical inoculation, most of them are mainly transmitted plant-to-plant in a semipersistent manner by insects. Ampeloviruses are transmitted by mealybugs, while closteroviruses and criniviruses are transmitted by aphids and whiteflies, respectively. Transmission for the genus *Velarivirus* are still unknown, however in recent studies has been shown that the Areca palm velarivirus 1 (APV1), identified as a primary causative agent of the Yellow Leaf Disease (YLD), is also transmitted by mealybugs, specifically by *Ferrisia virgata* and *Pseudococcus cryptus* (Zhang *et al.*, 2022; Zhao *et al.*, 2024). So far, APV1 is the sole member of velarivirus with known transmission vectors.

The biological and molecular complexity of the *Closteroviridae* family stems from its modular evolutionary history, which involved a series of successive modifications. These include genome segmentation, gene duplication, sequence loss through deletion, and the acquisition of genetic material from external sources such as other viruses or host organisms, including plants (Martelli 2019).

The genus *Crinivirus*

To date, the genus *Crinivirus* comprises 14 described species. It is unique among *Closteroviridae* family for being exclusively transmitted by whiteflies of the genera *Trialeurodes* and *Bemisia* (Ng and Falk, 2006). As others closterovirids, criniviruses are transmitted in a semipersistent manner: they are phloem-restricted, and long acquisition periods (24-48h) are required for effective transmission. These virus-vector interactions are highly specific: while some criniviruses are transmitted only by a single whitefly species, others can be transmitted by diverse species across different genera. Indeed, the persistence and specificity of the vector transmission have been used as taxonomic criteria for species differentiation (Tzanetakis *et al.*, 2013). The severity and geographic distribution of diseases caused by members of this genus largely depend on the

abundance and dispersal of their vector, which is particularly concern in tropical and subtropical regions.

Their genome is divided in two linear +ssRNA genomes of 15.6-17.9 kb, with the only exception to Potato yellow vein virus (PYVV) which comprises three +ssRNA segments (Livieratos *et al.*, 2004). All molecules are needed for infectivity and are encapsidated separately into flexuous filaments averaging 650-900 nm in length (Kiss *et al.*, 2013). RNA1 encompasses genes for the replication-related proteins, including RdRP, whereas RNA2 contains several gene products with diverse functions. Lettuce infectious yellows virus (LIYV), as a type member of the *Crinivirus* genus, has been deeply studied. Numerous experiments have been performed on LIYV, helping to elucidate the mechanisms underlying vector retention sites and virus transmission determinants (Tian *et al.*, 1999; Stewart *et al.*, 2010; Chen *et al.*, 2011).

Criniviruses infect mainly herbaceous hosts, including plants of several botanical families. Symptomatology caused by these viruses comprises from extensive chlorosis to yellow discoloration of the leaves, often accompanied by thickening, which primarily affects the older parts and gradually expands to the younger leaves, to slight or no symptomatology. Mild or lack of symptoms used to be found in crinivirus single infections; nonetheless, in co-infections with other unrelated viruses, the symptoms can be aggravated (Loebenstein 2015). A typical example is SPCSV, that in combination with potyvirus SPFMV or ipomovirus SPMMV, causes severe symptoms as a result of viral synergism (Moreno and López-Moya, 2020).

Sweet potato chlorotic stunt virus (SPCSV)

Sweet potato chlorotic stunt virus (SPCSV, genus *Crinivirus*, family *Closteroviridae*) is a widespread globally RNA virus, which infect mainly *Ipomoea* spp. but also it may infect other plants such as *Arabidopsis thaliana*, *N. tabacum*, *N. benthamiana* even *Solanum lycopersicum* (unpublished data from our laboratory). Symptoms vary depending on the host species and their geographically distribution. Slight vein yellowing or upward curling are common in SPCSV single infection, whereas in complex infection with SPFMV or other viruses yield losses increase to 50% and more (Gibson *et al.*, 1998; Untiveros *et al.*, 2007). This virus is phloem-limited and transmitted semi-persistently by several whitefly species: *B. tabaci*, *T. abutilonea* and *B. afer* (Loebenstein 2012).

SPCSV possess the second largest identified +ssRNA genome within *Closteroviridae* family, only after CTV (Kreuze *et al.*, 2002). RNA1 and RNA2 (9.4 and 8.2 kb, respectively) are encapsidated into separate particles of circa 900-1000 nm in length, forming the 'rattlesnake' structure previously mentioned. The RNA1 encompasses four

Introduction

ORFs; ORFs 1a and 1b encode for the replication module, (1a with conserved domains of the viral methyltransferase, helicase, and 1b being the RNA dependent RNA polymerase) while the ORF2 and 4 are the counter-defence of the virus (Fig. I4). The RNA silencing suppression system comprises at least two components: the p22, a 22-kDa protein, which shows no significant sequence similarity to any known protein, and the RNAase III homologue (Kreuze *et al.*, 2002, 2005; Cuellar *et al.*, 2009). In contrast, RNA2 contains up to seven ORF involved in diverse functions including virion assembly, virus cell-to-cell movement and vector-mediated dispersal (Cuellar *et al.*, 2008; Kreuze *et al.*, 2002).

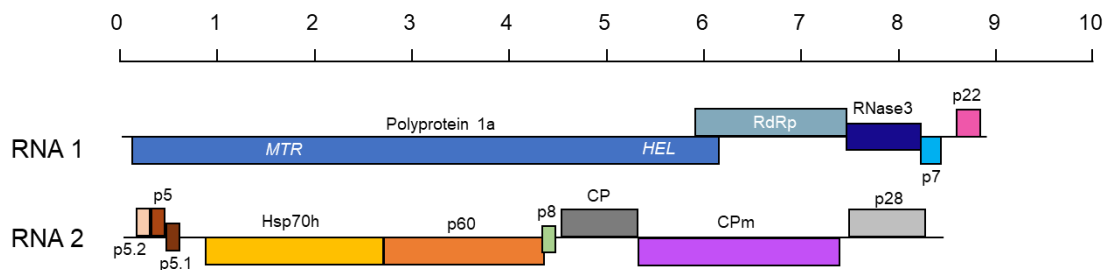


Figure I4. Schematic representation of the SPCSV genome organization. The genome consists of two +ssRNA components, RNA1 and RNA2, depicted as lines with open reading frames (ORFs) shown as boxes. ORFs positioned above, below, or along the line indicate different reading frames. Functional domains are indicated within boxes: MTR (methyltransferase), HEL (helicase), and RdRp (RNA-dependent RNA polymerase). Expression of specific gene products involves a +1 ribosomal frameshift and the production of subgenomic RNAs (not shown).

1.2.1.3 The family *Geminiviridae*

The family *Geminiviridae* is the largest as well as one of the most diverse family of viruses. Circa 520 species are comprised in fourteen different genera, based on their genome structure and organization, type of insect vector, host range and phylogeny. These genera are *Becurtovirus*, *Begomovirus*, *Capulavirus*, *Citlodavirus*, *Curtovirus*, *Eragrovirus*, *Grablovirus*, *Maldovirus*, *Mastrevirus*, *Mulcrilevirus*, *Opunvirus*, *Topilevirus*, *Topocuvirus*, *Turncurtovirus* (<https://ictv.global/report/chapter/geminiviridae/geminiviridae>). Geminivirids are responsible for significant losses on food, feed, and fiber crops worldwide (e.g., maize, wheat, cassava, cotton), particularly in most tropical and subtropical regions (Rojas *et al.*, 2018). The genus *Begomovirus* has the greatest number of species, and members have monopartite or bipartite genomes (Rojas *et al.*, 2005). *Begomovirus* are classified into Old World (OW) and New World (NW) groups, which differ in their geographical distribution, genome organization, and genetic diversity. OW begomoviruses generally exhibit greater genetic variability (Fortes *et al.*, 2025); and are predominantly monopartite, containing a single genomic DNA component (DNA-A),

although bipartite species such as Tomato leaf curl New Delhi Virus (ToLCNDV) have also been described (Zaidi *et al.*, 2017). In contrast, NW begomoviruses are mainly bipartite (Rojas *et al.*, 2018).

The genome of geminiviruses consist of circular single-stranded DNA which can be monopartite, with a DNA of ~2.5 kb-3.2 kb, or bipartite, which consist in two ssDNA molecules of ~2.5-2.6 kb in length (termed as DNA-A and DNA-B). Members of the *Begomovirus* genus can be either monopartite or bipartite, and the genome of monopartite begomoviruses closely resemble the DNA-A component of bipartite ones. All members of the family have a 5' noncoding intergenic region (IR) which is part of the origin of replication (Fig I5). Except for becurtoviruses and eragroviruses where the IR sequence is 5'-TAAGATTCC-3' (Yazdi *et al.*, 2008), the IR of all geminiviruses presents the conserved sequence TAATATTAC contained within a stem-loop structure and is commonly found in mono- and bipartite genomes. Moreover, the IR is embedded within a sequence defined as the common region (CR) which varies among geminivirus members but remains identical between the two genomic components of begomovirus, DNA-A and DNA-B (Lazarowitz and Shepherd, 1992; Fiallo-Olivé *et al.*, 2021). Geminivirus genomes are arranged with coding and non-coding regions; coding regions contain divergent transcription units that can be transcribed bidirectionally, differing in number according to the geminivirus genus, whereas non- coding regions contain regulatory sequences for replication and expression (Bonnamy *et al.*, 2023). Virus particles are twinned quasi-icosahedral structures ("geminated") resulting from the fusion of two icosahedral subunits. Each geminated particle (18-20 x 30-38 nm) is expected to contain a single DNA component for monopartite as well as bipartite species, or satellite components in certain cases (Hesketh *et al.*, 2018; Saunders *et al.*, 2020).

The genome structure varies according on the type of geminivirus and typically encodes six to eight proteins, that in some cases can be multifunctional, and play essential roles in essential steps of the viral cycle, including replication, movement, transmission, and also pathogenesis functions (Hanley-Bowdoin *et al.*, 2013). ORFs are designated using letters and numbers that indicate their positions on the viral (V) or complementary (C) strands, with the prefixes A or B to identify the genome component in bipartite viruses. DNA-A encodes in the complementary strand a replication- protein (ORF AC1, Rep), a transcriptional activator (ORF AC2, TrAP), a replication enhancer (ORF AC3, RE_n), and a C4 protein (ORF AC4, C4), and in the viral strand a capsid protein (ORF AV1, CP), and a putative movement protein (ORF AV2, MP, although this is absent in New World begomoviruses). DNA-B present in bipartite geminiviruses encodes a nuclear shuttling protein (ORF BV1, NSP) and a movement protein (ORF BC1, MP) (Fig I5). These are

Introduction

the canonical ORFs found in most members of the *Geminiviridae* family, however novel ORFs are being described, starting with the one termed “C5” identified in some mono- and bipartite begomoviruses (Li *et al.*, 2015), and more recently, several studies have provided evidence for the existence of additional ORFs (Gong *et al.*, 2021; Chiu *et al.*, 2022; Zhao *et al.*, 2022; Wang *et al.*, 2022; Liu *et al.*, 2023) potentially transforming our understanding of the entire geminivirus family.

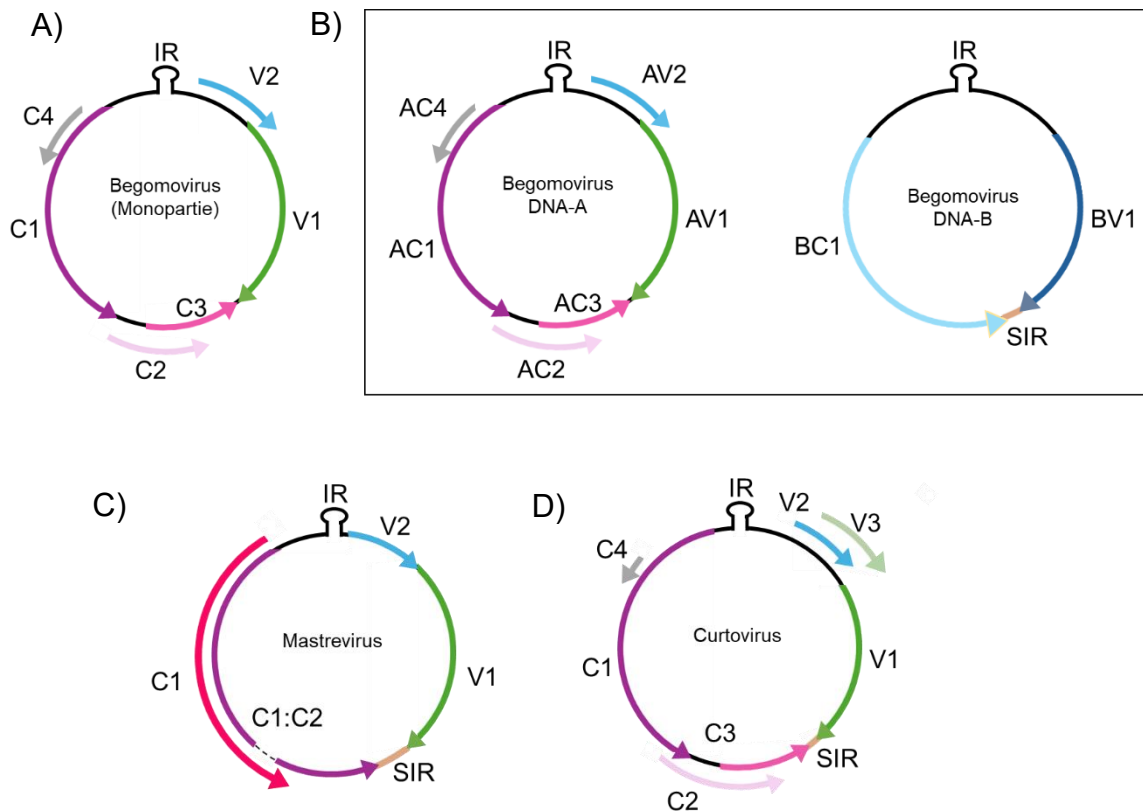


Figure I5. Genome organization of representative geminivirus genera. A) Begomovirus (monopartite), B) Begomovirus (bipartite, Old World), C) Mastrevirus, and D) Curtovirus. The genomes are bidirectionally transcribed and are color-coded according to the different open reading frames (ORFs). The intergenic region (IR) is indicated as a stem loop structure containing the conserved sequence that functions as the origin of replication. A short intergenic region (SIR) is also present between two of the mature products in the opposite transcripts.

Mastrevirus, *Curtovirus* and *Begomovirus* genera are the most well-characterized genus within *Geminiviridae* family, for this reason functionalities of their different proteins will be display next:

Some proteins are highly conserved across the family *Geminiviridae*, such as replication initiator protein (Rep). Rep (C1/AC1) is required for viral DNA replication by performing multiple specific functions during the rolling circle replication (RCR) process. Replication begins when the synthesis of the complementary-strand DNA (csDNA) is initiated within the CR, using the ssDNA as template. The resulting csDNA is converted into a double-

stranded (ds) intermediated which subsequently is replicated via the RCR (Rojas *et al.*, 2005; Fiallo- Olivé *et al.*, 2021). Rep recognizes and cleaves the conserved sequence between nucleotides T and A in bold in the IR (5'-TAATATT**AC**-3'), and it remains covalently linked to the 5'- phosphate end of the cleaved positive single-strand (Fontes *et al.*, 1994; Laufs *et al.*, 1995). The free 3'- OH extremity is released to serve as primer for the synthesis of the new complementary-strand by action of host polymerases. Rep also helps to unwind the dsDNA from the 3'- to the 5'-end of the csDNA by its helicase activity as the replication complex progresses. Once the complete full-length DNA is polymerized, the newly synthesized nonanucleotide sequence is recognized and cleaved by the Rep protein, then it joins the 3'- and 5'-ends of the new synthesized DNA constituting a free circular single-stranded DNA. When a free circular single-stranded DNA is released, a new csDNA initiation event is likely to occur. The newly formed csDNA can then re-enter the replication cycle, generating dsDNA and perpetuating replication either reusing the same Rep polymerase complexes or recruiting new Rep and polymerase complexes. In mastreviruses, a splicing event of the Rep transcript generated a truncated form, RepA, which functions as cell cycle regulator (Hanley-Bowdoin *et al.*, 2013). Finally, the DNA molecules would be encapsidated and/or move outside of the host cell (Hanley-Bowdoin *et al.*, 2013; Bonnamy *et al.*, 2023). Additionally, to the complete DNA molecules, large amounts of heterogenous sub- and extra-genome length geminivirus DNA (hdDNA) are generated during the RCR and used as template for the production of new viral DNA molecules in the infected cells (Preiss and Jeske, 2003).

C2, also known as AC2/AL2, acts as transcriptional activator protein (TrAP) activating virion sense gene expression, interfering with transcriptional gene silencing (TGS) by multiple mechanisms and as an efficient suppressor of posttranscriptional gene silencing (PTGS) (Voinnet *et al.*, 1999; Wezel *et al.*, 2002). TrAP contains an N-terminal basic domain, a C-terminal acidic domain implicated in the transcriptional activation, and also at the core region, a zinc-binding phosphoprotein domain which interacts with ssDNA in preference to dsDNA in a sequence-nonspecific manner (Hartitz *et al.*, 1997; Noris *et al.*, 1996; Sung and Coutts 1996; Wezel *et al.*, 2002). TrAP/C2 is also a transcription factor required for CP and NSP expression by bipartite begomoviruses (Brough *et al.*, 1992; Haley *et al.*, 1992; Groning *et al.*, 1994; Hartitz *et al.*, 1997; Hong *et al.*, 1996; Sunter and Bisaro 1991, 1992, 1997).

Replication enhancer protein (temerd "REn" or also known as C3/AC3) is involved in viral replication. In begomoviruses and curtoviruses, REn hugely enhances the viral DNA accumulation by interacting with plant cell transcription machinery (Selth *et al.*, 2005;

Introduction

Settlage *et al.*, 2005). It also can interact with Rep and host replication factors and be part of the replisome (Wu *et al.*, 2021).

C4 or AC4 in some viruses, is a protein coded by an embedded ORF within the C1/AC1 ORF. It functions as a suppressor of PTGS and in several viruses interact and silence the expression of shaggy related kinases, delaying the infection. However, C4 or AC4 proteins do not behave uniformly across geminiviruses: they can differ in the strength of their effects. For instance, in bipartite viruses, AC4 binds to and interfere with BIN2, activating the brassinosteroid pathway, whereas in monopartite viruses, BIN2-mediated phosphorylation of the C4 prevents BIN2 inactivation and thus maintains inhibition of the brassinosteroid pathway (Piroux *et al.*, 2007; Ascencio-Ibanez *et al.*, 2008; Dogra *et al.*, 2009; Mills-Lujan and Deom, 2010; Lozano-Durán *et al.*, 2011; Hanley-Bowdoin *et al.*, 2013). This difference highlights the importance of not assuming that all virus–host interactions are conserved.

CP (V1/AV1 ORF) forms the viral capsid, which plays a crucial role in the infection success of geminiviruses. Beyond its main structural function as protection of viral genome, the CP is involved in multiple processes including virus movement within the plant and determination of the insect vector specificity in plant-to-plant transmission process (Briddon *et al.*, 1989). Geminivirus particles typically consist of 110 copies of a single type of CP (each of symmetry $T=1$) assembly largely in two hemispheres (Hipp *et al.*, 2017; Hesketh *et al.*, 2018). Nevertheless, alternative particle morphologies have been reported, such as isometric or single particles in *Begomovirus* and *Mastrevirus* plant infections (Stanley and Townsend, 1985; Casado *et al.*, 2004), and even, a triple capsid structure isolated from a *Curtovirus* (Beet curly top virus, BCTV) infecting *Capsella bursa-pastoris* (Larsen et Duffus, 1983). The N-terminal region of the CP of several geminiviruses contains a DNA-binding domain that facilitates interaction with both ss- and dsDNA, in a sequence non-specific manner (Liu *et al.*, 1997a; Qin *et al.*, 1998; Ingham *et al.*, 1995; Palanichelvam *et al.*, 1998; Henle *et al.*, 2004). However, the presence of genomic DNA is essential as a structural scaffold to induce conformational changes in the CP, which are required for the proper assembly of viral particles (Hesketh *et al.*, 2018). Also, in monopartite viruses, CP functions as nuclear shuttle protein (NSP) (Priyadarshini *et al.*, 2011).

All monopartite and some bipartite geminiviruses (only in Old World begomoviruses) encode a small ORFs upstream of CP gene, termed V2/AV2 (Rojas *et al.*, 2005). This protein is multifunctional and serve to several functions including anti-defence proteins to inhibit PTGS, host cell death induction and determinant of pathogenicity (Glick *et al.*,

2008; Mubin *et al.*, 2010; Amin *et al.*, 2011; Zhang *et al.*, 2012). V2 also provides the movement function for monopartite viruses (Priyadarshini *et al.*, 2011).

Finally, and previously mentioned, DNA B of bipartite geminiviruses contains two additional proteins: nuclear shuttle protein (NSP) and movement protein (MP) which are involved in the DNA viral movement into and out of the nucleus and between cells (Gilbertson *et al.*, 2003; Krenz *et al.*, 2012; Patil *et al.*, 2022; Torralba *et al.*, 2024).

A distinctive feature of geminiviruses is their ability to associate with circular ssDNA satellites molecules, particularly alphasatellites, betasatellites or deltasatellites. Begomoviruses represent the most extensively documented group in this context, with numerous studies confirming their consistent association with both types of satellites. Nevertheless, recent studies have revealed that alphasatellites can also be found in association with the genomic DNA of mastreviruses (Briddon *et al.*, 2001; Briddon *et al.*, 2018, Claverie *et al.*, 2020; Navas-Castillo *et al.*, 2021).

Insect vectors play an important role for the transmission of geminiviruses. Leafhoppers transmit members of the genera *Mastrevirus*, *Curtovirus*, *Becurtovirus*, *Mulcrilevirus* and *Turncurtovirus*, while treehoppers are responsible for the spread of *Topocuvirus* and *Grablovirus*. *Capulavirus* species are vectored by aphids and begomoviruses are exclusively transmitted by whiteflies. The vectors of other genera within the family still remain unknown. The host range of geminiviruses varies depending on the genus members: some species infect a large number of plant species whereas other are limited to a narrow spectrum. Members of *Becurtovirus*, *Begomovirus*, *Capulavirus*, *Citlodavirus*, *Curtovirus*, *Grablovirus*, *Mulcrilevirus*, *Opunvirus*, *Topilevirus*, *Topocurivrus* and *Turncurtovirus* infect dicotyledonous plants. In contrast, most *Mastrevirus* species, the sole representative of *Eragrovirus*, and one of the three known *Maldovirus* species are restricted to monocotyledonous hosts (Fiallo-Olivé *et al.*, 2021).

Plant diseases caused by geminiviruses result in significant economic losses worldwide, particularly in tropical and subtropical regions where susceptible crops are widely cultivated. Among them, diseases caused by species of the genus *Begomovirus* are the most devastating, often leading to complete crop failure (Rojas *et al.*, 2018).

The genus *Begomovirus*

The genus *Begomovirus* is the largest within the family *Geminiviridae* comprising 445 species recognized by the ICTV. These viruses infect a wide range of dicotyledonous hosts, including cultivated crops, non-cultivated plants and ornamental plants (e.g., common bean, cotton, cucurbits, okra, papaya, peppers, tomatoes, weds, honeysuckle) (Rojas *et al.*, 2018). In some cases, begomovirus infections can lead to complete crop

Introduction

loss, posing a major constraint to the production of open-field vegetable, root, and fiber crops in the warmer parts of the world, as well as in greenhouse systems worldwide (Stanley *et al.*, 2005; Fiallo-Olivé and Navas-Castillo, 2023).

Begomovirus genomes consist of one (monopartite) or two (bipartite) circular ssDNA molecules, each of 2.5-2.7 kb in size. They share the geminiviral characteristic genomic organization (see above), including several bidirectionally oriented ORF and a CR containing the IR, which is identical in both DNA-A and DNA-B of bipartite genomes (Fig. I5). Virions display the typical twinned morphology. Cryo-EM analysis by Hesketh *et al.*, (2018) suggested that a ~2.7 kb genomic DNA fits the internal space of geminate particles. Subsequently, Saunders *et al.*, (2020) experimentally demonstrated that the CP of begomoviruses may encapsidate molecules of varying lengths and sequences. They adopt the hypothesis that begomovirus flexibility is due to the frequent association of smaller DNA elements, such as the alpha and beta satellite DNAs, defective genomic DNAs and recombinant DNA, a phenomenon commonly observed in the *Begomovirus* genus.

Alpha- beta- or deltasatellites are circular ssDNA molecules frequently associated with begomovirus genomes. These satellites act as facultative subviral agents, depending on their helper virus for essential viral functions such as replication, encapsidation and transmission (Saunders and Stanley, 1999; Mansoor *et al.*, 1999). Alphasatellites (previously referred as DNA-1) are approximately half the size of begomovirus genomic components and possess the ability to replicate autonomously in host plants by encoding an initiator protein (alpha-Rep), which is similar to the Rep protein of nanoviruses (Zhou 2013). However, alphasatellites are not considered strict satellites, as they rely on their helper begomoviruses for intra- and intercellular movement and do not contribute to symptom development, unlike betasatellites (Briddon *et al.*, 2003; Lozano *et al.*, 2016). The first DNA satellite molecule was identified in 1997 by Dry *et al.*, in association with the monopartite begomovirus Tomato leaf curl virus (ToLCV). Two years later, Saunders and Stanley reported the first alphasatellite in association with Ageratum yellow vein virus (AYVV). Since then, over 100 alphasatellites sequences have been deposited in public databases. Alphasatellites are traditionally associated with monopartite begomoviruses from the OW, often alongside betasatellites, although a few have also been found associated with bipartite begomoviruses from the NW (Paprotka *et al.*, 2010; Briddon *et al.*, 2018). Betasatellites, previously known as DNA β , are also typically half the size of their helper begomoviruses. Unlike alphasatellites, they do not encode a Rep protein and are therefore dependent on their helper virus for replication; however, they encode a major pathogenicity and symptom determinant, the β C1 protein, which is a

suppressor of gene silencing and act enhancing the accumulation of the helper viruses, and in some cases, being essential for the induction of characteristic disease symptoms, as observed with AYVV (Saunders *et al.*, 2000; Briddon *et al.*, 2001; Yang *et al.*, 2019). In terms of genome, alpha- and betasatellites differ from begomoviruses, except for the presence of conserved a stem-loop structure required for the initiation of RCR. ssDNA satellites also are encapsidated by the coat protein of their helper, which enables their efficient transmission by the insect vector, in a similar manner to the helper virus (Briddon *et al.*, 2008; Zhou 2013).

Begomoviruses are transmitted in a circulative, non-propagative manner, meaning that viral particles circulate through the insect vector without replicating. However, whereas begomoviruses replicate or not in the insect vector has been and still is a topic of debate, as thoroughly reviewed by Wang and Blanc, 2021 (section 6). Several studies have supported the hypothesis that TYLCV was able to replicate in its insect vector (Pakkianathan *et al.*, 2015; Wang *et al.*, 2016), while others have reported contradictory findings (Becker *et al.*, 2015; Sánchez-Campos *et al.*, 2016). To date, only He *et al.*, (2020) have provided direct evidence of TYLCV replication within the vector, and this phenomenon appears to be unique for this particular virus TYLCV. The still not completely resolved question of whether begomoviruses exhibit propagative or non-propagative transmission, together with the lack of fully identified viral receptors in insects represent critical gaps that will require further investigation to clarify virus-vector interactions.

Begomovirus infections induce a wide range of symptoms across different host species, including mosaics and mottle patterns, leaf yellowing, curling and deformation, stunted plant growth, and reduced fruit number and quality. Some examples of economically important diseases include: cassava mosaic disease in the Sub-Saharan Africa, cotton leaf curl disease (CLCuD), and tomato yellow leaf curl disease (TYLCD), and diseases caused by other begomoviruses as Tomato chlorotic mottle virus (ToCMoV), Tomato severe rugose virus (ToSRV) and Tomato leaf curl New Delhi virus (ToLCNDV) (Moriones *et al.*, 2017; García-Arenal and Zerbini, 2019; Ahmed *et al.*, 2021).

Tomato leaf curl New Delhi Virus (ToLCNDV)

ToLCNDV (genus *Begomovirus*, family *Geminiviridae*) represents one of the most important constraints to tomato production being responsible for a disease called Tomato leaf curl disease (ToLCD). It causes significant economically disease affecting tomato in Indian sub-continent, reducing production in the range from 18% to 100%. However, in recent years, ToLCNDV has been extended its host range and spread to new

Introduction

geographical regions including the Middle East and the western Mediterranean Basin (Yazdani-Khameneh *et al.*, 2016; Fortes *et al.*, 2016) (Fig. I6). ToLCNDV was primarily described in northern India in 1995 (Padidam *et al.*, 1995). Reports of its presence were limited to the Indian subcontinent and other countries in Asia until 2012, when it was first detected in the western Mediterranean Basin (Juárez *et al.*, 2014). The genetic analysis of the viral isolates presents in Spain, closely related to those characterized in other Mediterranean countries, showed that they all belong to a novel strain, named “*Spain strain*” (ToLCNDV-ES) which is prevalent in cucurbitaceous and rarely infect tomato (Fortes *et al.*, 2016; Ruiz *et al.*, 2015, 2017). ToLCNDV was initially found infecting tomato (*Solanum lycopersicum* L.) and other solanaceous crops such as eggplant, pepper and potato, but it has also been reported in cucurbit crops (cucumber, melon, zucchini squash, pumpkin) and in various other cultivated and wild plant species (Juárez *et al.*, 2014; Zaidi *et al.*, 2017; Moriones *et al.*, 2017). In tomato it causes stunting with upward and downward rolling and crinkling of the leaves, interveinal yellowing and vein clearing. In members of *Cucurbitacea* family common symptoms include leaf distortion, mosaic, short internodes and curling, yellowing in young leaves in zucchini plants, vein swelling in cucumber plants, and severe mosaic in melon plants (Chakraborty, 2008; Juárez *et al.*, 2019). Under natural conditions, ToLCNDV, as the rest of begomoviruses, is transmitted by whiteflies, exclusively by *Bemisia tabaci* (Gennadius), in a circulative persistent manner and mostly restricted to the phloem of infected plants (Farina *et al.*, 2023). It is relevant to add that both experimental and host plant can also be infected by mechanical inoculation (López *et al.*, 2015), a rather uncommon feature for phloem restricted viruses, although it was observed in other cases with cucurbit-infecting begomoviruses (Romay *et al.*, 2015).

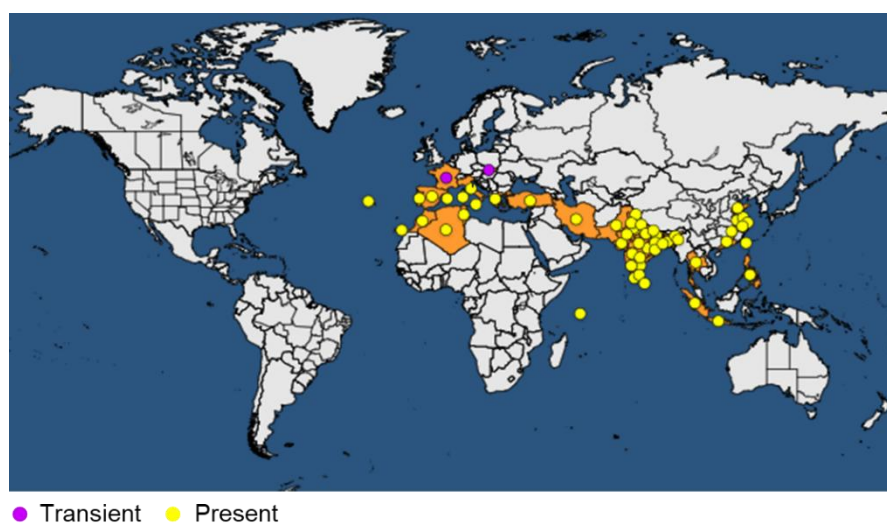


Figure I6. Geographical distribution of ToLCNDV. Colored areas indicate regions where the virus is currently present. (Source EPPO, 2026. <https://gd.eppo.int/taxon/TOLCND/distribution>)

ToLCNDV is a distinctive bipartite begomoviruses from Old World, characterized by the presence of an AV2 gene in its DNA-A component. The DNA-A component encodes six ORF: AC1, AC2, AC3, AC4, AV1 and AV2, while the DNA-B component contains two ORF: BC1 and BV1 (protein functions are described in previous subsections). The complete genomic organization comprises eight viral proteins, suggesting that many of them possess multifunctional roles. Similar to monopartite begomoviruses from the OW, ToLCNDV has also been found in association with DNA satellite molecules in natural infections, including both alphasatellites and betasatellites (Anwar 2020).

The emergence and spread of ToLCNDV are increasingly favoured by human activities and global warming. Effective management requires an integrated approach combining chemical, biological, and cultural control strategies to ensure both efficacy and environmental sustainability. In Europe, restrictions on the widespread use of pesticides for vector control create an urgent need for innovative strategies against begomoviruses, particularly emerging diseases. In this context, biotechnological approaches offer valuable alternatives for disease management.

1.2.2 Crops

1.2.2.1 Impact of plant viruses in sweet potato crops

Sweet potato

Sweet potato (*Ipomoea batatas*) is considered one of the oldest domesticated crops, with evidence suggesting its domestication occurred approximately 5,000 years ago. It is widely believed to have originated in Central or South Americas, from where it was subsequently introduced to Polynesia, Europe, Africa and Asia (Austin *et al.*, 1987; Loebenstein *et al.*, 2009; Roullier *et al.*, 2013). As a dicotyledonous specie within the family *Convolvulaceae*, sweet potato holds significant agronomic value due to its high biomass and nutrient yield per hectare, ranking as one of the most important food root crops, alongside potato and cassava. Its cultivation spans diverse zones, mainly in tropical and subtropical regions, with approximately 80% of global production concentrated in Asia. Notably, the highest production levels are concentrated in China and East African countries such as Malawi and Tanzania (FAOSTAT, 2022).

Sweet potato plant is an herbaceous perennial plant which can be grown as an annual crop. It is primarily propagated vegetatively using stem cuttings from production fields or from sprouted storage roots (Loebenstein *et al.*, 2009). Consequently, virus-infected planting stock will almost inevitably transmit infections to newly established crops, leading to viral accumulation over successive cycles and progressive losses in yield and quality (Clark *et al.*, 2012).

Sweet potato viral diseases

Numerous pests and pathogens, including whiteflies, fungi or most predominantly viruses, have affected or limited sweet potato production for decades. Recent advances in molecular diagnostics have enabled the identification of more than 30 different viruses infecting this crop, mostly transmitted by the aphid *Myzus persicae* or the whitefly *B. tabaci* (Clark *et al.*, 2012). Among these, DNA viruses belonging to the *Geminiviridae* family are the most frequently detected, followed by members of the *Potyviridae* family. Apart from CMV (*Bromoviridae* family), a virus which is known for its exceptionally broad host range, the rest of species affecting sweet potato exhibit high specificity to the genus *Ipomoea*. This host specificity suggests that sweet potato might possess a cellular environment that restricts a limited group of viruses for a successful viral infection (Kreuze *et al.*, 2021).

SPFMV and SPMMV, both members of the *Potyviridae* family, are the most prevalent viruses affecting sweet potato. In contrast, the crinivirus SPCSV is considered the most damaging, particularly when co-infecting with other unrelated viruses. Such mixed infections often resulted in a pronounced synergistic effect, exacerbating disease severity and yield losses. A notable example of this phenomenon is SPVD. As it has been mentioned previously, SPVD causes the most devastating virus disease of the sweet potato crop, with yield losses reaching up to 85% (Karyeija *et al.*, 2000; Gibson and Kreuze, 2015). Symptoms derived from SPVD include severe leaf mosaic, strapping and malformation, vein clearing, puckering, stunting and substantial reductions in tuberous root yield (Salazar *et al.*, 2001). SPVD emerges from a dual infection involving SPCSV and a member of the *Potyviridae* family, usually SPFMV or SPMMV. SPFMV titers increase several hundredfold greater in SPVD compared to SPFMV-infected plants, while SPCSV levels remain stable or are slightly reduced (Mukasa *et al.*, 2006; Cuellar *et al.*, 2009). This imbalance is potentially linked to the RNA silencing activity of SPCSV RNAase-III enzyme (Karyeija *et al.*, 2000; Cuellar *et al.*, 2009; Cuellar 2015). Furthermore, a similar kind of synergism has been reported between SPCSV and other potyviruses such as SPV2, SPVC, SPVG, the ipomovirus SPMMV and even with sweet potato begomoviruses (Mukasa *et al.*, 2006; Untiveros *et al.*, 2007; Cuellar *et al.*, 2015). These findings support the hypothesis that SPCSV may interfere with the plant's antiviral defence mechanisms, thereby facilitating co-infection and exacerbating disease outcomes (Cuellar *et al.*, 2009).

1.2.2.2 Impact of plant viruses in cucurbit crops

Cucurbit crops

Cucurbits represent one of the most significant crop groups in global agriculture. All major cucurbit crops are cultivated primarily for their fruits, a particular type of berry called 'pepo' which is consumed in various forms depending on the species: ripe (e.g. pumpkin) or unripe (e.g. zucchini), raw (watermelon), cooked (squash), or pickled (gherkins). Among these, *C. pepo* is the unique in being also cultivated for its seeds and the oil extracted from them (Chomicki *et al.*, 2020).

In the Mediterranean region, the most widely cultivated species include cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* Thunb.), zucchini (*Cucurbita pepo* L.), and pumpkin and gourd (*Cucurbita* spp.) (Robinson and Decker-Walters, 1997). Spain stands out as a leading producer within the Mediterranean basin, producing mainly melon (16 570 ha, 516 520 tonnes), watermelon (21 460 ha, 1 168 930 tonnes) and zucchini and pumpkin (16 620 ha, 706 880 tonnes), according FAOSTAT 2023. Given the economic relevance of these crops, producing countries are forced to continuously enhance the quality and efficiency of their production systems.

Domestication of cucurbit crops started approximately c. 11 000 years ago in the New World and Asia, and more recently in Africa. Some species, such pumpkin, appear to have undergone a single domestication event, as archaeological indicates its earliest cultivation in northern South America and Central America around 10000 years ago (Smith 1997). Similarly, *C. pepo*, native to North America (specifically Northeast Mexico, and the Southeast and Central United States), was likely domesticated between 8000-10000 years ago in Mexico. In contrast, melon, previously believed to have an exclusively Asian origin, is now understood to have been domesticated independently at least twice, in both Asia and Africa (Chomicki *et al.*, 2020; Martín-Hernandez and Picó, 2021).

Cucurbits viral diseases

Although cucurbits are among the most economically important horticultural vegetables, the sustainability of their production is increasingly at risk by viral diseases. Indeed, these crops are persistently threatened by several pest and pathogens, including many viruses. Over 70 virus species affecting cucurbits has been described worldwide, and specifically 28 virus species within the Mediterranean region (Lecoq and Desbiez, 2012; Lecoq and Katis, 2014; Radouane *et al.*, 2021). This high viral diversity likely results from the genetic and ecological variability of their hosts plant (Lecoq and Desbiez, 2012). Most of the viruses infecting cucurbits are transmitted by insect vectors including aphids and

Introduction

whiteflies and belong to a wide range of families such as *Potyviridae*, *Bromoviridae*, *Luteoviridae*, *Closteroviridae*, *Geminiviridae*, *Secoviridae*. Other viruses infecting cucurbits can be transmitted by contact, by seeds and by soil-organisms like fungi. The Table I2 summarized the main viruses described in recent reviews on the subject.

Table I2: Viruses infecting cucurbits (based in Lecoq and Desbiez, 2012; Martín-Hernández and Picó, 2021; Radouane *et al.*, 2021).

Virus	Family	Genus	Vector/mode of transmission
Cucumber mosaic virus (CMV)	<i>Bromoviridae</i>	<i>Cucumovirus</i>	Aphid
Beet pseudo-yellows (BPYV)	<i>Closteroviridae</i>	<i>Crinivirus</i>	Whitefly
Cucurbit chlorotic yellows virus (CCYV)	<i>Closteroviridae</i>	<i>Crinivirus</i>	Whitefly
Cucurbit yellow stunting disorder virus (CYSDV)	<i>Closteroviridae</i>	<i>Crinivirus</i>	Whitefly
Melon chlorotic mosaic virus (MeCMV)	<i>Geminiviridae</i>	<i>Begomovirus</i>	Mechanically
Squash leaf curl virus (SqLCV)	<i>Geminiviridae</i>	<i>Begomovirus</i>	Whitefly
Tomato Leaf Curl New Delhi Virus (ToLCNDV)	<i>Geminiviridae</i>	<i>Begomovirus</i>	Whitefly
Watermelon chlorotic stunt virus (WmCSV)	<i>Geminiviridae</i>	<i>Begomovirus</i>	Whitefly
Chickpea chlorotic dwarf virus (CpCDV)	<i>Geminiviridae</i>	<i>Mastrevirus</i>	Leafhopper
Cucurbit aphid-borne yellows virus (CABYV)	<i>Luteoviridae</i>	<i>Polerovirus</i>	Aphid
Cucumber vein yellowing virus (CVYV)	<i>Potyviridae</i>	<i>Ipomovirus</i>	Whitefly
Algerian watermelon mosaic virus (AWMV)	<i>Potyviridae</i>	<i>Potyvirus</i>	Aphid
Clover yellow vein virus (CIYVV)	<i>Potyviridae</i>	<i>Potyvirus</i>	Aphid
Melon vein-banding mosaic virus (MVBMV)	<i>Potyviridae</i>	<i>Potyvirus</i>	Aphid
Moroccan watermelon mosaic virus (MWMV)	<i>Potyviridae</i>	<i>Potyvirus</i>	Aphid
Papaya ringspot virus (PRSV)	<i>Potyviridae</i>	<i>Potyvirus</i>	Aphid
Telfairia mosaic virus (TeMV)	<i>Potyviridae</i>	<i>Potyvirus</i>	Aphid
Turnip mosaic virus (TuMV)	<i>Potyviridae</i>	<i>Potyvirus</i>	Aphid
Watermelon leaf mottle virus (WLMV)	<i>Potyviridae</i>	<i>Potyvirus</i>	Aphid
Watermelon mosaic virus (WMV)	<i>Potyviridae</i>	<i>Potyvirus</i>	Aphid
Zucchini yellow fleck virus (ZYFV)	<i>Potyviridae</i>	<i>Potyvirus</i>	Aphid

Zucchini yellow mosaic virus (ZYMV)	<i>Potyviridae</i>	<i>Potyvirus</i>	Aphid
Eggplant mottled dwarf virus (EMDV)	<i>Rhabdoviridae</i>	<i>Nucleorhabdovirus</i>	Leafhopper
Squash mosaic virus (SqMV)	<i>Secoviridae</i>	<i>Comovirus</i>	Beetle
Cucumber leaf spot virus (CLSV)	<i>Tombusviridae</i>	<i>Aureusvirus</i>	Fungi
Cucumber soil-borne virus (CuSBV)	<i>Tombusviridae</i>	<i>Carmovirus</i>	Fungi
Melon necrotic spot virus (MNSV)	<i>Tombusviridae</i>	<i>Carmovirus</i>	Fungi
Tobacco necrosis virus (TNV)	<i>Tombusviridae</i>	<i>Necrovirus</i>	Fungi
Cucumber fruit mottle mosaic virus (CFMMV)	<i>Virgaviridae</i>	<i>Tobamovirus</i>	Mechanically
Cucumber green mottle mosaic virus (CGMMV)	<i>Virgaviridae</i>	<i>Tobamovirus</i>	Mechanically
Kyuri green mottle Mosaic virus (KGMMV)	<i>Virgaviridae</i>	<i>Tobamovirus</i>	Mechanically
Zucchini green mottle mosaic virus (ZGMMV)	<i>Virgaviridae</i>	<i>Tobamovirus</i>	Mechanically

Some viral pathogens, such as CMV, exhibit broad host range and can infect multiple cucurbit species. Other viruses have more specific host preferences but cause substantial damage. For instance, CYSDV affects severely to melon and cucumber, while ToLCNDV is especially damaging in zucchini and pumpkin. In our work we are particularly interested in ToLCDNV, which in our crop conditions can lead to yield losses of up to 30% for greenhouses-grown zucchini, and causes significant damage to pumpkin squash under open-field conditions (Romay *et al.*, 2019; Crespo *et al.*, 2020; Martín-Hernández and Picó, 2021).

In addition to the diseases caused by individual viruses, mixed infections are a common scenario and recently are attracting the attention of the scientific community because their importance (De Moya-Ruiz *et al.*, 2021; Ontiveros *et al.*, 2022; Domingo-Calap *et al.*, 2020).

1.2.2.3 Plant defence responses

Plants are sessile organisms that need to interact with the environment and with surrounding communities of other plants, animals and microbes, which often can challenge the plant well-being. Once pathogenic organisms attack plants, a multilayer immune system is activated and can trigger several responses. These responses can be divided in passive and inducible defence pathways. Passive responses include the reinforcement of physical or chemical barriers such as waxy cuticles or rigid cell walls,

Introduction

that act as a first protection layer, preventing the pathogen entry (Nishad *et al.*, 2020). The second layer of protection is activated by the pathogen perception thanks to recognizing elements known as pathogen associated molecular patterns (PAMPs). This recognition occurs through specific molecular elements named pattern recognition receptors (PRR). The PRR are often located on the surface exposed membranes and trigger the activation of the first line of host-induced defence denominated as PAMP-triggered immunity (PTI) (Saijo and Loo, 2020). After PTI, plants initiate signalling cascades, including the production of reactive oxygen species (ROS), calcium ion signalling (Ca^{2+}), production of antimicrobial compounds to contain the infection or biosynthesis of defence-related factors (Lee *et al.*, 2020). Nevertheless, in a classical arms race, there are pathogens capable to escape the PTI by producing virulence factors and effectors which evade their recognition or block the multilayer plant defence system. To face this situation, plants have co-evolved having a second line of defence triggered by resistance (R) proteins that usually have intracellular localization and belong to the nucleotide-binding site/ leucine-rich repeat family of receptors (NLRs). R proteins recognize and interact with the pathogen effectors giving a new defence response which is called effector-triggered immunity (ETI). Although there is evidence indicating that PTI and ETI share numerous signalling components, ETI is stronger and more specific than PTI (Ding *et al.*, 2022). After ETI induction, often a hypersensitive response (HR) and programmed cell death (PCD) can be stimulated on the area of infection as an effective manner to contain the microbial spread.

In the case of viruses, and in addition to these variety of strategies to defend against pathogens, specific mechanisms can be activated in response to viral infections including immune receptor signalling-mediated signalling, hormone-regulated defence pathways, protein degradation, metabolic reprogramming and RNA silencing or RNA interference (RNAi), which has a significant role in plant-virus interaction (Guo *et al.*, 2019).

Antiviral RNAi

RNA silencing is an evolutionary conserved system that control gene expression via sequence specific RNA neutralization by small RNAs. This system, present in mostly eukaryotic organisms, plays a significant role in many biological processes such as regulation of gene expression and protection against viruses and transposons (Baulcombe 2004). During a viral infection, the produced double-stranded RNAs (dsRNAs), including viral replicative intermediates, structure parts of transcripts for both RNA and DNA viruses, or *de novo* double-stranded molecules synthesized by the RNA-dependent RNA polymerase (RdRp), can be processed by Dicer-like (DCL) dsRNA-specific endoribonucleases into 21-25nt virus-derived small RNAs (viral sRNAs). The

types of sRNAs that can be found in plants include endogenous microRNAs (miRNAs) and small interfering RNAs (siRNAs) of 21, 22 and 24nt. In their biogenesis, dsRNAs are processed into sRNAs of 21nt, 22nt and 24nt by DCL4, DCL2 and DCL3, respectively, while DCL1 mainly processes miRNAs. Subsequently, Argonaute (AGO) proteins provide slicing activity to the RNA-induced silencing complex (RISC) and bind these siRNAs to guide them either for cleavage or translation inhibition of the corresponding target mRNA sequences (Diaz-Pendón *et al.*, 2007; Ding 2010).

Since its discovery in the late years of the past century, the phenomenon of RNAi has been intensively studied, and in the case of plant and viruses there are many recent excellent reviews summarizing our current knowledge (Jin *et al.*, 2021; Lopez-Gomollon and Baulcombe, 2022; Matsumura and Kormelink, 2023; Li *et al.*, 2025).

Virus counter defence mechanisms

Plant can directly use the silencing pathway to inactivate viral genomes. However, plant viruses have co-evolved to find diverse strategies for escaping from plant host defences. It is remarkable that all known plant viruses encode at least one RNA silencing suppressor (RSS) protein, which collectively can interfere with almost all steps of the silencing pathway, acting specifically or at multiple levels of the pathway, being the dsRNA, the vsiRNAs and the AGO proteins the most common targets (Csorba *et al.*, 2015; Li *et al.*, 2025).

However, an equilibrium must be maintained between viral propagation and the suppression of the host defence mechanisms. To achieve this, viruses have developed diverse strategies to regulate the activity of their RSS. These proteins exhibit substantial variability in sequence, structure and mode of action across unrelated viruses. As a result, numerous studies have focused on identifying and characterizing their molecular functions (Lakatos *et al.*, 2004; Chiu *et al.*, 2010; Giner *et al.*, 2010; Cheng and Wang, 2017; Pollari *et al.*, 2020; Kenesi *et al.*, 2021; Baulcombe, 2022).

The first viral RSS to be identified was the HCPro protein of potyviruses, described simultaneously in 1998 by several groups, including Kasschau and Carrington (Kasschau and Carrington, 1998) and the team of Vance (Anandalakshmi *et al.*, 1998;) and an unfortunately later retracted paper (Brigneti *et al.*, 2015). Initially, HCPro was believed to act as a universal silencing suppressor across all potyvirids. However, subsequent studies revealed that not all members of the family encode HCPro. As a result, the RSS function appears to have been assumed by alternative gene products in some viruses such as in certain ipomovirus members, where the P1 serine protease functions as an RSS (Valli *et al.*, 2006; Giner *et al.*, 2010). Moreover, in at least some

members of the genus *Potyvirus* like SPFMV, the transframe gene product P1N-PISPO, produced by polymerase slippage, exhibited silencing suppressor activity in standard co-agroinfiltration assays (Mingot *et al.*, 2016; Untiveros *et al.*, 2016). An additional case was described when a newly identified arepavirus, encoding two HCPros in tandem, only HCPro2 exhibited antisilencing activity (Qin *et al.*, 2020). Due to the high diversity of factors expressed from the 5'end of potyvirid genomes, part of this thesis aims to elucidate if proteins from different isolates of SPFMV share RNA silencing suppressor activity or if additional viral proteins are also involved in this function.

1.2.3 Players in virus transmission

One of the key factors contributing to the success of plant-infecting viruses is their ability to move efficiently between hosts, thereby ensuring their survival and propagation. As immobile organisms, plants are unable to facilitate active virus transmission, and thus, most plant viruses rely on biological vectors for their spread. Among these vectors, arthropods, particularly sap-sucking insect such aphids, whiteflies, leafhoppers and trips, are the more prevalent and effective. Their feeding strategies, which involve piercing plant tissues and accessing vascular elements like the phloem, provide an optimal route for virus acquisition and inoculation, making them responsible for the transmission of the majority of known plant viruses (Ng and Falk, 2006).

Aphids

Aphids are the most successful organism acting as plant virus vector s owing to their ability to transmit multiple viral specie across diverse taxonomic families. The family *Aphididae* (Order: *Homoptera*) comprises more than 5000 described species, although only a subset of around 100 are considered significant agricultural pests (Blackman and Eastop, 2007). Many aphid species exhibit polyphagy, feeding on a wide range of host plants. Morphologically, aphid possess a small and ovoid body ranging from 1-4 mm in length, divided into three main anatomical regions: the head, the thorax (prothorax, mesothorax and metathorax) and the abdomen (composed of eight segments) (Fig. 17A). They present a pair of antennae on the head and three pairs of legs on the thorax. Their sap-sucking mouthparts include a stylet, a needle-like structure composed of two mandibles and two maxillae, allowing two internal canals, one for food intake and other for saliva secretion. The most distal part of the stylet, known as acrostyle, has been identified as the binding site of non-persistent viruses (Jayasinghe *et al.*, 2022). Aphids display morphological and reproductive plasticity. Their coloration varies among species, being yellow, green or black colours the most prevalent. Interestingly, aphids can occur in both winged (alate) and wingless (apterous) forms, being able to colonize short- and

long-distance hosts, often in response to environmental conditions. Their development is ametabolous, involving incomplete metamorphosis from egg to several nymphal stages, culminating in adults capable to reproduce parthenogenetically for many generations. This reproductive strategy allows aphids to rapidly colonize new environments and escalate their numbers exponentially in short periods (Ng and Perry, 2004).

Taken together their polyphagous feeding behaviour, capacity for asexual reproduction, phenotypic plasticity, and the possession of a mouthparts for probing plant tissues with minimal physical damage while determining its suitability as food source, make aphids exceptionally well-adapted vectors of plant viruses. The most common species vectoring plant viruses and agricultural relevant pests are *Aphis gossypii* and *Myzus persicae* (Byers 2008). They can transmit different viral species either in a persistent or non-persistent manner, often depending on the viral genus. For example, members of the genus *Potyvirus*, *Caulimovirus* or *Carlavirus* are transmitted in a non-persistent mode; in contrast, viruses belonging to the genera *Luteovirus* or *Polerovirus* are transmitted persistently (Racchah and Fereres, 2009).

Whiteflies

In recent decades and in our latitudes, emerging viral diseases have increasingly impacted agriculture, largely due to viruses transmitted by insect vectors, being particularly important in this category of emergent pathogens many viruses disseminated through transmission by whiteflies, highly efficient vectors that are expanding its global distribution. Whiteflies were first detected by Gennadius on tobacco plants in Greece (Gennadius, 1889) and, although approximately 1500 species of whiteflies have been described, only a few shows the ability to transmit plant viruses. In terms of virus transmission, *B. tabaci* (Gennadius; order *Hemiptera*, family *Aleyrodidae*) stands out the most significant whitefly specie, due to its remarkable fitness, environmental adaptability and broad host range (Navas-Castillo *et al.*, 2011). To date, it is considered one of the most damaging insect vectors in agriculture, able to infest over 600 cultivated or wild species. Although globally distributed, it is particularly prevalent in tropical and subtropical areas, as well as in greenhouses environments within temperate regions (De Barro *et al.*, 2011).

B. tabaci is considered a cryptic complex of species, morphologically indistinguishable but with differences in genetics and biological characteristics such as the variation in their host range, the capacity to disperse widely and transmit viral species, the attraction behaviour towards different hosts, or their resistance to insecticides (Bedford *et al.*, 1994;

Introduction

Brown *et al.*, 1995; Brown *et al.*, 1996; Perring 2001; De Barro and Khan, 2007). According to the different mitochondrial cytochrome oxidase I (mtCOI) gene consensus sequence, *B. tabaci* can be grouped into distinct species, being Middle East-Asia Minor 1 (MEAM) and Mediterranean (MED) biotypes the most widespread and invasive isolates (Barbosa *et al.*, 2015). The first major global invasion by whiteflies was performed by the MEAM biotype in the late 1980s, spreading from its origins in the Middle East-Asia region to at least 54 countries. More recently, the biotype MED, which has its origin are in the countries bordering the Mediterranean Basin, has been spread to father regions such as China, Japan, Mexico and North and Central America (De Barro *et al.*, 2011).

Morphologically, they are tiny insects, only 1-3 mm long, and covered with a powdery or flour-like white wax (Fig. 17B). Their life cycle is relatively short, spanning between three to five weeks, but once reached their adulthood, individuals can lay up to 200 to 400 eggs during their lifespan. *B. tabaci* is a phloem-feeding insect that can provoke direct damage on its hosts. They reduce plant vigor by feeding in plant phloem and removing plant sap; in addition, its excretion of a honeydew subproduct promotes a sooty mold which interferes with photosynthesis. Nevertheless, the most critical issue from a phytopathological perspective is their role as efficient vectors of plant viruses (Navas-Castillo *et al.*, 2014). *B. tabaci* may transmit both semi-persistent and persistent viruses and serves as a vector for both DNA and RNA viruses. Begomoviruses, criniviruses, carlaviruses, ipomoviruses, torradoviruses and some viruses in the genus *Carlavirus* like cowpea mild mottle virus (CPMMV), are transmitted by whiteflies. Recently, the polerovirus Pepper whitefly-borne vein yellows virus (PeWBVYV) has been reported to be transmitted by whiteflies, representing the exception in the whole family *Luteoviridae*, whose members are typically transmitted by aphids (Zanardo and Carvalho, 2017; Ghosh *et al.*, 2019; Fiallo-Olivé *et al.*, 2020). Given the ability to transmit numerous plant viruses and the severe infestations caused by whiteflies globally, their management has become a central focus in plant health research and policy. Preventing further dispersal and developing effective control strategies for these insect vectors are now considered critical to mitigate their impact on agricultural systems and limit the spread of associated viral diseases.

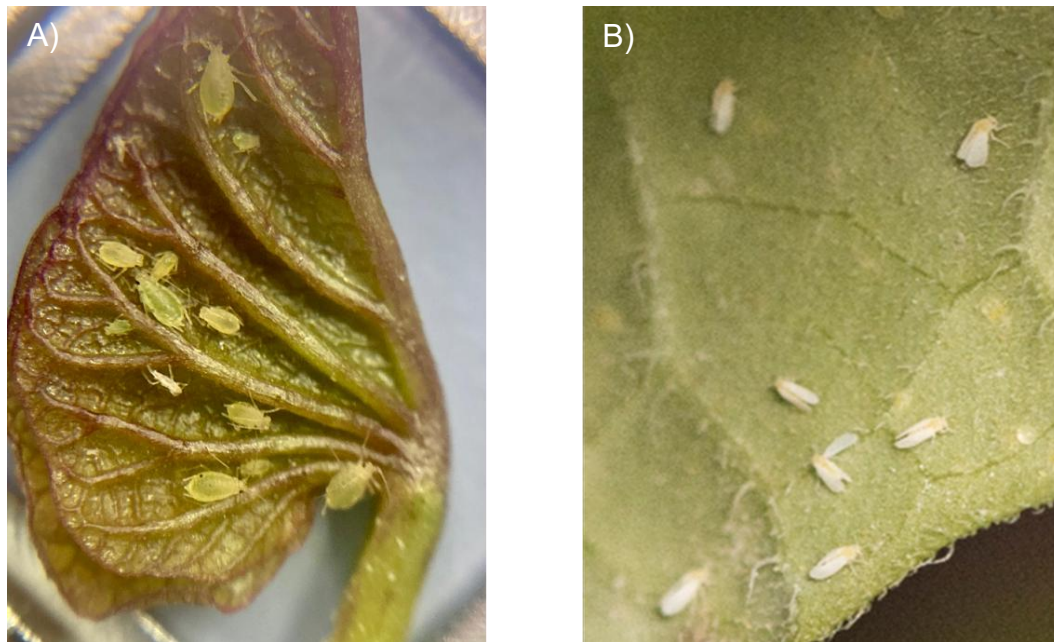


Figure 17. Insect vectors of plant viruses. A) Aphid *Myzus persicae*. B) Whitefly *Bemisia tabaci*

1.2.3.1 The vector transmission process

Due to the complexity and specificity of the virus-vector interactions, insect transmission is classified in four main categories based on the duration and nature of the interaction between the virus and its insect vector: non-persistent, semi-persistent, persistent circulative and persistent propagative (Bragard *et al.*, 2013).

First, non-persistent transmission occurs when the virus is acquired inoculated within a short period of time (seconds to minutes) whereas the insect probes or pierces plant tissues to identify and select the suitable feeding site. Historically, this manner to transmit was referred to as “stylet-borne”, in part because this mode involves virus retention on the surface of the stylet or within the foregut, without internal circulation through the vector’s body. Different studies suggested that virions retained at the distal tip of the stylet play a key role in transmission (Wang and Pirone, 1999; Fu *et al.*, 2025). To date, all viruses transmitted via this mode are exclusively vectored by aphids (Whitfield *et al.*, 2015).

The second mode of transmission that can be differentiated is the semi-persistent type. Unlike the non-persistent mode, it requires longer periods for viral acquisition and inoculation. In this case, the virus still does not need to be internalized within the insect internal cavities crossing gut cellular barriers, instead, it has been suggested that viral particles are retained on the exterior of the digestive apparatus and chitin-lined surfaces. Acquisition and inoculation periods range from minutes to some hours, and mostly involve phloem tissues. This may explain the challenges associated with mechanical

Introduction

transmission of semi-persistent viruses (Ng et Falk, 2006). Although aphids are common vectors, semi-persistent viruses can also be transmitted by whiteflies and leafhoppers; however, because the virus is retained externally or on chitinous parts of the insect, it is lost during vector insect moulting (Blanc *et al.*, 2014).

The third category includes viruses transmitted in a circulative, non-propagative manner. This transmission mode requires extended acquisition periods (for hours up to days), and after the acquisition the insect vector often remains viruliferous for the duration of its lifespan. In this category, the virus is internalized into the vector's body with virions circulating through the haemolymph until they reach the salivary gland cells where they are subsequently inoculated into host plants (Gutierrez *et al.*, 2013). Once acquired, a latent period is required for transmission, and it lasts since the acquisition to the first successful inoculation (Bragard *et al.*, 2013). Although the virus enters into the insect, it does not replicate within its tissues. This transmission strategy has been documented in several genera in the family *Luteoviridae*, *Geminiviridae* and *Nanoviridae*, and involves a range of insect vectors including aphids, whiteflies, leafhoppers, beetles and mirids (Gray *et al.*, 2014).

The fourth and final category comprises circulative, propagative viruses. In terms of acquisition, path of movement within the insect vector, and inoculation mechanisms, they are very similar to the circulative non-propagative viruses. However, circulative propagative viruses exhibit a distinctive feature: once acquired by the insect, the virus is capable of replicating within the vector, even prior transmission. Viruses in this category belong to taxonomic families that include members that in many cases can infect either animals or plants, such as *Rhabdoviridae*, *Bunyaviridae* and the *Reoviridae* (Ng and Perry, 2004; Whitfield *et al.*, 2015).

This classification provides a frame for understanding the mechanisms underlying virus acquisition, retention and transmission across diverse vector species.

1.2.3.1.1 Interference with transmission

The vector-borne transmission of viruses relies on a close and specific interaction between the virus, the insect vector and the plant. Consequently, disrupting any stage of the virus-vector-host interaction during the transmission or infection process, can potentially have significant impact to interfere or block virus dissemination and therefore be used as a control strategy. This section focuses on different strategies proposed to serve as the basis for interference with a successful viral transmission.

Identification of virus receptors: The characterization of vector elements acting as receptors for viruses has been extensively studied in recent years. These receptors are

implicated in transmission by conferring specificity to virus-vector interactions. In non-circulative viruses, additional virus-encoded proteins are required to complete the transmission process. A well-known example of relevant recent research in this field is related to potyviruses, aphid-transmitted in a nonpersistent manner, where the HCPro protein functions as molecular bridge or accessory factor facilitating the reversible retention of virions on the vector's mouthparts (Pirone and Blanc, 1996). Research in this field is currently trying to identify the receptor(s) required for potyvirus and other virus acquisition, presumably located in or near this site in vector stylets (Urizarna and López-Moya, 2014).

RNA interference (RNAi): Post-transcriptional gene silencing appears to be a conserved phenomenon across all eukaryotes, including plants and insects. This has enabled the use RNAi to induce loss-of function phenotypes in insect vectors, providing a powerful tool for identifying elements essential for virus transmission and offering promising approaches for insect pest management (Huvenne and Smagghe, 2010). However, susceptibility to RNAi varies significantly among species, particularly among insects, with some exhibiting greater sensitivity to systemic RNAi than others. Consequently, empirical optimization is required for each species, including the selection of the most suitable delivery method for the RNAi-triggering molecules, determination of effective doses for expected response, and characterization of the duration of the silencing effect (Urizarna and López-Moya, 2014). Several delivery routes have been assayed (Yu *et al.*, 2013; Lu *et al.*, 2023), including microinjection of in vitro synthesis dsRNA directly into the insect hemocoel, which can result in a transient knockdown of specific target genes and was tested with circulative viruses (Tamborindéguy *et al.*, 2008). Another strategy involves oral delivery, where insects are fed an artificial diet supplemented with vector-generated dsRNAs. This artificial feeding system, originally developed by Pirone in 1964, allows aphids and other piercing-sucking insects to ingest dsRNA through artificial membranes. The RNAi activity can also occur in cross-kingdom interactions, and since it has been described to potentially affect gene expression between plants and insects feeding on them, it could open new strategies for disease control in agriculture (Wang *et al.*, 2017; Matsumura and Kormelink, 2023).

Interference strategies have long been considered as an important area of study for understanding virus-vector interactions and remain a key focus in this field. Together with the previously mentioned approaches, the study of receptor-blocking molecules and cross-precipitation using attenuate variants, can have a strong potential to block or interfere with virus dissemination and, consequently, to be used as possible control strategy.

1.2.3.2 Single and mixed infections

The presence of multiple viruses in the same plant is a common situation in both nature and agricultural environments, often causing significant complex diseases (Moreno and López-Moya, 2020). Mixed infections are favoured by the polyphagous condition of insect vectors, their efficient transmission mechanisms, and the broad range of susceptible hosts, leading to overlapping host ranges for many viruses (Syller 2014). According to the timepoints of infection, these interactions can be distinguished as co-infections, when two viruses infect the plant simultaneously, or superinfections when a second virus infects a plant that is already infected by another virus (Saldaña *et al.*, 2003).

Mixed infections can involve viruses either genetically related or unrelated and they can interact in different ways. These interactions can vary from neutrality to synergism and antagonism (Syller 2012). The neutral interaction occurs when coexisting viruses do not influence on each other, not producing changes in viral accumulation, pathogeny, or other aspects in comparison with single infections. However, synergistic and/or antagonistic viral interactions are more frequent, and they can significantly impact in the yield of crops by altering the pathogenicity. In most cases, antagonistic interactions occur when the presence of one virus is detrimental for at least one of the other present viruses, for example with a decrease in the replication or viral accumulation (Moreno and López-Moya, 2020). This phenomenon could be exploited in cross-protection strategies, although in practical terms the preferred scenario is based in the use of attenuated strains (Úbeda *et al.*, 2026).

On the other hand, in synergistic interaction, the fitness of at least one of the viruses involved in a mixed infection, is enhance by the presence of the other(s). This may result in an increase in the viral titers and usually in a development of more severe symptoms compared to those caused by single infections of the same viruses (Mascia and Gallitelli, 2016; Ontiveros *et al.*, 2022). Therefore, synergistic interactions can have a substantial economic impact, particularly when they affect commercial and agricultural crops. A well-studied example of synergism is the co-infection between the potyvirus Potato virus Y (PVY) and the potexvirus Potato virus X (PVX) in tobacco plants, where the PVY assists the replication of the PVX, increasing the virulence and the severity of symptoms (Vance, 1991; Rochow and Ross, 1955). Another notable case involves sweet potato crops, where the co-infection between the SPFMV and the SPCSV affect dramatically to their production, and where the crinivirus enhances the viral loads of the potyvirus. Apart from the viral titers, mixed infections can also influence vector attraction and feeding behaviour, and consequently, the transmission efficiency of the involved viruses.

Ontiveros *et al.*, (2022) observed in tomato plants that, the co-infection by *Tomato chlorosis virus* (ToCV, genus *Crinivirus*, family *Closteroviridae*), and *Tomato yellow leaf curl virus* (TYLCV, genus *Begomovirus*, family *Geminiviridae*) resulted in a synergism at later stages of plant development. Moreover, whiteflies showed a preference for leaves of plants previously infected with TYLCV or mixed infections. In other cases, the outcome of mixed infections, such as symptoms or viral accumulation, may vary depending on the host plant. For instance, Wintermantel *et al.*, (2008) reported that the co-infection between the crinivirus Tomato infectious chlorosis virus (TICV) and ToCV led to host-specific changes in viral titers: in *N. benthamiana*, TICV titers increased while ToCV titers decreased, whereas in *Physalis wrightii*, both viruses showed reduced accumulation in comparison to single infections. More recently, an intriguing case of mixed infections has been reported in tomato plants by Vo *et al.*, (2022) showing that the strain ToLCNDV-ES, typically infecting *Cucurbita* spp. in Mediterranean countries, was able to infect tomato plants when co-infecting with TYLCV. However, later studies performed with different tomato accessions showed that the outcome of this mixed infection involving ToLCNDV-ES and TYLCV will require further analysis (Fortes *et al.*, 2025).

Given these and other examples of mixed infections, it is essential to expand our knowledge of virus-virus interactions and their influence on disease ecology and evolution. Elucidating these interactions and their mechanisms will be crucial for the development of effective control strategies.

1.3 Virus like-particles: advances in plant sciences

In the last decades, plants have emerged as powerful and versatile biofactories for the production of recombinant proteins, becoming an attractive alternative to traditional systems based on bacteria, yeast or animal cells (Buyel 2024). Producing proteins in plants offer several intrinsic advantages, including low production costs, scalability, and a high level of biosafety as plant pathogens do not infect mammals, and reciprocally mammalian pathogens do not replicate in plants (Kusnadi *et al.*, 1997; Lico *et al.*, 2008). Consequently, plants are highly advantageous compared with other organisms to produce pharmaceuticals, vaccines, and complex macromolecular assemblies.

Historically, two main strategies have been employed to produce heterologous proteins in plants: stable genetic transformation, and transient expression (Marsian and Lomonossoff, 2016). Stable transformation is achieved through the introduction and expression of foreign DNA in the plant nuclear genome, and it enables large scale, low-cost biomass production and potential for crossing transgenic lines to obtain multiple proteins expressed in the same plant (Santi *et al.*, 2006). However, this approach is often

Introduction

very time-consuming and less suitable for the rapid screening of a wide variety of different constructs (Kusnadi *et al.*, 1997; Peyret and Lomonossoff, 2015). On the other hand, transient expression systems allow high-level protein accumulation in a matter of days, making them particularly well suited for exploratory studies and fast protein production (Pogue *et al.*, 2002). Among these systems, *N. benthamiana* has become the host of choice due to its non-food crop status, a rapid growth in high density, the availability of *A. tumefaciens* mediated gene delivery, and compatibility with a wide range of expression vectors (Goodin *et al.*, 2008; Peyret and Lomonossoff, 2013). Efficient viral expression vectors have further enhanced the potential of plant transient expression systems providing autonomously replication for the expression of recombinant proteins plants (Mardanov *et al.*, 2024). Both RNA- and DNA-based viral vectors have been adapted to produce robust protein accumulation in plants; the most popular plant expression vectors are based on RNA viruses such as Tobacco mosaic virus (TMV), Potato virus X (PVX), cowpea mosaic virus (CPMV) as well as bean yellow dwarf virus (BeYDV), a DNA-containing geminivirus (Peyret and Lomonossoff, 2015; Mardanov *et al.*, 2024). The need for a rapid and efficient production of candidate vaccines and vaccine carriers in plants has driven the development of novel expression systems. In this context, the CPMV derived pEAQ vector was developed to significantly increase protein expression (Sainsbury *et al.*, 2009). Subsequently, a second vector, the pHREAC, achieved particularly high recombinant protein yields, with important implications for the broader deployment of plant-based transient expression platforms (Peyret *et al.*, 2019). These systems have proven particularly effective for producing virus-like particles (VLPs).

Virus-like particles are a non-infective version of viral particles that mimic their morphology forming the viral structure of native virions but lacking infectious genomic material (Marsian and Lomonossoff, 2016). They possess exceptional features, including the ability of self-assembling into defined structures of known dimensions, showing a high degree genetic flexibility, a structural uniformity and they also can be used for conjugation to inorganic ligands (Steele *et al.*, 2017). In addition, the use of noninfective VLPs instead virions particles result in low risk to the environment. VLPs have great potential for a number of applications, from basic virus assembly and structure studies to the production of commercial vaccines for use in humans and animals (Zeltins 2013). Indeed, the major application of VLPs is their direct use as vaccines, especially as safer alternatives to attenuated live or inactive killed virus-based vaccines, and for many others applications in nanomedicine, with VLPs derived from plant viruses being particularly valuable as they originate from pathogens that do not infect humans or animals (Santi *et*

et al., 2006; Balke and Zeltins, 2020). Since the pioneering effort with CPMV-based constructs, the production of vaccine candidates through VLPs expressed in plants has been intensively pursued, and several recent reviews provide comprehensive summaries of current advances in this field (Peyret *et al.*, 2021; Mardanova *et al.*, 2024). In parallel to other plant molecular pharming approaches, the interest in potential applications of VLPs in agriculture is increasing, constituting a rather promising technology in this field (Xue *et al.*, 2023).

OBJECTIVES

2. OBJECTIVES

The overall aim of the present thesis is to advance the understanding of complex virus-plant-vector interactions, with particular emphasis on the infection and transmission mechanisms of different sweet potato viruses, as well as the exploration of novel viral-based platforms with potential applications in biological control. To accomplish this, the following specific objectives were defined:

1. Exploration of aphid-transmission of the potyvirus SPV2 and characterization of the role of particular mutations in two viral isolates.
2. Description of an isolate of SPFMV, characterizing its RNA silencing suppressor proteins, and comparing them with those of a previously characterized isolate.
3. Analysis of the sweet potato virome complexity and functionally and transcriptomical characterization of plant responses to single and mixed viral infection.
4. *In planta* production and purification of virus-like particles of the begomovirus ToLCNDV
5. Generation and validation of an infectious clone of the Spanish strain of ToLCNDV.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Biological materials

3.1.1 Virus sources

Four different plant viruses were used in the experiments described in this thesis. The potyvirus isolates SPFMV AM-MB1 and AM-MB2 were previously identified by Mingot *et al.*, (2016) through RNA-seq analysis of commercially acquired sweet potato (*Ipomoea batatas*) plants. Since their identification, these viruses have been maintained by vegetative propagation in the CRAG greenhouse facilities. SPV2 was identified alongside SPFMV isolates through RNA-seq analysis and it has been maintained in *Nicotiana benthamiana* plants by monthly mechanical inoculation passages.

To obtain sweet potato plants with super-infections, the crinivirus SPCSV-Can181-9 isolate, kindly provided by Dr. Jesús Navas (IHSM La Mayora, Málaga, Spain), was used. This isolate was maintained in *I. batatas* plants through vegetative propagation and occasional transmission experiments by *Bemisia tabaci*.

ToLCNDV (ND-661-2.0-A and ND-661-2.0-B of ToLCNDV-[ES-Alm-661-Sq-13]) (GenBank accession number DNA-A KF749223, DNA-B KF749226) was kindly provided by Dr. E. Moriones (IHSM La Mayora, Málaga, Spain). ToLCNDV-infected source plants were obtained using *Agrobacterium tumefaciens* inoculation technique (agroinfiltration) and whiteflies-mediated transmission assays. These plants were used for virion purification and transmission control experiments and maintained at CRAG under the required biosafety conditions.

3.1.2 Plants

Sweet potato plants infected with SPFMV, SPV2, or SPCSV were maintained under greenhouse conditions (25-20 °C Day/night; 14/10 h light/dark cycle, 45-55% humidity).

N. benthamiana plants were grown from the CRAG seed stock in a greenhouse at 24-22°C Day/night with a 14/10h light/dark photoperiod and 45-55% humidity.

Nicotiana tabacum cv. Xanthi and *Solanum melongena* plants were also used for rearing virus-free colonies of the green peach aphid *Myzus persicae* and the whitefly *B. tabaci*, respectively, and they were grown from seeds (provided by CRAG greenhouse service) and maintained under the same growing conditions as *N. benthamiana* plants.

Cucurbita pepo plants were grown from seeds (cv. Diamant, provided by Semillas Fitó) in a greenhouse at 22°C and with a 16h/8h light/dark photoperiod and 65% humidity.

3.1.3 Transmission vectors

Aphids

The green peach aphid (*Myzus persicae*-MP89) colony used for transmission assays was originally provided by Dr. A. Fereres (ICA/CSIC, Madrid, Spain). Aphid colonies have been reared on *N. tabacum* cv. Xanthi plants and maintained in chambers with constant controlled environmental conditions (25°C, 16/8 hours light/darkness, 50% humidity).

Whiteflies

The MED biotype of *Bemisia tabaci* (Gennadius), previously designated as Q (De Barro *et al.*, 2011), was employed as a vector in transmission experiments involving the phloem-limited crinivirus SPCSV and the begomovirus ToLCNDV. Colonies of *B. tabaci* MED were supplied by personnel from IRTA Cabrils (Spain) from field individuals originally collected by Dra. R. Gabarra, and they were maintained under controlled laboratory conditions on *S. melongena* plants in growth chambers set at 25 °C with a 16/8 h light/dark photoperiod, 50% humidity.

3.1.4 Bacterial strains

Escherichia coli* and *Agrobacterium tumefaciens

TOP10 strain of *E. coli* was used routinely for transformation, multiplication, and purification of plasmids constructs. It was grown at 37°C at 200 rpm for 24 h in LB medium.

A. tumefaciens strains EHA 105 strains (used for plasmids dedicated to plant expression) and LBA4404 (used for transient expression of ToLCNDV VLPs) of were routinely used for transformation, multiplication and transfer of gene constructs into plant cells via agroinfiltration. All *Agrobacterium* cultures were grown at 28°C at 200 rpm for 24-48 h in LB or YEB medium.

- LB medium (10 g/L triptone, 5 g/L yeast extract and 10 g/L NaCl; Agar 15 g/L was complemented for preparation of solid medium)
- YEB medium (5 g/L beef extract, 1 g/L yeast extract, 5 g/L peptone, 5 g/L sucrose, 0.48 g/L MgCl₂)

Media were supplemented with appropriate antibiotics depending on the resistance genes for each specific plasmid (kanamycin, streptomycin, rifampicin) at the required concentrations of use.

Bacterial strains were maintained at 4°C during short period of time and stored at -80°C for long-term storage in 25% glycerol preparations.

3.1.5 Plasmids and cloning vectors

A set of viral and/or binary vectors have been employed for cloning and expression of constructs (Table M1). Gateway technology (Invitrogen, United States) was used to generate plasmids starting with pENTRY/D-TOPO and mobilizing into two pDEST destination vectors (pGWB702Ω and pGWB718) through LR recombination reactions, as described in the commercially available kits (Invitrogen).

Two engineered expression vectors, pEAQ-*HT* (Sainsbury *et al.*, 2009) and pHREAC (Peyret *et al.*, 2019), kindly provided by Professor George Lomonosoff (John Innes Centre, Norwich, UK), were used for the transient expression of begomovirus VLPs. The first system was propriety of Leaf Systems Ltd until its cease of activity, and later transferred to Plant Bioscience Ltd, and it was used only for academic research purposes as established in a Material Transfer Agreement.

The pCAMBIA binary vector containing DNA-A and DNA-B ToLCNDV infectious clones (ND-AL-661-2.0-A and ND-AL-661-2.0-B, respectively) kindly provided by Dr. E. Moriones, were employed as template for different cloning approaches and to agroinoculate *N. benthamiana* and zucchini squash plants in order to purify ToLCNDV virions. Additionally, pCAMBIA 0380 vector (Cambia, Canberra, Australia) was used to generate pCAMBIA:GFP-ToLCNDV and pCAMBIA:CP-ToLCNDV binary vectors. Other used plasmids were based in pBIN (van Engelen *et al.*, 1995) and pMA (commercially available when ordering GeneArt constructs).

Table M1. Used expression constructs for experimental purposes of the present thesis dissertation.

Plasmid generated	Vector	Cistron	Virus origin	Condition
pENTRY-P1_AM-MB1	pENTRY/D-TOPO	P1	AM-MB1 SPFMV	De novo generated
pENTRY-P1ONLY_AM-MB1	pENTRY/D-TOPO	P1-ONLY	AM-MB1 SPFMV	De novo generated
pENTRY-P1N-PISPO_AM-MB1	pENTRY/D-TOPO	P1N-PISPO	AM-MB1 SPFMV	De novo generated
pENTRY-HCPro_AM-MB1	pENTRY/D-TOPO	HCPro	AM-MB1 SPFMV	De novo generated
pENTRY-P1HCPro_AM-MB1	pENTRY/D-TOPO	P1HCPro	AM-MB1 SPFMV	De novo generated
pGWB702Ω-P1_AM-MB1	pGWB702Ω	P1	AM-MB1 SPFMV	De novo generated
pGWB702Ω-P1ONLY_AM-MB1	pGWB702Ω	P1-ONLY	AM-MB1 SPFMV	De novo generated

Materials and methods

pGWB702Ω-P1N-PISPO_AM-MB1	pGWB702Ω	P1N-PISPO	AM-MB1 SPFMV	De novo generated
pGWB702Ω-HCPro_AM-MB1	pGWB702Ω	HCPro	AM-MB1 SPFMV	De novo generated
pGWB702Ω-P1HCPro_AM-MB1	pGWB702Ω	P1HCPro	AM-MB1 SPFMV	De novo generated
pGWB718-P1_AM-MB1	pGWB718	P1	AM-MB1 SPFMV	De novo generated
pGWB718-P1ONLY_AM-MB1	pGWB718	P1-ONLY	AM-MB1 SPFMV	De novo generated
pGWB718-P1N-PISPO_AM-MB1	pGWB718	P1N-PISPO	AM-MB1 SPFMV	De novo generated
pGWB718-HCPro_AM-MB1	pGWB718	HCPro	AM-MB1 SPFMV	De novo generated
pGWB718-P1HCPro_AM-MB1	pGWB718	P1HCPro	AM-MB1 SPFMV	Mingot <i>et al.</i> , 2016
pGWB702Ω-P1_AM-MB2	pGWB702Ω	P1	AM-MB2 SPFMV	Mingot <i>et al.</i> , 2016
pGWB702Ω-P1ONLY_AM-MB2	pGWB702Ω	P1-ONLY	AM-MB2 SPFMV	Mingot <i>et al.</i> , 2016
pGWB702Ω-P1N-PISPO_AM-MB2	pGWB702Ω	P1N-PISPO	AM-MB2 SPFMV	Mingot <i>et al.</i> , 2016
pGWB702Ω-HCPro_AM-MB2	pGWB702Ω	HCPro	AM-MB2 SPFMV	Mingot <i>et al.</i> , 2016
pGWB702Ω-P1HCPro_AM-MB2	pGWB702Ω	P1HCPro	AM-MB2 SPFMV	Mingot <i>et al.</i> , 2016
pGWB718-P1_AM-MB2	pGWB718	P1	AM-MB2 SPFMV	Mingot <i>et al.</i> , 2016
pGWB718-P1ONLY_AM-MB2	pGWB718	P1-ONLY	AM-MB2 SPFMV	Mingot <i>et al.</i> , 2016
pGWB718-P1N-PISPO_AM-MB2	pGWB718	P1N-PISPO	AM-MB2 SPFMV	Mingot <i>et al.</i> , 2016
pGWB718-HCPro_AM-MB2	pGWB718	HCPro	AM-MB2 SPFMV	Mingot <i>et al.</i> , 2016
pGWB718-P1HCPro_AM-MB2	pGWB718	P1HCPro	AM-MB2 SPFMV	Mingot <i>et al.</i> , 2016
pCAMBIA:DNA-A-ToLCNDV (ND-661-2.0-A)	pCAMBIA	DNA-A	ToLCNDV	Fortes <i>et al.</i> , 2016
pCAMBIA:DNA-B-ToLCNDV (ND-661-2.0-B)	pCAMBIA	DNA-B	ToLCNDV	Fortes <i>et al.</i> , 2016
pEAQ-HT-D1-AYVVCP	pEAQ-HT	CP	AVYY	Hesketh <i>et al.</i> , 2018
pBinAYVV 1/7	pBIN	alphasatellite DNA	AVYY	Saunders <i>et al.</i> , 1999
pHREAC:CP-ToLCNDV	pHREAC	CP	ToLCNDV (DNA-A)	De novo generated
pMA-DNA-A/GFP	pMA	DNA-A	ToLCNDV	De novo generated
pMA-DNA-A/CP	pMA	DNA-A	ToLCNDV	De novo generated
pCAMBIA:GFP-ToLCNDV	pCAMBIA	DNA-A	ToLCNDV	De novo generated

pCAMBIA:CP-ToLCNDV	pCAMBIA	DNA-A	ToLCNDV	De novo generated
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3.2 Virus inoculation

3.2.1 Mechanical inoculation

SPV2 was maintained by monthly mechanical inoculation of *N. benthamiana* plants. For inoculation, abrasive carborundum powder was applied on leaves of healthy plants approximately 2 weeks old to gently rub the inoculum and cause wounds in epidermal cells that facilitated viral entry. Inoculum was prepared by grinding 1 part of symptomatic upper leaf tissue from previously infected plants with 2 parts of phosphate buffer (0.02M, pH 7.2) using a cooled mortar and pestle to ensure proper homogenization. Active carbon was also added to the homogenate to enhance infection efficiency. The resulting sap was gently rubbed onto the leaves and plants were covered with wet tissues to keep under high humidity. Plants recover during overnight before moving to the greenhouse or growth chambers under controlled conditions. Viral symptoms typically developed are visible after 10-12 days post inoculation (dpi). When mechanical inoculation was conducted with virions, SPV2 purified virions have been gently rubbed onto the leaves.

3.2.2 Insect-mediated inoculation

Aphid-mediated inoculation / Aphid mediated non-persistent transmission

M. persicae individuals were used to conduct transmission experiments of SPV2 in *N. benthamiana* plants. Apterous adult aphids were collected from *N. tabacum* plants, where they were reared, and starved for 2 h in a glass vial. Subsequently, they were placed on virus-infected leaves for a 10-minute virus acquisition access period (AAP). After this period, the aphids were carefully transferred to test plants using a fine paintbrush and allowed an inoculation access period (IAP) of at least 1h (Ontiveros *et al.*, 2024; Fig. M1). After inoculation period, aphids were eliminated by spraying the plants with a freshly prepared insecticide solution of commercial Imidacloprid (0.05% v/v). Plants were then monitored for symptom development and tested for virus infection.

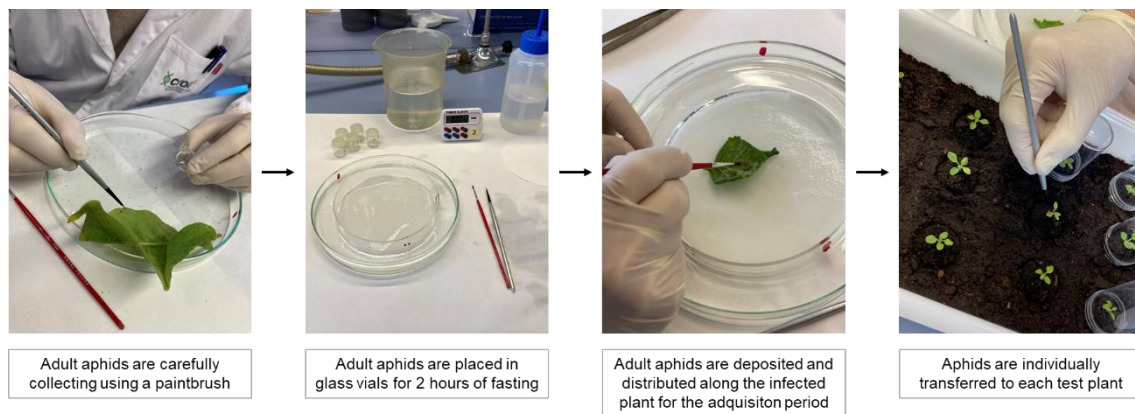


Figure M1. Experimental procedure for aphid-mediated transmission of SPV2 from infected *N. benthamiana* to healthy *N. benthamiana* plants.

Whitefly-mediated inoculation

Adults of whitefly *B. tabaci*, MED biotype, were used for whitefly-mediated virus inoculation. For obtaining viruliferous whiteflies, insects reared on virus free plants, were mass given a 48h AAP on virus source infected plants (for SPCSV and for ToLCNDV). Viruliferous whiteflies were transferred to an insect-proof cage with healthy test plants for a 48h IAP. Then the insects were eliminated by spraying insecticide Imidacloprid at least 3 treatments, as described for aphids. Plants were then monitored for symptom development and tested for virus infection.

3.2.3 Agrobacterium-mediated virus inoculation

Agrobacterium cultures were used to inoculate ToLCNDV for whitefly transmission experiments. Bacterial cultures of DNA A and DNA B from ToLCNDV were grown on YEB liquid medium at 28°C, and the growth was monitored by optical density assessment at $\lambda = 600$ (OD₆₀₀). They were centrifuged at 7500 rpm for 12 mins and then re-suspended in inoculation medium (10 mM MES/NaOH, pH 5.6, 10 mM MgCl₂, 150 μ M acetosyringone) and induced for 2-3h at 28°C. The cultures were adjusted to OD₆₀₀ = 0.5 before infiltration with a needleless syringe. Squash plants were inoculated in the fully extended cotyledons and *N. benthamiana* plants where inoculated in two-leaf growth stage. Plants were maintained under controlled greenhouse conditions.

3.3 Nucleic acid manipulation

3.3.1 DNA extraction from plant material

DNA extraction was performed following the protocol described by Gilbertson *et al.*, (1991). Leaf tissue samples were collected by excising leaf discs using the cap of a sterile microcentrifuge tube (2 mL) and flash frozen in N₂. After adding 500 μ L of

extraction buffer (50mM EDTA, 500mM NaCl, and 10mM β -mercaptoethanol) samples were homogenized by grinding and vortexing. Subsequently, 33 μ L of 20% SDS was added and the mixture was vortexed and incubated at 65°C for 10 minutes. After incubation, 160 μ L of 5M potassium acetate was added and samples were centrifuge at 10,000 rpm for 10 mins. The resulting upper aqueous phase was recovered into fresh tubes and 230 μ L of isopropanol was added, followed by vortex. Samples were centrifugated at 10,000 rpm for 5 mins and the supernatant was carefully discarded. Pellet was washed with 500 μ L of 70% ethanol, centrifuged and subsequently resuspended in 200-500 μ L MiliQ water. The resulting pellets were used directly for ToLCNDV detection.

3.3.2 RNA extraction from plant material and reverse transcription

Leaf patches were cut with the cap of a sterile microcentrifuge tube (2 mL) and immediately flash frozen in N₂. The tissue was grinded in the same microcentrifuge tube either using glass beads in a TissueLyser (Qiagen) homogenizer device, or using a prechilled micro pestle adequate for microcentrifuge tubes. RNA extraction of plant samples was done using Maxwell® RSC plant RNA kit (Promega Corporation, United States) for total RNA isolation. This method provided pure RNA free of DNA contaminants since the reaction included a DNase I treatment that facilitated the use of the RNA as template in virus detection RT-PCR, qRT-PCR measurements and construction of RNA-seq libraries. The High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, United States) was used to reverse transcribe samples, corresponding to 1 μ g of total RNA, using a mix of random or gene specific primers depending the experiment's objective, in accordance with the manufacturer's instructions.

3.3.3 PCR amplification

- ExTaq polymerase amplification

For virus detection, the commercial TaKaRa Ex Taq kit (Takara Bio Inc., Japan) was employed to amplify target sequences using virus specific-primers and approximately 0.05 μ g of RT-derived cDNA as template. Purified plasmid DNA served as positive control for amplification. Annealing temperatures and elongation times were optimized on each reaction based on expected amplicon sizes and the melting temperature of each pair of specific primers used. The specific primers used for molecular detection of SPFMV AM-MB1, SPFMV AM-MB2, SPV2 and SPCSV are listed on Supplementary Table S1.

- **Phusion polymerase amplification**

For the amplification of viral gene products intended for cloning into different expression vectors, the proofreading High Fidelity Phusion Polymerase (Thermo Fisher Scientific, United States) was employed in HF buffer and with appropriate primers, following provider instructions. As described in the previous section, purified plasmid DNA served as a positive control for amplification. Annealing temperatures and elongation times were optimized for each reaction based on the melting temperature of the specific primer pairs employed.

- **Colony PCR analysis**

For the rapid screening and detection of positive colonies with the expected fragment cloned into the correspond vector, the GoTaq Hot Start Green Master Mix (Promega Corporation, United States) was employed together specific primers, following manufacturer recommendations. Temperature and cycles used for the different PCR reactions varied according to T_m of primers and amplicon length.

3.3.4 Nucleic acid gel electrophoresis

Separation of DNA and RNA fragments by size was performed by electrophoresis on ethidium bromide-stained agarose gels (1–2% agarose, depending on amplicon size) and visualized using a BIO-PRINT UV gel documentation system. Gels were prepared by melting the appropriate amount of agarose in 1× TAE buffer (0.4 M Tris-acetate, 0.001 M EDTA, pH 7.8), and following cooling and solidifying, samples were loaded into wells after adding loading buffer. Electrophoresis was conducted at 100V for 30 mins, using a commercially available molecular weight markers (GeneRuler 1 kb Plus DNA Ladder, Thermo Fisher Scientific, United States) to estimate fragment sizes.

3.4 Genome sequencing and sequence analysis

3.4.1 Genome sequencing of AM-MB1 SPFMV isolate

To obtain the complete coding sequence of AM-MB1 isolate, a genome walking strategy was employed. This involved amplifying viral segments using High-Fidelity Phusion Polymerase and visualizing the products on 1% agarose gels. Fragments of the expected size were excised from the gels and purified with the NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel, United States). Subsequently, the purified fragments were sequenced by Sanger capillary sequencing at the CRAG facilities (primers listed in Supplementary Table S1). The assembly of the sequenced fragments into a consensus was done using at least 2 verified reads for each section using the licensed SnapGene

software (Insightful Science). Complete sequence was generated overlapping the individual segments and followed by full annotation of the polyprotein cleavage sites.

5' and 3' RACE sequencing

The terminal regions of the SPFMV isolates were obtained using 5' and 3' RACE (Rapid Amplification of cDNA Ends). For 3' RACE, total RNA was reverse-transcribed with SuperScript II™ reverse transcriptase using an oligo-dT adapter primer (AP) to anneal to the poly(A) tail. The resulting cDNA was amplified with three different gene-specific primers (GSPs) together with the Abridged Universal Amplification Primer (AUAP) to recover the unknown 3' terminal sequence. For 5' RACE, first-strand cDNA synthesis was primed with the corresponded gene-specific antisense primer GSP1. After purification, homopolymeric tails were added to the cDNA using terminal deoxynucleotidyl transferase (TdT), and the tailed cDNA was amplified by nested PCR using the GSP2 together with the Abridged Anchor Primer (AAP) and subsequently reamplifying using additional GSPs and AUAP. This strategy enabled the recovery of the 5' terminal region. All procedures were applied to both SPFMV isolates and primers designed and used in each reaction are listed in Table M2.

Table M2. Primers designed for deciphering 5' and 3' UTR of AM-MB1 and AM-MB2 SPFMV isolates.

Isolate end	Primer name	Sequence (5'→ 3')
3' RACE AM-MB1	3'RACE-GSP1-AM-MB1-SPFMV	TGGATCTATTGTGACGCCGATGG
3' RACE AM-MB1	3'RACE-GSP2-AM-MB1-SPFMV	GTCGTACCACGTGTTAAGATGAAT
3' RACE AM-MB1	3'RACE-GSP3-AM-MB1-SPFMV	GCTTACATAGAGATGCGAAACCGT
5' RACE AM-MB1	GSP1_5RACE_FM_AMMB1_RV	ACGTGGCATGTTTCATTTGAGACCT TAAAC
5' RACE AM-MB1	GSP2_5RACE_FM_AMMB1_RV	CAATACCCTAGCAATGGCGTTCAT GTTG
5' RACE AM-MB1	GSP3_5RACE_FM_AMMB1_RV	G TTCAGACTCCCACTTGTATGTTT CGCC
5' RACE AM-MB1	GSP4_5RACE_FM_AMMB1_RV	CCCACGTGAACCATAATAGTATTT GTGCC
3' RACE AM-MB2	GSP1_Nib_8806_SPFMV_3'_RACE_FW1	TGGGTTTATTGTGACGCTGATGG
3' RACE AM-MB2	GSP2_CP_9913_SPFMV_3'_RACE_FW1	GTTGTGCCACGTGTCAAGATGAAT
3' RACE AM-MB2	GSP3_CP_10291_SPFMV_Seq_Fw	GCCTATATAGAAATGCGAAACCGT

3' RACE AM-MB2	GSP4_CP_10408_SPFMV_Seq_RV	TGAATGCAGCTCGTAAAAATCAAA TG
5' RACE AM-MB2	GSP1_5RACE_SPFMV_P1_879_RV	AGTCGATTCAATAATGGTTGTGGT AGGCAC
5' RACE AM-MB2	GSP2_5RACE_SPFMV_P1_597_RV	AGGTGCGACTTGGTCAAGCTCATA
5' RACE AM-MB2	GSP3_5RACE_SPFMV_P1_501_RV	GTTTCATGTTATCTGCGATGTTATCA TCGCG
5' RACE AM-MB2	GSP4_5RACE_SPFMV_P1_424_RV	ATCATCACATTTCAGCACAAAAAGT GAGCTC
5' RACE AM-MB2	GSP5_5RACE_Seq_SPFMV_P1_270_RV	GAGATATGCGGCCAGATGGTC

3.4.2 Sequence alignment and phylogenetic analysis

SPV2 HCPPro and CP amino acid alignments were performed with CLUSTAL Omega. Sequences obtained of HCPPro and CP proteins from SPV2 AM-MB2 and SPV2_Nb-CO, together with additional 14 SPV2 isolates available in GenBank were used to search putative recombination events. The recombination points were identified using Genetic Algorithm for Recombination Detection (GARD) with default parameters (Kosakovsky *et al.*, 2006).

Phylogenetic analysis of P1 gene were conducted using complete P1 nucleotide sequences of SPFMV AM-MB1 and SPFMV AM-MB2, together with P1 sequences selected from additional 24 SPFMV isolates available in the GenBank database. Phylogenetic tree was inferred using the maximum-likelihood method with 1000 bootstraps interactions available in MEGA12 (Kumar *et al.*, 2024). CLUSTAL W with default parameters was used to generate multiple sequence alignments for the P1 sequences.

3.5 Transient expression of heterologous proteins *in planta*

3.5.1 Assessment of RNA silencing suppressor activity

Construct of binary plasmids associated to viral proteins

AM-MB1 SPFMV-derived constructs were generated based on the AM-MB2 SPFMV construct described by Mingot *et al.*, (2016) to allow direct comparisons. The complete cistrons of P1, HCPPro and P1HCPPro (in *cis*) were amplified by RT-PCR employing total nucleic acids extracted from infected sweet potato plants. Amplifications were performed by using High Fidelity Phusion Polymerase and specific primers (Table M3). Additionally, a P1 variant, denominated P1-ONLY, was obtained by a recombinant PCR. A single

nucleotide substitution (G → T) was introduced, resulting in a synonymous mutation within P1 (CTG → CTT; Leu), which does not alter the encoded amino acid. In contrast, the same substitution within PISPO introduces a nonsynonymous change, translating a glycine codon (GGA) into a premature stop codon (TGA) thereby preventing any expression of P1N-PISPO, even in the event of polymerase slippage at the conserved G₂A₆ motif. The transframe gene product P1N-PISPO was also generated by recombinant PCR. An additional guanine was inserted in the conserved G₂A₆ motif (G₂A₆ → G₃A₆) and a thymine-to-adenine substitution was introduced. The guanine insertion shifts the reading frame to PISPO, while the nucleotide substitution forces the introduction of a premature stop codon within P1.

Table M3. List of primers used for amplification of AM-MB1 SPFMV proteins and cloning into pENTRY/D-TOPO.

Primer name	Sequence (5' → 3')	Application
pENTRY_P1_SPFMV_AMMB1_FW	CACCATGGCAACTATTG TGGCAT	Directional cloning of P1 wild type, recombinant P1-ONLY, recombinant P1N-PISPO, and P1HCPPro
pENTRY_P1_SPFMV_AMMB1_RV	CTAAACTGAGTGATGT ATGGTAAGACAGA	Cloning of SPFMV P1, P1-ONLY, and recombinant P1N-PISPO
recomb_P1only_SPFMV_AMMB1_FW	GGAAAAAAGTTGACGAA CAATTG	Site-directed mutagenesis of SPFMV P1 to generate P1-ONLY
recomb_P1only_SPFMV_AMMB1_RV	CAATTGTTCTGCAAGTTT TTTCC	Site-directed mutagenesis of SPFMV P1 to generate P1-ONLY
P1NPISPO_AMMB1_FW	CTCGTCTGGGAAAAAAC TGGACGAACAATAGGTT GC	Site-directed mutagenesis of SPFMV P1 to generate P1N-PISPO
P1NPISPO_AMMB1_RV	GCAACCTATTGTTCTGTC CAGTTTTTCCCAGACG AG	Site-directed mutagenesis of SPFMV P1 to generate P1N-PISPO
pENTRY_HCPPro_SPFMV_AMMB1_fw	CACCATGAGCTCCACCA TGGAAAGGTTTCTGTCA GG	Directional cloning HCPPro
pENTRY_HCPPro_SPFMV_AMMB1_RV	CTAACCAACAATATAGT GTTTCATTTCTCAGACTCTA	Cloning of SPFMV HCPPro and P1HCPPro

All PCR products were purified from agarose gels using the NucleoSpin Gel and PCR Clean-up Kit (Macherey-Nagel, USA) and directionally cloned into the pENTR™/D-TOPO® vector (Invitrogen) within the GATEWAY™ expression system, employing *E. coli* strain TOP10. Clones containing the correct inserts were identified by restriction enzyme analysis and verified by Sanger sequencing. For the silencing suppressor assay, pENTR constructs were subsequently transferred via LR recombination (Invitrogen) into pDEST plasmids, specifically pGWB702Ω and pGWB718 (N-4xMyc), both carrying the CaMV 35S promoter and NOS terminator for transcriptional termination (Tanaka *et al.*, 2011).

Plasmids confirmed to harbour the correct cistron sequences were introduced into *A. tumefaciens* strain EHA105 by heat-shock transformation.

Agrobacterium-mediated co-infiltration assay

A standard co-agroexpression assay was employed to identify potential RNA silencing suppressor (RSS) proteins. Briefly, a binary vector carrying the GFP (pBIN61S, 35S:GFP) reporter gene was used in co-agroinfiltration experiments to evaluate the ability of viral gene products to suppress RNA silencing. Proteins exhibiting RSS activity maintain high GFP expression levels, which are detectable under UV light. Agroinfiltration of the generated binary plasmids was performed essentially as described by Voinnet *et al.*, (2000) and Valli *et al.*, (2006). Wild-type or 16c *N. benthamiana* plants with fully expanded leaves (approximately 2-3 weeks old) were co-infiltrated with a mix at equal volume of two or three different EHA105 *A. tumefaciens* cultures, depending on the experimental design. One or two cultures harbored plasmids expressing viral gene products, while the other contained the GFP-expressing construct (pBIN61S, 35S:GFP) (Silhavy 2002; Valli *et al.*, 2006). Positive (WMV-HCPro) and negative (an inverted orientation form of WMV-HCPro, designated orPCH) controls (Domingo-Calap *et al.*, 2021) were included in all assays. *Agrobacterium* cultures were grown as described in the previous Material and methods section 3.2.3 prior to infiltration using a needleless syringe.

GFP extraction and measurements

GFP fluorescence was visually inspected under UV light at 2 and 4-days post agroinfiltration (dpa), and photographs were captured with a Nikon D7000 digital camera. For analysis of GFP expression levels, three patches (two from third leaf and one from second leaf), around 35,6 mg were harvested, immediately flash frozen in liquid N₂ and grinded and homogenized with 600 µL cold carbonate buffer pH 9,5. The tubes were stirring and subsequently centrifuged at maximum speed for 15 mins at 4°C in darkness. 150 µL were taken from the supernatant and placed in wells of translucent 96 well reading plate and the GFP fluorescence was measured in a SPECTRAMAX (bioNova científica, s.l.) at 488nm. In all cases, three technical and three or four biological replicates were processed per construct.

Protein extraction and immunoprotein techniques (Western Blot analysis)

Agroinfiltrated patches of *N. benthamiana* plants were homogenized in extraction buffer containing 125 mM Tris-HCl (pH 7.5), 2% sodium dodecyl sulfate (SDS), 6M urea and 5% β-mercaptoethanol). Derived homogenates were boiled for 5 mins at 95°C and centrifuged at maximum speed for 10 mins to remove cell debris. Supernatants (10 µL)

were mixed with 2 μ L of loading buffer and resolved by electrophoresis on a 12% SDS-PAGE gel for 1 hour at 200V. Electrophoresed proteins were transferred to a nitrocellulose membrane (Amersham protan 0.45 NC, GE Healthcare, United States) for immunoblotting detection. A commercially available anti-Myc Tag monoclonal antibody (clone 4A6, Millipore, United States) was used for binding to the tested Myc-tagged SPFMV (AM-MB1 and AM-MB2) proteins, followed by incubation with secondary goat anti-mouse IgG (conjugated to horseradish peroxidase). Immunostained proteins were visualized by chemiluminescence (Super Signal™ West Femto, Thermo Fisher Scientific, United States) according to manufacturer's instructions using the Amersham™ ImageQuant™ 800 device Fluor (Cytiva, United States). Total protein content of the samples was assessed by Ponceau-red staining of membranes.

Statistical analysis

Statistical analysis was performed using appropriate software packages (GraphPad Prism). Data were first examined for normality and homogeneity of variance using standard test. Depending on the data distribution and experimental design, groups were assessed using a specific parametric test. GFP absorbance results are represented by the average $\pm$ SEM for each variable. Two-way ANOVA test was applied for significance assessment in a 95% confidence level. (p value <0.05). To estimate statistical differences of SPFMV single or in co-infection with SPCSV data were analysed by the Student-t-test (p -value <0.05).

3.6 Molecular quantification and transcriptomic analysis of viral accumulation

3.6.1 Viral load quantification

Total RNA from single- and mixed-infected sweet potato plants was isolated from tissue samples collected at T0 and T30 (0 dpi and 30 dpi, respectively). Six biological replicas were used for each timepoint. RNA extraction was performed using the Maxwell® RSC Plant RNA Kit. The quality and concentration of the extracted RNA were assessed with a NanoDrop® spectrophotometer (ND-8000, Thermo Fisher Scientific, USA), and reverse transcription was carried out using the High-Capacity cDNA Reverse Transcription Kit. The resulting cDNA served as the template for qPCR reactions using Master SYBR Green I in a final volume of 10 μ L. Amplifications were performed on a LightCycler 480 system (Roche, Switzerland) with 384-well plates under the following cycling conditions: 95°C for 10 mins, followed by the 45 amplification cycles at 95°C for 15 secs and 60°C for 60secs.

The relative quantification of specific RNAs of SPFMV and SPCSV were normalized to the ribosomal protein L2, (*IbRPL2*; Supplementary Table S1) (Yu J, *et al.*, 2020). Gene-specific primers used to determine SPFMV AM-MB2 isolate and RNA1 and RNA2 of SPCSV are also listed in Supplementary Table S1. Each primer pair efficiency was evaluated by a standard curve with three points to obtain efficient rates (E) ($E = 10^{(1/\text{slope})-1}$, expressed as percentages) with $R^2 > 0.95$ correlation values for the curves.

3.6.2 Libraries construction and sequencing

Leaf tissue from eight sweet potato plants single- and eight sweet potato plants mixed-infected (the same plant set used for symptom evaluation and virus detection) was collected at 0 dpi (T0) and 30 dpi (T30) to generate RNA-sequencing libraries. Two leaf patches per sample were pooled, immediately frozen in liquid nitrogen and maintained at -80°C. ~1000 ng of total RNA was extracted from previously frozen and manually homogenized tissue by using the Maxwell® RSC plant RNA kit. All samples were analysed by RT-PCR to confirm the presence of viral RNA in infected sweet potato plants at each time point.

Library construction was performed using the TruSeq Stranded Total RNA with RiboZero Plant kit (Illumina, ref. 20020610) following the manufacturer's instructions. Briefly, after rRNA depletion, the remaining RNA was purified, fragmented, primed for cDNA synthesis and subsequent 3' adenylation was performed, followed by adapter ligation from the Truseq RNA UD Indexes v2 set (Illumina, ref. 20040871). Library quality and fragment size were assessed on a TapeStation 4150 (Agilent Technologies) using the D1000 assay (Agilent Technologies, ref. #5067-5582 and #5067-5583).

3.6.3 RNA-seq data processing and analysis

To identify viral sequences present in the RNA-seq datasets of *I. batatas*, the Genome Detective platform (v2.21.4) (Vilsker *et al.*, 2019) was used with the default metagenomic virus analysis protocol. For each sample, a table of detected viral sequences with at least 50% nucleotide (nt) similarity was generated, reporting the number of virus-associated reads, depth of coverage, genome coverage, and nt identity.

Raw paired-end RNA-seq reads were processed using nf-core/rnaseq v3.18.0 (doi: 10.5281/zenodo.1400710) of the nf-core collection of workflows (Ewels *et al.*, 2020), utilising reproducible software environments from the Bioconda (Grüning *et al.*, 2018) and Biocontainers (da Veiga Leprevost *et al.*, 2017) projects. The pipeline was executed with Nextflow v24.04.4 (Di Tommaso *et al.*, 2017) with the following command: "nextflow run nf-core/rnaseq -resume -r 3.18.0 -profile singularity -params-file nf-params.json". Adapter trimming and quality filtering were performed using *fastp* (v0.23.4). Reads were

subsequently screened for ribosomal RNA contamination using SortMeRNA (v4.3.7), and rRNA-derived reads were removed prior to downstream analysis. Cleaned reads were aligned to a custom reference genome using the STAR aligner (v2.7.11) in combination with Salmon quantification (v1.10.3) in STAR–Salmon mode.

The reference genome consisted of the *I. batatas* “Beauregard v1” consensus assembly obtained from the Sweetpotato Genomics Resource, supplemented with viral genome sequences of SPFMV and SPCSV (RNA1 and RNA2). Corresponding genome annotations were provided as a GTF file. The alignment was performed with both “Beauregard” and “Tanzania” cultivar reference (Wu *et al.*, 2025). “Beauregard” genome was selected after yielding superior alignment metrics compared to the “Tanzania” cultivar reference. The reference transcriptome achieved 53% average mapping rates. Quality control metrics were generated at multiple stages of the workflow, including read quality, alignment statistics, library complexity, strandedness inference, and gene body coverage. Quality control results from all samples were aggregated into a single report using MultiQC. MultiQC data, Software Versions and nf-core/rnaseq Workflow Summary are provided in the rna_seq_multiqc_report_beauregard.html link: https://cragenomica-my.sharepoint.com/:u:/r/personal/ignasi_andreu_cragenomica_es/Documents/RNA-seq_ClaraOnta%C3%B1on/Beauregard/rna_seq_multiqc_report_beauregard.html?csf=1&web=1&e=BWJTKb.

Differential gene expression analysis

Gene-level count matrices generated by the nf-core/rnaseq pipeline were used as input for differential expression analysis using the nf-core/differentialabundance v1.5.0 pipeline (doi: 10.5281/zenodo.7568000), executed with Singularity containers and default setting unless otherwise specified. The analysis compared co-infected (mixed infection by SPFMV+SPCSV) samples, collected at time point 30 dpi (T30), against their corresponding 0 dpi (T0) controls (single infection by SPFMV).

Only samples collected at T30 that showed a substantial accumulation of SPCSV, as determined from viral read abundance, were retained for differential expression analysis together with their corresponding T0 samples. The final dataset comprised four T30 samples (V_SP13, V_SP14, V_SP15 and V_SP16) and four T0 samples (V_SP5, V_SP6, V_SP7 and V_SP8). The merged gene-level count matrix produced by Salmon quantification and the corresponding gene length matrix were provided to the pipeline. Lowly expressed genes were filtered by retaining only those with a minimum count of 10 in at least three samples. Differential expression analysis was conducted using DESeq2, with time point (T0 vs. T30) specified as the contrast. Genes were considered

differentially expressed if they exhibited an absolute log₂ fold change ≥ 1 and an adjusted p -value < 0.05 .

Functional annotation and enrichment analysis

Functional annotation of *I. batatas* genes was performed using eggNOG Mapper based on the eggNOG database v5.0.2. Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway annotations were extracted from the eggNOG output and used to build custom gene-term mappings. Differentially expressed genes (DEGs) identified by DESeq2 were subjected to GO and KEGG enrichment analyses using the clusterProfiler R package. Enrichment analyses were performed using a hypergeometric test, with the background defined as all genes with at least one GO or KEGG annotation. Resulting p -values were adjusted for multiple testing using the Benjamini-Hochberg method, and terms with an adjusted p -value < 0.05 were considered significantly enriched.

To reduce redundancy among enriched GO terms, semantic similarity-based simplification was applied using the simplify function in clusterProfiler. Enriched GO terms were classified into Biological Process (BP), Molecular Function (MF), and Cellular Component (CC) categories and visualized using dot plots. KEGG pathway enrichment results were similarly visualized, and pathway descriptions were retrieved from the KEGG database.

Weighted gene co-expression network analysis (WGCNA)

Additionally to the previous analysis, weighted gene co-expression network analysis (WGCNA) (Langfelder and Horvath, 2008) was performed to identify gene modules associated with viral accumulation independently of time. All 16 RNA-seq samples were included in this analysis (T30 samples: V_SP9, V_SP10, V_SP11, V_SP12, V_SP13, V_SP14, V_SP15 and V_SP16 and T0 samples: V_SP1, V_SP2, V_SP3, V_SP4, V_SP5, V_SP6, V_SP7 and V_SP8). Variance-stabilized expression values were obtained from the DESeq2 object using the variance stabilizing transformation (VST). Prior to network construction, genes with low counts (fewer than 10 reads in at least three samples) were removed. To further reduce noise, genes in the lowest quartile of expression variance were also excluded. The resulting expression matrix was transposed to a sample-by-gene format for network analysis.

A signed co-expression network was constructed using the WGCNA R package. The soft-thresholding power was selected based on the scale-free topology criterion (power = 12). Modules were identified using the blockwiseModules function with a minimum module size of 30 genes, deepSplit = 2, and merge cut height of 0.4. Module eigengenes

were calculated and used to assess module-trait relationships. Trait data included log₁₀-transformed viral loads for SPFMV and SPCSV, as well as sampling time (T0 vs. T30). Pearson correlations between module eigengenes and traits were computed, and corresponding *p*-values were estimated using Student's asymptotic test. Modules significantly correlated with viral load (SPFMV or SPCSV) but not with time were selected for downstream analyses. Gene expression patterns within selected modules were visualized using Z-score scaled heatmaps annotated with viral load and time metadata. For modules of interest, designated as “lightyellow” and “royalblue”, gene lists were extracted and subjected to GO and KEGG pathway enrichment analysis using the same annotation and enrichment strategy described above (eggNOG-based mapping and clusterProfiler with Benjamini-Hochberg correction). Enrichment results and annotated gene lists were exported for downstream interpretation.

3.7 *In planta* VLPs production and construction of ToLCNDV infectious clone

3.7.1 Production of AYVV-VLPs in *N. benthamiana* tissues by agroinfiltration

Generation of AYVV VLPs were performed as previously described in Hesketh *et al.*, (2018). Cultures harbouring pEAQ-HT-D1-AYVVCP and pBinAYVV 1/7 were kindly provide by Professor Lomonossoff (Jonh Innes Center, Norwich, UK). Bacterial cultures were grown as described in the previous Material and methods section 3.2.3 before infiltration with a needless syringe. *N. benthamiana* plants in fully extended leaves. Equal volumes of bacteria were combined prior to agroinfiltration. Plants were maintained under controlled greenhouse conditions.

3.7.2 Production of ToLCDNV-VLPs in *N. benthamiana* by agroinfiltration

- Generation of pHREAC:CP-ToLCNDV construct

For the amplification and subsequent cloning of the CP of ToLCNDV into the pHREAC vector (complete sequence available under GenBank accession number MK521430.1), specific primers (CP_clon_F and CP_clon_R; Table M5) were designed for Type IIS restriction/ligation cloning, using *BsaI* recognition sites flanking the gene of interest (Peyret *et al.*, 2019). This approach enabled one-pot restriction/ligation reactions. The reaction mixture was incubated at 37 °C for 1 hour, followed by transformation of *E. coli* with 10-20 ng of the ligation product. Transformed cells were grown overnight at 37 °C in selective LB medium, and liquid cultures were propagated for plasmid isolation. Colony

PCR was performed to screen positive clones, and the integrity of the CP coding region was confirmed by Sanger capillary sequencing. ToLCNDV DNA-A material served as the template for obtaining the CP.

- Generation of pCAMBIA:GFP-ToLCNDV construct

The construction of pCAMBIA:GFP-ToLCNDV was initiated with the *in silico* design of a synthetic fragment, which was subsequently synthesized by the Thermo Fisher GeneArt® service. The design was based on the complete DNA-A component of the ToLCNDV Spanish strain (2,769 nt), linearized at the intergenic region (IR). Within this fragment, the mostly CP coding sequence (771 bp) was replaced by the GFP coding sequence (770 bp), flanked by two *SapI* restriction sites. *SapI* is a Type IIS restriction enzyme that recognizes asymmetric DNA sequences and cleaves outside its recognition site. At the 3' end of GFP and downstream of the *SapI* site, 17 nucleotides corresponding to the 3' end of CP were retained to preserve the natural overlap with AC3 in DNA-A. This fragment is denominated DNA-A/GFP.

In the 5' of the DNA-A/GFP, upstream of the IR, a partial DNA-A fragment (483 nt) was included, comprising the AC4 coding region and the 5'-end of AC1/Rep (335 nt). An *AclI* restriction site (GG/CGCGCC) was added at the 5' terminus of the previous construct. At the 3' end of the DNA-A/GFP sequence, another partial fragment (285 nt) was incorporated, containing a second IR and the complete AV2 coding region, followed by the insertion of a *Sall* restriction site (G/TCGAC) at its terminus. The complete synthetic fragment was generated by the GeneArt® service and cloned into the pMA vector. The plasmid pMA-DNA-A/GFP was digested with *AscI* and *Sall* and cloned into an empty vector pCAMBIA digested previously with the same restriction enzymes, generating the binary vector pCAMBIA:GFP-ToLCNDV.

3.7.3 Construction of ToLCNDV infectious clone for agroinoculation

The construction of pCAMBIA:CP-ToLCNDV was based on the previously generated pMA-DNA-A/GFP construct. The template plasmid (pMA-DNA-A/GFP) was digested with *SapI*, a Type IIS restriction enzyme that cleaves outside its recognition site, allowing precise removal of the GFP sequence without introducing additional nucleotides (scarless excision). In parallel, two primers (Table M4) were designed to amplify the ToLCNDV CP coding sequence, which was subsequently cloned into the *SapI*-digested pMA-DNA-A/GFP backbone to restore the complete native DNA-A component, generating pMA-DNA-A/CP. The resulting pMA-DNA-A/CP plasmid was then digested with *AscI* and *Sall* and ligated into an empty pCAMBIA vector previously digested with the same restriction enzymes, yielding the binary vector pCAMBIA:CP-ToLCNDV.

Table M4. List of primers used for amplifying ToLCNDV CP and generate different plasmids.

Primer name	Sequence (5'→ 3')	Applications
CP_clon_F	AACGGTCTCTAAAATGTCGAAGC GACCAGCAG	Primer Fw for cloning CP ToLCNDV into pHREAC
CP_clon_R	AACGGTCTCGAGCGCTAATTTGT GACCGAATCATAAA	Primer RV for cloning CP ToLCNDV into pHREAC
SAPI-CP-ND-fw	CTTAgtcttcaactATGTCGAAGCGA CCAGCAGAT	Primer FW for cloning CP ToLCNDV into pMA-DNA-A/GFP to replace GFP by the own CP of the virus
CRAG-SAPI-CP-ND-rv	CGCGCGCTCTTCCgAATCATAAA AATAGATCCGGATTTCAAAGT	Primer RV for cloning CP ToLCNDV into pMA-DNA-A/GFP to replace GFP by the own CP of the virus

Plasmids containing the correct sequences were identified by restriction enzyme analysis and verified by Sanger sequencing. Then they were introduced into *A. tumefaciens* strain EHA105 by heat-shock transformation or to *A. tumefaciens* (LBA4404 strain) by electroporation depending on the construction.

***In planta* expression, protein extraction and Western Blot analysis**

Agrobacterium cultures grown in selective LB medium supplanted with rifampicin and kanamycin. Bacteria cultures with plasmids were grown to OD₆₀₀ of 2.0 or 4.0 and induced by acetosyringone for at least 2-3h, before infiltration to leaves of *N. benthamiana* plants (3-weeks old, grown under constant 16/8h light/darkness, 25°C) using a needleless syringe. Equal volumes of co-infiltrating bacteria were combined prior to infiltration. Infiltrated leaves were harvested at 7 dpa and the expression of ToLCNDV-CP was confirmed by Western blotting using a polyclonal antibody raised against ToLCNDV CP (ToLCNDV (AS-1109) Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) by incubation with secondary goat anti-rabbit horseradish peroxidase conjugated (HRP) secondary antibody was used at 1:10.000 dilution, and revealed using chemiluminescence substrate (Super Signal™ West Femto, Thermo Fisher Scientific, United States) according to manufacturer's instructions using the Amersham™ ImageQuant™ 800 device Fluor (Cytiva, United States). Total protein content of the samples was assessed by Ponceau-staining of membranes.

Molecular hybridization by tissue printing

To verify infection in virus-inoculated plants, DNA samples were blotted onto a positively charged nylon membrane (Roche, Germany), and nucleic acids were fixed by UV crosslinking at 1,200J for 1 min using a UVC500 cross-linker (Amersham Biosciences, United Kingdom). Membrane hybridization was carried out following previously described protocols (Fiallo-Olivé and Navas-Castillo, 2025). Primers used for the specific

detection of ToLCNDV were designed to target the IR of the DNA-A component of the virus and they are listed in Supplementary Table S1. The immobilized DNA was hybridized with digoxigenin (DIG)-labeled probes specific to the target sequence, and detection was performed using an alkaline phosphatase-conjugated anti-DIG antibody. The chemiluminescent signal was captured with a ChemiDoc™ imaging system (Bio-Rad Laboratories, United States) after incubation with CDP-Star reagent (Roche, Switzerland).

3.8 Virions and Virus-like particles purification

3.8.1 Purification of SPV2 virions

Fresh symptomatic *N. benthamiana* tissue infected with SPV2 was harvested at 15 days post inoculation (dpi) and flash-frozen in liquid N₂. Leaves were homogenized in three volumes of extraction buffer (50 mM sodium phosphate, 5 mM EDTA, 0.1% β-mercaptoethanol, pH 7.5) supplemented with EDTA-free protease inhibitor tablets (Roche), 1 tablet/100 mL. The homogenate was filtered through two layers of Miracloth (Merck KGaA, Germany) and clarified by low-speed centrifugation (8,000×g, 15 min, 4°C). The resulting supernatant was incubated with 2% Triton X-100 for 1 h at 4 °C with gentle stirring, followed by an additional clarification step under the same centrifugation conditions (8,000×g, 15 mins, 4°C). For the first ultracentrifugation step, the clarified supernatant was layered onto a 20% (w/v) sucrose cushion and centrifuged at 130,000×g for 3h at 4 °C using either a 50.2 Ti fixed-angle rotor (Beckman Coulter, USA) or a TH-641 swinging-bucket rotor (Thermo Fisher Scientific, USA). The resulting pellet was resuspended in 1 mL of extraction buffer by gentle pipetting and further clarified at 3,000×g for 10 mins. The supernatant was then loaded onto a 10-40% (w/v) sucrose gradient and subjected to ultracentrifugation at 85,000×g for 2h at 4°C using an AH-650 swinging-bucket rotor (Thermo Fisher Scientific, USA). Fractions of 500 µL were collected by puncturing the bottom of the tubes with a sterile syringe needle. Gradients were fractionated into 0.5 mL fractions and the fractions containing viral CP were identified by electrophoresis on 12% polyacrylamide gels, stained with InstantBlue (Abcam, United Kingdom), and selected samples were subsequently examined by transmission electron microscopy.

3.8.2 Purification of ToLCNDV virions

Fresh infected squash leaves displaying symptoms were removed and frozen in liquid N₂ after 21 dpi, and ground at 4°C in 3 volumes (1g/3 mL) of fresh extraction buffer (250 mM sodium phosphate buffer pH 6.0, 10 mM Na₂SO₃, 0.1% (v/v) 2-mercaptoethanol, 0.5% (v/v) Triton X-100, 0.1% (w/v) Driselase (Sigma-Aldrich)), supplemented with

EDTA-free protease inhibitor tablets (Roche), 1 tablet/100mL. After overnight incubation at 4°C with constant stirring, the mixture was squeezed through 2 layers of Miracloth (Merck KGaA, Darmstadt, Germany) and clarified by centrifugation at 11,500×g for 20 mins at 5°C. To the resulting supernatant, one quarter volume of chloroform was added and mixed for 10 mins at constant stirring. The mixture was centrifuged again at 12,000×g for 20 mins at 5°C for separating phases. After recovering the aqueous phase with pipette to avoid disturbing the pellet, the volume was measured and a quarter volume of a 1 M NaCl, 20% (w/v) PEG 6000 solution was added. The resulting mixture was stirred constantly overnight at 4°C. Precipitated virus particles were collected by centrifugation at 16,500×g for 20 mins at 5°C. Pellets were resuspended in 100 mM sodium phosphate buffer pH 7.0 and the solution was clarified by centrifugation at 27,000×g. To the supernatant, Cs₂SO₄ was added to a final concentration of 36% (w/v) and the solution was ultracentrifuged at 247,103 ×g for 24h at 5°C, in a swinging bucket AH650 Rotor (Thermo Scientific, Waltham, USA). Gradients were fractionated into 0.5 mL fractions and the fractions containing viral CP were identified by electrophoresis in 12% (w/v) NuPAGE MOPS- buffered gels (Life Technologies) followed by staining with Instant Blue dy (Expedeon Ltd). Fractions containing begomovirus CP were dialyzed overnight at 4°C against 100 mM sodium phosphate buffer pH 7.0 using Spectra-Por® Float-A-Lyzer® G2, black, 1 mL, MWCO 100 kDa.

3.8.3 Purification of begomovirus VLPs

Fresh *N. benthamiana* agroinfiltrated leaves were removed and frozen in liquid N₂ after 7 dpa, and ground at 4°C in 7-10 volumes (1g/7-10 mL) of fresh extraction buffer (250 mM sodium phosphate buffer pH 6.0, 10 mM Na₂SO₃, 0.1% (v/v) 2-mercaptoethanol, 0.5% (v/v) Triton X-100, 0.1% (w/v) Driselase (Sigma-Aldrich)), supplemented with EDTA-free protease inhibitor tablets (Roche), 1 tablet/100 mL. Then the purification was done essentially as for virions (see above) Fractions containing begomovirus CP were dialyzed overnight at 4°C against 100 mM sodium phosphate buffer pH 7.0 using Spectra-Por® Float-A-Lyzer® G2, black, 1 mL, MWCO 100 kDa. Samples were concentrated using Amicon Ultra-50 50,000 MWCO spin concentrators as described by the manufacturer.

3.8.4 Negative staining and TEM imaging

Negative stained electron microscope (EM) grids were produced for visualization and morphology determination of virions purified and assembled VLPs. For virions purified and begomovirus VLPs, 8 and 3,5 µL of samples respectively were applied on formvar/carbon film copper 400-mesh grids (EM Resolutions, Sheffield, UK) and allowed

to be adsorbed for one minute before being washed with droplets H₂O while sample excess was removed using Whatman No. 1 filter paper. Subsequently, grids were stained with 2% (w/v) of uranyl acetate (8 µL) and incubated for 1 more minute, before allowed to dry at room temperature (RT). Images were taken using a Jeol electron microscope JEM-1400 (operated at 120kV) in case of virions purified and a Talos F200C transmission electron microscope operated at 200keV (Thermo Fisher Scientific, Eindhoven, The Netherlands) in case of begomovirus VLPs.

3.8.5 Mass spectrometry: Preparation of gel slices for trypsin digestion

For mass spectrometry analysis, protein bands visualized using Instant blue were excised from the gel. Gel slices were destained with 30% ethanol for 30 mins at 65°C and the procedure was repeat with fresh 30% ethanol until the stain was completely removed. Once destained, the slices were washed with 1 mL of 50 mM TEAB/50%ACN (50 mM TEAB in 50% acetonitrile) with gentle vortexing for 20 mins. Samples were then incubated with 10 mM dithiothreitol (DTT) for 30 mins at 55°C. After removing DTT solution, iodoacetamide (IAA) previously diluted on TEAB, was added and incubated for 30 mins at RT, vortex and keep in a dark place. The IAA solution was removed, and the gel pieces were washed again with 50 mM TEAB/50%ACN for vortexing 20 mins, followed by an additional wash with 50 mM TEAB for 20 mins vortexing.

Each gel slice was then transferred to a sterile Petri dish for estimating their volumes and cut into ~2 x 2 mm fragments using clean scalpels. The fragments were transferred into low-bind tubes and washed with 50 mM TEAB/50%ACN for 20 mins in vortex, and followed by two washes with 100% ACN for 20 mins each. After completely removing the ACN, a small hole was made in the tube lids and the samples were dried in speed vacuum for 30 mins. The dried gel pieces were then processed for trypsin digestion and submitted to analysis at the proteomics facility (JIC, Norwich).

RESULTS - CHAPTER I

SWEET POTATO PATHOSYSTEM

4. RESULTS - CHAPTER I

Deciphering mechanisms underlying virus transmission and suppression of silencing in sweet potato potyviruses

Sweet potato plants frequently exhibit mixed viral infections, with multiple virus species co-infecting the same plant and collectively contributing to the disease severity. This complexity makes particularly difficult the assignment of specific symptoms or host responses to individual viral agents. To address this issue and establish a tractable experimental framework, we considered several viruses known to infect sweet potato, including sweet potato virus 2 (SPV2). SPV2 was initially selected as the primary target of our work for three main reasons: First, unlike many other sweet potato infecting viruses, SPV2 reliably establishes systemic infections and induces clear symptoms in *N. benthamiana* plants, providing a robust surrogate host for controlled infection and transmission assays. Second, SPV2 is known to be aphid-transmissible from sweet potato to other plant species (Ateka *et al.*, 2004), making it an appropriate model for studying vector-mediated transmission and virus-host interactions under laboratory conditions. Third, SPV2 virus-like particles (VLPs) had previously been produced in our laboratory, offering a valuable platform for future applications such as interference assays aimed at modulating or blocking virus transmission. Taken together, these considerations initially supported the choice of SPV2 as a candidate model for our experimental work.

4.1 Exploring the requirements for insect-mediated transmissibility of SPV2

4.1.1 Evaluation of SPV2 transmission: Infected host plants and purified virions

To investigate the transmission behaviour of SPV2, we carried out two approaches in parallel: (i) plant to plant transmission assays using aphid vectors, and (ii) transmission attempts after acquisition of purified virions by aphids. For the latter, SPV2 infected and symptomatic *N. benthamiana* leaves (Fig. R1A) were harvested and processed using a purification protocol developed and optimized in our laboratory (Chase *et al.*, 2023), derived from Ateka *et al.*, (2004) with modifications. This method produced sucrose gradient fractions containing a single protein band in SDS-PAGE analysis corresponding

to the SPV2 coat protein (~37 kDa; Fig. R1B), and transmission electron microscopy (TEM) confirmed the presence of elongated virions (50 nm -1 μ m in length and approximately 12-15 nm in diameter) (Fig. R1C). Purified particles were subsequently used for mechanical inoculation, and their infectivity was evaluated after different periods of storage at 4°C or -80°C. SPV2 virions remained fully infectious after one month at 4°C (100% infectivity), and partially infectious after three months at -80°C, as verified by inoculations onto *N. benthamiana* plants (Table R1).

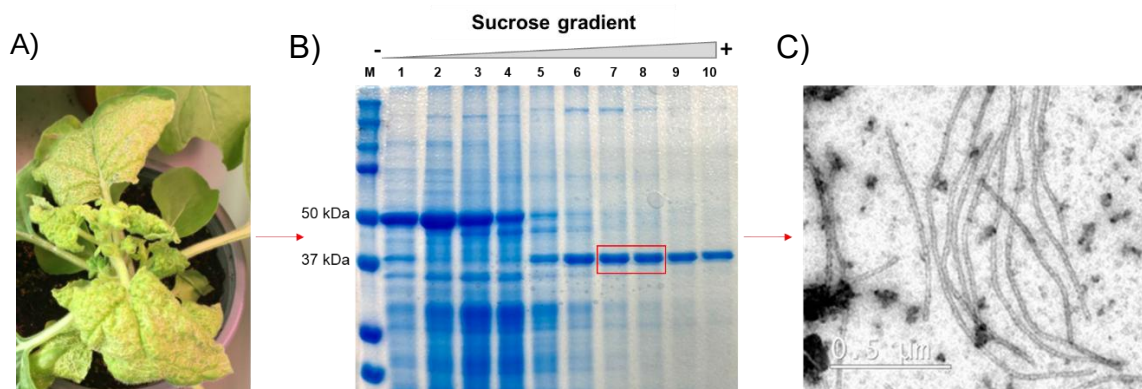


Figure R1. Symptoms in SPV2-infected *N. benthamiana* and purification of SPV2 virions. A) Symptoms associated with SPV2 infection in *N. benthamiana* plants. B) InstantBlue-stained SDS-PAGE gel showing sucrose gradient fractions (10–40%; fractions 1–10) of purified SPV2 virions. A single band of approximately 37 kDa is detected across most fractions, consistent with the expected size of the SPV2 coat protein (SPV2-CP). Additional bands observed in fractions 1–5 likely correspond to residual host proteins from the purification (e.g., Rubisco at ~50 kDa). The SPV2-CP band is clearly enriched in fractions 5–10. C) Fractions 7 and 8 were selected for transmission electron microscopy (TEM) analysis and reveal flexuous filamentous particles corresponding to SPV2 virions.

Table R1. Infectivity of different dilutions of purified SPV2 virions employed for mechanical inoculation.

Storage temperature	Time after purification	Dilution (in buffer phosphate)	Infected plants
4°C	1 month	1:1	2/2
		1:10	2/2
		1:100	2/2
-80°C	3 months	1:1	3/4
		1:10	3/3
		1:100	1/3

In relation to the first approach (i), previous studies had reported successful aphid transmission of a particular SPV2 isolate (Ateka *et al.*, 2004). Therefore, we evaluated aphid-mediated transmission using whole plants infected with our material corresponding to the native isolate SPV2 AM-MB2 (Accession number KU511270.1), originally obtained from commercial sweet potato (*Ipomoea batatas*) roots (Mingot *et al.*, 2016). Prior to my arrival, transmission assays performed by laboratory members in 2017

resulted in only 16% of plants testing positive for SPV2 (2/12), indicating a low but detectable aphid transmission efficiency. After many monthly mechanical passages, aphid-transmission assays conducted in 2022 (the SPV2 AM-MB2 isolate virus variant was renamed as SPV2_Nb-CO) yielded negative results, suggesting a loss of detectable transmissibility over time (Table R2).

Table R2. Aphid transmission and mechanical inoculation experiments conducted throughout the study period.

	Inoculum: <i>N. benthamiana</i>	Transmission	Rate (%)
Aphid transmission	SPV2_Nb-CO (2022)	0/50 ¹	0%
Mechanical inoculation	SPV2_Nb-CO (2017-2022)	25/25 ²	100%

¹ Five independent experiment were performed, with results 0/10, 0/10, 0/10, 0/10 and 0/10.

² Groups of 2-3 plants were used at different times with monthly periodicity, to keep the inoculum, and always with 100% infectivity.

Sequence analysis and recombination of SPV2 sequences

As shown in the previous section, although SPV2 remained fully infectious when mechanically inoculated, SPV2 infected plants failed to allow transmission using aphid-mediated under our controlled laboratory conditions, discouraging its use as a model for studying vector transmission. The observations along the time raised the possibility that the loss of transmissibility could be related to sequence changes accumulated during consecutive mechanical passages, as suggested in other cases with potyviruses (Legavre *et al.*, 1996). To examine this hypothesis, we initially compared the sequences of the isolate SPV2 AM-MB2 (Accession number KU511270.1) previously reported by Mingot *et al.*, (2016) and completed through 3'RACE, with that of the *N. benthamiana* adapted variant (SPV2_Nb-CO), focusing specifically on the HCPro and CP regions. For this analysis, HCPro and CP were amplified by PCR from infected *N. benthamiana* plants (SPV2_Nb-CO) and sequenced by Sanger capillary sequencing. Once obtained, amino acid sequences were compared between the two isolates. Protein alignments were performed using CLUSTAL Omega. In HCPro, only a single polymorphism was detected at aa position 394, the original SPV2 AM-MB2 encoded an isoleucine, whereas SPV2_Nb-CO encoded a threonine. In CP, two polymorphisms were identified at positions 87 and 162, corresponding to valine/asparagine in SPV2 AM-MB2 and leucine/lysine in SPV2_Nb-CO, respectively. Particular attention was given to the conserved K/RITC and PTK motifs in HCPro and the DAG motif in CP, since they are known to play key roles in aphid transmission, finding no substitutions observed in those motifs.

Results - Chapter I

Subsequently and to place these results in a broader context, HCPro and CP sequences from the six SPV2 isolates currently available in GenBank, including the SPV2 reference sequence (NC_017970.1), were aligned and compared with those SPV2 AM-MB2 and SPV2_Nb-CO. Clustal Omega analyses revealed substantial amino acid variation near the N-terminal regions of both proteins regarding to the SPV2 reference sequence, including positions adjacent to the K/RITC, PTK (HCPro) and DAG (CP) motifs (Fig. R2), which can suggest that natural variability in these regions may influence transmissibility in SPV2.

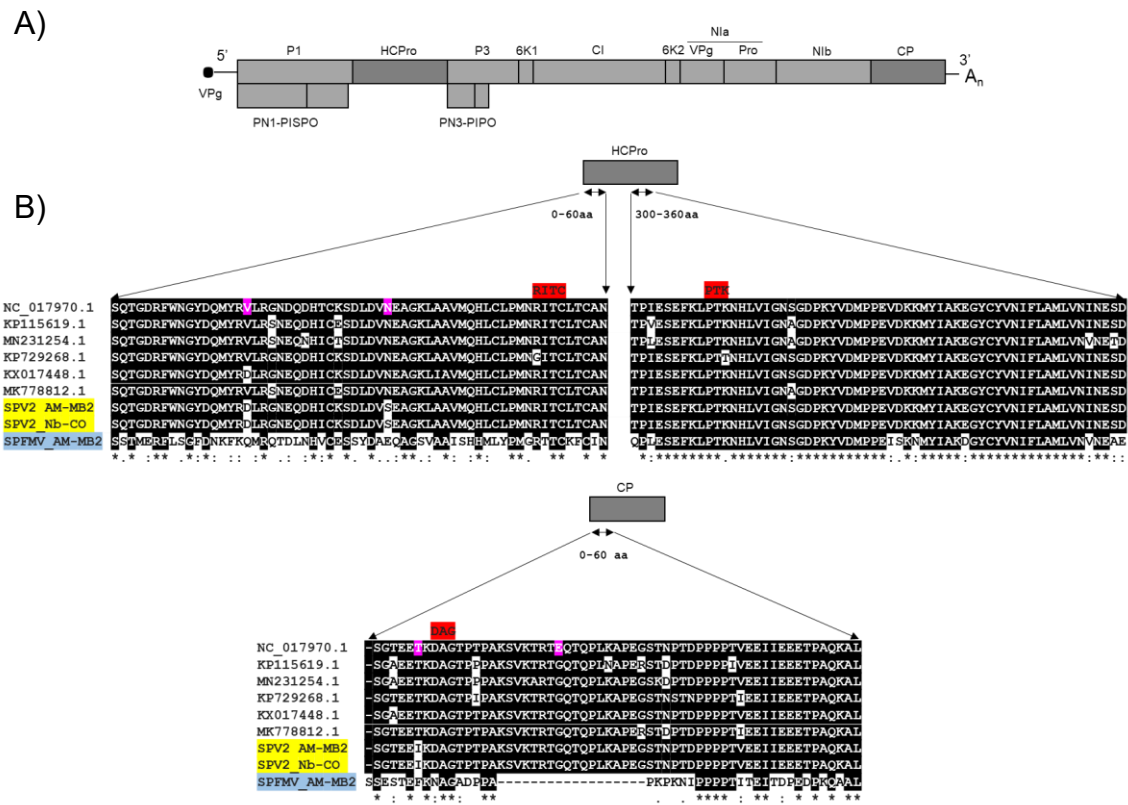
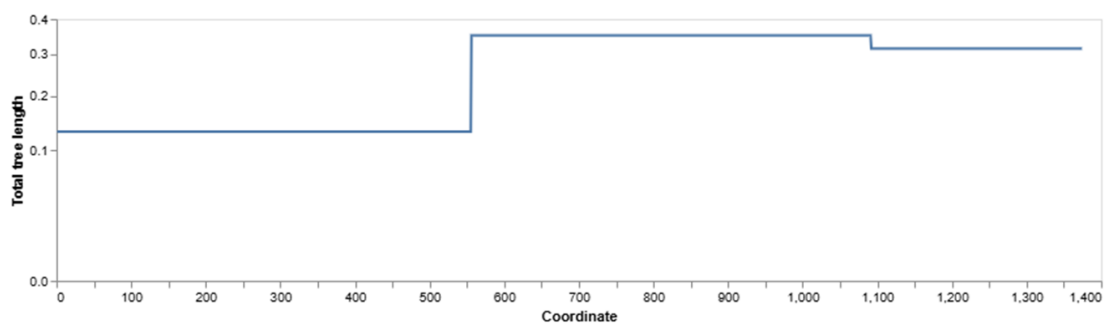


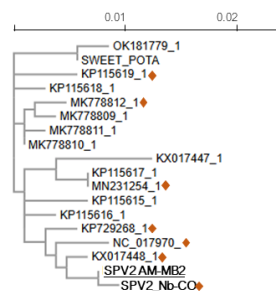
Figure R2. Schematic representation of the SPV2 genome and amino acid sequence analysis of HC-Pro and CP regions. A) Schematic map of the SPV2 genome. Boxes indicate the mature proteins, with protein names shown above and below. B) Amino acid alignment of HCPro and CP regions from nine related sequences. Six sequences correspond to SPV2 isolates available in GenBank, while SPV2 AM-MB2 and SPV2_Nb-CO (highlighted in yellow) are included for comparison. The SPFMV AM-MB2 sequence (blue) was used as an outgroup. Conserved motifs (RITC, PTK, and DAG) are indicated in red. Amino acid positions of the compared regions are shown below the arrows in the maps of the proteins. Black shading indicates identical residues, whereas white shading indicates differences. The consensus line shows asterisks (*) for identity, colons (:) for strong conservation, periods (.) for weak conservation, and spaces for no conservation. Pink boxes highlight amino acid differences between SPV2 AM-MB2 and SPV2_Nb-CO relative to the SPV2 reference sequence (NC_017970.1).

Additionally, a Genetic Algorithm for Recombination Detection (GARD) analysis was performed with 16 SPV2 isolates, comprising the six previously analyzed sequences, the SPV2 AM-MB2 and SPV2_Nb-CO, and other eight additional SPV2 sequences available in GenBank. This analysis identified two putative recombination breakpoints within HCPPro and one within the CP region (Fig. R3). HCPPro and CP of SPV2 AM-MB2 and SPV2_Nb-CO consistently clustered together, indicating as expected minimal evolutionary divergence despite mechanical passages. This high degree of similarity was further supported by amino acid alignments, which showed only the mentioned substitutions within the HCPPro and CP sequences.

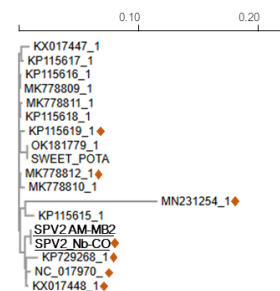
A)



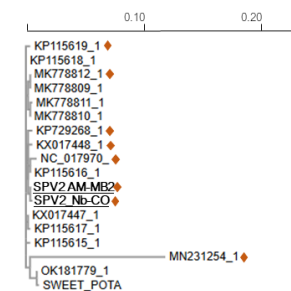
Tree 1, coordinate range 1-555



Tree 2, coordinate range 556-1091



Tree 3, coordinate range 1092-1374



B)

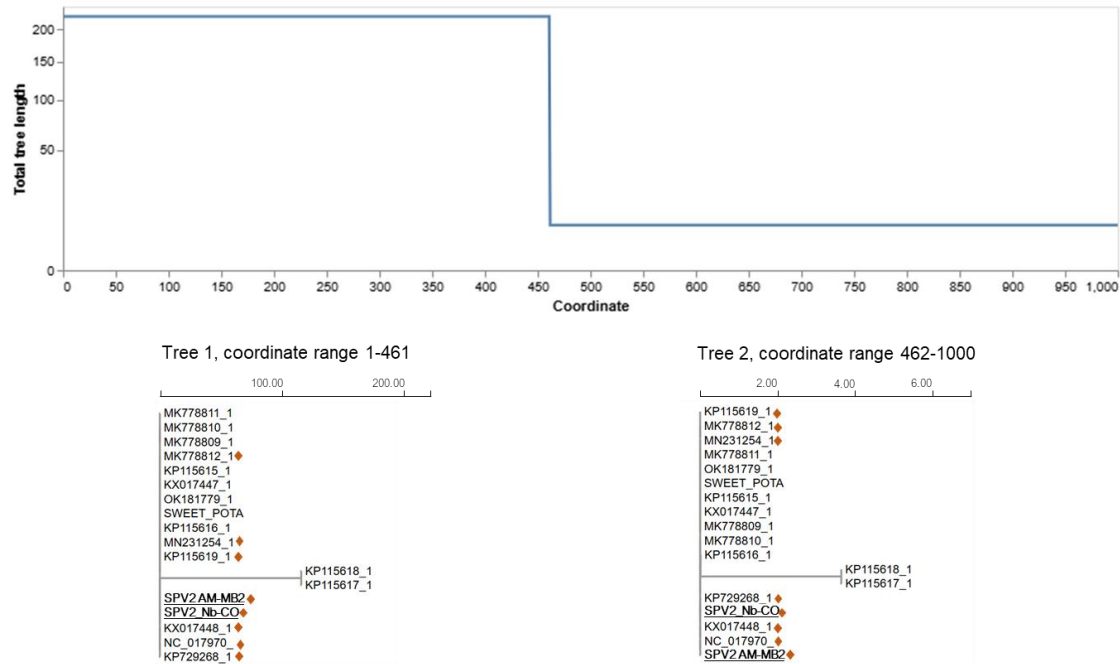


Figure R3. Recombination analysis of HCPro and CP sequences from SPV2 isolates. HCPro and CP sequences from 16 SPV2 isolates obtained from GenBank, together with SPV2 AM-MB2 and SPV2_Nb-CO, were analyzed using the Genetic Algorithm for Recombination Detection (GARD). A) Upper panel: total tree length by partition, indicating genomic coordinates associated with predicted recombination breakpoints in HC-Pro (segments spanning positions 1–555, 556–1301, and 1302–1901). Lower panel: phylogenetic trees for each segment, illustrating relationships among isolates across the corresponding regions. B) Upper panel: total tree length by partition, indicating genomic coordinates associated with predicted recombination events in CP (segments spanning positions 1–461 and 462–1000). Lower panel: phylogenetic trees for each segment, showing the phylogenetic relationships among isolates. Sequences obtained in this study (SPV2 AM-MB2 and SPV2_Nb-CO) are underlined, and orange diamonds indicate sequences selected for the amino acid alignment shown in Fig. R2.

Given the inability to establish a reliable experimental model for the vector-based transmission studies, and the absence of a sequence-level obvious explanation for this phenotype, we redirected our efforts focused on sweet potato potyviruses towards sweet potato feathery mottle virus (SPFMV), a biologically and epidemiologically relevant potyvirus. This enabled us to continue exploring essential processes in the chosen host, including RNA silencing suppression, polymerase slippage events, and single and mixed infection dynamics, providing a relevant framework for subsequent sections.

4.2 Characterization of different SPFMV isolates and their relevant functions of plant viruses such as silencing suppressor activity

Our characterization of SPFMV isolates focused in the materials available in the laboratory, which were initially found in commercial sweet potato plants, which had been maintained in the greenhouse for several years. Two isolates of SPFMV, named AM-MB1 and AM-MB2 isolate, were partially characterized, and the second one was selected and used for previous studies (Mingot *et al.*, 2016). Using primers designed for the AM-MB2 isolate (GenBank: KU511268.1), we confirmed the infections by SPFMV in plants by generating PCR amplicons of the expected size. Sanger sequencing revealed that in addition to plants only infected by the SPFMV AM-MB2 isolate, other closely related SPFMV-like sequences were also present in other samples, either alone or in multiple infections, a finding consistent with the well-known capacity of sweet potato to host simultaneously a wide diversity of virus species and also variants and isolates of a given virus. Since we observed many nucleotide changes between the sequences corresponding to the fully sequenced AM-MB2 isolate (GenBank: KU511268.1) and the equivalent regions in the isolate AM-MB1, we decided to complete its characterization to allow comparisons.

4.2.1 Comparative genomic sequencing of two SPFMV isolates

4.2.1.1 Complete genome sequencing of AM-MB1 isolate

To complete the genome sequence of the isolate AM-MB1, partial genomic sequences were amplified through RT-PCR in different fragments. PCR fragments were purified and submitted to Sanger capillarity sequencing, firstly using primers designed on the reference AM-MB2 sequence but later on internal primers designed based on the reads corresponding to sequenced fragments (Fig. R4). The assembly of the sequenced fragments into a consensus was done using at least 2 verified reads for each section, and the sequence annotation was performed considering the conserved Potyvirus proteolytic cleavage sites (Adams *et al.*, 2005).

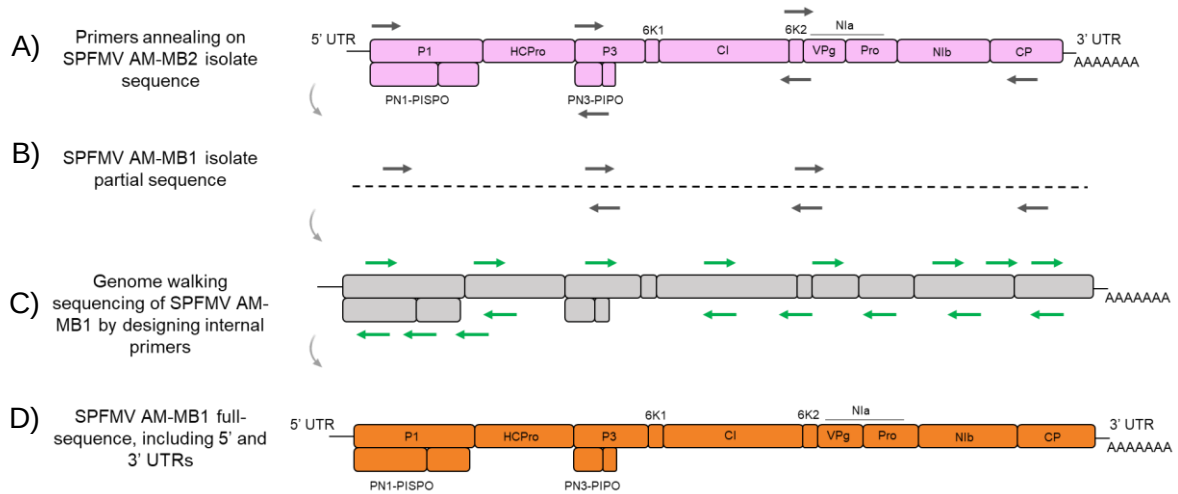


Figure R4. Genome walking strategy followed to obtain the full-length sequence of SPFMV AM-MB1 isolate, including 5' and 3' UTRs determined by rapid amplification of cDNA ends (5' and 3' RACE, respectively). A) Previously available sequence of SPFMV AM-MB2 isolate. B) Initial partial sequence of SPFMV AM-MB1 isolate obtained using primers designed on the AM-MB2 reference sequence. C) Extended SPFMV AM-MB1 sequence generated using internal primers designed from the previous partial sequence. D) Complete genomic sequence of SPFMV AM-MB1 isolate. Black arrows represent primers designed from the AM-MB2 isolate sequence, while green arrows represent primers designed from the AM-MB1 isolate sequence.

The complete genome sequence of the isolate AM-MB1 was found to be 10,896 nt long (the complete sequence is currently being prepared for submission to GenBank) and showed the typical genomic organization of potyviruses, presenting the 5' and 3' non-coding regions and the polyprotein ORF spanning 10,557 nt and encoding the canonical potyvirus gene products: P1 (2067 nt), HCPro (1374 nt), P3 (1056 nt), 6K1 (156 nt), CI (1929 nt), 6K2 (159 nt), NIaVPg (576 nt), NIaPro (529 nt), NIb (1563 nt) and CP (945 nt), as well as two additional partially out of frame proteins predicted for potyviruses infecting sweet potato plants: P1N-PISPO (2037 nt) and P3N-PIPO (669 nt) (Supplementary Table S2). Most cleavage sites of the SPFMV AM-MB1 polyprotein were conserved with other isolates (all available in the dedicated database: <https://www.dpvweb.net/potycleavage/species.html>), including also the AM-MB2 isolate. Differences between AM-B1 and AM-MB2 were detected only at two positions: the P1/HCPro junction, which corresponds to ITQF/S in AM-MB1 and IIQY/S in AM-MB2, and the P3/6K1 junction, defined as VEHQ/G in AM-MB1 and VKHQ/G in AM-MB2.

• 5' and 3' RACE sequences

For the complete characterization of the SPFMV AM-MB1 isolate, we carried out 5' and 3' RACE (rapid amplification cDNA ends) analyses. Total RNA extracted from an infected plant was used to synthesize cDNA with gene-specific primers (GSPs; Table M2,

Material and methods section; Fig. R4), allowing the amplification of the terminal regions of the viral genome. Amplification of the 3' end with primer 3'-RACE-GSP3- AM-MB1-SPFMV yielded a fragment of approximately 504 nt, which included the expected artificial poly(A) tail generated during cDNA synthesis. For the 5' end, cDNA primed with GSP1_5RACE_FM_AMMB1_RV followed by nested PCR using primers GSP2-, GSP3, and GSP4_5RACE_FM_AMMB1_RV produced the expected ~331 nt fragment, containing the dC-tail introduced during terminal transferase treatment. Sequencing of both RACE amplicons confirmed the integrity of the genomic termini and enabled the precise definition of the 5' and 3' UTR regions of the AM-MB1 genome. The resulting sequence displayed the characteristic non-coding regions of potyviruses with a 117-nt 5'UTR and 222-nt 3' UTR. These validated terminal sequences were subsequently incorporated into the full genome assembly described below. Additionally, and for comparative purposes, 5'/3' RACE analysis was conducted to characterize the terminal nucleotide sequences of the AM-MB2 isolate (GenBank: KU511268.1), following the same procedure used for AM-MB1. These analyses identified terminal regions of 116 nt at the 5'- and 221 nt at the 3'-end. The complete genome length was determined to be 10,819 nt, of which 10,482 nt corresponded to the polyprotein open reading frame (ORF). Comparison of the 5' UTRs of both isolates revealed that AM-MB1 contains five adenine residues at the extreme 5' terminus, whereas AM-MB2 contains four. In the 3' UTR, AM-MB1 appears with six thymine residues (position 200-206), while AM-MB2 contains five (Fig. R5A). Both terminal regions from the two isolates were aligned with 84 SPFMV sequences available in GenBank to validate the annotation of the genomic ends (Fig. R5B). Other minor differences were observed in the comparison with other isolate sequences.

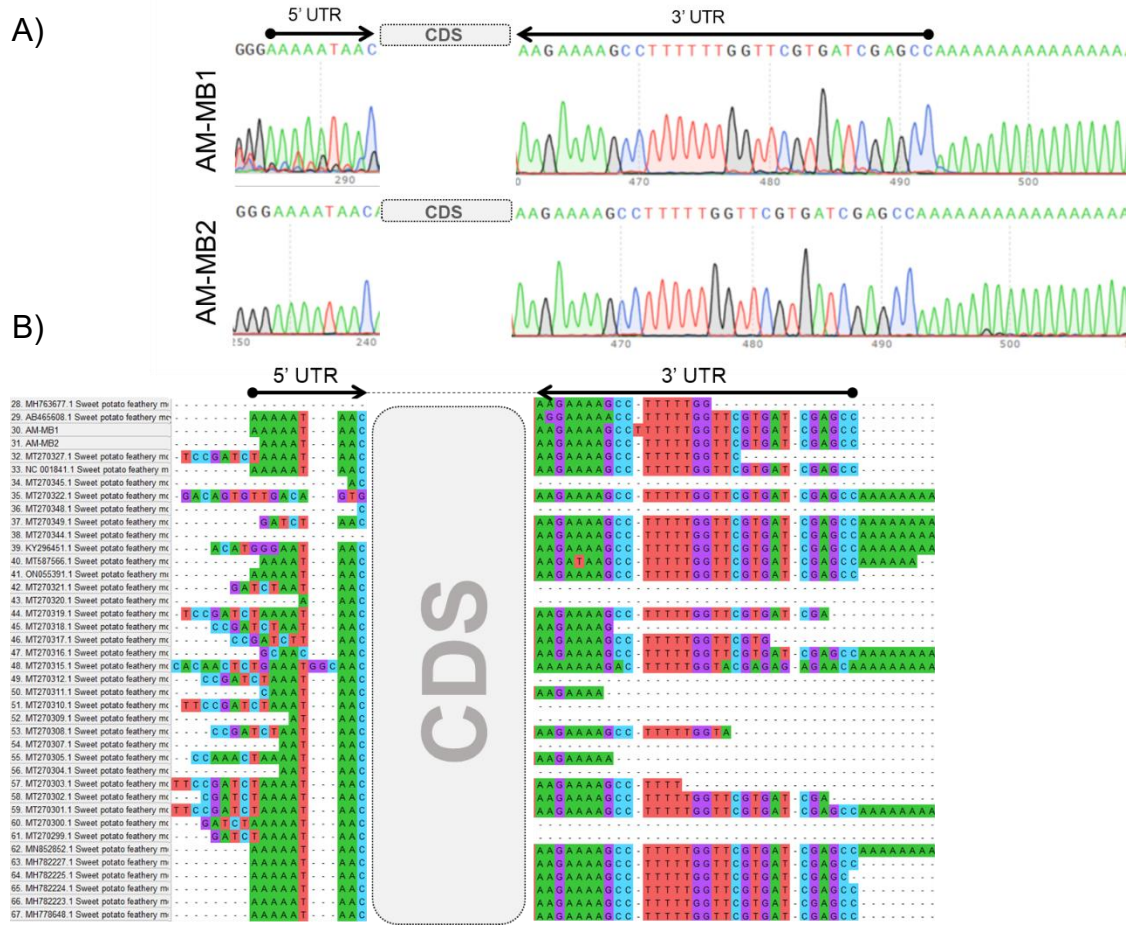


Figure R5. Analysis of the 5' and 3' ends of SPFMV by RACE. A) Sanger capillary sequencing results of the 5' and 3' untranslated regions (UTRs) of SPV2 isolates AM-MB1 and AM-MB2. B) Alignments of the 5' and 3' UTR sequences of AM-MB1 and AM-MB2 with 37 SPFMV sequences available in GenBank. Black arrows indicate the lengths of the 5' and 3' UTRs among SPFMV isolates. The central part of the genomes are represented by boxes including the rest of UTRs and the coding sequences (CDS) for the protein-coding regions of the genomes.

4.2.1.2 Comparative analysis of P1 and PISPO region

Blast suit-2sequences was used to perform nucleotide and amino acid sequence alignments for the two viral isolates, AM-MB1 and AM-MB2. Analysis of the complete coding sequences revealed 91.62% amino acid sequence identity between two isolates. Additionally, the identity of each gene product individually was performed in separate analysis and is summarised in Table R3. Among all viral proteins, the P1 coding region, which includes P1, P1N-PISPO and PISPO, showed the greatest divergence between isolates, with amino acid identities below 80%. Thus, we decided to explore these discrepancies, considering the possible role in RNA silencing suppression activity attributed to P1 and P1N-PISPO in SPFMV (Untiveros *et al.*, 2016; Mingot *et al.*, 2016). Nucleotide and amino acid alignments of the P1 region revealed that the AM-MB1 isolate

contains an additional 75 nucleotides (positions 985-1059), corresponding to 25 extra amino acids (positions 290-314), compared to the AM-MB2 isolate (Fig. R6). Logically, the same insertion was present in the P1N-PISPO, since these additional 25 aa are in the central region of the protein, upstream of slippage motif required for P1N-PISPO translation (Fig. R6). Additionally, PISPO protein have also an interesting level of difference between both isolates being this product the most divergent viral product because both proteins are embedded in the highly variable P1 region (Table R3) After them, the next most variable gene products were 6K2 and P3.

Table R3. Amino acid identity percentage of SPFMV AM-MB1 gene products compared with SPFMV AM-MB2.

Amino acid identity percentage (%)	
P1	79.39
P1N-PISPO	74.52
[PISPO]	68.10
HCPPro	93.45
P3	89.49
P3N-PIPO	90.58
6KI	90.38
CI	94.25
6K2	88.68
NlaVPg	97.92
NlaPro	97.53
Nlb	96.35
CP	97.14

Results - Chapter I

A)

P1

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AM-MB1-P1  MATIVASAKPASKRKLTWKECCNKWGRAAMEQQKEQSRKTVHLGRDHLAAYLMAFVPET 60
AM-MB2-P1  MATVMASAKPAGKRKLTWKECCNKWGRAAMEQQQQQSKKTVHLGRDHLAAYLLAFVPET 60
          ***:*****.*****:*****:*****:*****

AM-MB1-P1  EWHKYYYGSRGGPSPALLILKGAIECGETYKWESELTFCACDDVLDGHKCDSCGYNHIK 120
AM-MB2-P1  EWHKYYYGSRGGPSPALLILKGAIEHGEVYKWESELTFCACDDVLDGHNCDSCGHRHIK 120
          ***** **.******:*****:***

AM-MB1-P1  HDDNITNNMNAIARVLGGYDAFYASNWAVYETAKYELDQVAPTAGMLYKQAKEAEKLLGK 180
AM-MB2-P1  RDDNIADNMNAIARALGGYDAYYASNWAVYETAKYELDQVAPTAGMLYKQAKEAEKLLGK 180
          :****:*****.*****:*****:*****

AM-MB1-P1  RPTREIQEVEDLWAEYEEAAAREAAEAFKVSNEHATSEVANKTAYLSDEEDDEAFPLV 240
AM-MB2-P1  RPTREIQEVEDLWAEYEEAAAREAVEASEASNGHATSEVANKNAYLSDEEDDEAFPLV 240
          *****.*:.* *****.*****

AM-MB1-P1  TTIEKVALATTIIESVSEVNKTPEVQAPIVEAPEVSVAAATFVEATNNEGDEQLLTQEQA 300
AM-MB2-P1  VTIVEKVPTTTIIESTPEVGKTIIEVQTPLEVPVEVLAATTFVEATIEGK----- 289
          .:***. :*****. **. ** *:***.***. :*****:
          [ ]

AM-MB1-P1  KNANCSAANLTTLGDVSIQFGTISCVLGQTKVQETETAKESTTGFFFGTIPATIPLP 360
AM-MB2-P1  -----DAPTGSIQFGTIVCALEPIKASEAGIVKEPTTGFFFGTIPAIIVPLP 335
          [ ]*. ***** *. * *..*: .** *****:***

AM-MB1-P1  TIPIMKLESAIVEPIATPVAEITSNEIVEVPVIAAPTEMAKAPKTPLPKHLYPWTSKTQTF 420
AM-MB2-P1  TIPLLKLEPTIVEPIATPVVEVTSSEIVKVPIATPTEVEKASKAPLPKHLYPWTAKTQTF 395
          ***:*** :*****.*:*. ***:***:***: ** *:*****:*****

AM-MB1-P1  GEVHHKMVRRWVQKTQQAAAEKEKLWKKLDEQLVARNEARKDLKVKWRWGLYRLVRKTR 480
AM-MB2-P1  GEVHHKMVRKWVQKTQQAAAEKEKLWKKLDEQLATRNEIRKGLKVKWRWGLYRLVRKTR 455
          *****:*****:*** **.******:***

AM-MB1-P1  KDNQRQRQKRMEKEQKLLTAMPPQILTSISIAGGPAASLEMTPTLNGKIFCTPSMKKKR 540
AM-MB2-P1  KDNQRQRQRMEKEQQLLMAMPPQVLTGISIAGGPSASLEMTPTPNGKIFCTPSMKKKK 515
          *****:*****:*. *****:*. *****:***** *****:

AM-MB1-P1  TLKSPILTQEKIHELMQAVLKIANKKEMNVELVGKKPTRGQYKKFQGAKHLFLHLKHKMG 600
AM-MB2-P1  TLKSPKLTQEKIHELTQAVLKIACRKRMSIELVGKKSTKGQYRKFGGANYLFLHLKHMEG 575
          *****.***** ***** :*.*:***** *:***:*****:*****:

AM-MB1-P1  LRESVDLRVHPPTQDIVLQAAKVGAWERSIKTITLSKGSSGLVMNPDKLLGPRGHAFQGM 660
AM-MB2-P1  LRESVDLRIHHTTQNLVLQAAKVGAWKRPVKTMTLSKGSSGMVLNPDKLLGPRGHAFHGM 635
          *****:* ***:*****.* :** *****:*.*****:***

AM-MB1-P1  FVVRGAFKGVLYDARMKLGSRVLPYITQF 689
AM-MB2-P1  LVVRGALRGVLYDARMKLGSRVLPYITQY 664
          :*****:*****.*:

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B)

P1N-PISPO

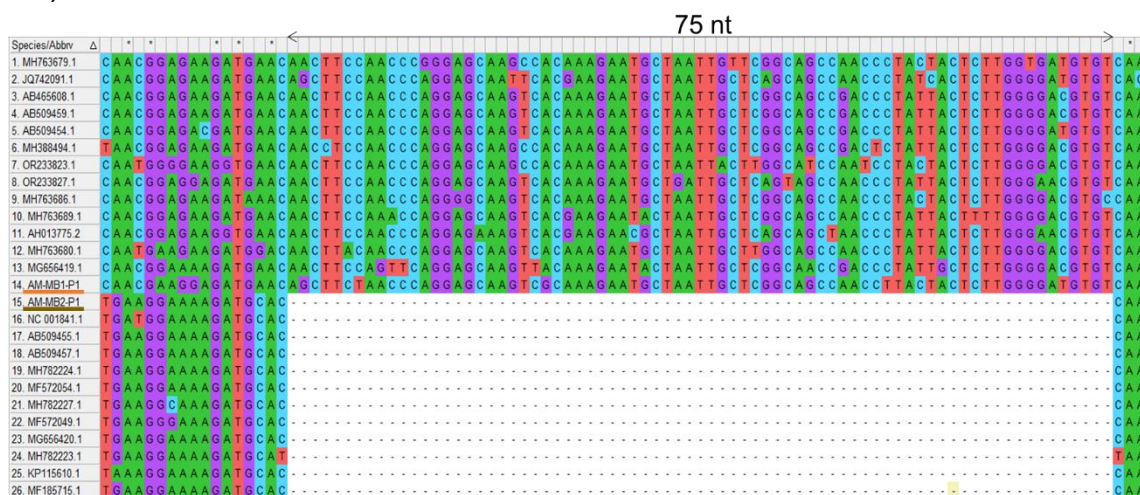
AM-MB1	MATIVASAKPASKRKLTWKECCNKWGRAAMEQQKEQQSRKTVHLGRDHLAAYLMAFVPET	60
AM-MB2	MATVMSAKPAGKRKLTWKECCNKWGRAAMEQQQQQQSKKTVHLGRDHLAAYLLAFVPET	60
.:**.*****:***:*****:*****		
AM-MB1	EWHKYYYGSRGGPSPALLILKGAIECGETYKWESELTFCACDDVLDGHKCDSCGYNHIK	120
AM-MB2	EWHKYYYGSRGGPSPALLILKGAIEHGEVYKWESELTFCACDDVLDGHNCDSCGHRHIK	120
*****.***.*****:*****:***		
AM-MB1	HDDNITNNMNAIARVLGGYDAFYASNWAVYETAKYELDQVAPTAGMLYKQAKEAEKLLGK	180
AM-MB2	RDDNIADNMNAIARALGGYDAYASNWAVYETAKYELDQVAPTAGMLYKQAKEAEKLLGK	180
:***.:*****.*****:*****:*****		
AM-MB1	RPTRREIQEVEDLWAEYEEAAAREAAEFKVSNEHATSEVANKTAYLSDEEDDEAFPPLV	240
AM-MB2	RPTRREIQEVEDLWAEYEEAAAREAVEASEASNGHATSEVANKNAYLSDEEDDEEFPPLV	240
*****.***.:** *****.***** *****		
AM-MB1	TTIEKVALATTIIESVSEVNKTPEVQAPLVPPEVSVAAATFVEATNNEGDEQLLTQEQVA	300
AM-MB2	VIVEKVVPTTTIIESTPEVGKTIIEVQTPLEVPPEVLAATTFVEATIEGK-----	289
.:***.:*****.***.*** ***:** *.* ***.:*****:		
AM-MB1	KNANCSAANLTTLGDVSI CDIQFGTISCVLGQTKVQETETAKESTTGFFFGTIPAIIPLP	360
AM-MB2	-----DAPTGSIQFGTIVCALEPIKASEAGIVKEPTTGFFFGTIPAIIVPLP	335
..*****.*.*.*.:** *****:***		
AM-MB1	TIPIMKLESAIVEPIATPVAEITSNEIVEVPIAAPTEMAKAPKTPLPKHLYPWTSKTQTP	420
AM-MB2	TIPLLKLEPTIIVEPIATPVVEVTSSEIVKVIATPTEVEKASKAPLPKHLYPWTAKTQTP	395
.::*****.*:*.*.**:***:***: ** *:*****:*****		
AM-MB1	GEVHHKMVRRWVQKTQAAAAEKEKLVWEKTGRTIGCKERGQKGPQGEMEVGALQTGKKNQ	480
AM-MB2	GEVHHKMVRKWVQKTQAAAAEKEKLVWEKTGRTIGHKERDQKRSQSKMEVGTLOTQSEDQ	455
*****:*****:***** ***.** *:***:***.:** *		
AM-MB1	EGQPKAAPTEAHGEGAKITDCHATANSDEYLHRWGSCESGNDSNSEWEDFLHAFYEEEE	540
AM-MB2	EGQPKTAPTEAHGEGTAIIDGYATSSSDGHLHCWGSIGESGNDSNSEWEDFLHAFHEEEE	515
*****:*****: ** *:***: ** *:** ***.*****:*****		
AM-MB1	NFKISHANTRENPRAHASSLENSLEKGDECRAGGQETHKRPIQEISRSKAFVPSFKTYER	600
AM-MB2	NFKISQINTRENSRAHAGSSENCVQEKDEHRTGGQEVHKRAVQEISRSKLFVPSFKTYGR	575
*****: ***** ***.** ***:** *:***:***:***** ***** *		
AM-MB1	PQRISGFKSPPNNSRYRTASRKGWGMGKVNKNNIIQRFKWSSNEPRQTAGSSGSRITRN	660
AM-MB2	LKRVSFGFKNSHNNSKPRTS SCQGWSMEKTCNDNNVQRFKWHGAESRQTVGPKRSCTTRN	635
.:***.***:***:*:*.*.*.*:***:*****.* ***.**.* *****		
AM-MB1	VRGTWGFQSAIRRTDEAW	679
AM-MB2	ARGAWGETRSAIRRTNETW	654
.*:*** *****:*** *		

Figure R6. Alignment of P1 (A) and P1N-PISPO (B) proteins from SPFMV isolates AM-MB1 and AM-MB2. Black shading indicates identical amino acids, whereas white shading indicates differences. The consensus line shows asterisks (*) for complete identity, colons (:) for strong conservation, periods (.) for weak conservation, and spaces for no conservation. The pink framebox highlights an additional 25–amino acid insertion in the AM-MB1 isolate. The red star indicates the site of the ribosomal slippage event resulting in the transframe P1N-PISPO protein, and the red shaded region corresponds to the PISPO domain.

4.2.1.3 Phylogenetic analysis of P1

The P1 genomic region of isolates AM-MB1 and AM-MB2 was selected for further comparative analysis, including homologous SPFMV sequences available in the GenBank database, using the BLAST tool to select the closest ones for each case. P1 nucleotide sequences showing high similarity to each isolate were retrieved, verified, and subsequently aligned using the CLUSTAL W algorithm implemented in MEGA12 (Kumar *et al.*, 2024). The multiple sequence alignment of the P1 nucleotide region revealed the high degree of variability among the 26 SPFMV isolates included in the analysis (Fig. 7A). In addition to the numerous polymorphic sites observed, the existence of additional 75 nt in 14 isolates of the 26 isolates analysed, showed the remarkable divergence between SPFVM isolates regarding to P1 region. This is consistent with the high evolutionary rate reported for this gene in potyviruses (Adams *et al.*, 2005b; Valli *et al.*, 2007). Both isolates analysed in this study, AM-MB1 and AM-MB2 displayed distinct nucleotide patterns across the aligned region. This divergence was also reflected in the phylogenetic tree generated from the same datasheet (Fig. R7B), where both isolates were separated into two main clusters. Within these two major clusters, isolates AM-MB1 and AM-MB2 were positioned into distinct branches, indicating that, at least for the P1 gene, they might have different origins.

A)



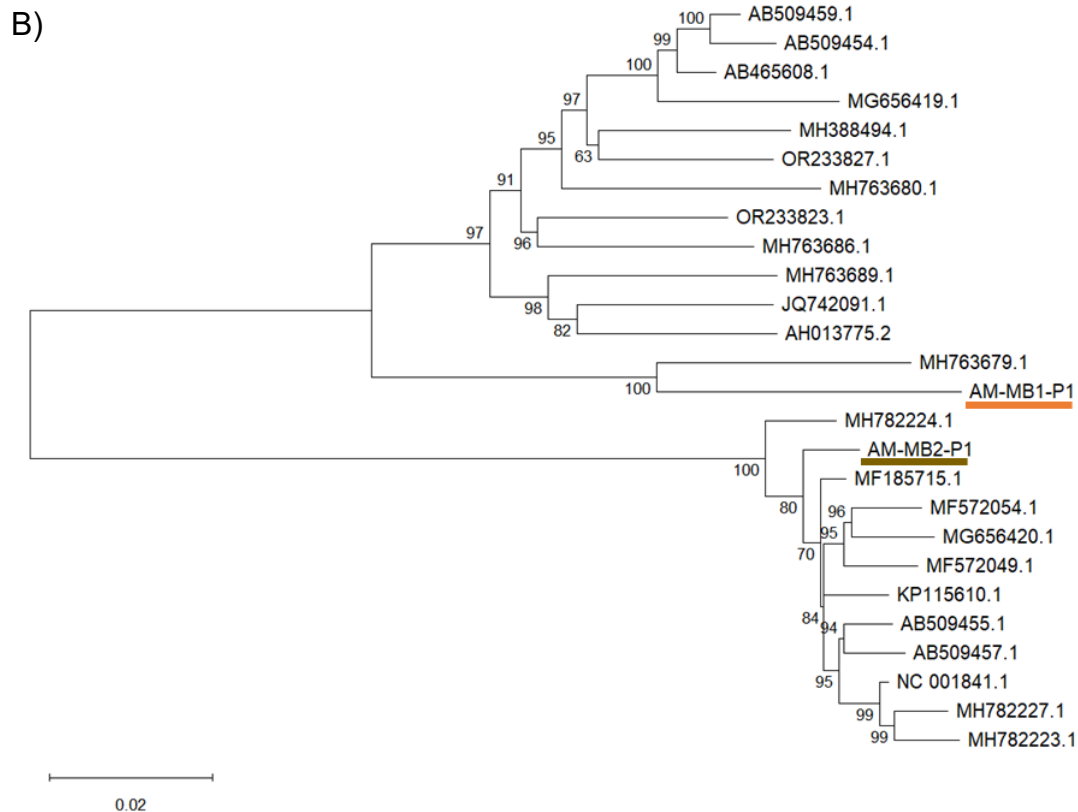


Fig. R7. Alignment and phylogenetic analysis of the SPFMV P1 region using MEGA12. A) Nucleotide alignment of the P1 region from 26 SPFMV sequences; 14 sequences, including AM-MB1, contain an additional 75 nt within the P1 coding region. B) Phylogenetic tree based on the nucleotide sequences corresponding to the P1 region of the same 26 SPFMV isolates. AM-MB1 is underlined in orange, and AM-MB2 is underlined in brown.

4.2.2 Comparison of RSS activity in gene products of both isolates of SPFMV

4.2.2.1 SPFMV proteins conferring local RNA silencing activity

The genome of SPFMV encodes the typical proteins found in members of the *Potyvirus* genus, including the predicted P1N-PISPO (Mingot *et al.*, 2016), which is present only in some potyviruses (Fig. R8A), mainly in viruses infecting sweet potato. Previous studies have shown that proteins encoded at the 5' ends of potyviral genomes often exhibit RNA silencing suppression activity (Kasschau and Carrington, 1998; Anandalakshmi *et al.*, 1998; Lakatos *et al.*, 2006; Mingot *et al.*, 2016). Based on this, five constructs expressing 5' encoded proteins from SPFMV isolates AM-MB1 and AM-MB2 were tested by co-agroinfiltration with GFP-expressing construct (Voinnet *et al.*, 2000; Mingot *et al.*, 2016; Untiveros *et al.*, 2016). The tested proteins include P1, HCPro, P1HCPro (both gene products in the same *cis* construct, expected to undergo self-cleavage by P1 protease), P1-ONLY (P1 with an out-of-frame stop codon preventing any expression of PISPO) and

P1N-PISPO (generated by inserting an extra G nucleotide in the conserved G_2A_6 motif to mimic a “forced” slippage). These products were cloned into suitable binary vectors (Fig. R8B) and introduced into *A. tumefaciens* for subsequently co-infiltration with a second *A. tumefaciens* strain carrying the GFP- construct used as reporter in *N. benthamiana*.

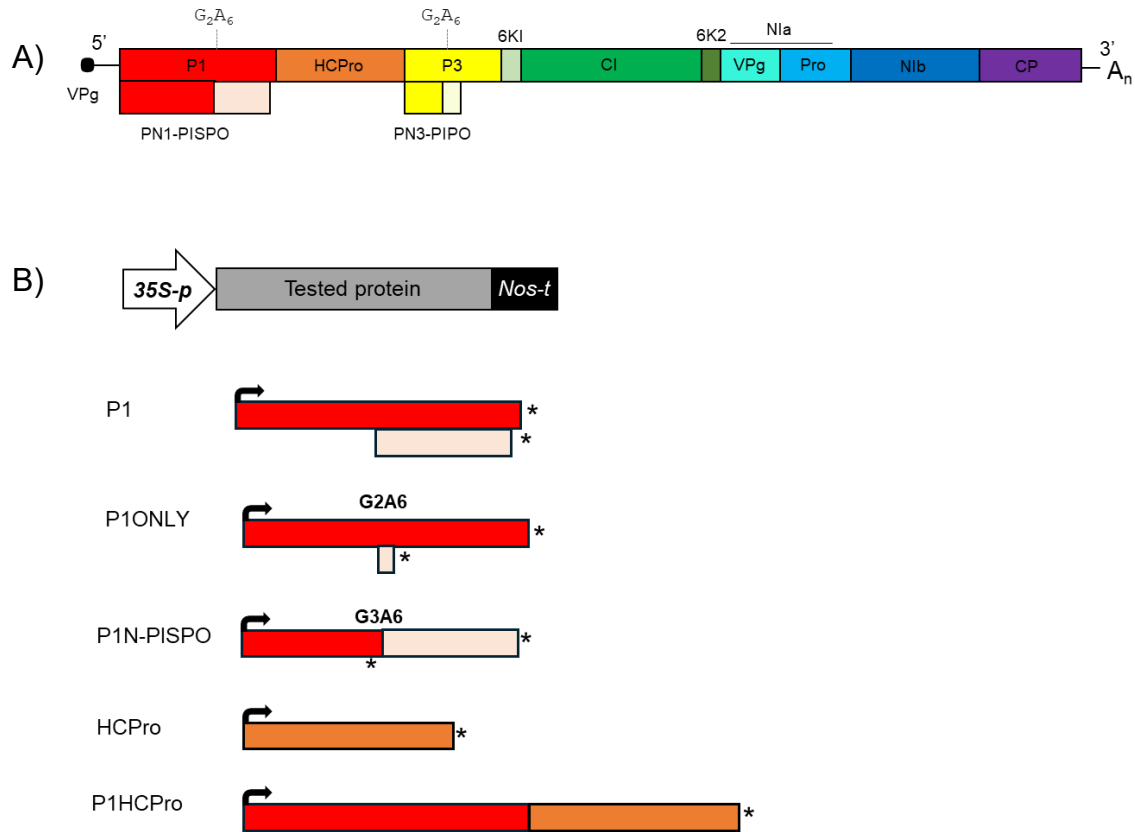


Fig. R8. Schematic representation of the SPFMV genome and constructs used for functional analysis. A) Genome map, with the viral protein genome-linked (VPg) covalently attached to the 5' end of the genome depicted as a black circle, while the poly(A) tail at the 3' end is indicated as A_n. The viral open reading frame (ORF) for the polyprotein is proteolytically cleaved into ten mature proteins (shown as boxes). Two additional cistrons, P1N-PISPO and P3N-PIPO, are generated by RNA polymerase slippage at conserved G_2A_6 motifs within the P1 and P3 regions, respectively. B) 5'-proximal SPFMV-encoded proteins tested for RNA silencing suppressor activity. Black arrows at the beginning of each cistron indicate the translation start codon (AUG), and asterisks denote introduced stop codons. P1N-ONLY and P1N-PISPO constructs contain site-directed mutations in the G_2A_6 motif to prevent or promote respectively the translation of the polymerase slippage-derived products. All constructs were cloned into appropriate binary vectors for *in planta* expression under the control of the 35S promoter.

This standard silencing suppression assay relies on the co-expression of GFP mRNA with viral proteins. Overexpression of GFP normally triggers sense-mediated silencing, which leads to a loss of fluorescence under UV light after a period of time, unless the viral protein tested suppresses RNA silencing in the plant (Fig. R9A). In such cases, GFP

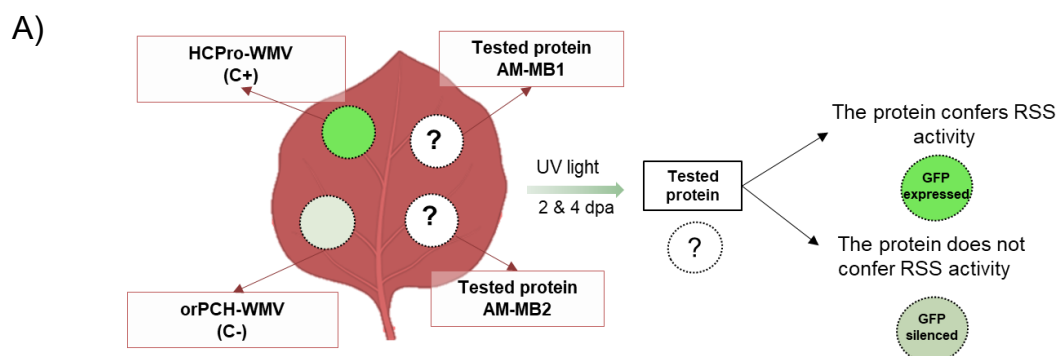
fluorescence persist for a longer period of time (2-7 dpa). To verify which protein(s) could act as RNA silencing suppressors (RSSs) and to compare in this activity between SPFMV isolates, AM-MB1 and AM-MB2, the assay was carried out in *N. benthamiana* plants. The well-characterized HCPro-WMV was used as positive control, whilst an untranslatable HCPro of WMV in inverted orientation, designated orPCH, was used as negative control. Both positive and negative controls were included side-to-side with the tested constructs in the same leaves and GFP fluorescence was monitored at 2 and 4 dpa under UV light (Fig. R9A).

At 2 and 4 dpa patches infiltrated with negative control exhibit weak GFP fluorescence, in contrast, patches expressing positive control displayed an increase in fluorescence over time (Fig. R9B). Among the tested proteins, P1HCPro from both isolates consistently presented the most intense fluorescence, exceeding the signal observed for P1, P1-ONLY, P1N-PISPO and HCPro at 2 dpa (Fig. R9B, 2 dpa). But when comparing isolates at this time, most proteins showed similar fluorescence intensities, with exception of P1N-PISPO; the AM-MB2 version of this construct accumulated slightly more GFP than the AM-MB1 variant. By 4 dpa, fluorescence decreases across most of the constructs, including P1, P1-ONLY, HCPro and P1HCPro, although the reduction was less pronounced than in the negative control. Notably, P1N-PISPO from AM-MB2 maintained strong silencing suppression, whereas the corresponding AM-MB1 protein did not display a comparable increase in fluorescence (Fig. R9B, 4 dpa).

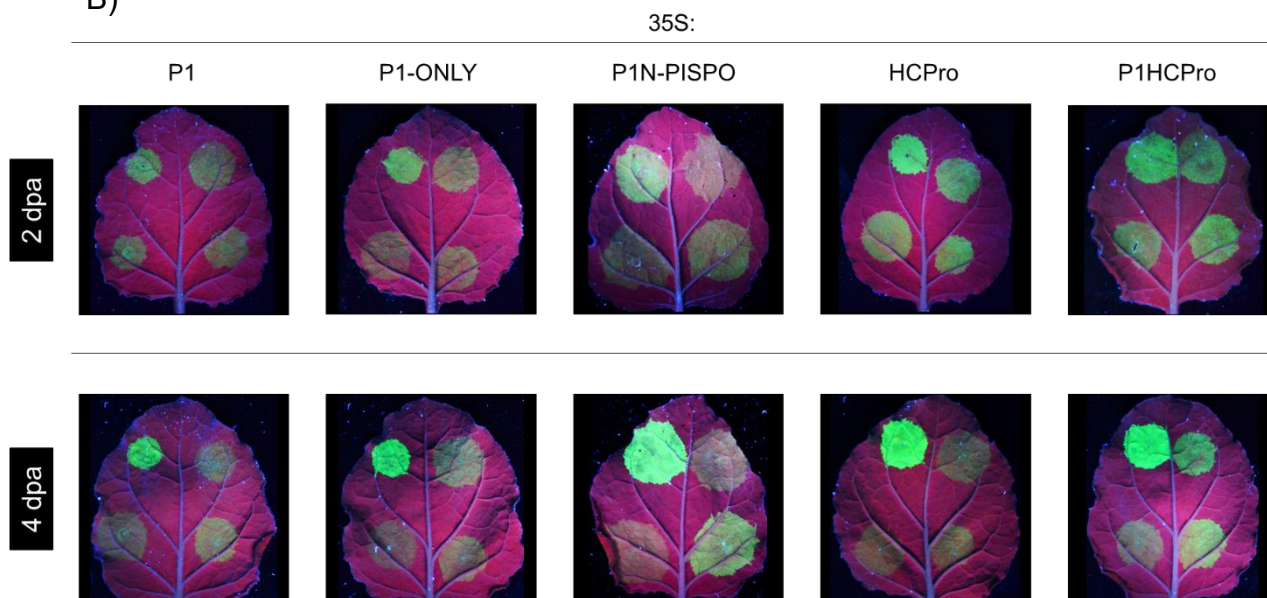
To complement these qualitative observations, GFP fluorescence was quantified for each construct. For this analysis, three patches were collected from per plant, pooled per sample, and evaluated across three biological replicates. Quantification of the fluorescence per sample showed that at 2 dpa only P1HCPro from AM-MB2 isolate displayed a significant increase in GFP accumulation compared with the negative control, whereas the AM-MB1 equivalent did not reach significance (Fig. R9C). At 4 dpa, fluorescence values decreased globally across most constructs, except the positive control, which remained significantly higher than all proteins tested of both isolates. Notably, comparison between isolates revealed a significant difference for P1N-PISPO at 4 dpa: the AM-MB2 version exhibited markedly stronger RNA silencing suppressor activity than AM-MB1, in agreement with the qualitative observations (Fig R9B). The absence of a strong suppressor comparable to the P1N-PISPO in any of the individual AM-MB1 gene products suggested that the full RNA silencing suppression capacity of this isolate may rely on the coordinated activity of multiple viral proteins rather than on individual components alone.

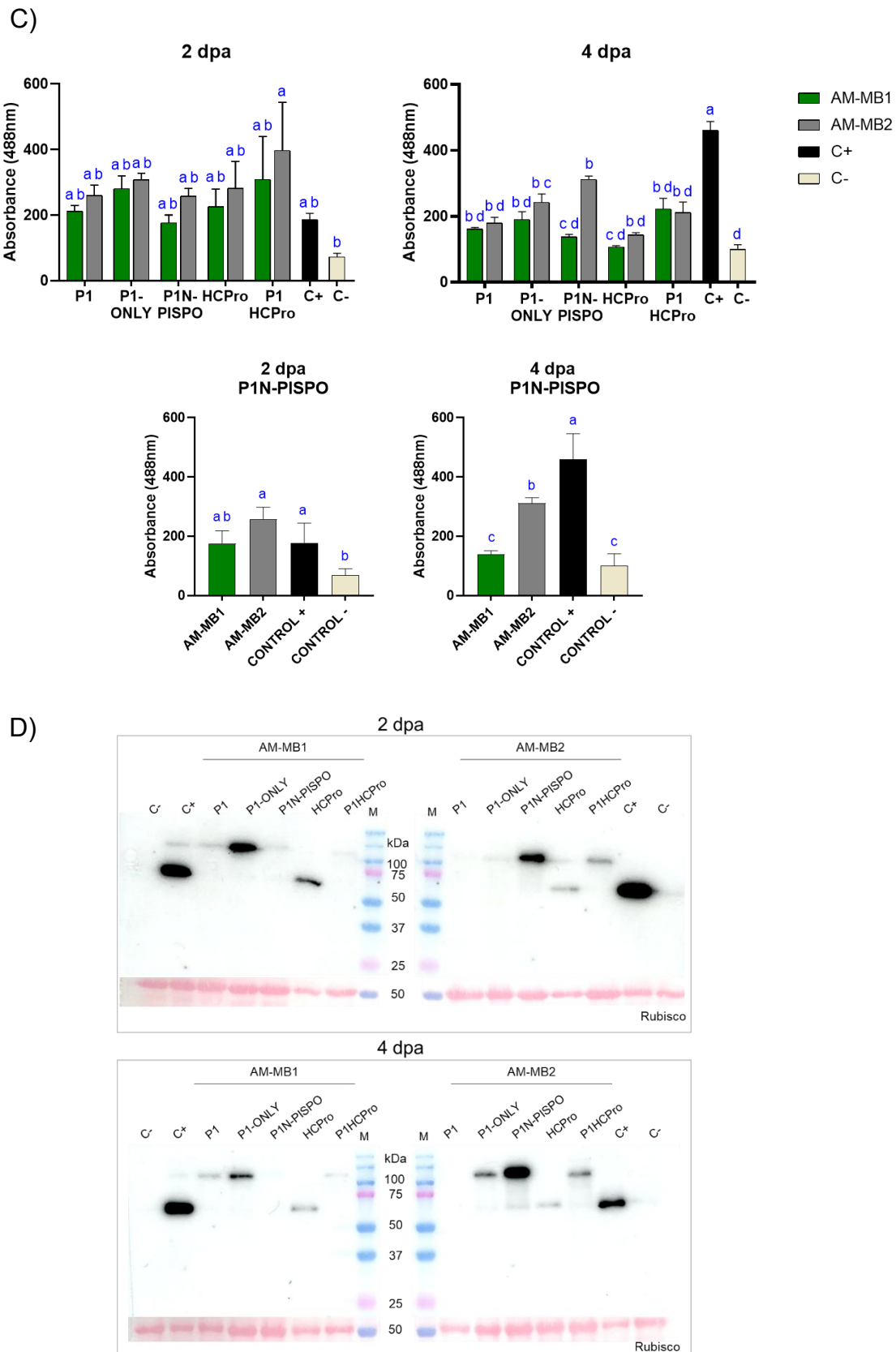
Results - Chapter I

To ensure the expression of all viral proteins tested were correct, MYC tagged versions were prepared by adding the tag at the N-terminal part of the different proteins. All constructs were agroinfiltrated in *N. benthamiana* leaves for further analysis of protein extracts by SDS-PAGE and Western Blot. Except the P1 of AM-MB2 isolate, which was not detectable at 2 dpa nor at 4 dpa, all viral gene products were detected (Fig R9D), showing the strongest signals in the positive control, in the P1-ONLY of AM-MB1 and P1N-PISPO of AM-MB2 at 2 and 4 dpa. The results observed in the Western blot analysis roughly correlated with the activity levels for the different constructs measured by GFP accumulation.



B)





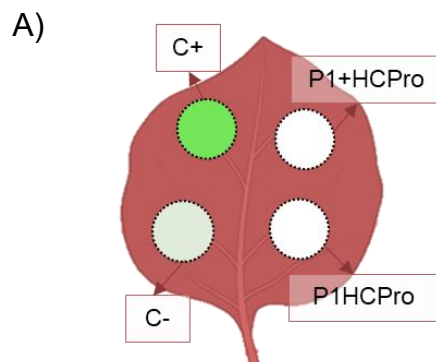
RSS proteins: AM-MB1 (top) and AM-MB2 (bottom). The left side of the leaf displays the controls: the positive control (HCPPro-WMV, top) and the negative control (inverted HCPPro-WMV, which prevents its expression, bottom). GFP fluorescence was monitored under UV light at 2 and 4 days post-agroinfiltration (dpa) to evaluate the presence or absence of silencing suppression activity. B) RNA silencing suppressor (RSS) activity of SPFMV AM-MB1 and AM-MB2 proteins in *N. benthamiana*, showing representative images of agroinfiltrated leaves expressing the indicated constructs (P1, P1-ONLY, P1N-PISPO, P1HCPPro, and HCPPro), visualized under UV light at 2 days post-agroinfiltration (top) and 4 days post-agroinfiltration (bottom). Positive (HCPPro-WMV) and negative (inverted HCPPro-WMV) controls were always included on the same leaf. C) Quantification of GFP accumulation in protein extracts from leaf patches agroinfiltrated with the GFP reporter construct in combination with the indicated RSS gene constructs. GFP was extracted using carbonate buffer and quantified by spectrophotometric analysis at 488 nm. Bars represent mean values from 3 independent samples at 2 dpa (top left) and 4 dpa (top right) for candidate RSS proteins. GFP accumulation for P1N-PISPO proteins of the two isolates at 2 and 4 dpa are compared in the bottom panels. Blue letters above the bars indicate statistically significant differences determined by two-way ANOVA ($p < 0.05$). Error bars represent the $\pm$ SEM of three independent samples. D) Western blot analysis of N-terminal MYC-tagged SPFMV-tested proteins at 2 dpa (top) and 4 dpa (bottom). The corresponding molecular weights are indicated in the center. Blots were probed with a specific anti-MYC primary antibody, followed by an anti-mouse secondary antibody. Total protein extract from non-agroinfiltrated *N. benthamiana* used as a negative control (C-), and positive control (C+) correspond to a MYC-tag version of HCPPro-WMV. The M lane shows the molecular weight marker (kDa). Ponceau red staining of the Rubisco large subunit (53 kDa) was used as a loading control.

4.2.2.2 *Cis* or *trans* co-expression of P1 and HCPPro does not affect their local RSS capacity

Given that the P1N-PISPO protein from isolate AM-MB1 displays weaker RNA silencing suppression activity than the corresponding protein from AM-MB2, and considering that P1HCPPro showed detectable silencing suppression in previous assays, we investigated whether other combinations were responsible for the silencing suppression in AM-MB1. This was of particular interest considering that, in other potyviruses, P1 has been proposed to potentiate the RNA silencing suppression activity of HCPPro when both proteins are expressed together (Kasschau and Carrington, 1998; Valli *et al.*, 2006). Since SPFMV P1 and P1HCPPro have been shown to possess silencing suppression activity (Untiveros *et al.*, 2016; Rodamilans *et al.*, 2021; present work, respectively), we aimed to determine whether HCPPro could in turn enhance P1 activity when the proteins are expressed in *trans* (P1+HCPPro) in this system. Although P1 and HCPPro from AM-MB1 are naturally expressed in *cis* (P1HCPPro) as part of the viral polyprotein, our previous co-agroinfiltration assays showed that neither protein displayed strong suppression when expressed individually with GFP, particularly HCPPro. Therefore, we decided to test whether their activity might change when they are expressed together in *trans*.

To address this, we compared the silencing suppression capacity of P1HCPPro with that of P1+HCPPro, evaluating both isolates. GFP was co-expressed with each construct alongside positive and negative controls (Fig. R10A), and fluorescence was quantified at 2 and 4 dpa. Because P1+HCPPro required mixing of three separate cultures (P1, HCPPro and GFP), the equivalent P1HCPPro mixture included as an additional component a negative control culture to assure equal final concentrations, and controls were prepared accordingly. For this analysis, three patches were collected per plant, pooled per sample, and evaluated across four biological replicates.

The expression of GFP under UV light at 2 and 4 dpa showed that the leaf patches expressing P1HCPPro produced similar GFP fluorescence than the patches expressing P1+HCPPro, and increasing of the GFP accumulation from 2 to 4 dpa in the case of positive control (Fig. R10B). When P1HCPPro and P1+HCPPro of each isolate were co-expressed in the same leaf, no significant differences were observed between the two constructs for AM-MB1 at either 2 or 4 dpa, although both differed significantly from the positive and negative controls (Fig. R10C). For AM-MB2 at 2 dpa, only P1HCPPro showed a significant difference compared to the negative control, whereas P1+HCPPro did not. At 4 dpa, both constructs differed significantly from the positive control but not from the negative control (Fig. R10C). Therefore, the GFP accumulation analysis revealed that P1HCPPro and P1+HCPPro produced comparable levels of suppression within each isolate, indicating that HCPPro does not substantially enhance the silencing suppression activity when combined with P1 under the experimental conditions used, and regardless of their co-expression in cis or trans.



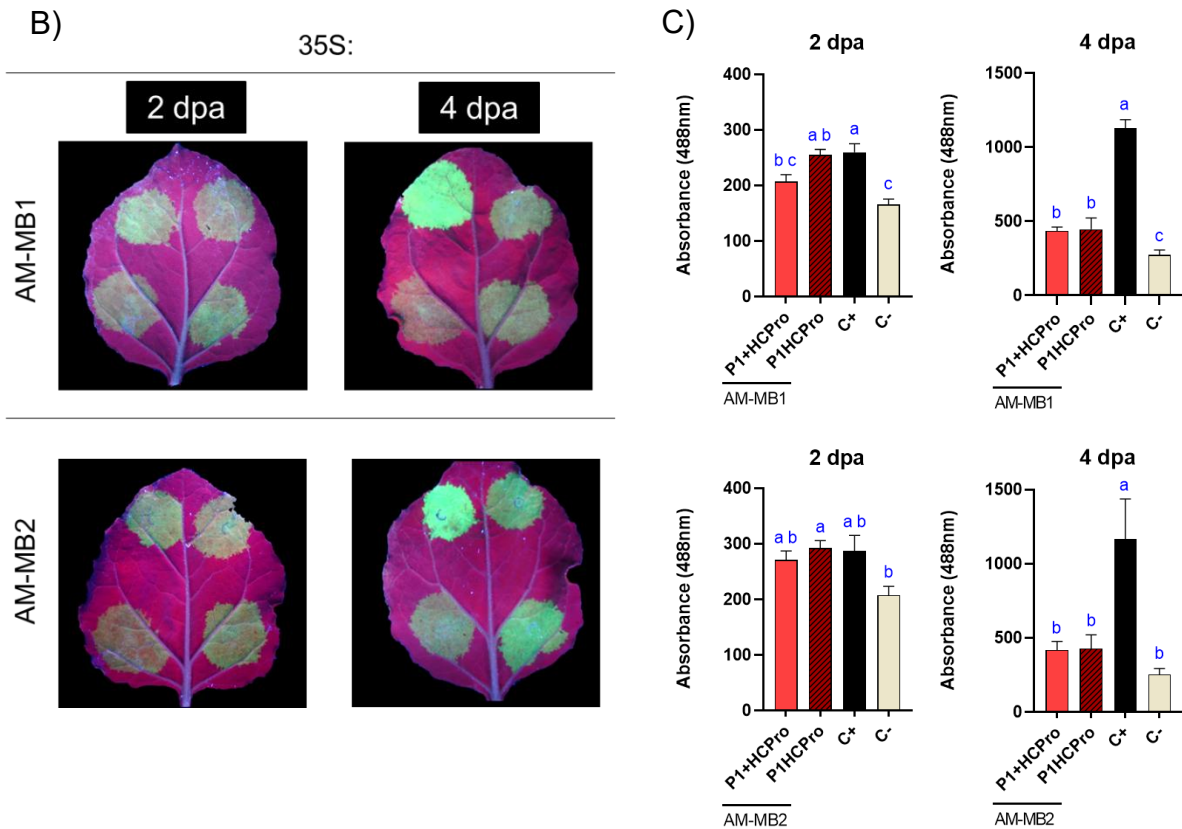


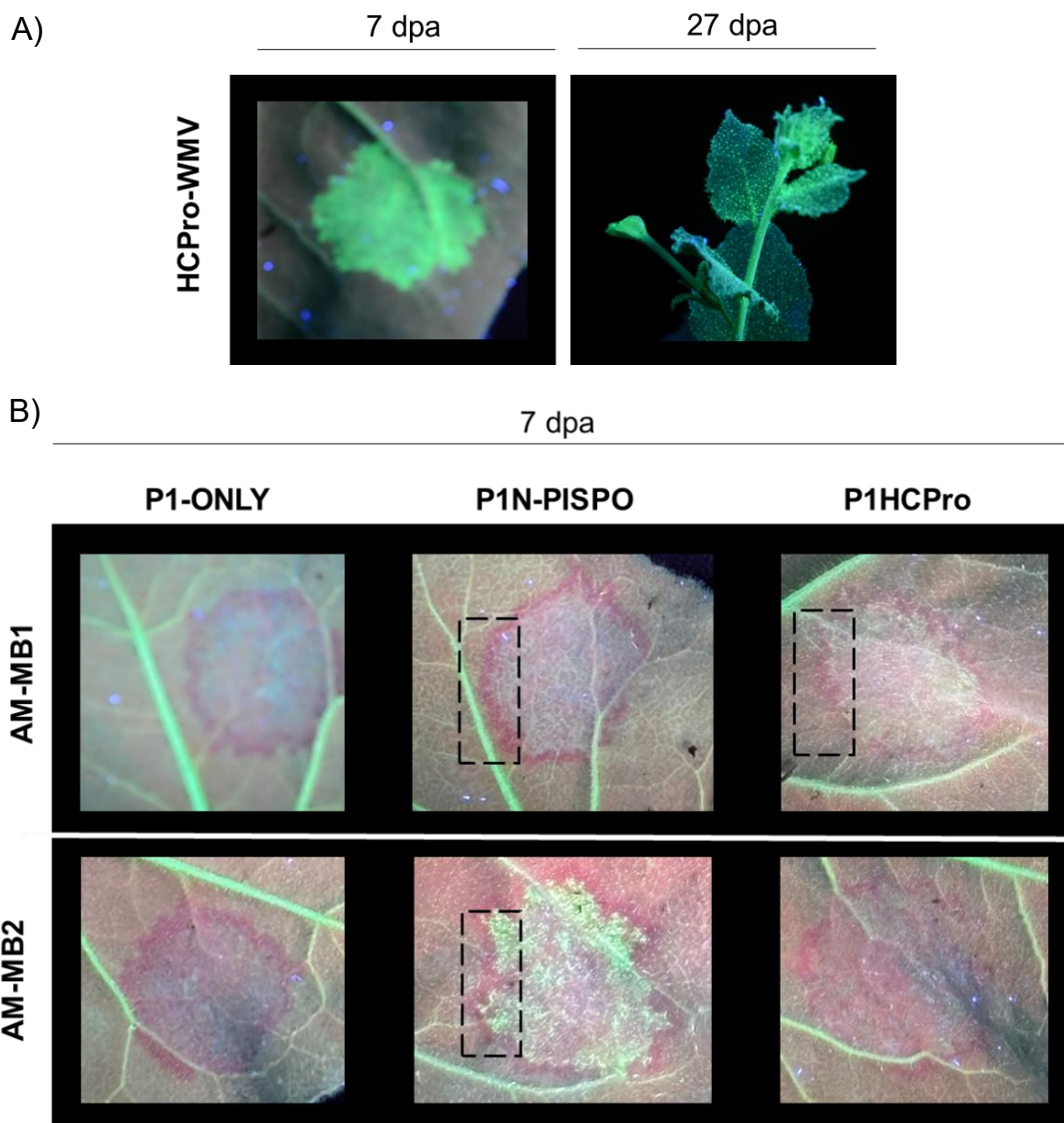
Figure R10. RNA silencing suppressor (RSS) activity of the P1HCPPro construct in *cis* versus *trans* expression. A) Schematic representation of RSS constructs in leaves, expressed either separately (P1+HCPPro, *trans*; top right) or as a single unit (P1HCPPro, *cis*; bottom right). Controls are shown on the left as in Figure R9. B) Representative UV images of agroinfiltrated leaves at 2 (left) and 4 dpa (right) using constructs from isolates AM-MB1 (top) and AM-MB2 (bottom). Positive (HCPPro-WMV) and negative (inverted HCPPro-WMV) controls were included. C) GFP quantification from protein extracts of infiltrated leaf patches, as described in Figure R9. Bars represent mean values ($n = 4$) at 2 (left) and 4 dpa (right). Different letters indicate significant differences (two-way ANOVA, $p < 0.05$). Error bars show mean $\pm$ SEM.

4.2.2.3 Effect of viral proteins in local and systemic movement of the silencing signal

A complementary analysis was performed using the well-characterized GFP-transgenic *N. benthamiana* line 16c. These plants constitutively express GFP and are used to check whether RNA silencing suppressor proteins interfere with the cell-to-cell or systemic spread of silencing signals from the infiltrated leaves to distal tissues. When RNA silencing is initiated in a local patch, the spread of the silencing signal into neighbouring cells results in the progressive loss of GFP expression, visible as a red halo surrounding the infiltrated area, and eventually leads to systemic silencing throughout the plant (Voinnet and Baulcombe, 1997).

In this experiment, P1-ONLY, P1N-PISPO and P1HCPPro from each isolate, as well as with HCPPro from WMV, previously used as positive control, were co-expressed with the

GFP reporter in basal leaves of 16c plants, and these plants were monitored over 27 dpa. A faint red halo around the infiltrated region was observed around all patches infiltrated (Fig. R11A, 7 dpa) excepted HCPPro from WMV (Fig. R11B, 7 dpa), indicating the activation of transgene silencing and cell-to-cell movement of the silencing signal. At 27 dpa, the upper leaves of plants co-agroinfiltrated with GFP and P1-ONLY, P1N-PISPO and P1HCPPro from AM-MB1 and AM-MB2, and HCPPro from WMV (Fig. R10A, B, 27 dpa) did not showed any apparent reduction in GFP expression, tentatively indicating a failure in the systemic long distance spread of silencing suppression. Similar results were observed in Valli *et al.*, (2006).



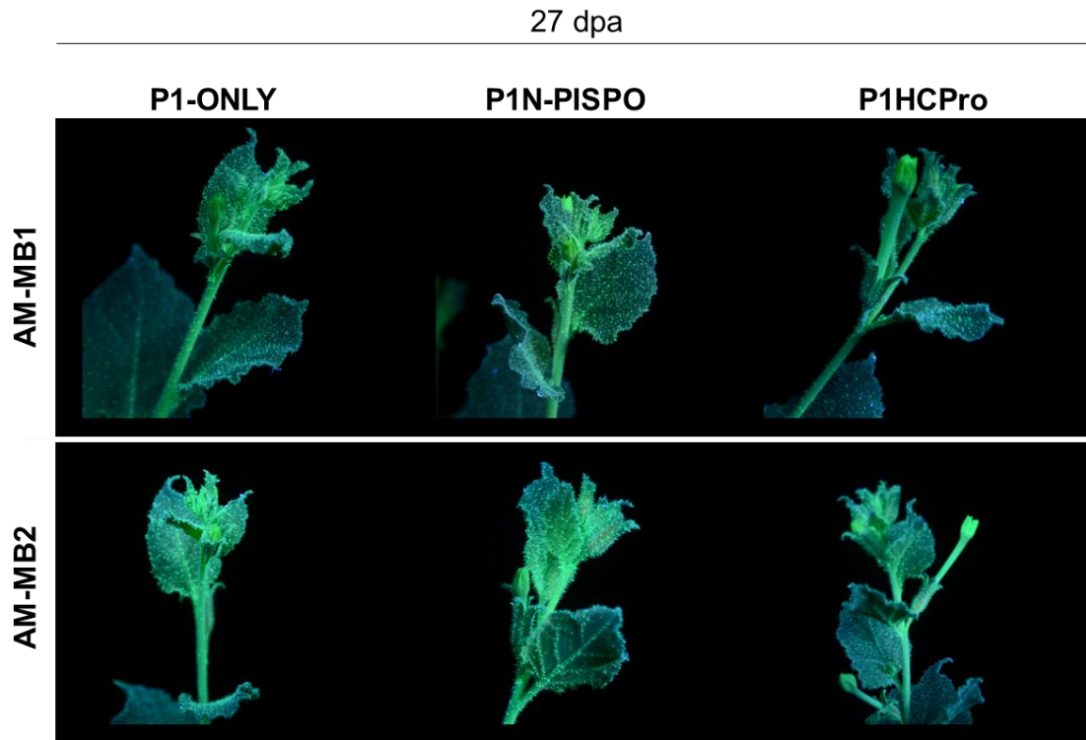


Figure R11. Effect of viral proteins in local and systemic RNA silencing suppressor (RSS) activity. A) GFP fluorescence in of *N. benthamiana* 16c under UV light after agroinfiltration with the control construct WMV-HCPro co-expressed with the GFP reporter, locally at 7 dpa (left) in agroinfiltrated patches, and systemically at 27 dpa in upper tissues. B) GFP fluorescence in agroinfiltrated leaves and systemic tissues of *N. benthamiana* 16c at 7 (top) and 27 dpa (bottom) under UV light. P1-ONLY, P1N-PISPO, and P1HCPPro from isolates AM-MB1 and AM-MB2 were co-expressed with the GFP reporter. Dashed rectangles indicate red halos surrounding agroinfiltrated patches.

DISCUSSION - CHAPTER I

Sweet potato provides a particularly rich and complex pathosystem for plant virology, being a host susceptible to a broad group of viruses, including several members of the *Potyvirus* genus. Among these, sweet potato potyviruses form a distinct taxonomic cluster within the genus, extensively documented (Clark *et al.*, 2012; Moreno and López-Moya 2020). Potyvirus transmission relies on specific molecular interactions enabling noncirculative and nonpersistent spread by aphids (Gray 1996; Blanc *et al.*, 1997), although some isolates display inefficient or even absent aphid transmissibility (Inoue-Nagata *et al.*, 2022). The peculiarities of the sweet potato cultivation system, in particular the frequent vegetative propagation, might help to explain the prevalence of many viruses along generations, even without an efficient insect transmission. Within this biologically intricate context, part of our efforts has been focused to characterize potyviruses infecting this crop.

A first task in this section was to examine the transmissibility of the potyvirus SPV2, testing an available isolate. Our results demonstrated that this particular isolate, maintained through repeated mechanical inoculation passaging in the laboratory, remained fully infectious when inoculated mechanically into *N. benthamiana* plants, whether using crude sap or purified virions, which also retained structural integrity after long-term storage at 4° and -80°C. In contrast, our attempts to perform aphid plant-to-plant transmission assays using *M. persicae* as the putative vector organism consistently failed; despite earlier reports demonstrating successful transmission of isolates of this virus (Ateka *et al.*, 2004). This discrepancy aligns with previous observations indicating that, although potyviruses can be experimentally transmitted by mechanical inoculation, repeated passages often lead to a progressive loss of aphid transmissibility (Simons, 1976), a phenomenon often linked to mutations in the HCPro-CP transmission core elements. According with the classical “bridge hypothesis” (Govier and Kassanis, 1974b; Pirone and Blanc, 1996), which proposes that aphid transmission requires the involvement of K/RITC and PTK motifs in HCPro and DAG motif in the CP, our sequence analysis revealed that all three canonical motifs remained conserved in both the original sequence of SPV2 AM-MB2 and the laboratory adapted SPV2_Nb-CO variant (Fig. R2), and therefore the loss of aphid transmissibility cannot be readily attributed to disruption of these described elements required for the vector-HCPro and HCPro-CP interactions. Accordingly, we compared the sequences of the SPV2 AM-B2 and SPV2_Nb-CO isolates with the SPV2 reference sequence (NC_017970.1) to identify other potential determinants. We identified several amino acid substitutions in the SPV2 AM-MB2 and

SPV2_Nb-CO sequences, at positions close to the conserved HCPro and CP transmission motifs, relative to the SPV2 reference sequence (NC_017970.1). These substitutions may influence aphid transmission efficiency, in agreement with observations reported for other potyviruses (Llave *et al.*, 1999). The replacement of Val (hydrophobic non-polar amino acid) and the Asn (hydrophobic polar amino acid) at position 17 and 34, respectively, present in the reference sequence (NC_017970.1) with a negatively charged Asp at position 17 and a hydrophobic polar Ser at position 34 in SPV2 AM-MB2 and SPV2_Nb-CO sequences (Fig. R2), could mirror previously described cases in which changes in charge or polarity near conserved HCPro domains drastically altered or abolished aphid transmission, despite leaving the K/RICT motif intact (Llave *et al.*, 1999; Blanc *et al.*, 1998). As well as in other potyviruses, the mentioned mutations are located near a cysteine-rich domain which can play a critical role in the efficiency of potyvirus aphid transmission, as shown for TEV (Llave *et al.*, 2002).

In the case of the CP, numerous studies have demonstrated a clear correlation between the loss of aphid transmissibility and mutations within the DAG motif (Atreya *et al.*, 1991; Gal-on *et al.*, 1992), however alterations in residues adjacent to this motif can also impair transmissibility; that happens in the case of TVMV, where a substitution of the lysine following the DAG motif with a glutamic acid abolished aphid transmission (Atreya *et al.*, 1995) or where the seven amino acids preceding the DAG motif were involved in the aphid transmissibility (Blanc *et al.*, 1997). Similar effects have been reported for changes within or near the DAG region of TEV (López-Moya and Pirone, 1998) and of ZYMV (Dombrovsky *et al.*, 2007). In addition, and according to the early structural model of Shukla and Ward (1989), recently reinterpreted with abundant experimental data in several virus species of the family (Agirrezabala *et al.*, 2015; Cuesta *et al.*, 2019; Chase *et al.*, 2023; Bonnet *et al.*, 2025; Koritnik *et al.*, 2026) the N-terminus of the CP of potyviruses is surface-exposed, ensuring accessibility of the DAG motif to interact with HCPro; thus, even subtle conformational changes caused by nearby mutations may affect this interaction. Then, these findings support the idea of surrounding amino acids influence the exposure and functionality of the DAG motif during the transmission process (López-Moya *et al.*, 1999). In both SPV2 AM-MB2 and SPV2_Nb-CO, we observed a substitution preceding the (K)DAG motif, an Ile (hydrophobic) instead of the Thr (noncharged polar) present in the reference sequence and rest of the sequences aligned (Fig. R2). This change, together with additional alterations near the conserved RITC-HCPro motif, could have contributed cumulatively to reduce or abolish aphid transmission.

To summarize, these observations are compatible with the loss of aphid transmissibility in SPV2-Nb_CO, as it might be the result of mutations accumulated during serial mechanical passaging for adaptation to the *N. benthamiana* host. Consistent with the broader literature, a single amino acid substitution close to HCPro and CP conserved motifs can noticeably disrupt the molecular interactions required for vector-mediated transmission. However, further experimentation should be required to clarify this situation. In any case, the interest of using the SPV2 isolate as model for the study, prompted by its capacity to infect *N. benthamiana*, was drastically reduced once we observed that the initial poor transmissibility later leads to a complete loss of the capacity of being aphid transmitted in our conditions.

The presence of multiple sweet potato viruses from same or different species within a single sweet potato plant, allowed us to focus on SPFMV to characterize the AM-MB1 isolate, that we confirmed can co-exist in the same plant with the previously characterized AM-MB2 isolate (GenBank: KU511268.1). To explore the genomic variability between these two isolates, we completed the full genome sequence of SPFMV AM-MB1 and compared the coding region sequences and both UTRs with those of AM-MB2. These analyses revealed several differences, and the most relevant are discussed in the following paragraphs.

First, regarding the non-coding UTRs flanking the viral polyprotein, the 5'RACE analysis showed that AM-MB1 contained a stretch of five consecutive adenines (A₅) in this region (positions 1-5 nt), whereas in the AM-MB2 isolate the equivalent region contained only four. The integrity in the initial and final positions of potyvirus genomes has been considered prone to template-independent repair mechanisms during in vivo RNA synthesis (Takahashi and Uyeda, 1999; Simón-Buela *et al.*, 2000), making this particular variability an interesting feature with potential biological effects. Likewise, in the 3' UTR of AM-MB1 a stretch of six Uracils (U₆) is present (positions 10875-10880 nt), compared with only five in AM-MB2 (Fig. R5). Although such differences are located in UTRs, they could be relevant as they might affect regulatory elements implicated in translation efficiency and infectivity (Carrington and Freed, 1990; Haldeman-Cahill *et al.*, 1998; Valli *et al.* 2015). It should be noted that the U₆ motif in the 3' UTR of AM-MB1 (U₅ in AM-MB2), can derive from a transcriptional slippage event. In SPFMV, RNA slippage was found responsible to the production of P1N-PISPO and P3N-PIPO (Rodamilans *et al.* 2015). In a work focusing on TuMV it was shown that the introduction of artificial U₆ motifs induced slippage (Olspert *et al.*, 2016). Recent work has shown that natural slippage motifs (long homopolymeric runs of As or Us) can promote polymerase slippage at multiple positions along potyvirid genomes, independently of the presence of

downstream ORFs (Valli *et al.*, 2024), leading to expression of either partially out-of-frame or truncated gene products. Taking all this together, it is tempting to speculate that slippage events could also happen out of the coding region, and it might have some biological impact that should be evaluated case by case. In our specific observed variability in the 3' UTR between AM-MB1 and AM-MB2, it cannot be discarded the possibility that slippage-associated mechanisms are contributing to the functional diversification of these two sweet potato potyvirus isolates.

Second, in the polyprotein region, the sequence comparisons between SPFMV AM-MB1 and AM-MB2 revealed several differences. Among the changes of amino acids, at least two proteolytic junctions might be affected: the P1/HCPPro junction and the P3/6K1 junction. In the P1/HCPPro junction two amino acids differed between isolates: in the case of AM-MB1 isolate the motif comprises a Thr in position 687 and a Phe in 689, resulting in ITQF/S junction, whereas in the case of AM-MB2 the motif contains an Ile in position 662 and a Tyr in 664, resulting in IIQY/S junction. It has been previously reported that both cases, Phe₆₈₉/Ser₆₉₀ as well as Tyr₆₆₄/Ser₆₆₅, conserved the proteolytic activity (Mavankal and Rhoads, 1991; Verchot *et al.*, 1992; Yang *et al.*, 1998; Adams *et al.*, 2005). In contrast, at the P3/6K1 junction appears a Glu (E), which has a negative charge in AM-MB1 instead of a Lys (K) with positive charge, in AM-MB2, resulting in VEHQ/G and VKHQ/G, respectively. The introduction of this charge-change in an essential cleavage position can affect to the proteolytic processing of both proteins involved. We can hypothesize that this variation may influence in the cleavage efficiency and, consequently, alter the correct dynamic production of the P3 and 6K1 mature proteins. Notably, the VEHQ/G motif present in AM-MB1 is not reported in the cleavage site P3/6K1 sites in other potyviruses according to the dedicated database (<https://www.dpvweb.net/potycleavage/species.html>), but it was present in SPFMV and in Rymoviruses, while the VKHQ/G is unique for the AM-MB2 isolate, suggesting that this divergence may have functional implications, potentially affecting essential processes like the viral replication, pathogenesis and symptomatology that are mediated by P3 and 6K1 (Klein *et al.*, 1994; Luan *et al.*, 2016; Cui and Wang, 2016).

While looking for more potential discrepancies between isolates, is important to highlight that the N-terminal region of monopartite potyviruses, which includes P1 and HCPPro proteins, is usually the most variable and least conserved region of the potyviral polyprotein (Adams *et al.*, 2005; Cui and Wang, 2019). HCPPro is a multifunctional protein, and one of its most extensively examined roles involves its interactions with the CP during aphid transmission, as previously discussed (Revers and García, 2015). Beyond this function, HCPPro has also been widely described as an RNA silencing suppressor

(RSS) in most members of the genus *Potyvirus* (Anandalakshmi *et al.*, 1998; Kasschau and Carrington, 1998; and retracted paper Brigneti *et al.*, 2015). Meanwhile, the extensive literature describing and studying the multiple biological functions attributed to HCPro (Pirone and Blanc, 1996; Valli *et al.*, 2018), the contribution of the potyviral P1, have remained unclear for many years. More recent work has started to highlight the broader relevance of P1 in potyvirus infection (Pasin *et al.*, 2014; Martinez and Darós, 2014; Shan *et al.*, 2018), including evidence that P1 can modulate the RNA silencing suppressor activity of HCPro when both are expressed together (Valli *et al.*, 2006; Tena-Fernandez *et al.*, 2013). Interestingly, P1 also have been reported as RSS: in SPMMV, although HCPro is present, the main RNA silencing suppressor function resides exclusively in P1 (Giner *et al.*, 2010). Untiveros and colleagues (2016) reported that RNA silencing suppressor activity relied on the P1 as well as the P1N-PISPO, a transframe product generated through polymerase slippage at the conserved G₂A₆ motif upstream of PISPO, and contrasting with findings from Szabó *et al.*, (2012) and Mingot *et al.*, (2016), who did not report this activity for P1, likely due to differences in experimental approaches or isolates examined. Together, these studies illustrate the functional diversity and evolutionary versatility of the P1-HCPro module within potyviruses and related genera.

Our analysis comparing sequences from AM-MB1 and AM-MB2 SPFMV revealed that PISPO (68.10% amino acid identity), P1N-PISPO (74.52%) and P1 (79.39%) were the most divergent proteins between isolates, consistent with previous reports highlighting the recent evolution and variability of this genome region (Cui and Wang, 2019). Importantly, AM-MB1 isolate contained a 25 amino acid insertion (aa position from 290 to 314) upstream of PIPO region, which was absent from AM-MB2 and the reference sequence (GenBank NC_001841.1). Phylogenetic analysis of this P1 sequences and others available in GenBank, showed that there was a phylogenetic separation of the AM-MB1 and AM-MB2 isolates into distinct clusters (Fig. R7). SPFMV isolates are commonly classified based on the nucleotide and amino acid levels of the CP region into four phylogenetic lineages: East African (EA), Russet Crack (RC), Ordinary (O), and Common (C) based on CP sequence variability (Kreuze *et al.*, 2000) and posterior studies categorized the C strain members as a separate virus species, designated as *Sweet potato virus C* (SPVC) (Untiveros *et al.* 2010). However, when the P1 protein was used for phylogenetic comparisons, these lineage divisions were not clearly recovered, reflecting that the genetic differences present in this coding region do not correlate with the proposed lineages (data not shown). Analysis of multiple SPFMV sequences revealed that the central region of the P1 coding sequence frequently contains nucleotide

insertions of different sizes, ranging from 60 to 180 nt. Notably, several isolates from the EA lineage were found to share a consistent 25 aa insertion at same position, and alignment of additional sequences confirmed that this indel represents a characteristic feature of EA isolates, including those originating in Peru (Untiveros *et al.*, 2010). Then we can suggest strongly the AM-MB1 isolate belongs to the EA strain. Beyond these insertions, SPFMV isolates are known to exhibit recombination events, mostly in the 5' terminal of their genomes, reflecting not only interactions among strains but also the broader evolutionary history of the *Potyviridae*. Indeed, the P1 of SPFMV has been proposed to originate from an intergeneric recombination event, involving the ipomovirus sweet potato mild mottle virus (SPMMV), which encode a P1 protein with RNA silencing suppressor activity (Valli *et al.*, 2007; Giner *et al.*, 2010). Furthermore, instances in which the P1-N domain appears to have been exchanged between isolates of differences strains or even different species suggest that the P1-N domain is a functional element. This P1-N seems to be unique to potyviruses infecting convolvulaceous hosts, separated from P1-Pro by a hypervariable stretch that may facilitate such evolutionary exchanges (Untiveros *et al.*, 2010).

Given the functional importance in terms of RNA silencing suppressor activity of P1 and HCPro proteins in potyvirus and specifically in SPFMV, we decided to explore whether these 25 extra aa have a functional relevance in the activity of the proteins encoded at the 5' ends of the AM-MB1 potyviral genome, and compare with them with the equivalent from the AM-MB2 isolate. Our findings revealed a clear RNA silencing suppressor activity for the P1N-PISPO product of the AM-MB2 isolate, whereas P1 and HCPro showed only a residual or barely detectable suppression capacity, consistent with previous observations for SPFMV (Szabó *et al.*, 2012; Mingot *et al.*, 2016). In contrast, all AM-MB1 constructs tested displayed markedly weaker RNA silencing suppressor activity than their AM-MB2 counterparts, with neither P1, P1N-PISPO nor HCPro acting as clear RSSs in the standard transient expression assay. Although P1-ONLY and P1HCPro showed slightly higher activity within the AM-MB1 RSSs set, their effects remained residual, suggesting that the silencing suppression activity of this isolate may rely on the combined action of several viral proteins rather than on a single dominant component. The presence of a 25 amino acid insertion upstream of PISPO in AM-MB1 might be contributing to this reduced activity, by potentially altering the regulatory balance between P1 and P1N-PISPO. This possibility can be somehow similar to the findings in other potyviruses like PPV, where N-terminal alterations in P1 revealed an autoinhibitory mechanism that modulates protease activity (Pasin *et al.*, 2014; Shan *et al.*, 2018; Pasin *et al.*, 2020). Our results for the AM-MB2 isolate showed a clear role for P1N-PISPO as

RSS; interestingly, from the rest of the constructions P1 and HCPro were the least active ones, leaving P1-ONLY and P1HCPro as putative intermediate partners in the silencing activity (Fig. R9). Data showing no RNA silencing suppressor activity for P1 nor for HCPro in the transient expression mediated by agroinfiltration assay are consistent with those reported by Szabó *et al.*, (2012) and Mingot *et al.*, (2016). However, in the case of AM-MB1, all proteins tested in our constructs presented a lower RNA silencing suppressor activity than those belonging to the AM-MB2. In terms of silencing suppressor activity in the AM-MB1 system, P1, P1N-PISPO and HCPro can be considered barely active, and the topmost construct with some activity were P1-ONLY and P1HCPro; however, none of them showed a clearly sufficient RSS activity, and this suggest that if any of the individual AM-MB1 gene products can be considered responsible for the full RNA silencing suppression capacity of this isolate, the function may rely on the coordinated activity of multiple viral proteins rather than on individual components alone.

To investigate the molecular basis of the observed differences in RNA silencing suppressor activity, we examined the presence of AGO-binding WG/GW motifs, as key determinants of the function (Mingot *et al.*, 2016; Untiveros *et al.*, 2016). Both isolates possess the same number and a similar distribution of the WG/GW motifs, indicating that motif variation is unlikely to explain the reduced activity of P1 and P1N-PISPO of AM-MB1. The role played by the 25 aa additional upstream PISPO in regulating down the activity of AM-MB1 P1N-PISPO remains to be explored. An important consideration is that silencing suppression can vary substantially depending on the experimental system used; for example, the HCPro of SPFMV exhibits RSS activity when expressed in the context of a PPV chimeric virus (Rodamilans *et al.*, 2018), suggesting that its functionality may require viral infection conditions or interactions with additional viral components. Future testing of P1, P1N-PISPO or HCPro of the AM-MB1 isolate using a viral complementation system in the same experimental plant, either using a chimeric system combining with AM-MB2 sequences, or using a co-infection context with other potyviruses or sweepoviruses (begomoviruses infecting *Ipomoea* spp.), or even testing other susceptible host may therefore reveal activities not detectable through classical transient expression in *N. benthamiana*. This last point is particularly relevant given the association of P1 with host adaptation and specificity (Salvador *et al.*, 2008; Shan *et al.*, 2015; Cui and Wang, 2019), raising the possibility that the AM-MB1 25aa-insertion influences its functionality in a host-dependent manner. Additionally, previous work in our laboratory has revealed that in SPV2, HCPro, and to lesser extend P1N-PISPO proteins have RNA silencing suppressor activity (Chase *et al.*, unpubl. data). In addition, and to test their relevance as RSS in the context of a viral infection they used

the PVX-based vector (Valli *et al.*, 2008). Their results indicated that that different SPV2 encoded proteins (P1, HCPro and P1N-PISPO) enhance PVX pathogenicity like other previously described RSS proteins (Valli *et al.* 2008; Feng *et al.* 2018), and suggest that this effect is likely related to the capacity of these proteins to interfere with the RNA silencing machinery of the plant. Interestingly, SPV2 HCPro did not enhance considerable PVX pathogenicity at the same level as P1N-PISPO, reinforcing the idea that there are other mechanisms underlying this behaviour, and that the choice of assay conditions might critically shape the observed results.

In other studies, the levels of translation of HCPro were reported to be increased by P1 acting as a cis-enhancer of expression (Tena-Fernández *et al.*, 2013). To test if the suppression levels observed for the two leader proteins of SPFMV changed when they were expressed *in trans* (P1+HCPro) or *in cis* (P1HCPro) we performed a comparative experiment, finding that the two alternative situations for co-expression (cis and trans) were not significantly different.

A further comparison was done to determine whether any of the identified RSS candidates from both isolates could suppress RNA silencing at long-distance. Among the previously tested constructs, P1-ONLY, P1N-PISPO and P1HCPro were selected because they exhibited the strongest suppressor activity in both isolates. All these putative RSSs acted restrictively at the single-cell or local suppression level, as indicated by the characteristic red halo around all the infiltrated patches; having only AM-MB2 P1N-PISPO a clear ability to transiently suppress RNA silencing locally (Fig R11). In addition, and despite the small number of plants in the experiment (n=3), the data also showed that none of the tested constructs exhibited long distance silencing, although the behaviour of the plant material in the experimental conditions does not allow to derive definite conclusions.

Finally, although HCPro is the canonical suppressor in most members of the *Potyviridae*, and P1 and P1N-PISPO have been reported in other potyvirids to contribute to the RSS activity (Anandalakshmi *et al.*, 1998; Kasschau and Carrington, 1998; Giner *et al.*, 2010; Young *et al.*, 2012; Tatineni *et al.*, 2012; Mingot *et al.*, 2016; Untiveros *et al.*, 2016), we cannot exclude that other viral gene products might participate as well. For instance, the viral protein VPg has also been reported to exhibit RNA silencing suppressor activity, supporting the idea that several suppressors may act synergistically at different stages or cellular locations to counteract host defence mechanisms (Cuellar *et al.*, 2009; Rajamäki *et al.*, 2014). This level of functional redundancy could be particularly relevant in sweet potato, a host that frequently harbours mixed viral infections (Moreno and

López-Moya, 2020), building conditions that favour interaction and dynamics of several viral proteins. Altogether, our findings highlight the complexity of RNA silencing suppression in SPFMV and underscore the need to evaluate putative suppressors from different isolates across multiple experimental contexts.

Notably, the frequency of slippage in SPFMV decreases in mixed infections with SPCSV (Mingot *et al.*, 2016), the combination responsible for SPVD, suggesting that the presence of a synergistic partner virus can modulate slippage and, consequently the balance of RNA silencing suppressor activities (Rodamilans *et al.*, 2018). This is consistent with the profound disruption of the ecological equilibrium normally maintained between sweet potato and its well-adapted potyvirids, which becomes destabilized upon the arrival of the crinivirus (Kareyija *et al.*, 2000). Although it is tempting to attribute these outcomes to the distinctive RSS properties of SPCSV, the mechanistic basis for the altered behaviour of sweet potato potyviruses under mixed infection has remained elusive until recently. Future work will be required to clarify the interplay between slippage, viral suppressors and mixed-virus interactions, trying to explain, and ultimately mitigate, the severe pathogenicity associated with SPVD.

RESULTS – CHAPTER II

SWEET POTATO PATHOSYSTEM

5. RESULTS - CHAPTER II

RNA-seq profiling of sweet potato plants under SPFMV single infection and whitefly-mediated SPCSV-SPFMV co-infection

5.1 Characterization of the sweet potato virome using RNA-seq uncovers diverse plant-infecting viruses with SPFMV predominance

Sweet potato is widely recognized for its capacity to accumulate diverse viral agents, a consequence of its predominantly vegetative mode of propagation and the frequent exchange of planting material among growers, which together promote the long-term persistence and dissemination of viral infections within local varieties (Kreuze *et al.*, 2021). Consistent with this biological context and with the aim of characterizing the viral communities associated with *I. batatas* under mixed-infections conditions, RNA-seq libraries were generated from eight sweet potato samples maintained in CRAG greenhouse. The samples were collected at two time points before and after superinfection (T0 and T30, experimental details described below). Analysis of the resulting RNA-seq of T0-samples revealed the presence of a diverse virome, aligning with the well-documented tendency of *I. batatas* to harbour multiple viruses simultaneously (Moreno and López-Moya, 2020). Following library preparation and sequencing, reads were processed through the Genome Detective virus identification platform, yielding per-sample viral profiles. For each sample, a table of detected viral sequences with at least 50% nucleotide (nt) similarity was generated, reporting the number of virus-associated reads, depth of coverage, genome coverage, and nt identity. An initial taxonomic identification was conducted directly on the filtered reads of samples. Data represented were analysed and calculated by using the read count percentage of mapped reads for each sample (Supplementary Table S3). Aggregation of T0-sample reads enabled a comparative assessment of plant-infecting vs. non-plant-infecting viral taxa (Fig. R12). Notably, the total read count of non-plant viruses accounted for 1.015 of the read mapped (representing the 11.78% of total read count percentage of virus-associated mapped reads), predominantly representing viral families typically associated with fungi or arthropods or other invertebrates, included in Table R4, and reflecting the complex microbiological and pest-related environment of the crop. In contrast, plant

infecting viruses represented 7.601 of the reads counted, corresponding to 88.22% of the total read count percentage of virus-associated read mapped. To refine the analysis and focus exclusively on the plant-associated virome, only viral sequences infecting plants were retained for downstream comparisons (viruses included in Table R4). Within this plant-associated virus fraction, the majority of reads clustered within the families *Potyviridae* and *Geminiviridae* (Fig. R12), both of which were present in every sample analysed (Supplementary Table S4). Potyvirus species constituted the most abundant group showed in T0-samples, with SPFMV dominating the viral landscape. Begomoviruses constituted the second most represented group, whereas reads belonging to other plant-infecting genera, including *Badnavirus* and *Mastrevirus*, were detected only at low levels (Fig. R12). These results, along with the consistent detection of SPFMV in each sample analysed, confirmed a plant virome with a strong predominance by SPFMV within the plant-virus subset.

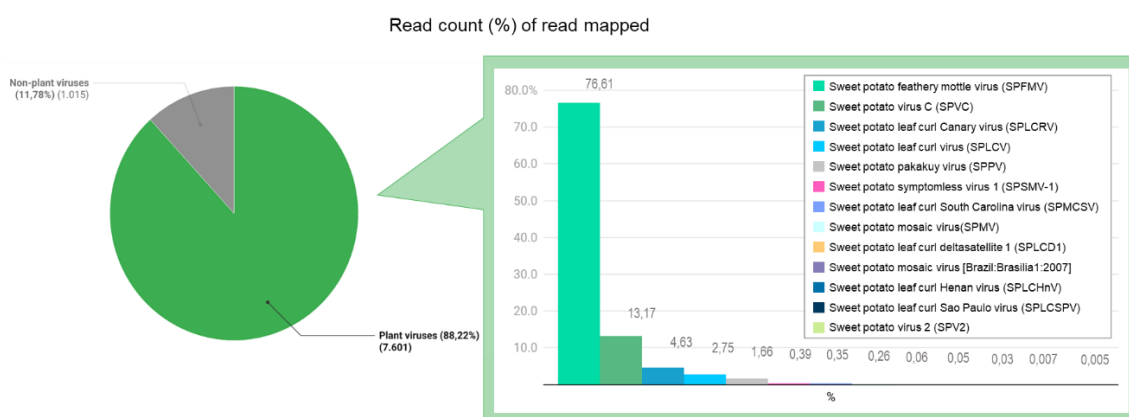


Figure R12. Abundance of virus-associated reads in RNA sequencing (RNA-seq) libraries from *I. batatas* T0 samples. The pie chart shows the proportion of reads mapped to virus-associated contigs, expressed as a percentage of total mapped reads. Virus-associated contigs are classified into plant viruses (green) and non-plant viruses (grey). The bar chart shows the relative abundance of contigs assigned to individual virus species within the plant virus fraction, expressed as a percentage of virus-mapped reads.

Table R4. List of viruses present in the RNA-seq samples analyzed at T0.

Virus	Genera	Family	Host
Cotesia glomerata bracovirus	<i>Bracoviriform</i>	<i>Polydnaviriformidae</i>	Insect
Diachasmimorpha longicaudata entomopoxvirus	Unclassified	<i>Poxviridae</i> Subfamily: <i>Entomopoxvirinae</i>	Insect
Fusarium andiyazi mitovirus 1	<i>Unuamitovirus</i>	<i>Mitoviridae</i>	Fungi
Fusarium andiyazi mitovirus 2	<i>Unuamitovirus</i>	<i>Mitoviridae</i>	Fungi
Wenzhou tombus-like virus 1	Tombusvirus-like	<i>Tombusviridae</i>	Invertebrates
Wenzhou tombus-like virus 3	Tombusvirus-like	<i>Tombusviridae</i>	Invertebrates
Shahe tombus-like virus 1	Unclassified	Unclassified	Invertebrates
Sweet potato feathery mottle virus (SPFMV)	<i>Potyvirus</i>	<i>Potyviridae</i>	Plants
Sweet potato virus C (SPVC)	<i>Potyvirus</i>	<i>Potyviridae</i>	Plants
Sweet potato virus 2 (SPV2)	<i>Potyvirus</i>	<i>Potyviridae</i>	Plants
Sweet potato leaf curl Canary virus (SPLCRV)	<i>Begomovirus</i>	<i>Geminiviridae</i>	Plants
Sweet potato leaf curl virus (SPLCV)	<i>Begomovirus</i>	<i>Geminiviridae</i>	Plants
Sweet potato leaf curl Henan virus (SPLCHnV)	<i>Begomovirus</i>	<i>Geminiviridae</i>	Plants
Sweet potato mosaic virus (SPMV)	<i>Begomovirus</i>	<i>Geminiviridae</i>	Plants
Sweet potato leaf curl Sao Paulo virus (SPLCSPV)	<i>Begomovirus</i>	<i>Geminiviridae</i>	Plants
Sweet potato leaf curl Sichuan virus (SPLCSiV)	<i>Begomovirus</i>	<i>Geminiviridae</i>	Plants
Sweet potato leaf curl South Carolina virus (SPMCSV)	<i>Begomovirus</i>	<i>Geminiviridae</i>	Plants
Sweet potato mosaic virus - [Brazil:Brasilia1:2007]	<i>Begomovirus</i>	<i>Geminiviridae</i>	Plants
Sweet potato symptomless virus 1 (SPSMV-1)	<i>Mastrevirus</i>	<i>Geminiviridae</i>	Plants
Sweet potato leaf curl deltasatellite 1 (SPLCD1)	<i>Tolecusatellitidae</i>	<i>Deltasatellite</i>	Plants
Sweet potato pakakuy virus (SPPV)	<i>Badnavirus</i>	<i>Caulimoviridae</i>	Plants

5.2 Temporal dynamics of SPFMV accumulation and symptom development in single and mixed infection SPCSV

SPFMV is widely distributed across *I. batatas* and related *Ipomoea* spp., typically causing mild or latent infections. Symptom severity increases substantially when SPFMV co-occurs with SPCSV, as demonstrated by strong synergistic interactions during co-

infection that lead to leaf narrowing and distortion, vein chlorosis, laminar rugosity and pronounced plant stunting (Salazar and Fuertes, 2001; Kokkinos and Clark, 2006a). Because SPFMV accumulates at low titers, it is often difficult to detect in singly infected plants (Hahn 1976; Mukiibi 1977; Ngeve and Bouwkamp, 1991), whereas SPCSV co-infection greatly enhances SPFMV accumulation (Karyeija *et al.*, 2000).

To date, most molecular and physiological studies on SPFMV-SPCSV synergism have relied on mechanical inoculation or grafting and have not incorporated *B. tabaci*, the natural vector of SPCSV, as a mean to achieve superinfection. Consequently, these approaches depart from the ecological and epidemiological context in which SPVD develops. To overcome this limitation and better approximate field epidemiology, we decided to investigate SPFMV-SPCSV interactions on vector-mediated mixed infections and compared them with SPFMV single infections in plants exhibiting contrasting viral loads.

To assess viral titers and symptom progression, SPFMV-infected sweet potato plants were generated by taking stem cuttings from plants maintained in the laboratory and used for the mixed infection experiment. The presence of SPFMV in all plants was first verified by conventional PCR using primers targeting AM-MB2 SPFMV (Supplementary Table S1). Once SPFMV infection was confirmed, tissue samples were collected and pooled from three sites per plant to better uniformize viral distribution, corresponding to 0 dpi (T0). Super-infections were subsequently established by transferring *B. tabaci* adults, previously allowed a 48h acquisition access period (AAP) on SPCSV-infected plants to become viruliferous (Fig. R13), onto the SPFMV-infected plants for 48h inoculation access period (IAP). Whiteflies were then eliminated by repeated Imidacloprid treatments applied every two hours.

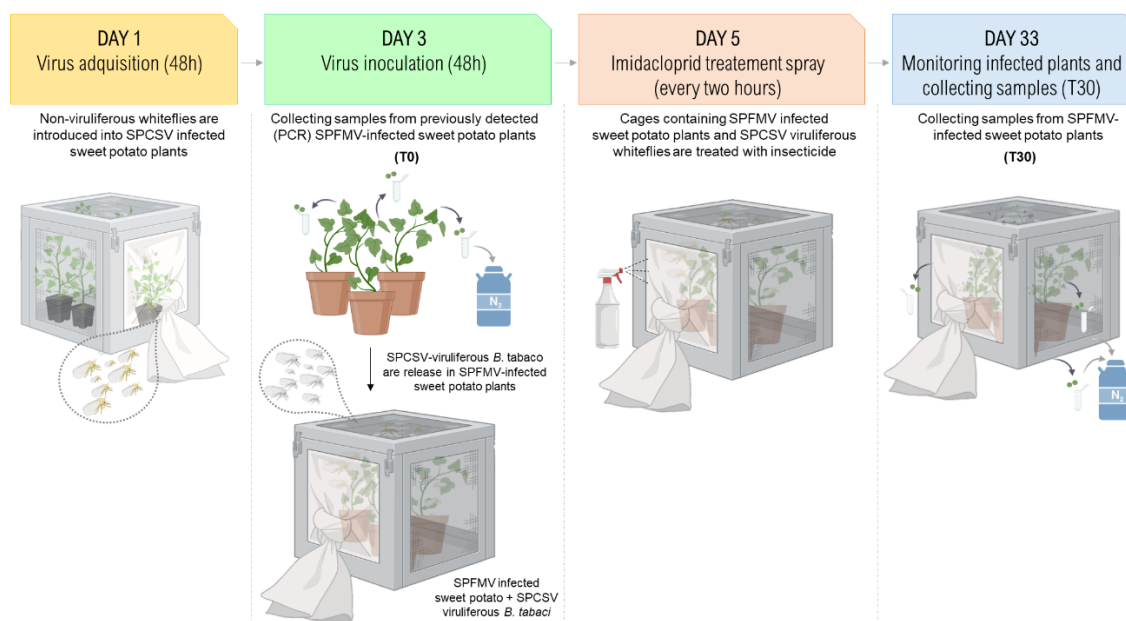


Figure R13. Schematic illustration of the whitefly-mediated SPCSV inoculation assay. Day 1: Non-viruliferous whiteflies reared on virus-free plants were transferred to SPCSV-infected sweet potato plants for a 48h acquisition access period. Infection of source plants was previously confirmed by PCR. Day 3: Samples from PCR-confirmed SPFMV-infected sweet potato plants (T0) were collected, frozen in liquid N₂, and stored at -80 °C. These plants were placed in insect-proof cages, and SPCSV-viruliferous whiteflies were transferred for a 48h inoculation access period. Day 5: Whiteflies were eliminated by repeated imidacloprid application until complete removal. Day 33: Plants were monitored, and samples (T30) were collected, frozen in liquid N₂, and stored at -80 °C for subsequent analysis.

Infection progress was monitored through sequential photography, total RNA extractions and qRT-PCR quantification of SPFMV and SPCSV titers. At 0 dpi (T0), SPFMV-infected plants displayed only very mild symptoms: purple pigmented margins and chlorotic discoloration along the leaf midrib and in occasional discrete spots (Fig. R14A). At 16 dpi, SPFMV and SPCSV presence was confirmed by RT-PCR. However, at neither 16 nor 30 dpi were symptoms distinguishable from those at T0, except in a single plant that showed early signs of co-infection. Clear and consistent co-infection symptoms were observed later at 60 dpi (T60), when all plants developed characteristic manifestations such as vein clearing, downward leaf curling, leaf deformation and severe stunting (Fig. R14B), revealing as expected that co-infection of SPFMV and SPCSV resulted in more exacerbated symptoms, compared to SPFMV single infection. By 90 dpi, symptom severity increased further, and some co-infected plants ultimately died. Time point T30 was chosen for the second sampling, as it precedes the clear onset of severe symptoms, when changes in the host plant are expected to occur.

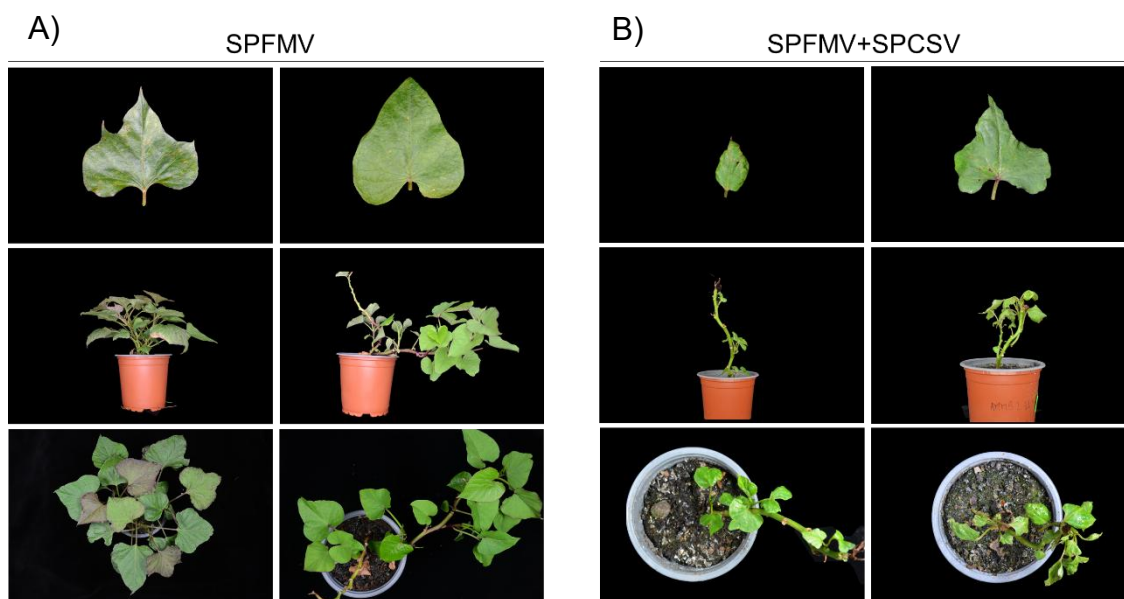


Figure R14. Symptoms observed on sweet potato leaves with SPFMV and SPFMV+SPCSV mixed infections. A) SPFMV infected plants showed regular leaf size (details in the upper lane) and normal growth (lateral and cenitals views of two representative plants) along the entire experiment. B) Sweet potato plants co-infected with SPCSV and SPFMV at a late time point (T60) displaying characteristics SPVD symptoms: reduced leaf size (upper lane), vein clearing, mosaic and downward leaf rolling, and plant dwarfing (lateral and cenital views of two representative plants).

Next, the influence of SPCSV on SPFMV accumulation was evaluated by measuring relative viral titers using qRT-PCR to quantify RNA levels. Results were divided into two groups (Group 1 and Group 2), each comprising three replicates corresponding to individual plants. Under single infection (0 dpi), SPFMV titers were uniformly low in both groups (Fig. R15). At 30 dpi, plants from Group 1, characterized by low SPCSV accumulation at this time (Fig. R15), showed SPFMV titers comparable to those measured at 0 dpi (Fig. R15). In contrast, plants from Group 2 that exhibited accumulation of RNA2-SPCSV at 30 dpi, and this was accompanied by clear increases in SPFMV titers at T30 relative to their T0 values, although the differences were not statistically significant. Nevertheless, the positive correspondence between high SPCSV and high SPFMV RNA levels supports the expected synergistic interaction and is consistent with previously described enhancement of SPFMV accumulation in SPCSV co-infected plants (Kokkinos and Clark, 2006a).

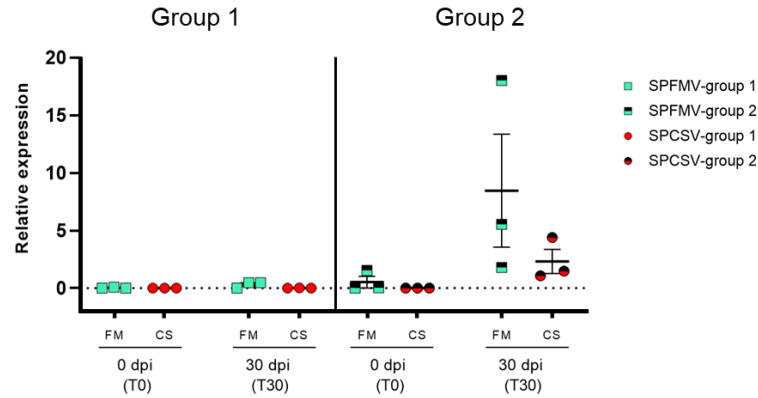


Figure R15. Dynamics of SPFMV and SPCSV viral titers in single- or double-infected sweet potato plants. Relative quantification of genomic RNA copies of the SPFMV (AM-MB2 isolate) and SPCSV RNA2 at 0 and 30 dpi. Each symbol represents an individual biological replicate. Error bars indicate mean $\pm$ SEM. Squares denote SPFMV accumulation, and circles denote SPCSV accumulation.

5.3 Transcriptomic signatures underpin the SPFMV accumulation enhancement during SPCSV co-infection

To characterize transcriptome alterations in sweet potato plants under single and mixed viral infections, we sequenced Illumina RNA-seq libraries generated from total RNA of SPFMV-infected plants collected at 0 dpi (T0) and SPFMV+SPCSV co-infected plants at 30 dpi (T30). To obtain a representative sample size, eight plant samples at T0 and their corresponding samples at T30 were sequenced. The raw reads were analysed using the Genome Detective virus identification pipeline with the parameters already described to focus on plant viruses. Data were analysed by using the read count percentage of mapped reads for all samples, extracted from Genome Detective platform (Supplementary Table S5). Aggregating sample reads and focusing specifically on SPFMV- and SPCSV-derived reads, the proportion of virus-mapped reads at both times is represented in Fig R16. As expected, T0 samples did not display SPCSV derived reads (0) and a low proportion of SPFMV (5.82%). In contrast, co-infected samples at T30 exhibited detectable SPCSV reads (6.38%) together with a substantial increase in SPFMV read abundance (46.52%), indicating a marked enhancement in SPFMV viral RNA accumulation under SPCSV-mixed infection, and supporting our RT-qPCR results.

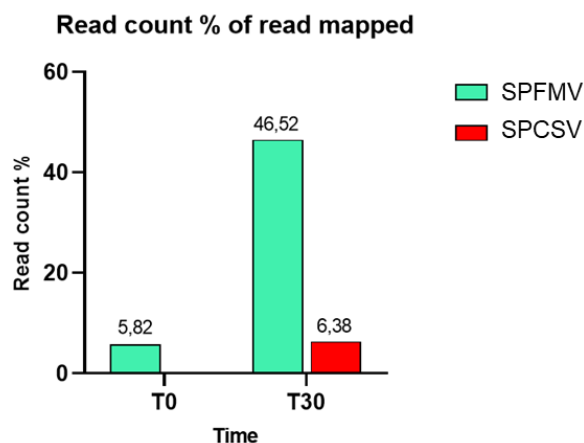


Figure R16. Proportion of virus-associated reads in single- and double-infected plants at T0 and T30. Bars show the percentage of reads mapped to SPFMV (green) and SPCSV (red). Values above bars indicate the percentage contribution of each virus.

5.4 From genome mapping to transcription structure: exploratory RNA-seq analysis of SPFMV and SPCSV co-infection

Reads were mapped to two available *I. batatas* reference genomes, “Beauregard v1” and “Tanzania”. Because the cultivar identity of the sequenced samples was unknown, both references were evaluated, and the “Beauregard” genome was selected after yielding superior alignment metrics compared to the “Tanzania” cultivar reference. To assess transcriptomic responses associated with SPCSV infection, only four samples from eight samples collected and sequenced at T30, exhibited substantial SPCSV accumulation, as indicated by viral read abundance. Consequently, the final dataset comprised four T30 samples (V_SP13, V_SP14, V_SP15 and V_SP16) and their four corresponding T0 samples (V_SP5, V_SP6, V_SP7 and V_SP8). Read counts per sample ranged from 18 to 26 million (average 22 million), and the reference transcriptome achieved an average mapping rate of 53%.

5.4.1 Exploratory Principal Component Analysis (PCA) of transcriptomic profiles in virus-infected sweet potato plants

Principal component analysis (PCA) revealed that PC1 accounted of 50.5% of the total variance and separated samples according to timepoint (T0 vs T30), which also reflect the absence or presence of SPCSV, respectively (Fig R17). PC2 explained an additional 18,5% of the variance but did not provide additional discrimination among samples. Thus, PCA reflects a high variability among replicates.

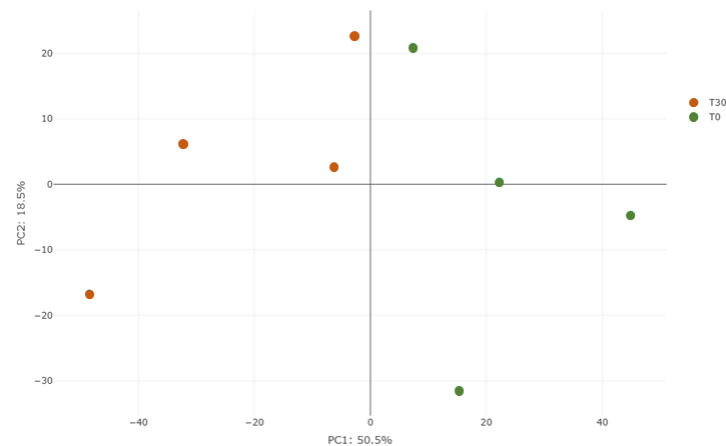


Figure R18. Principal component analysis of sweet potato response to viral infection. Samples are colored by time point: T0 (green) and T30 (orange).

5.4.2 Hierarchical clustering identifies time-associated transcriptional response patterns

To identify coordinated gene expression patterns, we performed unsupervised hierarchical clustering of the 50 most statistically significant differentially expressed genes (DEGs), after removing SPFMV- and SPCSV-derived genes from the DEG's list. This analysis revealed clear transcriptional response modules according to the two timepoints (Fig. R18). Analysis showed bifurcation into two primary gene clusters: a lower module with low transcriptional expression at T0 and high expression at T30, and an upper module displaying the opposite pattern. Column-wise clustering according to their timepoint with T0 and T30 replicates forming distinct branches in the dendrogram, indicated that time is a major determinant of the global expression profile. However, as the heatmap is based on variance-stabilized and row-scaled expression values, it enables comparison of expression patterns across samples, but not direct comparison of expression magnitudes between different genes.

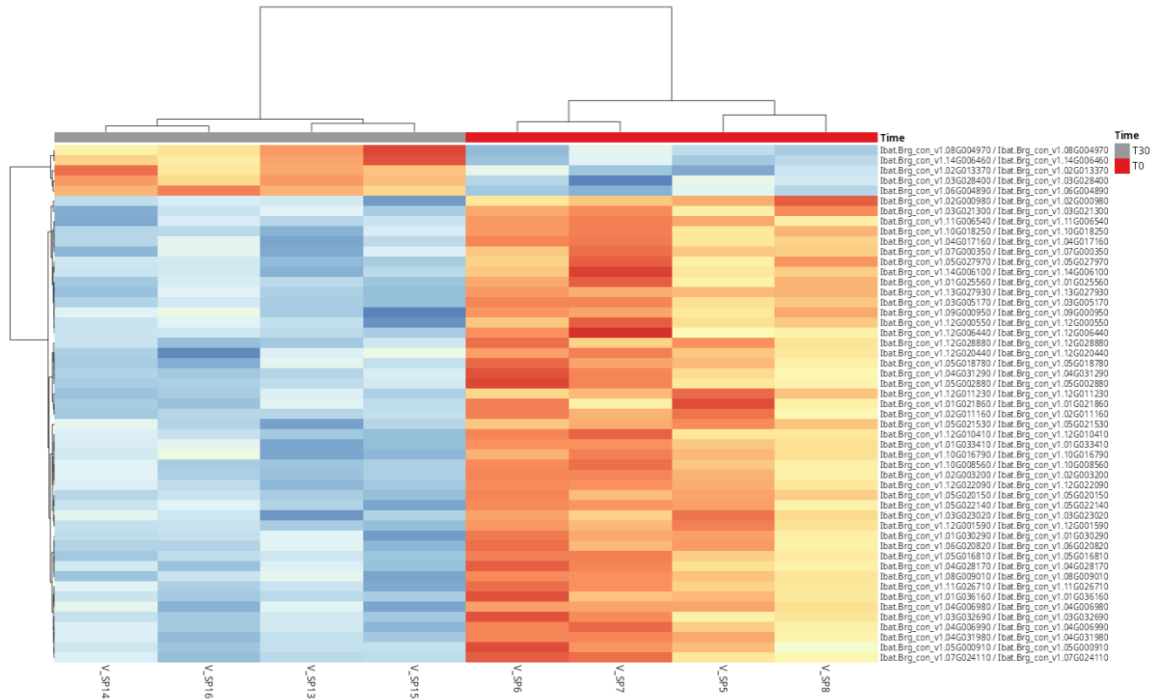


Figure R18. Expression patterns of the top 50 differentially expressed genes (DEGs). The heatmap shows row-scaled log2 fold change values for the 50 most significant DEGs across time points. Columns represent individual samples (V_SP13, V_SP14, V_SP15, V_SP16, V_SP5, V_SP6, V_SP7, and V_SP8). Hierarchical clustering was performed on genes (rows).

5.4.3 Identification and functional characterization of differentially expressed genes revealed transcriptional changes

5.4.3.1 Functional classification and enrichment analysis of DEGs

The differential expression analysis comparing SPFMV infected plants at T0 with plants co-infected with SPFMV and SPCSV at T30, identified a total of 719 differentially expressed genes (DEGs), comprising 575 downregulated and 144 upregulated. As expected, no SPFMV-derived genes appeared in the DEG list, since the virus was already present at T0; by contrast, ten SPCSV-derived genes (corresponding to mostly SPCSV annotated viral genes in the specie; Kiss *et al.*, 2013) were detected exclusively at T30, consistent with its presence only in co-infected plants. To further explore the biological significance of the transcriptional changes observed between T0 and T30, DEGs were subjected to functional enrichment analysis based on Gene Ontology (GO) classifications. GO enrichment analyses were performed and classified across the three major categories: Biological Process (BP), Molecular Function (MF), and Cellular Component (CC) in order to obtain a comprehensive overview of the biological pathways, molecular activities, and subcellular localizations associated with the regulated genes. This integrative approach allows the functional interpretation of the DEG dataset beyond

individual genes, highlighting coordinated changes at multiple functional levels. The results of these analyses are summarized in the Fig. R19, where enriched GO terms are displayed together for each category.

The majority of the GO BP terms identified were associated with photosynthetic electron transport, light reactions, plastid organization, pigment metabolic process and carbon fixation, indicating a coordinated transcriptional regulation of the photosynthetic apparatus (Fig. 19A). GO enrichment analysis focusing on the MF category revealed that DEGs are predominantly associated with enzymatic activities and binding functions related to photosynthesis, pigment metabolism and plastid-associated pathways (Fig. R19A). In addition, multiple oxidoreductase-related functions were represented, these terms reflect changes in redox-related enzymatic activities, which are central to photosynthetic electron transport and plastidial metabolism. The enrichment analysis also identified functions related to isoprenoid and carotenoid metabolism, suggesting regulation of pathways contributing to pigment biosynthesis and hormone-related metabolites (Fig. R19A). The GO enrichment analysis of the CC category showed DEGs associated with chloroplast- and photosynthesis-related subcellular compartments (Fig. R19A). The most significantly enriched cellular components included thylakoid, chloroplast thylakoid, plastid thylakoid, membrane and photosynthetic membrane, highlighting the thylakoid system as a major target of transcriptional regulation. Beyond the thylakoid system, other plastid-related compartments were significantly enriched, indicating that transcriptional changes affect multiple membrane systems within the plastid. Overall, these results highlight a modulation of photosynthesis-related and plastid-associated biological process between the two time points, T0 and T30. These categories match what can be expected as a host plant response after virus infections leading to chlorotic symptoms.

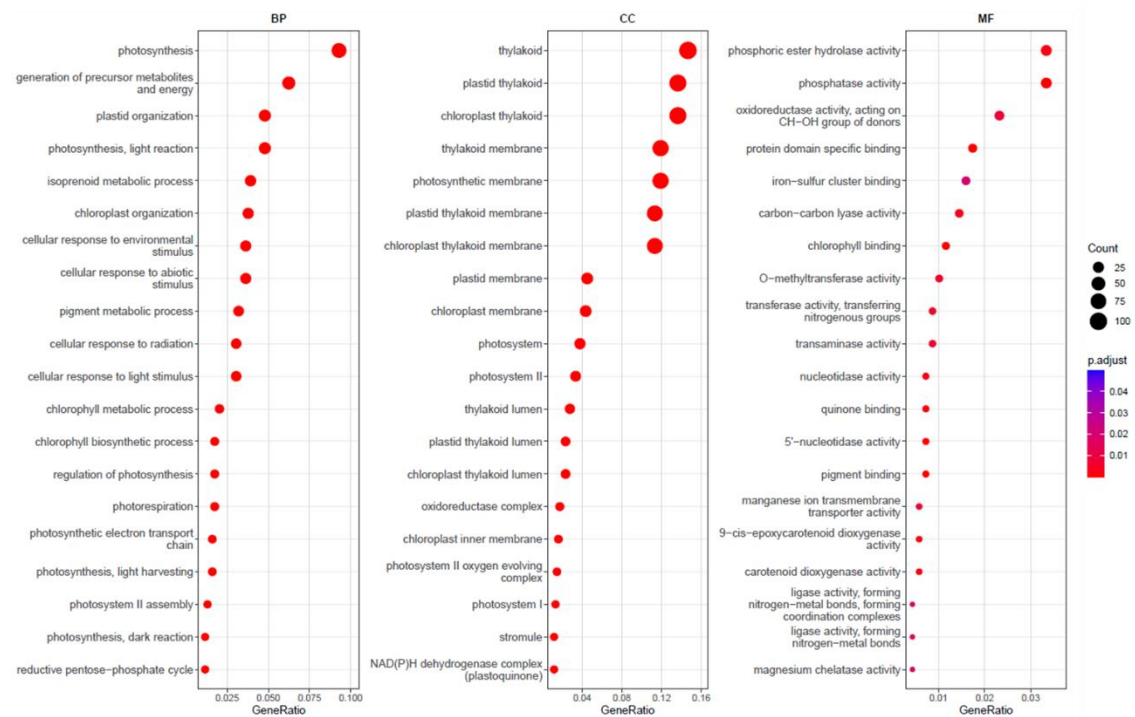
5.4.3.2 KEGG pathways influenced analysis

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis was performed using the same bioinformatic framework applied in the GO enrichment analysis to further identify biological pathways affected by the observed transcriptional changes. Overall, the KEGG results were highly consistent with the GO analysis, with enriched pathways predominantly related to photosynthesis, primary carbon metabolism, and associated biosynthetic and metabolism processes (Fig. R19B). Among the most significantly enriched pathways, photosynthesis and carbon fixation by the Calvin cycle were perceptively represented, indicating coordinated regulation of genes encoding core components of the photosynthetic machinery. The enrichment of porphyrin metabolism, terpenoid backbone biosynthesis, and carotenoid biosynthesis further supports a

substantial regulation of pigment and isoprenoid related pathways, which are essential for chlorophyll biosynthesis, photoprotection, and the production of signaling molecules.

Alterations in photosynthesis are a well-established and well-known response in plant viral infections often resulting from direct interference with proteins associated to or from indirect effects of altered energy allocation and defense responses (Zhao *et al.*, 2016; (Shahriari, *et al.*, 2025). Meanwhile, enrichment of Circadian rhythm suggests that temporal regulation may contribute to the transcriptional differences observed between T0 and T30.

A)



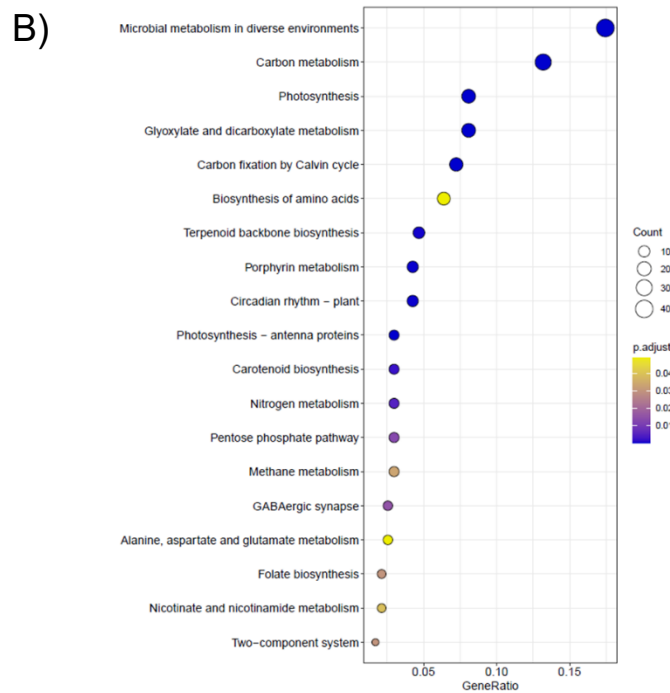


Figure R19. Functional enrichment analysis of differentially expressed genes (DEGs). A) Dot plots showing significantly enriched Gene Ontology (GO) driver terms, representing the most statistically robust and biologically relevant enrichments. Terms are grouped by GO domains: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). B) Top Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched in response to mixed infection with SPFMV and SPCSV at T30. Dot size indicates the number of DEGs contributing to each pathway, and dot colour represents the adjusted p -value

5.4.4 Weighted gene co-expression network analysis (WGCNA) reveals gene modules associated with viral accumulation

Several factors suggest that pairwise differential expression analysis alone may not fully capture the transcriptional dynamics associated with SPVD infection. First, virus-specific GO terms and KEGG pathways were not directly enriched, whereas functional enrichment analyses were dominated by pathways related to general responses, including photosynthesis, metabolic processes, and regulatory functions. Second, the differential expression patterns appear to be moderately influenced by time-dependent effects, suggesting that some transcriptional changes observed between T0 and T30 might be not fully attributable to viral co-infection. Ultimately, the substantial inter-sample variability observed in viral accumulation suggests that expression changes associated with differing viral loads may be obscured in conventional pairwise comparisons. To overcome these limitations and to identify gene modules specifically associated with viral accumulation, a weighted gene co-expression network analysis (WGCNA) was performed using all 16 RNA-seq samples (eight at T0 and their corresponding counterparts at T30). This approach enables the identification of co-expressed gene

modules and their association with quantitative traits, providing a complementary framework to uncover transcriptional networks potentially linked to virus accumulation during SPFMV and SPCSV co-infection.

The module-trait relationship heatmap (Fig. R20) summarizes the correlations between module eigengenes and quantitative traits, including $\log_{10}$ -transformed SPFMV and SPCSV viral loads, as well as sampling time. Distinct gene modules exhibited differential association patterns with these traits, indicating that viral load and time contribute differently to the observed transcriptional variation. Notably, the “royalblue” module displayed a strong and highly significant association with SPFMV accumulation, whereas the “lightyellow” module showed a significant correlation with SPCSV viral load. Importantly, these two modules were not significantly associated with time, indicating that their expression patterns are specifically linked to viral accumulation rather than temporal effects. In contrast, some modules exhibited significant association with time but weak or non-significant associations with viral load, suggesting that their expression patterns are predominantly time-dependent. Based on these criteria, the “royalblue” and “lightyellow” modules were selected for subsequent analysis. Gene lists from those modules (61 genes for “lightyellow” module, and 35 genes for the “royalblue” module) were extracted and subjected to GO and KEGG (data not shown) pathway enrichment analysis. For each module, the three most significantly enriched GO terms, the Biological Process (BP), Molecular Function (MF) and Cellular Component (CC) categories, were identified and analysed.

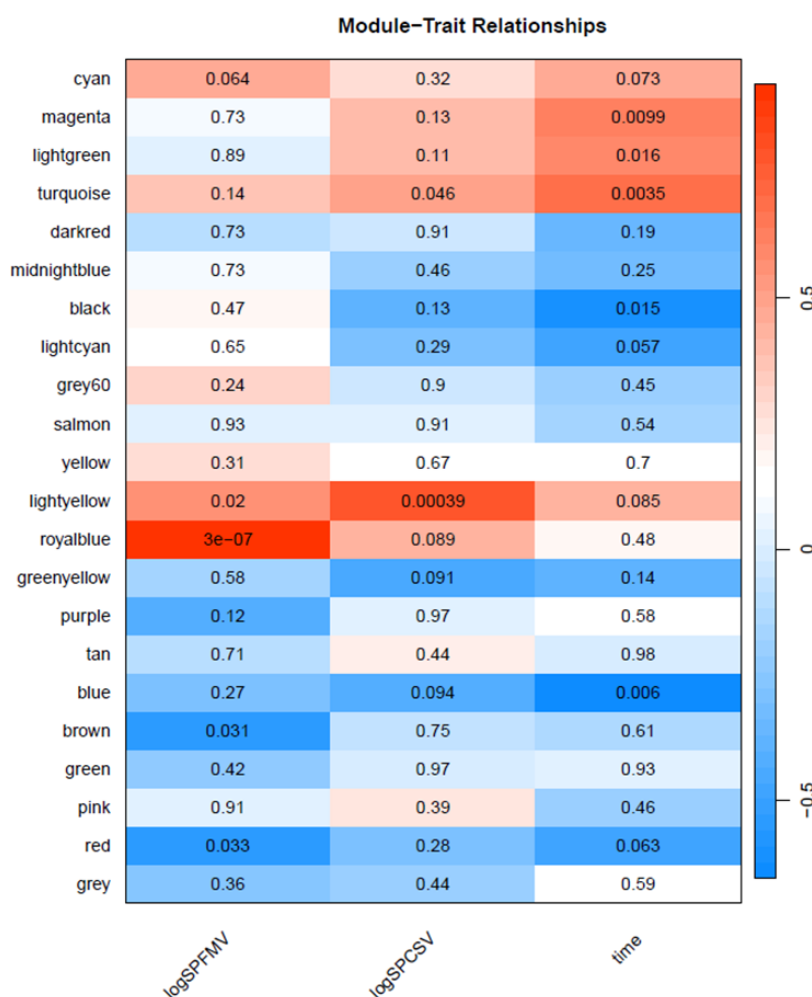


Figure R20. Heatmap showing correlations between gene modules and SPFMV accumulation, SPCSV accumulation, and time (T0–T30). Rows represent gene modules, and columns represent traits. Each cell displays the corresponding *p*-value. Red indicates positive correlation, whereas blue indicates negative correlation.

For the lightyellow module which showed a significant association with SPCSV accumulation, the information is summarized in Fig. R21. In the BP category, enriched terms include cellular response to external stimuli, phosphate starvation, and nutrient levels, as well as processes related to ion homeostasis, lipid biosynthetic process and hormone metabolic process (salicylic acid metabolism). In the MF category, enrichment was primarily observed for phosphatase-related activities. For the CC category, a single enrichment corresponding to plant-type vacuole membrane was identified.

The enriched GO terms within this module do not indicate pathways specifically linked to SPCSV accumulation, instead, they reflect broad host defense responses, largely associated to photosynthesis process, general stress responses, and metabolic or physiological changes. These responses may be influenced by the combined effects of the viral complex present during SPVD. Accordingly, the “lightyellow” module appears to

capture general host responses that are modulated by overall viral pressure, rather than pathways uniquely linked to the accumulation of a single viral species.

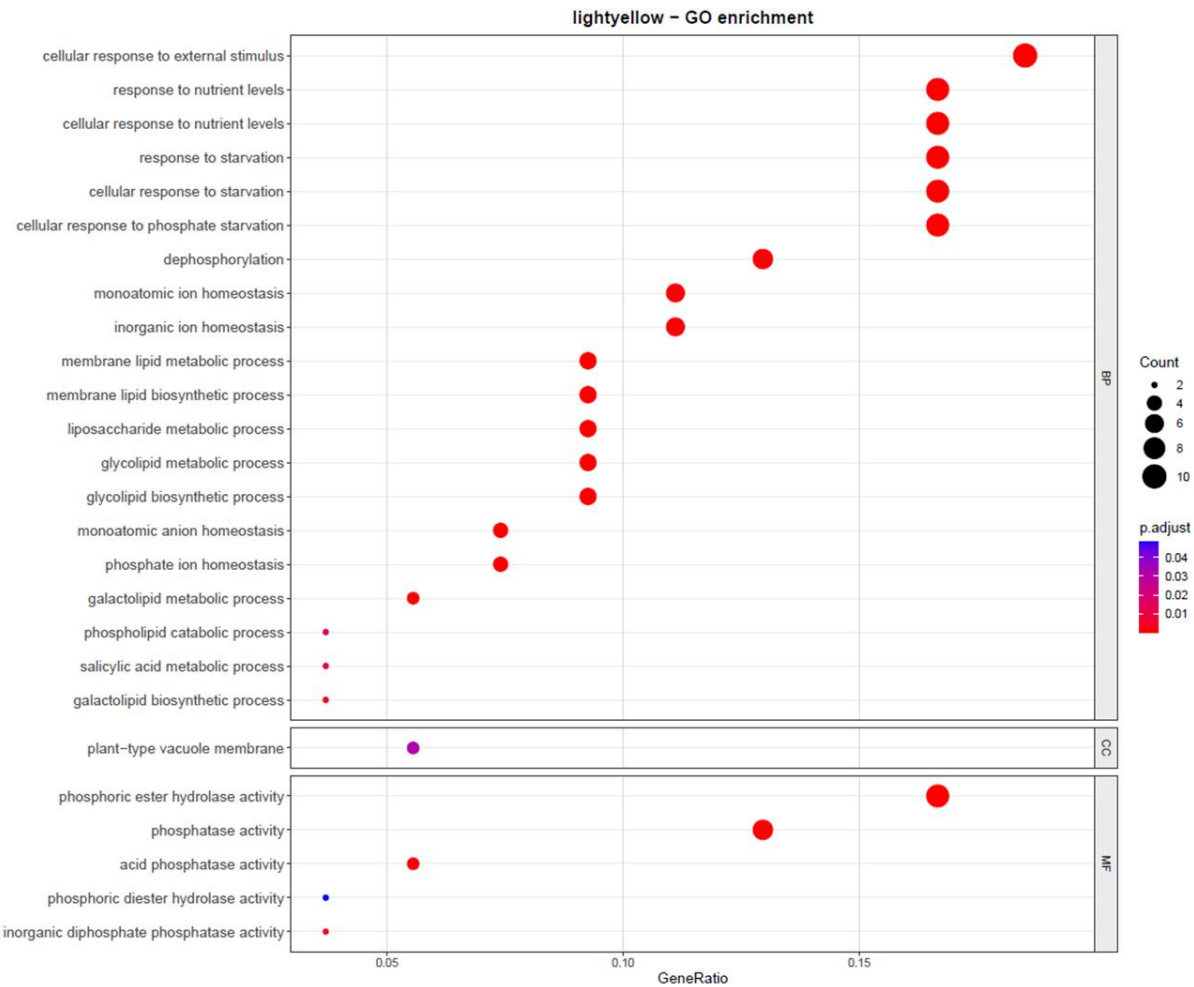


Figure R21. GO functional enrichment analysis of the “lightyellow” module. Dot plots show significantly enriched Gene Ontology (GO) driver terms, representing the most statistically robust and biologically relevant enrichments. Terms are grouped by GO domains: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). The y-axis represents GO terms. Dot size indicates the number of genes contributing to each term, and dot color represents the adjusted p -value.

The “royalblue” module showed a strong and significant association with SPFMV viral accumulation and no significant with the time. In the BP category, the most significantly enriched terms were predominantly related to antiviral defense and host-virus interactions, including immune effector process, defense response to virus, biological process involved in symbiotic interaction, RNAi-mediated antiviral immune response, cellular response to virus, and response to dsRNA. These enrichments indicate that genes within this module are functionally associated with cellular processes activated during viral challenge. In the MF category, enrichment terms were mainly related to RNA metabolism and nucleic acid processing, including double-stranded RNA-specific

ribonuclease activity, ribonuclease III activity, and RNA endonuclease activity. In the CC category, enriched was limited but specific, with components of the RNA-induced silencing complex (RISC) and RNAi effector complex being the most significant terms (Fig. R22). The GO enrichment profile of the “royalblue” module reveals a functional signature related on RNA-mediated antiviral defense and host responses to viral infection, consistent with its identification as a gene co-expression module associated with SPFMV accumulation. This module also likely represents a broader host antiviral defense program rather than a response uniquely specific to SPFMV, and its association may reflect the intensity of viral challenge in the hosts rather than processes exclusive to SPFMV biology.

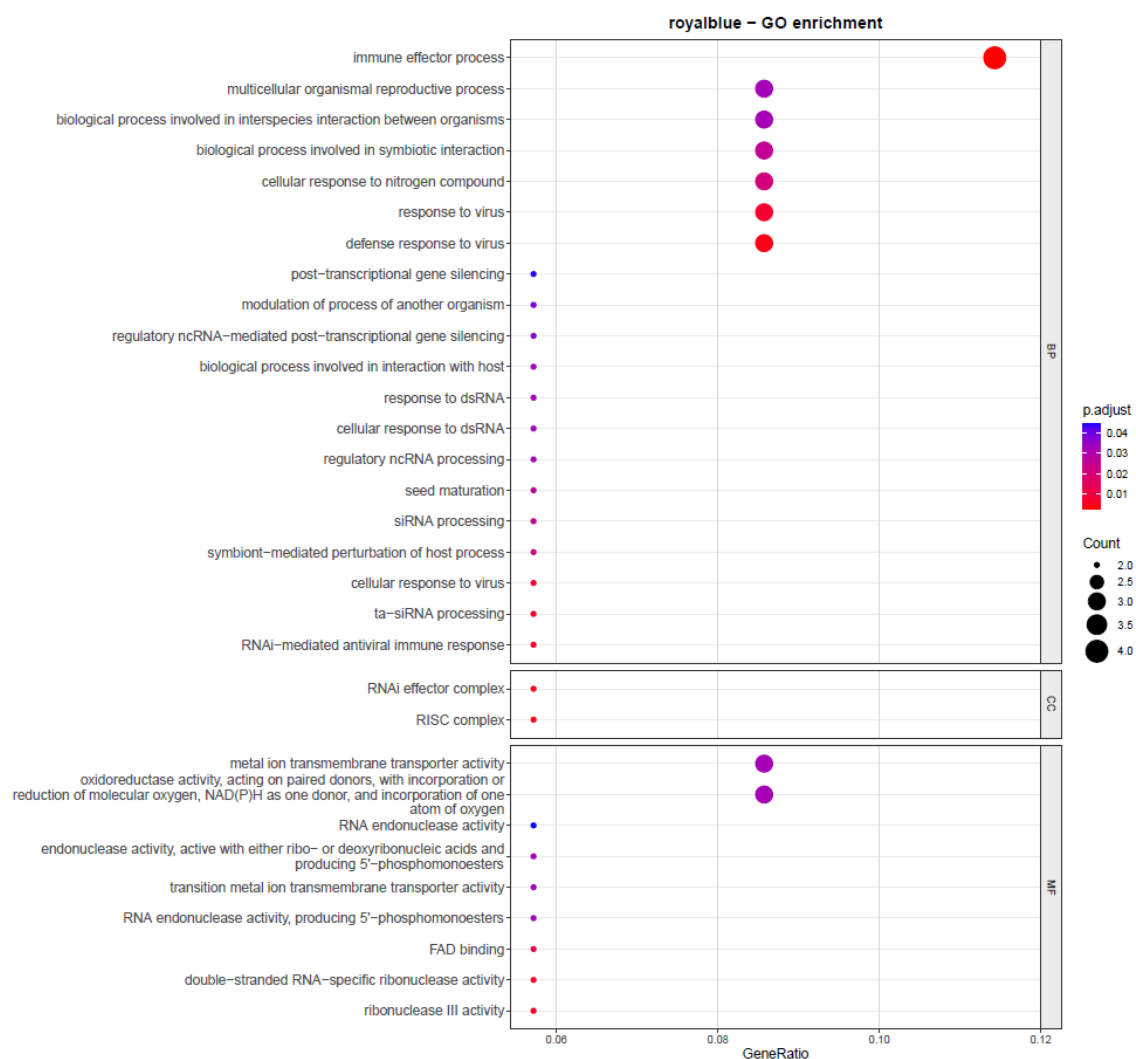


Figure R22. GO functional enrichment analysis of the “royalblue” module. Dot plots show significantly enriched Gene Ontology (GO) driver terms, representing the most statistically robust and biologically relevant enrichments. Terms are grouped by GO domains: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). The y-axis represents GO terms. Dot size indicates the number of genes contributing to each term, and dot color represents the adjusted p -value.

DISCUSSION - CHAPTER II

Sweet potato production can be challenged by a wide range of biotic constraints, including fungal and bacterial pathogens, insect pests, and most acute, viral diseases, which collectively can induce host responses ranging from latent infections to severe disease syndromes (Tarun *et al.*, 2025; Johnson and Gurr, 2016). Among these, viruses represent the most detrimental threat, largely due to their frequent asymptomatic nature and low accumulation levels during single infections, which complicate reliable detection and management strategies (Adam *et al.*, 2015; Kreuze *et al.*, 2021). This challenge is exacerbated by the predominantly vegetative propagation of sweet potato through stem cuttings, a practice that facilitates the progressive accumulation and long-term persistence of viral populations (Untiveros *et al.*, 2007; Valverde *et al.*, 2007). To date, more than 30 viruses have been reported to infect sweet potato, including DNA viruses primarily belonging to the families *Geminiviridae* or *Caulimoviridae* as well as many RNA viruses from the *Potyviridae*, *Closteroviridae* or *Bromoviridae* families (Clark *et al.*, 2012). Consistent with this extensive viral diversity, our RNA-seq based virome profiling revealed a complex viral landscape dominated by members of the *Potyviridae* and *Geminiviridae* families (Fig. R12). In particular, the predominance of SPFMV and SPVC supports the widespread and persistent presence of these viruses across major sweet potato growing regions, from East Africa regions as well as North and South America, Europe, Asia and Australasia (Maina *et al.*, 2018; Wang *et al.*, 2021; Kiemo *et al.*, 2022; Orfanidou *et al.*, 2022; Adero *et al.*, 2024). Among DNA viruses, begomovirus infecting sweet potato, named “sweepoviruses” (Fauquet and Stanley, 2003; Trenado *et al.*, 2011), were the most prevalent in our samples, with SPLCV and SPLCCV being the most frequently detected. This observation is uniform with the broad geographical distribution of SPLCV (Fuentes and Salazar, 2003; Briddon *et al.*, 2006; Miano *et al.*, 2006; Luan *et al.*, 2006, 2007; Paprotka *et al.*, 2010; Ling *et al.*, 2011), as well as with the original description of SPLCCV in Spain (Lozano *et al.*, 2009). Together, these findings reinforce the notion that potyviruses and begomoviruses constitute the most widespread and representative components of the sweet potato virome. In addition to these major virus groups, the badnavirus SPPV was also detected. This virus has been repeatedly detected in several studies in apparently healthy plants (Kashif *et al.*, 2012; Mbanzibwa *et al.*, 2014; Mingot *et al.*, 2016; Qin *et al.*, 2016; Orfanidou *et al.*, 2022) and is characterized by irregular detection patterns, with positive and negative results often observed across tissue samples (Kreuze *et al.*, 2020). Its presence in many sweet potato plants is likely attributable to the vegetative propagation, however seed transmission has also been demonstrated under experimental conditions (Kreuze *et al.*, 2020). Given its

limited effects of SPFMV-SPCSV co-infection on SPPV titers (Kreuze *et al.*, 2020) and their apparent universal presence, the biological relevance of SPPV to sweet potato production remains unresolved and was not further explored in this study. Finally, the presence of the mastrevirus SPSMV-1 across all samples agrees with previous reports describing its occurrence in multiple Spanish regions (Fiallo-Olivé *et al.*, 2022). The absence of visible symptoms associated with SPSMV-1 infection in several studies (Kreuze *et al.*, 2009; Cao *et al.*, 2017; Fiallo-Olivé *et al.*, 2021) illustrates the frequent existence of symptomless viral infections within the sweet potato virome. For these reasons, we did not pursue an in-depth analysis of the asymptomatic or latent viruses detected in our samples, focusing instead on the impact of co-infection by the potyvirus SPFMV and the crinivirus SPCSV.

Co-infections involving unrelated viruses have been extensively studied due to their frequent occurrence in both natural and agricultural systems (Alcaide *et al.*, 2020; Moreno and López-Moya, 2020), including previous studies in our group (Domingo-Calap *et al.*, 2020; Ontiveros *et al.*, 2022). In many cases, mixed infection results in synergistic interactions which lead to a drastic increase in symptom severity compared to either of the single infections (Abraham 2024). This viral synergism in a mixed infection of a plant with two or more viruses, often results in an increase multiplication of one or more of the viruses, intensifying disease phenotypes (Syller 2012). One of the most striking examples is the sweet potato viral disease (SPVD), which arises from the co-infection of SPFMV and SPCSV (Karyeija *et al.*, 2000a; Clark *et al.*, 2012). While SPFMV alone typically induces mild or no symptoms in sweet potato plants (Gutiérrez *et al.*, 2003; Loebenstein, 2012), in co-infection with SPCSV, it causes severe symptoms. SPVD is considered the most economically important disease affecting sweet potato (Jones 2021), causing yield losses up to 90% in susceptible varieties (Mukasa *et al.*, 2006; Adikini *et al.*, 2015; Gibson and Kreuze, 2015). This dramatic increase in severity caused by the synergistic interaction has been attributed to the ability of SPCSV to suppress RNA silencing-mediated viral defense in sweet potato (Cuellar *et al.*, 2009; Weinheimer *et al.*, 2014), thereby promoting an increase in viral replication, enhanced viral movement or any combination of these (Abraham 2024). Beyond to SPVD, SPCSV also participates in synergistic interactions with other sweet potato viruses, such as the ipomovirus SPMV, resulting in the denominated sweet potato severe mosaic disease (SPSMD) with also remarkable sweet potato yield reductions (Mukasa *et al.*, 2006). In addition to the potyvirids interactions, SPCSV can also synergize with sweepoviruses; however, the resulting symptoms are generally much milder than those observed in potyviral synergism (Cuellar *et al.*, 2015).

SPVD has been extensively characterized in sweet potato, nevertheless most physiological and transcriptomic studies have relied on grafting as the primary inoculation method to establish single and mixed viral infections (Gibson *et al.*, 1998; Karyeija *et al.*, 2000; Kokkinos and Clark, 2006; Tibiri *et al.*, 2020; Bednarek *et al.*, 2021; Adero *et al.*, 2025). In contrast, relatively few studies have incorporated *Bemisia tabaci*, the natural vector of SPCSV, despite its central role in disease epidemiology under field conditions (Navas-Castillo *et al.*, 2014). For these reasons, the present study was designed to more closely reflect natural infection scenarios by employing whitefly-mediated transmission for SPCSV. Moreover, the use of plants with a known pre-existing viral background, in which SPFMV was already established, allowed a more accurate representation of the biological processes underlying SPVD in nature after super-infection, and enabled a more realistic assessment of host-virus interactions under epidemiologically realistic conditions.

To achieve this objective, mixed infections were established as superinfections by exposing SPFMV-infected plants to SPCSV-viruliferous for a 48 h, corresponding to the time required for an efficient vector-mediated inoculation. Unfortunately, this approach does not ensure synchronous and regular superinfection across all tested plants, and variability in the time of SPCSV establishment cannot be excluded. Furthermore, because the plants harboured pre-existing SPFMV and other viruses, viral titers might not be uniform at the time of SPCSV inoculation. Notwithstanding these sources of variability, we adopted these experimental conditions as they better reflect the complexity of natural infection dynamics. At T0, SPFMV infected plants exhibited very mild symptoms, characterized by weak phenotypic alterations including purple pigmentation at leaf margins and mild chlorosis spots. By T30, a subset of plants symptoms progressed slightly, with the appearance of subtle leaf distortion and mild stunting (data not shown) and by T60, plants developed clear disease symptoms, including pronounced vein clearing, leaf curling and severe stunting. To account for this variability, plants were classified into two groups based on symptoms and viral accumulation at T30: Group 1, where plants exhibited low SPCSV levels, with a minimal increase of SPFMV titers and very mild symptoms, and Group 2, where plants showed higher SPCSV accumulation, increased SPFMV viral load and a more pronounced disease phenotype (Fig. R15). Appreciably, these results were supported by comparative transcriptomic analyses at T0 (SPFMV single infection) and T30 (SPFMV-SPCSV co-infection), which confirmed low SPFMV derived reads in single infected plants and a clear increase following SPCSV co-infection (Fig. R16). These results support findings of other studies previously reported, confirming once more the synergistic situation when co-infection between

SPFMV and SPCSV (Karyeija *et al.*, 2000a; Kokkinos and Clark, 2006). The analysed samples revealed substantial inter-sample variability, however, this symptomatology and viral load variability observed, was consistent with the PCA results. Such differences can be explained because biological factors, including plant age and size (Karyeija *et al.*, 2000a), and may also partially reflect methodological aspects inherent to the experimental system.

During the infection, plant viruses after superinfection depend on host cellular machinery to replicate and spread, thereby inducing extensive metabolic changes in the host (Nelson and Citovsky, 2005; Llave 2016; Zanardo *et al.*, 2019). Equally, plants can perceive viral invasion and activate defense responses by the induction of the expression of determined genes. In the present study, enriched GO terms and KEGG pathways identified that sweet potato viral infection affects multiple biological processes, ranging from general host functions to virus-specific responses.

Our results revealed that photosynthetic pathways, chlorophyll biosynthesis, pigment metabolism, and multiple components of chloroplast structure were significantly affected in co-infected plants, with a predominant trend toward transcriptional downregulation. GO enrichment analysis showed a repression in genes associated to photosynthesis and chloroplast components, indicating a substantial disruption of their functionality. This pattern is consistent with previous reports describing repression of genes associated to photosynthesis during viral infection (Hanssen *et al.*, 2011; Lu *et al.*, 2012; Zhao *et al.*, 2016; Bhattacharyya and Chakraborty, 2018; Shahriari *et al.*, 2025). Additionally, similar downregulation was observed for genes involved in carotenoid biosynthesis, which play a key role in protecting the photosynthetic apparatus against oxidative damage (Ramel *et al.*, 2012). Collectively, as observed in this study and reported elsewhere, the repression of these primary metabolism components produced an imbalance in redox homeostasis, in addition favouring virus infection (Li *et al.*, 2016; Zhao *et al.*, 2016) and constrained the plant growth (Zhang *et al.*, 2020). This interpretation is further supported by physiological evidence demonstrating reduced chlorophyll content and photosynthetic capacity in sweet potato plants affected by SPVD (Njeru *et al.*, 2004; Wang *et al.*, 2019a). In addition, similar repression of photosynthetic pathways has been described for SPVD in the susceptible cultivar “Beauregard” (Bednarek *et al.*, 2021; Adero *et al.*, 2025), associated with vein clearing and yellowing symptoms, which also were observed in the tested plants in the present study. Likewise, pathways related to carbon metabolism, carbon fixation through the Calvin cycle, and nitrogen metabolism were also altered, in line with transcriptomic analysis results of sweet potato plants infected by several sweet potato viruses (Shi *et al.*, 2019). All of this, together with the observed alterations in zinc

ion binding and other enzymatic functions, indicate a broad impairment of primary metabolism, consistent with reports in which viral infection induces coordinate alterations in primary and secondary metabolic processes as part of the general host response to infection (Fernández-Calvino *et al.*, 2014; Bednarek *et al.*, 2021; Jiang *et al.*, 2025).

The WGCNV analysis revealed that “lightyellow” and “royalblue” modules capture hosts responses associated with SPVD that extends beyond general host defense and includes processes more closely linked to viral infection. In lightyellow module, enriched GO terms reflect a reprogramming of nutrient, metabolic and regulatory processes, including the modulation of phosphate homeostasis, which could be associated with increased energy demand due to viral accumulation. Others processes, however, seems to be more directly associated with viral infection, particularly membrane lipid metabolism and salicylic acid (SA) metabolism. The representation of membrane lipid biosynthesis and catabolic processes, is consistent with lipid metabolism being extensively reprogrammed to support viral replication and evasion of host defences (Okazaki and Saito 2014), as cellular membranes provide essential scaffolds for viral replication complexes (VRCs) and can shield viral components from RNA silencing and innate immune responses (Ahlquist *et al.* 2003; Hyodo and Okuno, 2014). Membrane reorganization is especially characteristic of positive-strand RNA viruses (Xu and Nagy, 2014), and this process can facilitate plant viral replication and movement, supporting the enhanced accumulation of SPFMV and SPCSV in plant cells. In parallel the enrichment of SA metabolic processes highlights the involvement of defense-related hormonal signalling, suggesting a modulation of antiviral responses (War *et al.*, 2011; Jiang *et al.*, 2025). In this context, recent transcriptomic data indicates that a downregulation of the plant SA-mediated defense response may contribute to the SPVD severe disease outcome (Gibson and Kreuze 2015; Bednarek *et al.* 2021). In the royalblue module, enriched GO terms are directly associated with antiviral defense, including response to virus, immune effector processes, and multiple components of RNA-mediated gene silencing, including siRNA and ta-siRNA processing, ds-RNA response and RISC-associated complexes. This pattern highly correlates with the transcriptomic and small RNA signatures reported by Bednarek *et al.*, (2021). Similar RNA silencing pathway activation has been reported by other studies, for example in Adero *et al.*, (2025) where “Beauregard” showed early suppression of critical functions, reflecting a stress-induced “panic” response. RNA silencing is a critical point in sweet potato defense response, and “Beauregard”, as a highly susceptible cultivar, might suffer specially under SPVD co-infection in comparison with other cultivars more tolerant to viruses (Mwanga *et al.*, 2002; Adero *et al.*, 2025).

To summarize, our data confirms that while SPFMV alone causes mild or barely symptoms in sweet potato with no major alterations in gene expression, the arrival of SPCSV through whitefly transmission (our work) or grafting (other studies), triggers a severe and synergistic disease outcome characteristic of SPVD, activating a general host defense and specific-viral response. These observations underscore the need for in-depth genomic analyses aimed at identifying genes and pathways involved in RNA silencing suppression within the SPVD context. Nevertheless, effective disease management will require integrated strategies beyond the molecular characterization of the response, including the identification of molecular markers for breeding of tolerant SPVD cultivars. Early virus detection remains a critical component for mapping virus distribution together with bringing support and guidance to farmers for future agricultural practices. Strategies focusing on insect-vector are also essential, particularly those targeting whiteflies, which can transmit not only SPCSV but also multiple sweepoviruses and ipomoviruses such as SPMMV. Whitefly control still involve traditional approaches such as the use of chemical insecticides, widely used though often ineffective, highlighting the pressing demand for alternative approaches, such as new technologies designed to interfere with vector transmission, for instance the potential use of virus-like particles that we will consider in the last chapter of this thesis. Meanwhile, and in absence of better virus control strategies against viruses, it is important to acknowledge that the assessment of the SPVD remains a really challenging task due to multiple factors, some of them deriving from the intrinsic properties of the pathosystem. The attempts of breeding for virus-resistance is difficult in the hexaploid *I. batatas*, aggravated by the poorly functional annotation available for *Ipomoea* spp. Furthermore, resistances or tolerances can be overcome by emerging viral strains sweet potato viruses and the frequent occurrence of mixed viral infections adds another layer of complexity. Therefore, this study reinforces the value of application of integrative omics and combinatorial approaches to identify and functionally characterize host determinants in the virus infection. In particular, WGCNA analysis offers clear advantages over traditional differential expression analysis by identifying coordinated gene modules and their relationships with phenotypic traits. In this broad framework, the required combined analysis of transcriptomic, phenotypical, physiological and genomic data will offer a powerful strategy to elucidate the gene networks and molecular mechanisms underlying resistance to SPVD and to other viral infections detrimental for sweet potato production.

Chapters I and II (Sweet potato pathosystem) of the present thesis focused on the study of potyviruses infecting sweet potato, with particular emphasis on RNA silencing suppression, mixed viral infections and vector-mediated transmission. This pathosystem provided a biologically rich framework to investigate virus-host-vector interactions and revealed the molecular and ecological complexity of sweet potato viromes, in which members of both RNA and DNA virus families coexist, including *Potyviridae* and *Geminiviridae*. In addition to investigate fundamental knowledge on viral infection dynamics, this research line has also explored aspects related to plant virus transmission. Virus-like particles (VLPs) emerged as an experimental tool to decipher structural virus features, but also for exploring transmission related processes from viral infectivity. VLPs of several sweet potato viruses, including SPFMV, SPV2 and SPMNV, were successfully generated in our laboratory, however its low accumulation in single infections in susceptible plants (Chase *et al.*, 2023), their fragile structure, the challenging of transmission experiments, and the practical difficulties associated with working in sweet potato, significantly constrained their experimental application. These limitations prompted the search for an alternative and complementary viral model. In this context, Tomato leaf curl New Delhi virus (ToLCNDV), a whitefly-transmitted DNA virus belonging to the *Geminiviridae* family (genus *Begomovirus*), emerged as a major and expanding threat to horticultural production in many European countries, causing a general concern. This virus is composed by a small genome size and a well-defined capsid architecture, and interestingly, geminivirus VLPs studies were recently reported (Hestekh *et al.*, 2018; Saunders *et al.*, 2020). Taken together, Chapter III was focused on the study of ToLCNDV and the development of begomovirus VLPs as potential platform to investigate insect-mediated virus transmission.

RESULTS – CHAPTER III

STRUCTURAL BIOTECHNOLOGICAL TOOLS

6. RESULTS - CHAPTER III

***In planta* platforms of ToLCNDV particles as exploratory tools toward vector transmission mitigation.**

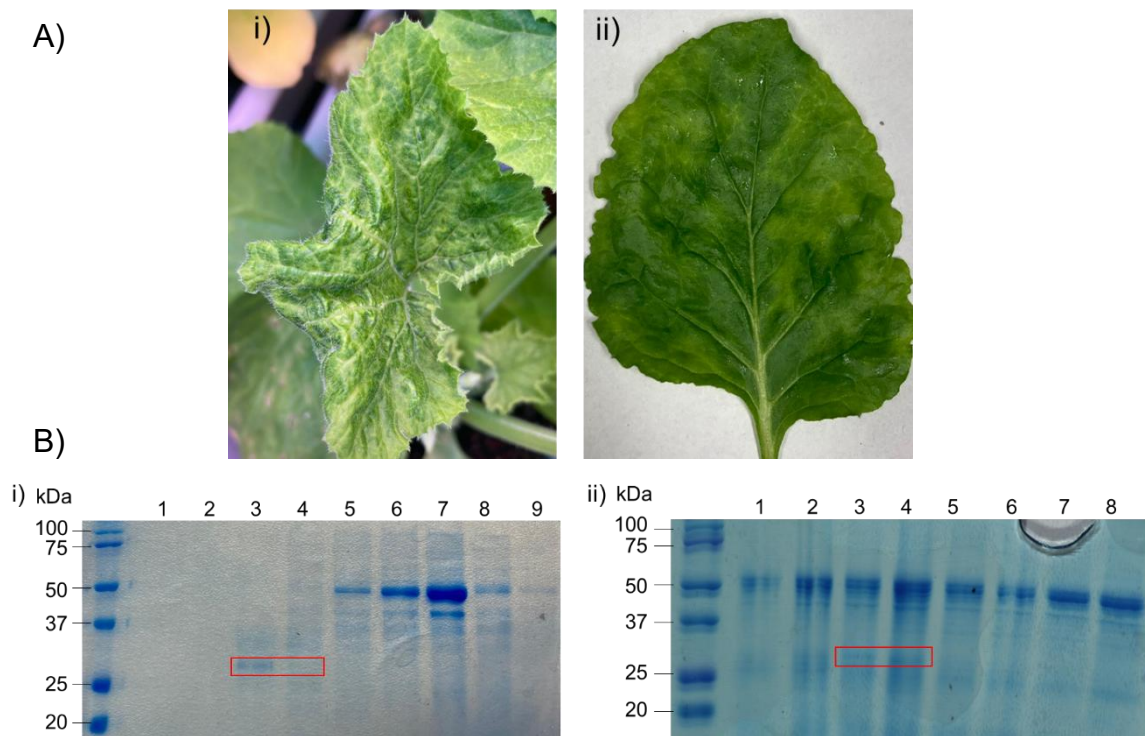
6.1 Assessment of ToLCNDV infectivity and virion purification across different host species

An infectious clone of ToLCNDV (DNA-A, ND-661-2.0-A; DNA-B, ND-661-2.0-B) was employed to inoculate different plant species with the objective to obtain purified virions. Equal volumes of the DNA-A and DNA-B components were agroinoculated into *N. benthamiana* and zucchini squash plants. Agroinoculation resulted in a 100% infection rate in *N. benthamiana*, and ~50% in zucchini squash) and symptoms development was monitored from 7 to 21 dpi in both host species. Between 7 and 12 dpi, agroinfiltrated leaves exhibited necrotic lesions (data not shown), and by 21 dpi both developed systemic symptoms. At this stage, zucchini squash plants exhibited severe mosaic patterns and pronounced apical leaf curling (Fig. Fig. R23Ai), consistent with previous reports (Fortes *et al.*, 2016), whereas *N. benthamiana* plants showed mild leaf curling and yellowing (Fig. R23Aii).

To assess whether agroinoculated inoculated zucchini squash plants could serve as a suitable inoculum source, *B. tabaci* transmission assays were performed. Non-viruliferous whiteflies were allowed a 48-h AAP on symptomatic ToLCNDV-infected zucchini plants. After this period, the insects were transferred to healthy susceptible host (zucchini or melon) plants for a 48h IAP. Whiteflies were then eliminated by spraying insecticide. At 30 dpi, *B. tabaci*- inoculated plants developed symptoms such as stunted growth, leaf curling, chlorosis and mosaicism, confirming successful virus acquisition and transmission. In our hands the transmission was confirmed for 11 plants out of 18 inoculated in a dedicated experiment, a rate similar to other experiments by laboratory colleagues (for instance, 6 out of 17). In addition, the presence of DNA-A of ToLCDNV was verified by PCR amplification using the primer pairs ToA-301F and ToA-453R (Supplementary Table S1).

Results - Chapter III

Virion purification was performed from either zucchini squash or *N. benthamiana* plants, following Hesketh *et al.*, (2018), generally omitting the final sucrose-gradient step. For zucchini squash, we processed approximately 30 g of infected leaves for purification. After caesium sulphate density-gradient separation and subsequent SDS-PAGE analysis, fractions 3 and 4 contained a ~29 kDa protein band, consistent with the expected molecular mass (29,6 kDa) of the ToLCNDV CP (Fig. R2Bi). Extracts from infected *N. benthamiana* plants also showed faint CP bands in the SDS-PAGE analysis of fractions 3 and 4 (Fig. R22Bii). These fractions were dialyzed and concentrated prior to visualization by TEM revealing the presence of geminate particles measuring approximately 22-29 x 38-40 nm, characteristic from *Geminiviridae* family, composed of two joined icosahedral-shaped units (<https://ictv.global/report/chapter/geminiviridae/geminiviridae>). Visual quantification of several TEM micrographs with 192 particles in zucchini squash, and 38 in *N. benthamiana* showed that the proportion of geminate (“bigeminate”) versus single (“icosaedric”) particles was approximately ~75% to ~25% in both host (Fig. R23C). Together with previous results and in terms of virion abundance, *N. benthamiana* extracts contained substantially fewer particles than those from zucchini squash, likely reflecting reduced viral accumulation in this non-natural host.



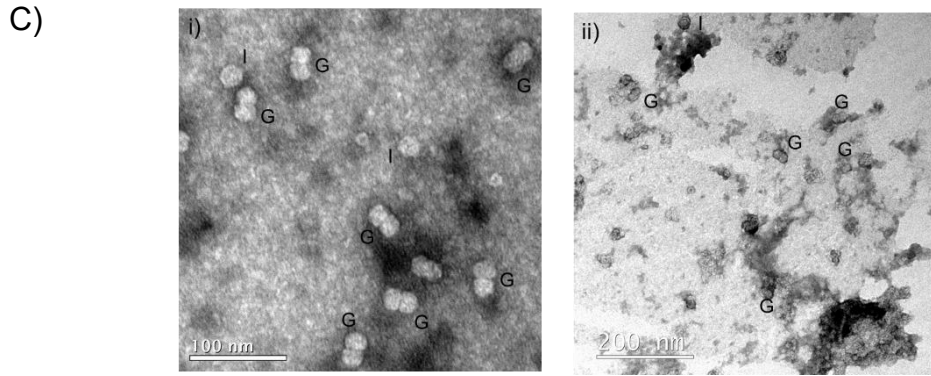


Figure R23. Symptoms associated with ToLCNDV infection and virion purification from zucchini squash and *N. benthamiana* plants. A) Symptoms in leaves of ToLCNDV-infected zucchini squash (i) and *N. benthamiana* (ii) at 21 dpi. B) InstantBlue-stained SDS-PAGE of cesium sulfate gradient fractions from purified ToLCNDV particles obtained from zucchini squash (i) and *N. benthamiana* (ii). Red rectangles highlight fractions 3 and 4, showing a band of ~29 kDa corresponding to the expected size of the ToLCNDV coat protein (29.6 kDa). Additional bands of ~50 kDa are observed in fractions 5–9 (i) and 1–8 (ii), likely corresponding to the Rubisco large subunit. The M lane indicates molecular weight markers (kDa). C) Transmission electron microscopy of negative staining corresponding to fractions 3 and 4 showing geminate (G) and isometric (I) particles from ToLCNDV-infected zucchini squash (i) and *N. benthamiana* (ii). Scale bars: 100 nm (i) and 200 nm (ii).

6.2 *In planta* production of AYVV and ToLCNDV VLPs using the self-replicating AYVV alphasatellite DNA

For the production of ToLCNDV VLPs, we adopted the strategy described by Hesketh *et al.*, (2018). Their work demonstrated that the circular ssDNA is essential for begomovirus capsid assembly and VLP formation. In their system, a self-replicating AYVV alphasatellite DNA (construct pBIN:AYVV 1/7), encoding a *Rep* protein that mediates its autonomous replication, was co-expressed with the AYVV CP (construct pEAQ-HT-D1-AYVVCp), resulting in the successful production of VLPs. Building on this strategy, we followed an equivalent approach for generating ToLCNDV VLPs, using the pHREAC vector, derived from pEAQ (Peyret *et al.*, 2019) for expressing the CP. Two primer pairs containing appropriate restriction sites were designed to amplify the ToLCNDV CP gene from the DNA-A component. After enzymatic digestion and cloning, the sequence of the resulting plasmid was confirmed by capillary sequencing. The validated construct, pHREAC:CP-ToLCNDV, was then used for transient expression experiments in *N. benthamiana* plants through agroinfiltration. Moreover, to validate the experimental conditions used for ToLCNDV VLP production, we performed a parallel process for AYVV VLPs formation as a positive control.

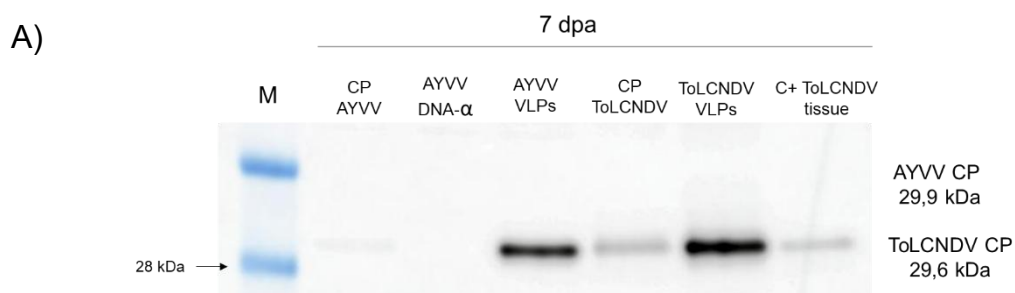
To compare the levels of coat protein accumulation under different expression configurations in *N. benthamiana*, several transient expression assays were performed

along the required controls, including: the AYVV CP construct alone (pEAQ-*HT*-D1-AYVVCP), the AYVV alphasatellite DNA alone (pBIN:AYVV 1/7), the combination of AYVV CP construct with the self-replicating AYVV alphasatellite (pEAQ-*HT*-D1-AYVVCP + pBIN:AYVV 1/7), the ToLCNDV CP construct alone (pHREAC:CP-ToLCNDV), and the ToLCNDV CP co-expressed with the self-replicating AYVV alphasatellite (pHREAC:CP-ToLCNDV+ pBIN:AYVV 1/7), all under identical agroinfiltration conditions. In all treatments except the AYVV alphasatellite DNA alone (pBIN:AYVV 1/7), infiltrated leaves showed extensive affected areas at 7 dpa (Supplementary Fig. S1A). To evaluate the CP accumulation in each condition, protein extracts from infiltrated leaves at 7 dpa were analysed by Western blot using a commercially available polyclonal antibody raised against ToLCNDV CP which presents cross-reaction with the AYVV CP consistent with their 81% amino acid identity between proteins (Supplementary Fig. S1B), suggesting the presence of conserved epitopes. A faint signal was detected when expressing AYVV CP alone and a slightly less prominent when the CP of ToLCNDV was expressed alone (Fig. 24A). In contrast, co-expression of either AYVV CP or ToLCNDV CP with the AYVV alphasatellite resulted in markedly stronger CP signals, with the highest accumulation observed for ToLCNDV CP co-expressed with the self-replicating AYVV alphasatellite (Fig. 24A). These results indicated that begomoviral CP accumulation was strongly enhanced when co-expressed with a cssDNA, whereas CP expression in the absence of such DNA replicon led only to a minimal accumulation.

We then evaluated whether the increased accumulation of ToLCNDV CP in the presence of the AYVV alphasatellite was derived from the assembly of VLPs, also testing in parallel the AYVV CP control. For this purpose, *N. benthamiana* leaf tissue infiltrated with the two VLP producing combinations was harvested and homogenized for virion purification: approximately 70 g from plants expressing ToLCNDV CP together with the AYVV alphasatellite (pHREAC:CP-ToLCNDV + pBIN:AYVV 1/7) and 35 g from plants expressing AYVV CP with the AYVV alphasatellite (pEAQ-*HT*-D1-AYVVCP + pBIN:AYVV 1/7). The purification protocol was adapted following Hesketh *et al.*, (2018), with modifications introduced trying to increase the yields (see in Material and Methods). Following this optimized procedure, predominant protein bands of ~29 kDa, corresponding to the expected size of the AYVV CP (29, 9 kDa) and ToLCNDV CP (29,6 kDa), were detected in fractions from the caesium sulphate density gradient was observed (Fig. 24B). The identities of the CPs of begomoviruses were confirmed in fractions displaying single prominent bands by Western blotting using the previously mentioned polyclonal antibody against ToLCNDV CP (data not shown).

Fractions 7-9 (in the case of AYVV), and 6-8 (for ToLCNDV), were dialyzed and concentrated for TEM visualization (Fig. 24C). TEM analysis revealed the assembly of heterogeneous VLPs in both preparations, including single (“icosaedric”) and geminate (“bigeminate”) forms. Moreover, some particles that resembled geminated, but they might be “double” particles, considered to be formed by a different assembly of two isometric particles, as described previously (Saunders *et al.*, 2020). AYVV VLPs generated under these conditions served as a positive control, validating the robustness of the assembly system. Our results confirmed that ToLCNDV VLPs can be obtained as expected by co-expressing ToLCNDV CP with the AYVV alphasatellite, and the VLPs displayed the characteristic morphology of begomovirus virions.

To complement the TEM observations and gain quantitative insight into the composition of the ToLCNDV VLP preparation, we next analysed purified particles by mass photometry. This technique was used to determine the relative abundance of single versus geminate particles. First, a large number of lower-mass events (158 kDa; σ = 99 kDa; ~4218 counts, 88%) were excluded from the analysis, as they likely represent uncharacterized specimens smaller than virions present in the preparation. For the remaining virion-compatible specimens, two main particle populations were distinguished by their molecular mass. The predominant population corresponded to particles with a molecular mass of ~1982 kDa (σ = 131 kDa), representing 354 counts (7% of detected particles), and it was consistent with single particles. A second, less abundant population of ~4010 kDa (σ = 159 kDa), representing 77 counts (2% of detected particles), was consistent with geminate particles. Altogether, these findings agree with previous observations for similar systems (Hesketh *et al.*, 2018), indicated that VLP production using this strategy yielded preparations enriched in single ToLCNDV particles, with geminate particles present at substantially lower abundance (approximately a 70:30 ratio of single to geminate particles, respectively). In addition, mass spectrometry of tryptic digests of the protein in the band confirmed its identity as the expected ToLCNDV CP. MALDI-TOF analysis showed 71% protein sequence coverage from purified ToLCNDV particles (Fig R24D).



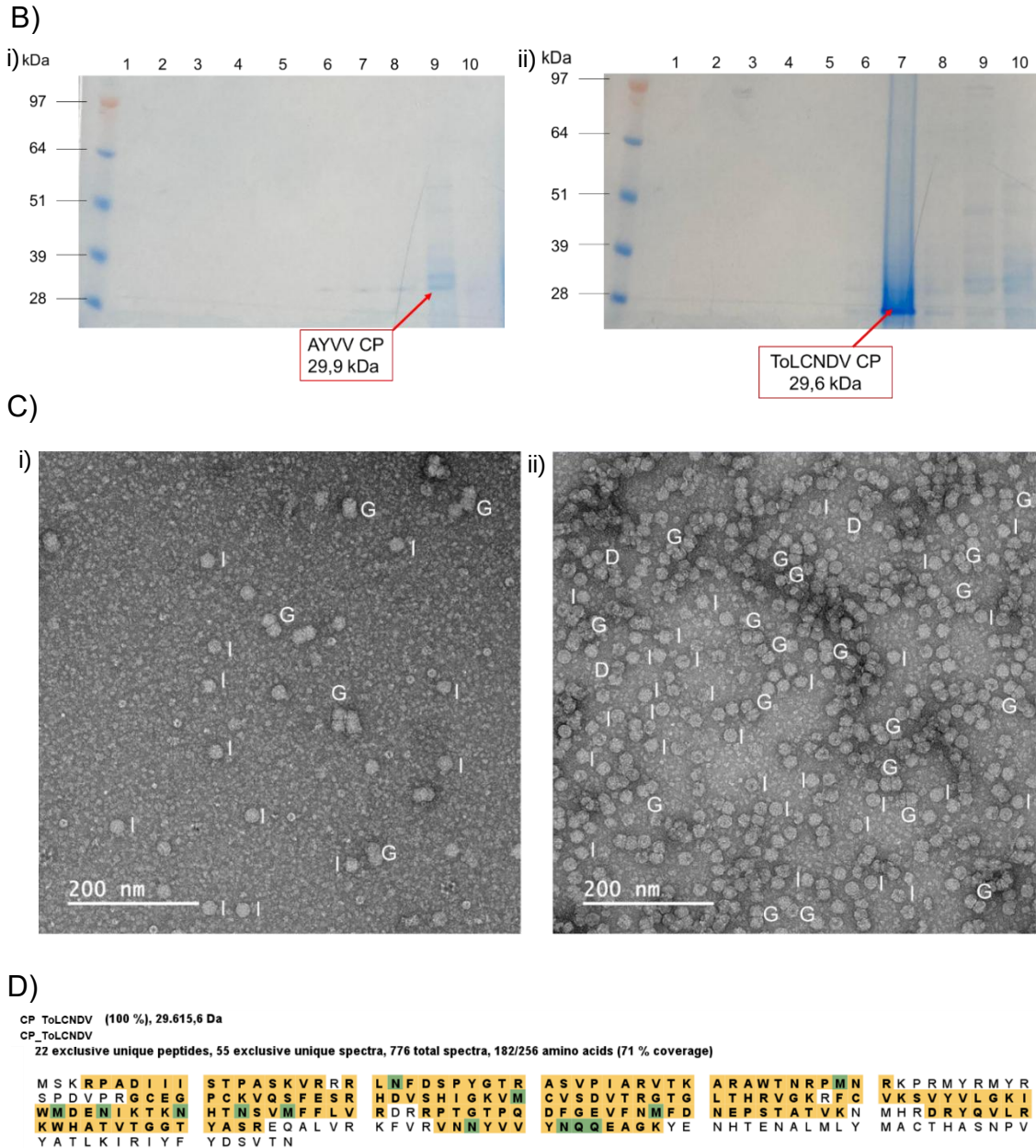


Figure R24. Transient expression and formation of virus-like particles (VLPs) of the begomoviruses AYVV and ToLCNDV. A) Western blot analysis showing accumulation of viral coat proteins (CPs) at 7 dpa. Detection was performed using a polyclonal antibody raised against ToLCNDV CP, which cross-reacted with AYVV CP, producing a band of ~28 kDa, consistent with its predicted size (29.9 kDa). ToLCNDV CP (29.6 kDa) was detected in samples expressing CP-ToLCNDV alone, ToLCNDV VLPs, and ToLCNDV-infected tissue (C+). AYVV CP corresponds to expression of pEAQ-HT-D1-AYVVCp alone, while AYVV DNA- α (pBIN:AYVV 1/7) was used as a negative control. AYVV VLPs were generated by co-infiltration of pEAQ-HT-D1-AYVVCp with pBIN:AYVV 1/7. ToLCNDV VLPs were generated by co-infiltration of pHREAC:CP-ToLCNDV with pBIN:AYVV 1/7. B) InstantBlue-stained 12% MOPS-buffered NuPAGE gels showing cesium sulfate gradient fractions of purified VLPs following co-agroinfiltration of (i) pEAQ-HT-D1-AYVVCp + pBIN:AYVV 1/7 and (ii) pHREAC:CP-ToLCNDV + pBIN:AYVV 1/7. Red arrows indicate bands of ~29 kDa in fraction 9 (i) and fraction 7 (ii), corresponding to AYVV and ToLCNDV CPs, respectively. Additional faint ~50 kDa bands likely correspond to the Rubisco large subunit. The M lane shows molecular weight markers

(kDa). C) Transmission electron microscopy of fractions 7–9 (AYVV, i) and 6–8 (ToLCNDV, ii) showing isometric (I), geminate (G), and double (D) particles resembling native virions. Scale bar = 200 nm. D) MALDI-TOF analysis of purified ToLCNDV CP showing 71% sequence coverage. Identified peptides are highlighted in yellow and green. The detection of AYVV CP-derived peptides in the analyzed sample suggests the presence of minor contamination, likely introduced inadvertently during the isolation procedure.

6.3 *In planta* assembly of ToLCNDV bigeminate VLPs using pCAMBIA:GFP-ToLCNDV

Once confirmed that co-expression of ToLCNDV CP with the self-replicating AYVV alphasatellite DNA supported the *in planta* assembly of VLPs, we next pursued modifications to promote the formation of ToLCNDV bigeminate particles more closely resembling native virions. To investigate whether the encapsidation of full-length genomic circular ssDNA could enable the production of VLPs of similar size and morphology to native ToLCNDV virions, aiming to get preparations specifically enriched in geminate particles rather than predominantly single particles, we generated a GFP-full-length version of the isolate ToLCNDV- [ES-Alm-661-Sq-13], DNA-A (2,738 nt). The construct design specifically deleted the viral coat protein gene and replaced it by a GFP gene, acting as a similar size filler, and it was intended to provide a replicating genomic DNA-A template available for encapsidation by ToLCNDV CP provided in trans, therefore being safer as the construct is drastically different of an infectious clone.

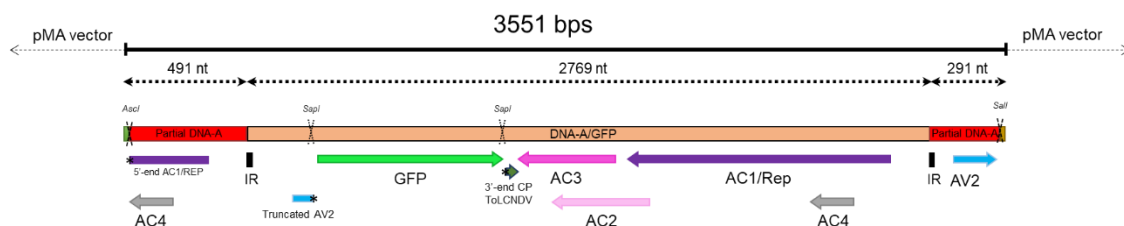
The native coat protein coding sequence (771 nt) was removed and replaced with a filler of comparable length (770 nt) that incorporated the GFP coding sequence, inserted downstream of the coat protein promoter and flanked by two type IIS restriction sites (*SapI*). As *SapI* cleaves outside of its recognition site, this configuration allows precise and scarless excision of GFP and its replacement with alternative sequences, such as the native ToLCNDV CP or other begomovirus CPs. 17 nucleotides corresponding to the 3' end of CP was intentionally retained downstream of GFP to preserve the natural overlap with AC3 gene. In addition, these modifications not only abolish CP expression but also disrupt the AV2 gene, which is associated with viral movement and it is found overlapping CP coding sequence. To generate molecular material suitable for subsequent encapsidation assays, the construct was designed to include one and a half copies of the DNA-A ToLCNDV genome. Infectious clones of begomovirus require the presence of two intergenic regions to initiate circular replication (Boulton 2008); therefore, an additional intergenic region was incorporated to the linear genome sequence, specifically a 291-nt region. This region contained the coding sequence AV2 and was inserted downstream of the DNA-A/GFP sequence. In addition, a 491-nt region,

containing partial AC1/Rep, was introduced upstream of one intergenic region included in the DNA-A/GFP region (Fig. 25A).

The resulting construct, pCAMBIA:GFP-ToLCNDV, was validated by capillary sequencing and subsequently used in co-infiltration experiments with the ToLCNDV CP expression construct pHREAC:CP-ToLCNDV, to examine the encapsidation of the full-length cssDNA genome. As an initial confirmation of construct activity, green fluorescence was observed under UV light in leaves infiltrated with pCAMBIA:GFP-ToLCNDV, confirming the expression of a full-length GFP gene under the control of the native CP promoter (Fig. 25B). To evaluate the accumulation of the ToLCNDV CP, a series of transient expression assays was performed including: the GFP-full-length version of the ToLCNDV alone (pCAMBIA:GFP-ToLCNDV), the ToLCNDV CP construct alone (pHREAC:CP-ToLCNDV), the combination of ToLCNDV CP with the GFP-full-length construct (pHREAC:CP-ToLCNDV + pCAMBIA:GFP-ToLCNDV), as well as negative and positive controls. As expected, no CP signal was detected in samples expressing pCAMBIA:GFP-ToLCNDV alone, consistent with the replacement design of CP by GFP coding sequence. A faint CP signal was observed when pHREAC:CP-ToLCNDV was expressed alone (Fig. 25C), in agreement with previous experiments. By contrast, co-expression of ToLCNDV CP with pCAMBIA:GFP-ToLCNDV at 7 dpa resulted in a markedly stronger CP signal (Fig 25C). Together, these results indicate that ToLCNDV CP accumulation is substantially enhanced in presence of a full-length circular ssDNA molecule, whereas CP expression in absence of genomic DNA leads only minimal accumulation, thereby providing a more favourable context for subsequent ToLCNDV VLP assembly.

A)

In silico fragment design of pMA-DNA-A/GFP



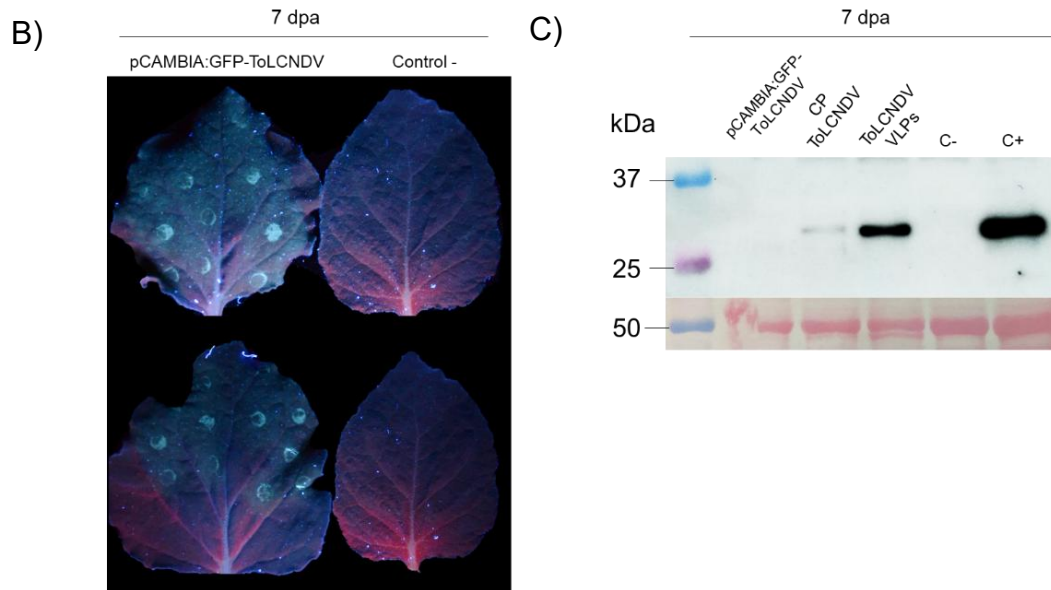


Figure R25. Transient expression of pCambia:GFP-ToLCNDV. A) Schematic representation of the pMA-DNA-A/GFP construct used for cloning into the pCambia0380 binary vector. Colored boxes indicate ToLCNDV DNA-A regions: the DNA-A/GFP fragment (orange), flanking partial DNA-A sequences (red), and restriction sites (Ascl, green; Sall, brown). Black boxes represent intergenic regions (IRs). Arrows indicate gene orientation and translation direction, with asterisks denoting truncated proteins. Dashed and dotted lines represent vector backbone elements and DNA-A fragments included in the final pCambia:GFP-ToLCNDV construct. Fragment lengths are also indicated. B) GFP fluorescence observed in leaves agroinfiltrated with pCambia:GFP-ToLCNDV at 7 dpa (left), imaged under UV light. A leaf from a negative control plant is shown on the right. C) Western blot analysis of ToLCNDV coat protein (CP) at 7 dpa. No CP signal was detected in pCambia:GFP-ToLCNDV samples, confirming deletion of the CP coding region. CP accumulation (~29 kDa) was weak in CP-ToLCNDV expressed alone, strong in ToLCNDV VLPs (co-infiltration with pCambia:GFP-ToLCNDV), and present in the positive control (ToLCNDV-infected zucchini squash). Ponceau S staining of the Rubisco large subunit (53 kDa) was used as a loading control. The M lane shows molecular weight markers (kDa).

Additionally, we confirmed that the co-expression of pCambia:GFP-ToLCNDV together with ToLCNDV CP in trans did not result in CP or GFP expression in non-agroinfiltrated leaves of *N. benthamiana*. Neither CP nor GFP expression were detected respectively by western blot (data not shown) or visualization under UV light in upper leaves of agroinfiltrated *N. benthamiana* plants at 7 dpa (data not shown).

6.4 Generation and functional validation of a native-length ToLCNDV DNA-A clone and assessment of its infectivity and transmission

The construct pCAMBIA:GFP-ToLCNDV was designed in a way that provided us with an opportunity to create a new version of the ToLCNDV DNA-A infectious clone Spanish strain (ToLCNDV- [ES-Alm-661-Sq-13]). The original DNA-A infectious clone (ND-661-2.0-A; Fortes *et al.*, 2016) ensures infectivity because it contains two IRs for starting the rolling circle replication; however, its whole-genome size tandem configuration poses a significant limitation for downstream molecular manipulations, as any modification in a given viral protein, would require introducing twice the same change affecting both duplicated copies, thereby increasing the complexity of cloning procedures. In contrast, the design of pCAMBIA:GFP-ToLCNDV facilitates the generation of a native-length DNA-A construct that still carries two IRs but contains only part of the genome, with a single CP coding region (Fig. R26). Because the GFP cassette is flanked by type IIS restriction sites, it can be replaced seamlessly by the native ToLCNDV CP, or by modified CP variants. This configuration means that pCAMBIA:GFP-ToLCNDV can be readily converted into pCAMBIA:CP-ToLCNDV yielding a functional, non-tandem DNA-A infectious clone, that is more suitable for future genetic and functional studies.

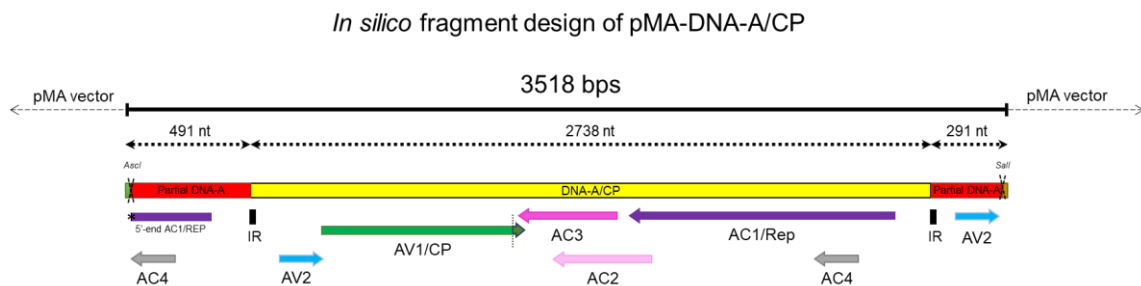


Figure R26. Schematic representation of the pCAMBIA:CP-ToLCNDV construct. The pMA-DNA-A/CP diagram illustrates the pMA vector designed for subsequent cloning into the pCAMBIA0380 binary vector (black line). Colored boxes indicate ToLCNDV DNA-A regions: the DNA-A/CP fragment (yellow), flanking partial DNA-A sequences (red), and restriction sites (*Ascl*, green; *Sall*, brown). Colored arrows represent gene orientation and direction of translation. Black boxes denote intergenic regions (IRs). Crossed dashed lines indicate introduced restriction sites (*Ascl*, *Sall*, *SapI*), and dashed arrows represent the vector backbone. Black dotted lines indicate the ToLCNDV DNA-A fragments included in the final construct. Fragment lengths are also indicated. Green dotted line indicate at AV1/CP protein indicates where the 17 nucleotides intentionally retained and corresponding to the 3' end of CP start to preserve its natural overlap with AC3 gene.

To generate the plasmid pCAMBIA:CP-ToLCNDV, the template with the construct carrying the GFP was digested with *SapI* to excise the GFP cassette. In parallel, the

ToLCNDV CP coding sequence was amplified using primers designed with *SapI*-compatible overhangs (see Table M4), allowing its insertion into the digested backbone to restore the complete native DNA-A component. After enzymatic digestion and cloning, the resulting plasmid was validated by capillary sequencing and subsequently transferred to *A. tumefaciens* for plant inoculation.

To assess whether the newly generated the full-length DNA-A construct, pCAMBIA:CP-ToLCNDV, could function as an infectious clone, we next verified its infectivity in combination with the DNA-B component. Agroinfiltration of *N. benthamiana* and zucchini squash plants with pCAMBIA:CP-ToLCNDV together with the DNA-B (ND-661-2.0-B; Fortes *et al.*, 2016) resulted in an infection success rate of 100% (6/6) in *N. benthamiana*, and of 30% (1/3) in zucchini squash, respectively. Plants were monitored over time, and tissue samples were collected from agroinfiltrated leaves at 5, 7 and 12 dpa, and from systemic leaves at 21 dpi. Protein extracts were analyzed by Western blot for detection of ToCLDNV CP. In *N. benthamiana* samples, CP accumulation was consistently detected at all time points, although showing mild symptom development (Fig. 27Ai). In zucchini squash plants, CP was detected exclusively in systemic tissues after 21 dpi, with severe symptom expression (Fig. 27Aii). This symptomatology closely resembled that induced by the original tandem DNA-A infectious clone (ND-661-2.0-A), confirming that pCAMBIA:CP-ToLCNDV behaves as a functional infectious clone. To further dissect the role of DNA-B in viral movement and ToLCNDV CP accumulation, we next examined whether the presence of DNA-B was strictly required for viral movement and CP accumulation. For this purpose, *N. benthamiana* plants were agroinfiltrated with pCAMBIA:CP-ToLCNDV alone, in the absence of DNA-B component. Noticeably, CP accumulation was detected in agroinfiltrated leaves at 5, 7 and 12 dpa, as well as in systemic leaves at 21 dpi, although CP signal was weaker than in the previous *N. benthamiana* plants co-inoculated with pCAMBIA:CP-ToLCNDV and DNA-B (Fig. 27B). In line with this reduced accumulation, symptom development was poorly than that observed in plants co-inoculated with DNA-B (Fig. 27B). These observations indicate that, while DNA-B enhances ToLCNDV infection symptom severity and CP accumulation, DNA-A alone is sufficient to support detectable CP accumulation and limited systemic spread in *N. benthamiana*, providing additional flexibility for its use in ToLCNDV assays.

Additionally to the immunodetection analysis, both *N. benthamiana* and zucchini squash plants inoculated with our constructs were analysed for the presence of ToLCNDV DNA-A by molecular hybridization. After 21 dpi, samples from non-inoculated apical leaves of infected plants were used for molecular hybridization signal corresponding to the DNA-

A component. Positive signal was confirmed in *N. benthamiana* and squash zucchini plants co-agroinfiltrated with pCAMBIA:CP-ToLCNDV and the DNA-B component (ND-661-2.0-B). In zucchini squash, the signal intensity was comparable to the positive control (10^{-1} ToLCNDV DNA-A purified from *E. coli*), whereas *N. benthamiana* exhibited a weaker signal, similar to the dilution 10^{-3} of ToLCNDV DNA-A positive control, probably indicating, a lower viral load (Fig R27C). In plants agroinfiltrated only with pCAMBIA:CP-ToLCNDV, *N. benthamiana* displayed an extremely faint hybridization signal and only in two plants from six analysed (Fig. R27C).

Furthermore, and to complete the information about new DNA-A component infectious clone (pCAMBIA:CP-ToLCNDV), ToLCNDV infected zucchini squash were used as a source for *B. tabaci* transmission assays. The virus was acquired and transmitted to healthy zucchini squash (one plant systemically infected out of four inoculated with the characteristic symptoms of ToLCNDV infections) corresponding to a 25% transmission, roughly on the rates usually observed in our conditions for cucurbit plants), confirming the ability of the new infectious clone to assembly fully functional particles and be transmitted.

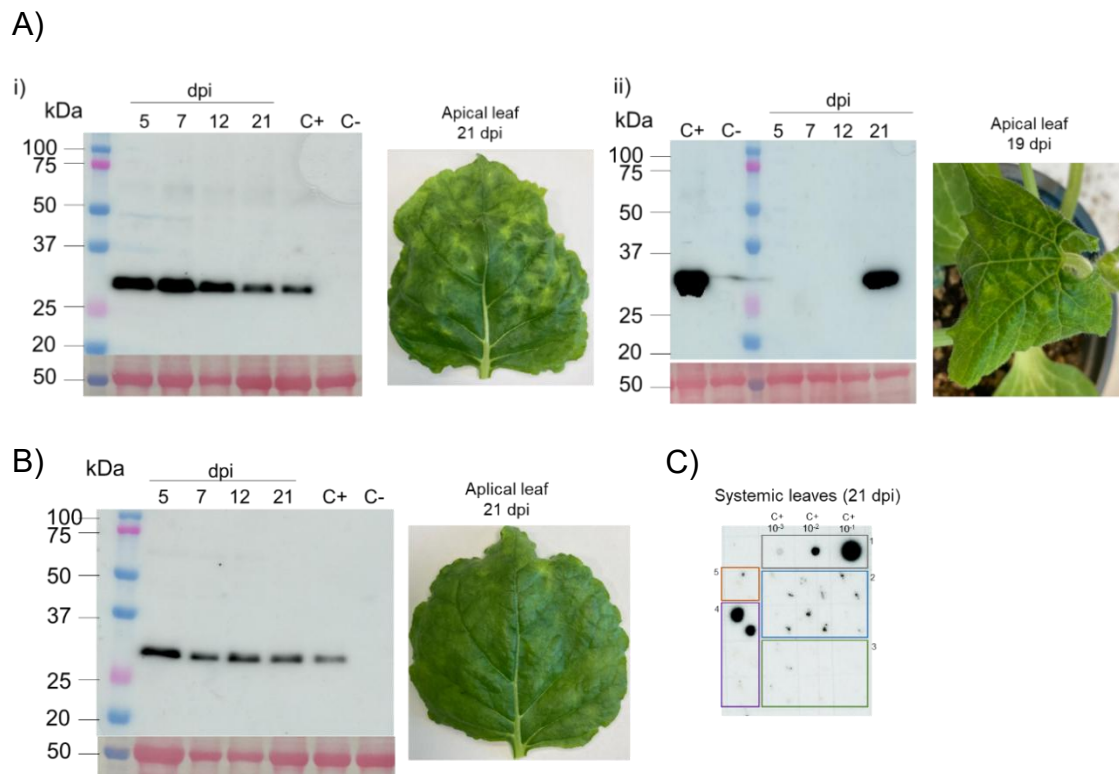


Figure R27. Western blot analysis of the ToLCNDV DNA-A infectious clone (pCAMBIA:CP-ToLCNDV). A) Total protein extracts from leaves agroinfiltrated at 5, 7, and 12 dpa, and from systemic leaves at 21 dpi, following co-infiltration of DNA-A (pCAMBIA:CP-ToLCNDV) and DNA-B (ND-661-2.0-B) in (i) *N. benthamiana* and (ii) zucchini squash. Pictures (right) show apical leaves of infected plants at 21 dpi. B)

Total protein extracts from leaves agroinfiltrated with DNA-A alone (pCAMBIA:CP-ToLCNDV) at 5, 7, and 12 dpa, and systemic leaves at 21 dpi. Picture (right) shows an apical leaf of *N. benthamiana* at 21 dpi. Positive controls (ToLCNDV-infected *N. benthamiana* (i) and zucchini squash (ii)) and negative controls (non-infected plants) were included. The M lane indicates molecular weight markers (kDa). C) Molecular hybridization of petiole cross-sections from young leaves of *N. benthamiana* and zucchini squash at 21 dpi. ToLCNDV DNA-A plasmid was used as a positive control (10^{-1} , 10^{-2} , 10^{-3} dilutions) (sector 1). Samples include: (sector 2) *N. benthamiana* co-infiltrated with DNA-A and DNA-B; (sector 3) *N. benthamiana* infiltrated with DNA-A alone; (sector 4) zucchini squash co-infiltrated with DNA-A and DNA-B; and (sector 5) non-infected zucchini squash (negative control).

DISCUSSION - CHAPTER III

Tomato leaf curl New Delhi virus (ToLCNDV) represents a major constraint to agricultural production worldwide, affecting a broad range of cultivated plant species belonging to the families *Cucurbitaceae*, *Euphorbiaceae*, *Fabaceae*, *Malvaceae* and *Solanaceae* (Moriones *et al.*, 2017). The virus is responsible for some of the most economically important viral diseases in the Indian subcontinent causing severe yield losses, particularly in tomato plants (Chakraborty, 2008; Zaidi *et al.*, 2016). In addition to tomato, ToLCNDV has emerged as significant pathogen of cucurbit crops, with outbreaks reported in several regions of the Mediterranean Basin, including Tunisia, Italy and Spain (Mnari-Hattab *et al.*, 2015; Panno *et al.*, 2016; Fortes *et al.*, 2016). Therefore, the establishment of ToLCNDV in this region constitutes a serious threat to economically important cucurbit crops. Symptom expression associated with ToLCNDV infection are quite similar to other begomovirus infection symptoms, such as stunting, yellowing and curling in apical leaves. When ToLCNDV is infecting tomato plants, these exhibit upward or downward curling and crinkling of leaves, vein clearing and yellow mottling among others; and similar symptoms are observed in ToLCNDV infected cucurbits (Moriones *et al.*, 2017).

In this chapter, we first examined the ability of the Spanish strain of ToLCNDV to infect zucchini squash and melon as natural hosts, as well as *N. benthamiana*, as an experimental host. Our results agree with previous findings reported by Fortes *et al.*, 2016, demonstrating that ToLCNDV Spanish strain can establish infection in zucchini squash and melon following agroinoculation, and also infects *N. benthamiana*. However, in contrast to the previous mentioned study, the infection rates in zucchini squash tested plants in our conditions were not of 100%. This variability may be influenced to differences in plant material used, as distinct accessions can display varying levels of susceptibility or resistance to the virus (Sáez *et al.*, 2016). Systemic symptoms development was observed in zucchini squash, including stunted growth, leaf curling, chlorosis and mosaicism, whereas symptoms in *N. benthamiana* were noticeably milder. This agrees with differences in the pathogenicity and symptom development that have been reported for ToLCNDV, likely reflecting differential host adaptation among viral isolates (Kushwaha *et al.*, 2015; Pérez-Rubio *et al.*, 2025).

In line with these host-dependent differences in symptom expression, we next investigated the structural characteristics and the production of native ToLCNDV virions from infected zucchini squash and *N. benthamiana* plants. Comparative observations

showed that in both hosts the virions were produced with similar appearance (22x38 nm bigeminated), but revealed a possible effect of the host species on viral accumulation and purification yield. Regardless of the high infectivity observed in inoculated *N. benthamiana* (100%), the purification of virions from this host consistently yielded lower amounts of particles than from equivalent weight of infected zucchini squash plants. Although we cannot exclude that part of these differences might come from the purification procedure, the fact that this reduced particle yield correlated with milder symptom and weaker accumulation of the viral coat protein in *N. benthamiana* (Fig R.23) suggested that this experimental host might not be the plant of choice for structural studies, despite its broad use due to its intrinsic permissivity towards a wide range of plants viruses (Goodin *et al.*, 2008).

Transmission electron microscopy (TEM) analysis of purified virions from both host species revealed a heterogenous particle population composed of geminate and isometric particles, with geminate forms representing the predominant fraction (~75%). The presence of single (isometric) particles alongside the canonical bigeminate virions contrast with observations reported for the wild-type AYYV (Hesketh *et al.*, 2018), in which virion preparations consist almost exclusively of geminate particles. Nevertheless, the coexistence of geminate and isometric particles has been previously described for a few geminiviruses and it has been associated with the encapsidation of subgenomic ssDNA molecules of reduced molecular weight (Casado *et al.*, 2004), as well as defective interfering (DI) DNAs or satellite ssDNAs, with approximately half the size of begomovirus genomic components (Frischmuth *et al.*, 2001; Bennet *et al.*, 2020). In line with this, Pérez-Rubio *et al.*, (2025) identified in one of their samples, a DNA-B-ES molecule of approximately 1.5 kb, containing a complete intergenic region (IR) and partial fragments of the BC1 and BV1 ORFs, supporting the natural occurrence of DI DNAs during ToLCNDV infections. Therefore, the occasional appearance of isometric particles in our preparations can be reasonably attributed to the nature of the encapsidated DNA, such as DI or satellite ssDNA. These results are consistent with the appearing flexibility in terms of size of DNA that can be encasidated by begomoviruses as well as to their frequent association with smaller DNA molecules, including alpha- and betasatellites, defective genomic DNAs, and recombinant DNA species (Saunders *et al.*, 2020; Briddon *et al.*, 2003, 2004).

Alpha- and betasatellites, which are approximately half the size of begomovirus genomic DNAs, have been associated with numerous begomovirus species. Betasatellites have been most commonly characterized in association with monopartite begomoviruses from the Old World; however, their presence has also been reported for the bipartite

ToLCNDV (Zaidi *et al.*, 2016). In particular, co-infection of ToLCNDV DNA-A and DNA-B with Cotton leaf curl Multan betasatellite (CLCuMB) has been shown to enhance pathogenicity in *N. benthamiana* (Sivalingam and Varma, 2012). Although the association of betasatellites with bipartite begomoviruses can contribute to increased disease severity, betasatellites are fully dependent on their helper virus for replication, cell-to-cell and systemic movement, encapsidation, and insect-mediated transmission (Zhou 2013). In contrast, alphasatellites encode a rolling circle replication (RCR) initiator protein (alpha-Rep), which enables their autonomous replication in plant cells, although they still require a helper begomovirus for movement within the host and for transmission by insect vectors (Saunders and Stanley, 1999; Mansoor *et al.*, 1999). This biological property of alphasatellites was exploited by Hesketh *et al.*, (2018), who demonstrated that co-expression of a begomoviral CP with a self-replicate alphasatellite, in the absence of viral infection, was sufficient to support begomoviral capsid assembly and the formation of a mixture of geminate and single virus-like particles (VLPs), whereas the CP expression alone did not result in particle formation. These findings established that the provision of a circular ssDNA is essential for geminivirus capsid assembly. Two years later, Saunders *et al.*, (2020) used this strategy to generate VLPs from other geminiviruses.

In light of these observations, we decide generate ToLCNDV VLPs as a possible experimental approach for future structural and insect-transmission studies. Based on previous works showing that a circular ssDNA molecule of half-size compared to genome components, in particular the AYVV alphasatellite, can be efficiently encapsidated by the CP of related begomoviruses, such as ACMV (Saunders *et al.*, 2020), we adopted an analogous system to generate ToLCNDV VLPs. We indeed succeeded to produce ToLCNDV VLPs by co-expression the ToLCNDV CP and AYVV alphastellite system. In the agroinfiltrated tissue we confirmed ToLCNDV CP accumulation, and furthermore, ToLCNDV VLPs purified were visualized by TEM, comprising both isometric and geminate particles in similar proportion to those reported by Hesketh *et al.*, (2018; Fig. R24). To provide an explanation for the coexistence of isometric and geminated structures, it was hypothesized that full-length begomovirus genomic DNA (~2.7 kb) might be needed to fill the entire internal volume of a geminated particle, whereas smaller satellite DNAs (~1.4 kb) were likely to be encapsidated separately into isometric structures (Hesketh *et al.*, 2018). Nevertheless, the observation that geminate structures can be formed with only presence of an alphasatellite indicates that the hypothesized correlation might not be absolute, and that two copies of the satellite DNA could be co-encapsidated in a bigeminated (Saunders *et al.*, 2020).

In addition to individual and geminated particles, few “double” particles were observed when co-expressing the AYVV alphasatellite together with the ToLCNDV CP. These structures named as “doubles” were also described by Saunders *et al.*, (2020), suggesting to derive from two closely associated isometric capsids rather than true (canonical) geminate particles. They speculate that these structures could arise from initiation of encapsidation at two distinct positions along the circular ssDNA, and are considered less stable than canonical geminate virions.

Begomoviruses are transmitted by whiteflies, specifically by *B. tabaci*. Together with begomoviruses, their associated satellite DNAs, alphasatellites and betasatellites, are also transmitted by *B. tabaci* whiteflies (Saunders and Stanley, 1999; Zhou 2013; Fiallo-Olivé *et al.*, 2020). Begomovirus transmission was initially considered circulative nonpropagative, meaning that the virions moved through the insect vector without replicating (Harrison 1985; Boulton and Markham, 1986), although there is a controversy because of some later conflicting results. For our purposes, and regardless of the possibility of replication in the vector, the CP is considered the only structural protein of geminiviruses (Harrison *et al.*, 2002) acting as the major and possibly the sole determinant of transmissibility (Wang and Blanc, 2021). The CPs of various begomoviruses have been reported to interact with several *B. tabaci* proteins, including BtHSP16 (Ohnesorge and Bejarano, 2009) and BtHSP70 (Götz *et al.*, 2012), and also with GroEL chaperonins produced by bacterial endosymbionts located in the whitefly midgut (Gottlieb *et al.*, 2010; Rana *et al.*, 2012). Additional proteins that have been shown to interact with begomoviruses CP *in vitro* are collagen, cyclophilin B (CypB), a midgut protein (MGP) and peptidoglycan recognition protein (PGRP) (Rana *et al.*, 2019; Kanakala and Ghanim, 2016; Rana *et al.*, 2016; Wang *et al.*, 2016). Regarding the mechanisms acting during transmission, clathrin-dependent endocytosis seems to facilitate begomovirus entry into midgut cells of *B. tabaci* (Pan *et al.*, 2017). Following this line, Zhao *et al.*, (2020) proposed that BtCUBN and BtAMN form a BtCubam receptor complex on the surface of midgut cells, which directly interact with the viral CP, allowing that virions along with the BtCubam complex are internalized via clathrin-mediated endocytosis. Then, the BtCubam receptor complex dissociates from virions in early endosomes and returns to apical membrane through recycling endosomes, while the virus continues intracellular trafficking (Xia *et al.*, 2018; Wang and Blanc, 2021). In addition to these midgut-occurring interactions, the transmission requires other CP-receptor interactions in other anatomical barriers within the insect during viral circulation. All these interactions with putative receptors and other whitefly proteins are considered essential for the circulative transmission of begomoviruses in *B. tabaci* (Czosnek *et al.*,

2017). However, the key binding sites in the CP to interact with insect-receptors remains unclear. Several studies have attempted to identify specific CP amino acids required for virion assembly and vector transmission. Hesketh *et al.*, 2018 demonstrated that substitutions at positions R48A and M59D affected the particle morphology, inducing a switch from geminate to icosahedral single particles; in addition, another alteration in single VLPs indicated that they appeared to have a missing pentameric capsomer. These structural deficiencies will be likely negative for the transmission process. Also, other mutational analyses had previously shown that amino acid changes in the CP can noticeably affect insect-transmissibility of begomoviruses: for example, alterations at position 129 and 134 of TYLCV CP resulted in a loss of whitefly transmission (Noris *et al.*, 1998), whereas a single amino acid substitution in the CP of a non-transmissible bipartite begomovirus, Watermelon chlorotic stunt virus (WmCSV), restored the transmission mediated by whiteflies (Kheyr-Pour *et al.*, 2000). Taken together, all these findings support the idea that begomovirus transmission specifically relies on coordinated interactions between their CPs and specific insect receptors, as well as on the interactions of the CP-DNA and CP-CP of the virus.

Despite these advances, the molecular machinery governing begomovirus transmission by whiteflies remains poorly understood, underscoring the importance of exploring complementary and innovative perspectives. In this regard, the study of Caciagli *et al.*, (2009) offers a particularly intriguing insight, as the authors reported a significant inhibition of transmission of the wild type (WT) TYLCSV when whiteflies were previously fed on plants infected with a non-transmissible TYLCSV mutant (PNHE). This inhibition was also reported when they were fed from purified PNHE TYLCSV preparations. Hence, these results could suggest that non-transmissible mutants compete with WT virions for binding to *B. tabaci* receptors, thereby interfering with transmission.

In this context, the need to explore alternative strategies to limit virus spread becomes evident. To address this challenge, part of this work was focused on the generation of non-infectious particles that closely resemble native ToLCNDV virions although obviously being non-infectious. ToLCNDV VLPs previously mentioned resulted predominantly in single (“isometric”) particles rather than the characteristic geminate morphology observed in natural virus infections. Therefore, we aimed to produce VLPs that more faithfully mimic native ToLCNDV virions. To achieve this, we constructed a full-length ToLCDNV genomic DNA highly similar to the WT virus but replacing the CP by the GFP sequence, and co-expressed with ToLCNDV CP in trans. The evaluation of this system revealed substantially higher ToLCNDV CP accumulation compared with CP expression alone, suggesting enhanced CP stability and, presumably, the formation of

stable geminate particles. These new tools open a promising way for generation of different VLPs that could serve as potential transmission interfering element adequate for biological control. ToLCNDV VLPs could be exploited in future studies, including membrane feeding assays to investigate interference with insect-mediated transmission.

Beyond the potential relevance for biological control strategies, the structural characterization of ToLCNDV virions and VLPs would represent another future valuable extension of this work. Previous studies have substantially advanced our understanding of begomovirus particle architecture and have provided important knowledge on the capsid assembly and the contribution of viral genomic DNAs and derived ssDNA molecules to the CP and virion stability (Hesketh *et al.*, 2018; Hipp *et al.*, 2017; Xu *et al.*, 2019). Applying similar approaches to ToLCNDV could therefore help to further elucidate CP-DNA interactions and their role in geminate particle formation. Moreover, the use of VLP based system would also facilitate the identification of putative CP determinants involved in interactions with insect receptors, while offering a safe experimental framework, avoiding the biosafety risks associated with the use of infectious begomoviruses.

An additional outcome of this work was the generation of a ToLCNDV DNA-A infectious clone containing only a single CP coding sequence (pCAMBIA:CP-ToLCNDV). This design was thought to preserve the ToLCNDV infectivity while overcoming the limitations associated with whole-genome tandems present in the initially used clone. The new clone was fully infectious in both zucchini squash and *N. benthamiana* when co-agroinoculated with DNA-B (ND-661-2.0-B; Fortes *et al.*, 2016), inducing characteristic symptoms, replicating and moving systemically in susceptible host plants, and being readily transmitted by its insect vector, *B. tabaci*. Interestingly, CP was detected in agroinfiltrated leaves only in *N. benthamiana*, whereas no signal was observed in zucchini squash, suggesting that host-dependent differences can exist in local viral expression. On the other hand, once a systemic infection is established, the cucurbit hosts seem to accumulate larger quantities of virions, which could explain the unusual property of ToLCNDV to be mechanically transmissible (Romy *et al.*, 2019). The dynamics of virus accumulation across different tissues of experimental plants and susceptible crops over time will require further analysis.

The newly generated infectious clone is also a promising tool for investigating infectivity. To determine whether the DNA-A alone could establish a monopartite-like infection in *N. benthamiana*, the newly generated infectious DNA-A clone was inoculated in absence of the DNA-B component. Despite that any visible symptoms were not observed in our

experiments, CP accumulation was detected in both agroinfiltrated and systemic leaves, although at lower levels than those observed when DNA-A and DNA-B were co-inoculated (Fig. R27). Our results are consistent with those reported by Pérez-Rubio *et al.*, (2025), who demonstrated that the DNA-A component of the Spanish strain was able to replicate autonomously in the absence of its cognate DNA-B, but at reduced levels. This is somehow reflected in our results by the lower CP accumulation and weaker signals detected by molecular hybridization. Moreover, the higher CP accumulation in *N. benthamiana* plants agroinfiltrated with DNA-A and DNA-B support the conclusion that proteins encoded by DNA-B contribute to enhance, rather than replace, viral accumulation and particle formation, underscoring the functional interplay between the two genomic components (Pérez-Rubio *et al.*, 2025).

A detailed understanding of the molecular mechanisms underlying virus-vector interactions is essential for the development of effective and sustainable control strategies. Approaches that integrate insights into key molecular interactions, particularly those related to viral transmission offer significant potential to interfere in the pathogen spread. The contribution of our study in this field is the generation of virus-like particles (VLPs) of the whitefly-transmitted ToLCNDV that will be further used in future studies. In addition, the generation of a novel infectious clone containing a single coat protein copy offers the possibility to launch mutational studies aiming to identify new CP residues involved in vector receptor interactions, expanding the repertoire of potential molecular targets for control. Ultimately, the development and application of VLPs across multiple virus-vector systems may represent a generalizable experimental strategy to dissect transmission mechanisms and to design innovative tools for the control of vector-borne plant viruses.

CONCLUSIONS

7. CONCLUSIONS

1. The sweet potato potyvirus SPV2 retains high infectivity, symptom expression and efficient virion accumulation in the experimental host *Nicotiana benthamiana* following mechanical inoculation, while preserving in its progeny the canonical aphid transmission-related motifs in HCPro (PTK and K/RITC) and CP (DAG). Nevertheless, a progressive decline in transmissibility was observed over successive mechanical passages, likely reflecting adaptative changes that favour viral replication and maintenance in the new host at the expense of vector transmission efficiency. The occurrence of amino acid substitutions in the surroundings of these motifs suggested that the sequence context may modulate their functionality during transmission.
2. The complete genome sequence of the AM-MB1 isolate of the potyvirus Sweet potato feathery mottle virus (SPFMV) was obtained from individual and mixed infection plants, and comparative analysis with the isolate AM-MB2 revealed remarkable differences within their UTRs and coding sequences, despite their overall 91,6% identity. The greatest variability was observed in the P1 and P1N-PISPO gene products, which exhibited amino acid identities of only 79,4% and 74,5% (down to 68,1% for the PISPO region), suggesting that these most divergent regions might contribute to the biological variability between isolates.
3. The P1N-PISPO protein of SPFMV isolate AM-MB1 exhibited significantly reduced local RNA silencing suppressor activity in transient expression experiments in *N. benthamiana* leaves when compared to its counterpart from SPFMV isolate AM-MB2. The silencing suppression capacity of SPFMV AM-MB1 appears to result from the combined contribution of multiple viral factors, with a particularly relevant functional role for the coordinated actions of P1 and HCPro, either in *cis* or *trans*.
4. Sweet potato plants support the accumulation of diverse viral populations due to their vegetative mode of propagation and the activity of multiple insect vectors. Our findings on laboratory samples revealed a highly complex virome, characterized by the coexistence of viruses from different families, frequently without producing detectable symptoms, highlighting the significant challenges for accurate diagnosis and effective disease management in this crop. Notably,

Conclusions

the introduction of new viral species may alter this balance, leading to enhanced viral accumulation and interspecific interactions.

5. Mixed infections of SPFMV and the crinivirus Sweet potato chlorotic stunt virus (SPCSV), following whitefly transmission of the second virus, resulted in a strong synergistic interaction in sweet potato plants, experimentally reproducing the previously described sweet potato viral disease (SPVD). The enhanced symptom severity correlated with increased SPFMV titers, and the transcriptomic analysis of superinfected plants revealed substantial alterations in gene expression, comprising both a general response to viral infection and virus-specific modulation of host pathways.
6. Weighted gene co-expression network analysis (WGCNA) effectively captured host responses resulting from the superinfection of SPCSV in SPFMV-infected sweet potato plants by identifying gene co-expression clusters. This approach enabled the exploration of the metabolic pathways involved and may contribute to the identification of gene sets associated with susceptibility or resistance mechanisms, offering new information for future virus control strategies in sweet potato crops.
7. Virus-like particles (VLPs) of the begomovirus Tomato leaf curl New Delhi virus (ToLCNDV) were successfully produced *in planta* using a previously described system based on the co-expression of the CP with a self-replicating alphasatellite DNA from Ageratum yellow vein virus (AYVV). Both virions and VLPs of ToLCNDV were purified and visualized by transmission electron microscopy, validating their structural integrity. Furthermore, the development of the pCAMBIA:GFP-ToLCNDV construct provides a promising approach for generating engineered VLPs that more closely resemble native viral particles.
8. A new infectious clone of ToLCNDV DNA-A was generated, and its infectivity was successfully validated. Notably, it was able to replicate autonomously and to accumulate in systemic leaves of *N. benthamiana*. Moreover, efficient infections of both *N. benthamiana* and zucchini plants were achieved through co-expression with the DNA-B component, and the resulting virus progeny was confirmed to be transmissible by its natural vector, *Bemisia tabaci*, demonstrating full biological functionality.

ANNEXES

8. ANNEX I

This annex compiles supplementary tables and figures corresponding to Material and methods, Chapters I, II and III of results.

Table S1. Primers used in the present thesis

Target	Name	Sequence (5' --> 3')	Amplicon size	Function
SPV2	FW-6_SPV2	CTTCCATCAGGCGCGCCATGTCAGGCACTGAAGAAAT	998	PCR virus detection and SPV2_Nb-CO CP amplifying
	RV-6_SPV2	TTACTTGTACACCCGGGTCACTGCACACCTCTCATTC		
	Fw_HCPro-SPV2-FWD	CACCATGTCACAAACAGGTGATAGATTCTGGAATGG	1380	SPV2_Nb-CO HCPro amplifying
	RV_HCPro-SPV2-RVS	TCATCCAACAAGATAGTGTTCATTTCTGAATCCAATG		
SPFMV AM-MB1	FW_seq_P1_MB1	CGTTTCAGAAGTAAACAAA	186	PCR virus detection
	RV_seq_P1_MB1	GACACATCCCCAAGAGTAGT		
SPFMV AM-MB2	PISPOMiSeqFW	GCACCACAAGATGGTGCGTAA	300	PCR virus detection
	PISPOMiSeqRV	GTCATTTCCAGACTCGCCGATG		
	FW_seq_P1_MB2	TGAGGTTTCAGACACCTCTTG	149	PCR virus detection
	RV_seq_P1_MB2	CTTCCGAGGCCTTAATTGGCT		
SPFMV AM-MB2	qPCR-FM-AMMB2-FW	CAGAAGTGGGTAAGACCAT	235	qPCR relative quantification
	qPCR-FM-AMMB2-RV	GGAAGTGGGACGATAGCAG		
SPFMV	P1_FM_AMMBX_detection_fw	TGARGTTTCAGRCACCTCTTG	339	PCR virus detection
	P1_FM_AMMBX_detection_rv	GCAATAGGTTCTACAATAG		
SPCSV	Poly1_CS_fw	ATGGCAACTGTTTGCTCATCCGC	948	PCR virus detection
	Poly1_CS_rv	ACCCTTATTACTATCGGTGCCTATTTTAGC		
	Hsp70_CS_seq_RNA2_FW	GGATCGGAATTTATCCCAAC	147	PCR virus detection
	Hsp70_CS_seq_RNA2_RV	AGACAGCCTATCAACCCCAAC		
SPCSV-RNA1	qPCR-CS-RNA1-FW	TAACGCCAGCCCGCCAAACAAA	200	qPCR relative quantification
	qPCR-CS-RNA1-RV	CTAACCTATCTTCGACGTTAAC		

SPCSV-RNA2	qPCR-CS-RNA2-FW	TTGGTGGTACTATGAAAGTCC	177	qPCR relative quantification
	qPCR-CS-RNA2-RV	ACAGCCTATCAACCCCAACC		
Ribosomal protein L2 (GenBank ID: AY596742.1)	lbRPL2_seq_FW	ATTAGCTAGAGCAGCGGGTG	125	qPCR relative quantification
	lbRPL2_seq_RV	CTTGTCCGACTGTTGCTGAG		
DNA-A ToLCNDV	MA1788	CGTGTCGTTTCGATCTGGTGTC	268	PCR-Molecular hybridization virus detection
	MA789	GTTTGTGGATCTAACTTGGTGAG		
	ToA-301F DNA-A CP	TCATCATTTCAACTCCCGCATC	174	PCR virus detection
	ToA-453R DNA-A CP	TTGGCACGTCAGGACTTCTATA		
SPFMV AM-MB2	Frag. P1+HCPro_fwd	GGAAAAATAACACAACACAATACAACACAACGAAAATCAATC	3495	Primers based on SPFMV AM-MB2 sequence to obtain preliminary sequences for AM-MB1
	Frag. P1+HCPro_rev	CTGTAACACCGCCTACTATGTAGTGTTTC		
	Frag. P3-CI_fwd	CATAGTAGGCGGTGTTACAGCATTGCCAG	3161	
	Frag.P3-CI_rev	AATCTTTTGTCTGATGAAGCACACAAGAG		
	CP_10408_SPFMV_Seq_RV	TGAATGCAGCTCGTAAAAATCAAATG	3799	
	Frag.6K2_3UTR_fwd	GCTTCATCAGACAAAAGATTCTCTCTCAAAGCAC		
SPFMV AM-MB1	GSP1_5RACE_FM_AMMB1_RV	ACGTGGCATGTTCAATTTGAGACCTTAAAC	-	Primers used for sequencing AM-MB1 isolate generating overlapping segments
	HCPPro_seq_AMMB1_RV	ATTCATTGAACGCCTTTACA		
	HCPPro_seq_FM-CO-AMMB1_FW	AGGAGTGGTGTGAATACGTAA		
	P3_SPFMV_AMMB1_RV	CGTACAAGATTTGGGTCAAA		
	HCPPro_FragB_seq_FM-CO_fw	ATGTTTGATGTAGCCACA		
	P3_seq_fw	AAGCTTTGCACTTTCACAGAA		
	CI_Seq_FW	TCACCAAAGCTTAGATGACA		
	CI*_seq_FM-CO_Fw	TTGTGGCTGTTGCTGACAACATATT		

Annexes

FragC_FM-CO	AAGCAAGCACGCACGATGTTGCAATTT
6K2_seq_FM-CO_fw	ATCTTGGTCTAAAGGGAGT
Nib_seq_FM-CO_rv	TGGTGGTTGTAAACATGT
FragC_FM-CO_SmaI_SphI	TGAACGATCTGCAGGCATGCcccGGGCTGTATCTGGCCTCATTGCAAGTA
NIb*_seq_FM-CO_FW	CACAGAGATAGTATACACACCA
CP*_seq_F- CO_RV/CP_SPFMV_AMMB1_RV	AAATGTGCTTCTTTTGCACG
GSP1_5RACE_FM_AMMB1_RV	ACGTGGCATGTTCAATTTGAGACCTTAAAC
GSP2_5RACE_FM_AMMB1_RV	CAATACCCTAGCAATGGCGTTCAT GTTG
GSP3_5RACE_FM_AMMB1_RV	G TTCAGACTCCC ACTTGTATGTTTCGCC

Table S2. Gene products from SPFMV isolate AM-MB1

Gene product	Size (aas)	Sequence
P1	689	MATIVASAKPASKRKL TWKECCNKWGRAAMEQQKEQQSRKTVHLGRDHLAAYLMAFVPETEW HKYYYGSRGGPSPALLILKGAIECGETYKWESELTFC AECDDVLDGHKCDSCGYNHKHDNDITNNMNAIARVLGGYDAFYASNWAVYETAKYELDQVAPTAGMLYKQAKEAEKLLGKRPTTRREIQEVEDLWAEYEE AAAREAAEAFKVSNEHATSEVANKTAYLSDEEDDEAFPLVTTIEKVALATTIIESVSEVNKTPEVQAPLPAPPEVSVAATFVEATNNEGDEQLLTQE QVAKN ANCSAANLTTLDGVSICDIQFGTISCVLGQTKVQETETAKESTTGFFFGTIPAIPLPTIPIMKLESAIVEPIATPVAEITSNEIVEVPAAAPTEMAKAPKTSLPKHL YPWTSKTQTPGEVHHKMVRRWVQKTQAAAAEKEKLVWKKLDEQLVARNEARKDLKVWWRWGLYRLVRKTRKDNQRQRQRKRMEKEQKLLTAMPPQIL TSISIAGGPAASLEMTPTLNGKIFCTPSMKKKRTLKSPTLTQEIKHELMQAVLKIWWKEMNVELVGKKPTRGQYKKFQGAHFLHLKHKMGLRESVDLRV HPTTQDIVLQAAKVGAWERSIKTITLSKGSSGLVMNPDKLLGPRGHAPQGMFVVRGAFKGVLYDARMKLGSRVLPYITQF
P1N- PISPO	679	MATIVASAKPASKRKL TWKECCNKWGRAAMEQQKEQQSRKTVHLGRDHLAAYLMAFVPETEW HKYYYGSRGGPSPALLILKGAIECGETYKWESELTFC AECDDVLDGHKCDSCGYNHKHDNDITNNMNAIARVLGGYDAFYASNWAVYETAKYELDQVAPTAGMLYKQAKEAEKLLGKRPTTRREIQEVEDLWAEYEE AAAREAAEAFKVSNEHATSEVANKTAYLSDEEDDEAFPLVTTIEKVALATTIIESVSEVNKTPEVQAPLPAPPEVSVAATFVEATNNEGDEQLLTQE QVAKN ANCSAANLTTLDGVSICDIQFGTISCVLGQTKVQETETAKESTTGFFFGTIPAIPLPTIPIMKLESAIVEPIATPVAEITSNEIVEVPAAAPTEMAKAPKTSLPKHL YPWTSKTQTPGEVHHKMVRRWVQKTQAAAAEKEKLVWKKLDEQLVARNEARKDLKVWWRWGLYRLVRKTRKDNQRQRQRKRMEKEQKLLTAMPPQIL DEYLHRWGSCESEGNDSNSEDWEDFLHAFYEEEEENFKISHANTRENPRAHASSLENSLEKGDCECRAGGQETHKRPIQEISRSKAFVPSFKTYERPQRISGF KSPNNNSRYRTASRKGWGMGKVNKNNNIQRFKWSSNEPRQTAGSSGSRTTRNVRGTWGFRSAIRRTDEAW
HCPPro	458	SSTMERFLSGFDNKFQMRQIDADHVCESYDAEQAGSVAATHHMLYPMGRTTCKSCINNIEDMSREEWCEYVKS FINKHKILCQFDYKDFVHLPQIMDF LSESLVNTNKNVKA FNEIQSLIGDRNDAPFTNVCEINKVLVKGGRAKSDELIRASENLELARYLKNRTENIKKGSLSFRNKISQKSSVNLALMCDNQLDKN GNLIWGERGYHSKRFFANYFDIIDPSQGYEKYVRRENPNNGSRKLAIGKLIVSTNFSVFREQMKGEPIQKQKLDNHCTSLRDGNFVYPCCCVTLDDGQPIES EFKLPTKNHLVIGNSGDPKYVDMPEISKMYIAKDG YCYVNI FLAMLVNVNEAEAKDFTKQVRDVLMEKLGRWPTMFDVATACAFMSVFYPETRNAELPR ILVDHTTKTMHVVD SFGSLSTGYHILKANTVSQLIQFASSSLESEMKHYIVG
P3	352	GITALPDIQERCIRTIKGVYKPRV MYTILSED PYALLLSVMSPRVLLALLNSGSLDRSMEVWITEDQEVA VIIGTLQELAKKVSTSRVLEQQ LKTIETQAHTLLF DPNLVRSRTPSFALSQKIIRGLAERRESNRVLYEQGHSITSYATSHELMEKIWDRLKKEEFGLPWHGKCAQIMQSSKRACGLLSIPIWPRTDALRDRATDL CTTLHTRSVTFKNTCKNGIVQRVVDTHVKCVRAIMRTSFAAIKFAIPDVLKFVNMLLVINLLLQIAKVAREMSTKHRQAQVDL NAYLFDQEIDKVNVIYDAYCL KIGGEPTMDEFLKHIEYINPTLLGTAKWLCYTADMNVEHQ
P3N- PIPO	223	GITALPDIQERCIRTIKGVYKPRV MYTILSED PYALLLSVMSPRVLLALLNSGSLDRSMEVWITEDQEVA VIIGTLQELAKKVSTSRVLEQQ LKTIETQAHTLLF DPNLVRSRTPSFALSQKIIRGLAERRESNRVLYEQGHSITSYATSHELMGKNLGSSFKGGVRRVALAWKMCTNHAIKACVWFTQYSNMAKNRRFRQSN RLMYFTHAECDIQEYL
6KI	52	GKSRKEMQYERIIAFISLLMIVDSERSDCVYKILQKLKGLMSTMNSDVYHQ
CI	643	SLDDITNVLEEKNLTVD FELQSGDHLVNPSTDFDEWWRRIETNNTITHYRTEGYFIEFTRGNAVSVVNNIATIDAKDILVRGAVGSGKSTGLPFYLSRK RVLLLEPTRPLAENVHKQLGGEPFMVQATLRMRGLTIFGSHPINIMTTGFAFHYANNPEQLREYDYIMFDECHVHDSQAMAFRCLLKEHEFNKGILKTSAT PPGREVEFTTQYPVQIKVEERLSFKAFVEAQGTGSNADVVAVADNILVYVASYNEVDELSRMLIEANHVKVTKVDGR TMKVGNAVIAQTGSPQKKHFIVATNII ENGVTL DIEAVDFGTVK TAYLDV DLRALHMSKGPISYGERIQR LGRVGRNKAGVALRIGFTEKGLTEIPQT TATEAAFLCFAYGLPVMTPNVSTSLSTCTV KQARTMLQFELTPFYMVNMVRYDGSMPHAIHNILKKYKLRDAETELNKMAIPNRGVTGWLSVGEYAKSGKKMDIDDSIRIPFLNPSMPEKLHVDIWDVITKY KHEAGFGRISCINSCKIAYTLQTDLYAIPRTIKIIDTLIADEMRRKEHYKTITGRTVSSSSFTLSIATLWRNRYAQDYTSENI AVLSSVKSQ LLEFQNL TMDASF NDIGTHALQAYVRETGATSCVLHQ
6K2	53	TRDSL SKHLGLKG VWNKSVITQDLLILAGVFAGGIWMIMAGVKDSFDQTVLHQ

Annexes

Nla-VPg	192	GKEKRQMQKLRFKARDNKLGFVHADDGTIEHFFGSAYTKKGKQKGKVTGMGSKNRKFINMYGFDPTSEYFVRFVDPLTGAVMDDSPYTDILLVQEKIG EARLNAIKEDELSREKVAQNPGIHAYYINEITNAALKVDLTPHNPLLACERHNTIAGHPEYEGVLRQTGPPIKMTLNDVPRNPEETSLVGHE
Nla-Pro	243	SKSLFRGLRDYNPIASVICHLVNEADGRTSDCFGIGYGGLIVTNRHLFKRNNGTLTIRSRHGEFVIKNTTQLGMKPCADRDILIIRMPKDIPPPQRLKFRVPK ENERICLVGSNFDKSTISTSETSVTCHVPNSHFWKHWIDTKDGHCGPLVSTTDGALLGVHSLSNLTNTQNFFASFPENFEVDYLKTPEAMDWIKRWSY NPDEICWGTLELKTGQPIAPFKVSKLITDLEGIQVYAQ
Nlb	521	TRSDRWVQDRLYGNLKAVGQCPAQLVTKHVVGKCMFLDLYLQQDQSEKEYFKPLMGAYGKSRLNKEAYNKDLFKYATQIQAGDVQVDMFELAERSVV SMLTAKGFEKCNITDPEEILKALNMKAAVGAMYSGKKKDYFEGMSDHDVEDHLFHSCKRLFMGYKGLWNGSLKAELRPMEKVELNKTRTFTAAPLDTLL GGKVCVDDFNNMFYNHHLKCPWTVGITKQYQGWDRLLTSLPEGWIYCDADGSQFDSSLSPYLINSVLNIRREFMEDWDVGDQMLRNLYTEIVYTPILTPD GTIVKKFKGNNSGQPSTVVDNTLMVVLAVHYTLLKLGIQSEFDECCVFFANGDDLLLAMRPDTAHLDDKFSECFSELGLNYDFSSRTSNKEELWFMSHCG VKRDGIFIPKLEPERIVSILEWDRSHEPIHRLAICAAMVESWGYDELLHHRKFYAWVLDQAPYNELARSGKAPYIAETALKALYTGVPQPSASELSAYAKVL NEMYDDSVLQENELEVYHQ
CP	315	SSERTEFKDAGAVPPAPKPKDIPPPPTITEVTDPEDPKQAALRAARAKQPATIPESYGRDTSKEKESIVGASSKGVKDKDVNVGTVGTFVVPVKMNANKK RQPMVNGRAINFQHLSTYEPEQFEVANTRSTQEQQAWYEGVKGDYGVDDTGMGILLNGLMWVCIENGTSPPNINGVWTMMMDGDEQVTYPIKPLLDHAV PTFRQIMTHFSDVAEAYIEMRNRTKAYMPRYGLQRNLTDMSLARYAFDFYELHSTTPARAKEAHLQMKAAALKNAKNRLFGLDGNVSTQEEDTERHTTTD VTRNIHNLLGMRGVQ

Table S3. Percentage of mapped read counts for each T0 sample, as determined using the Genome Detective platform.

Plant virus species	V_SP8	V_SP7	V_SP6	V_SP5	V_SP4	V_SP3	V_SP2	V_SP1	Total
<i>Badnavirus occultiipomeae</i>	0,009635776	0,000837163	0,001179842	0,020620033	0,006706354	0,031319919	0,045273358	0,010694868	0,126267312
<i>Begomovirus ipomoeacaryense</i>	0,023438713	0	0,00208949	0,008268772	0,049978866	0,22102738	0,015623409	0,031825416	0,352252045
<i>Begomovirus ipomoeae</i>	0,047553182	0,007669886	0,000810578	0	0,046743758	0,007891633	0,054811406	0,043557031	0,209037475
<i>Begomovirus ipomoeahenanense</i>	0	0	0	0	0,002337778	0	0	0	0,002337778
<i>Begomovirus ipomoeamusivi</i>	0,01071198	0	0	0	0,006446601	0	0,000474744	0,00231904	0,019952365
<i>Begomovirus ipomoeasaopauloense</i>	0	0	0	0	0,000188911	0	0	0,000341035	0,000529947
<i>Begomovirus ipomoeasouthcarolinaense</i>	0,001288942	6,16E-05	7,21E-05	0	0,016305413	0,006288645	0,001035806	0,001609687	0,0266621
<i>Deltasatellite ipomoeaprimi</i>	0,000738326	0	0	0	0,001357801	0	0,001898978	0,000886692	0,004881796
<i>Mastrevirus ipomoeae</i>	0,002778107	0,000824851	7,21E-05	0,007576824	0,001168889	0,014118625	0,001467392	0,001855232	0,029861972
<i>Potyvirus batataplumei</i>	0,914259986	0,217649947	4,2417915	0,0052588	0,210388236	0,00419243	0,227316278	0,002782848	5,823640026
<i>Potyvirus cebatatae</i>	0,935233442	0	0	0	0,065882842	0	0	0,000272828	1,001389112
<i>Potyvirus duobatatae</i>	0	0	0	0	0	0	0	0,00038196	0,00038196
<i>Sweet potato mosaic virus - [Brazil:Brasilia1:2007]</i>	0	0	0	0	0,003471247	0	0	0,000736636	0,004207883
<i>Crinivirus ipomeae</i>	0	0	0	0	0	0	0	0	0
TOTAL PLANT VIRUSES									7,60140177
Non plant virus species	V_SP8	V_SP7	V_SP6	V_SP5	V_SP4	V_SP3	V_SP2	V_SP1	Total
<i>Epsilonentomopoxvirus dlongicaudata</i>	0,014078245	0,009590435	0,00483645	0,040651911	0,007709945	0,107770114	0,065946321	0,014187069	0,264770491
<i>Unuamitovirus fuan1</i>	0	0	0	0	0	0	0	0,000272828	0,000272828
<i>Unuamitovirus fuci2</i>	0	0	0	0	0	0	0	0,00028647	0,00028647
<i>Wenzhou tombus-like virus 1</i>	0	0	0	0	0	0	0	0,017147256	0,017147256
<i>Wenzhou tombus-like virus 3</i>	0	0	0	0	0	0	0	0,068016084	0,068016084
<i>Bracoviriform glomeratae</i>	0,009022591	0,011326317	0,006502638	0,039579393	0,004392189	0,522882345	0,035951103	0,013368585	0,64302516
<i>Shahe tombus-like virus 1</i>	0	0	0	0	0	0	0	0,021839902	0,021839902
TOTAL NON-PLANT VIRUSES									1,015358192

Annexes

Viruses	Count of mapped reads	%
Plant viruses	7,60140177	88,216473
Non-plant viruses	1,015358192	11,783526
	8,616759962	100

Table S4. Aggregation of plant viruses reads in T0-sample extracted from Genome Detective platform.

Plant viral species in V_SP1,2,3,4,5,6,7,8	Aggregation	%
<i>Potyvirus batataplumei</i>	5,823640026	76,61271174
<i>Potyvirus cebatatae</i>	1,001389112	13,17374271
<i>Begomovirus ipomoeacanaryense</i>	0,352252045	4,634040613
<i>Begomovirus ipomoeae</i>	0,209037475	2,749985871
<i>Badnavirus occultipomeae</i>	0,126267312	1,661105619
<i>Mastrevirus ipomoeae</i>	0,029861972	0,392848227
<i>Begomovirus ipomoeasouthcarolinaense</i>	0,0266621	0,35075241
<i>Begomovirus ipomoeamusivi</i>	0,019952365	0,262482709
<i>Deltasatellite ipomoeaprimi</i>	0,004881796	0,064222311
<i>Sweet potato mosaic virus - [Brazil:Brasilia1:2007]</i>	0,004207883	0,055356669
<i>Begomovirus ipomoeahenanense</i>	0,002337778	0,030754568
<i>Begomovirus ipomoeasaopauloense</i>	0,000529947	0,006971697
<i>Potyvirus duobatatae</i>	0,00038196	0,005024857
<i>Crinivirus ipomeae</i>	0	0
Total	7,60140177	100

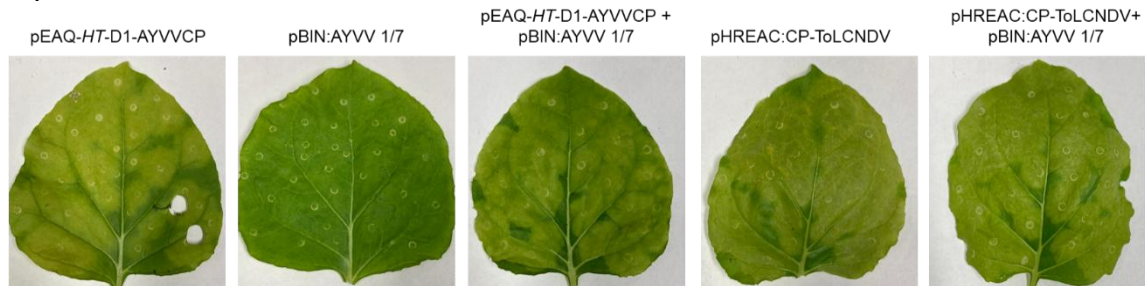
Table S5. Sample reads count percentage of mapped reads for all samples (T0 and T30) extracted from Genome Detective platform.

Plant virus specie (T0)	V_SP8	V_SP7	V_SP6	V_SP5	V_SP4	V_SP3	V_SP2	V_SP1	Total
<i>Potyvirus batataplumei</i>	0,914259986	0,217649947	4,2417915	0,0052588	0,210388236	0,00419243	0,227316278	0,002782848	5,823640026
<i>Crinivirus ipomeae</i>	0	0	0	0	0	0	0	0	0
Plant virus specie (T30)	V_SP16	V_SP15	V_SP14	V_SP13	V_SP12	V_SP11	V_SP10	V_SP9	Total
<i>Potyvirus batataplumei</i>	0,215193032	24,57058552	9,285678299	6,255454734	0,040124725	1,227273007	0,008986775	4,919542814	46,52283891
<i>Crinivirus ipomeae</i>	0,863062095	4,16541038	0,026311899	1,209573969	0,018192269	0	0	0,103927602	6,386478214

Read count % of read mapped		
	T0	T30
<i>Potyvirus batataplumei</i>	5,823640026	46,52283891
<i>Crinivirus ipomeae</i>	0	6,386478214

Figure S1. A) Detail of *N. benthamiana* agroinfiltrated leaves at 7 dpa. The constructs are indicated at the top of each image. Necrotic areas are observed in all treatments except for the AYVV alphasatellite DNA alone (pBIN:AYVV 1/7). B) Clustal Omega amino acid alignment of ToLCNDV CP and AYVV CP, showing 81% sequence identity.

A)



B)

CP_ToLCNDV	MSKRPADIIISTPASKVRRRLNFDSPYGTASVPIARV-TKARAWTNRPMNRKPRMYRMY	59
CP_AYVV	MSKRPADIVISTPASKVRRRLNFDTPVMSRAAAPTVLVTNRRRTWTNRPYRKPRLYRMY	60
	*****:*****:* :*:.* . * .: :***** *****:****	
CP_ToLCNDV	RSPDVPRGCEGPCVKQSFESRHDVSHIGKVMCVDVTRGTGLTHRVGKRFCVKS VYVLGK	119
CP_AYVV	RTPDVPKGCCEGPCVKQSYEQRHDI SHVGKVLCSVDVTRGNGLTHRVGKRFCVKS VYVLGK	120
	*:*****:*****:*.****:***:***:*****.*****	
CP_ToLCNDV	IWMDENIKTKNHTNSVMF FLVRDRRPTGT PQDFGEVFNMF DNEPSTATVKNMHRDRYQVL	179
CP_AYVV	IWMDENIKTKNHTNTVMFYLVRDRRPFGTAMDFGQVFNMYDNEPSTATIKNDLRDRYQVL	180
	*****:***:***** ** ***:***:*****:*** *****	
CP_ToLCNDV	RKWHATVTGGTYASREQALVRKFVRVNNYVVYNQQEAGKYENHTENALMLYMACTHASNP	239
CP_AYVV	RKFTSTVTGGQYASKEQALVKKFMKINNYVVYNHQEAAKYDNHTENALLYMACTHASNP	240
	: :*** ***:*****:***: :*****:***.*:*****:*****	
CP_ToLCNDV	VYATLKIRIYFYDSVTN*	256
CP_AYVV	VYATLKIRIYFYDSVQN*	257
	***** **	

BIBLIOGRAPHY

9. BIBLIOGRAPHY

- Abraham, A. (2024). Synergistic crop virus disease complexes in Sub-saharan Africa: causes, consequences and control. *Phytoparasitica*, 52(1). <https://doi.org/10.1007/s12600-024-01143-9>
- Abrescia, N. G., Bamford, D. H., Grimes, J. M., & Stuart, D. I. (2012). Structure Unifies the Viral Universe. *Annual Review of Biochemistry*, 81(1), 795-822. <https://doi.org/10.1146/annurev-biochem-060910-095130>
- Adam, R. I., Sindi, K., & Badstue, L. (2015). Farmers' knowledge, perceptions and management of diseases affecting sweet potatoes in the Lake Victoria Zone region, Tanzania. *Crop Protection*, 72, 97-107. <https://doi.org/10.1016/j.cropro.2015.02.010>
- Adams, M. J., Antoniw, J. F., & Beaudoin, F. (2005). Overview and analysis of the polyprotein cleavage sites in the family Potyviridae. *Molecular Plant Pathology*, 6(4), 471-487. <https://doi.org/10.1111/j.1364-3703.2005.00296.x>
- Adams, M. J., Antoniw, J. F., & Fauquet, C. M. (2005). Molecular criteria for genus and species discrimination within the family Potyviridae. *Archives of Virology*, 150(3), 459-479. <https://doi.org/10.1007/s00705-004-0440-6>
- Adero, J., Ssali, R., Segundo, F., Maria, D., Kitavi, M., Yada, B., Byarugaba, D. K., Dube, F., Aber, P. P., Opiyo, S. O., Fei, Z., & Kreuze, J. F. (2025). Virus-Specific Defense Responses in Sweetpotato: Transcriptomic Insights into Resistance and Susceptibility to SPFMV, SPCSV, and SPVD. *Biology*, 14(11), 1541. <https://doi.org/10.3390/biology14111541>
- Adero, J., Wokorach, G., Stomeo, F., Yao, N., Machuka, E., Njuguna, J., Byarugaba, D. K., Kreuze, J., Yencho, G. C., Otema, M. A., Yada, B., & Kitavi, M. (2024). Next Generation Sequencing and Genetic Analyses Reveal Factors Driving Evolution of Sweetpotato Viruses in Uganda. *Pathogens*, 13(10), 833. <https://doi.org/10.3390/pathogens13100833>
- Adikini, S., Mukasa, S. B., Mwanga, R. O., & Gibson, R. W. (2015). Sweet potato cultivar degeneration rate under high and low sweet potato virus disease pressure zones in Uganda. *Canadian Journal of Plant Pathology*, 37(1), 136-147. <https://doi.org/10.1080/07060661.2015.1004111>
- Agirrezabala, X., Mendez-Lopez, E., Lasso, G., Sanchez-Pina, M. A., Aranda, M., & Valle, M. (2015). The near-atomic cryoEM structure of a flexible filamentous plant virus shows homology of its coat protein with nucleoproteins of animal viruses. *Elife*, 4, e11795. <https://doi.org/10.7554/eLife.11795>
- Agranovsky, A. (2021). Enhancing Capsid Proteins Capacity in Plant Virus-Vector Interactions and Virus Transmission. *Cells*, 10(1), 90. <https://doi.org/10.3390/cells10010090>
- Agranovsky, A. A. (2016). Closteroviruses: molecular biology, evolution and interactions with cells. In *Plant viruses: evolution and management* (pp. 231-252). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-10-1406-2_14
- Agranovsky, A. A., Lesemann, D. E., Maiss, E., Hull, R., & Atabekov, J. G. (1995). «Rattlesnake» structure of a filamentous plant RNA virus built of two capsid proteins. *Proceedings of*

Bibliography

- The National Academy of Sciences*, 92(7), 2470-2473.
<https://doi.org/10.1073/pnas.92.7.2470>
- Ahlquist, P., Noueiry, A. O., Lee, W. M., Kushner, D. B., & Dye, B. T. (2003). Host factors in positive-strand RNA virus genome replication. *Journal of virology*, 77(15), 8181-8186.
<https://doi.org/10.1128/jvi.77.15.8181-8186.2003>
- Ahmed, N., Amin, I., & Mansoor, S. (2021). Cotton leaf curl disease (Geminiviridae).
<https://doi.org/10.1016/B978-0-12-814515-9.00011-4>
- Alcaide, C., Rabadán, M. P., Moreno-Pérez, M. G., & Gómez, P. (2020). Implications of mixed viral infections on plant disease ecology and evolution. *Advances in Virus Research*, 106, 145-169. <https://doi.org/10.1016/bs.aivir.2020.02.001>
- Almeyda, C. V., Abad, J. A., & Pesic-VanEsbroeck, Z. (2013). First report of Sweet potato virus G and Sweet potato virus 2 infecting sweetpotato in North Carolina. *Plant Disease*, 97(11), 1516-1516. <https://doi.org/10.1094/PDIS-04-13-0359-PDN>
- Amin, I., Patil, B. L., Briddon, R. W., Mansoor, S., & Fauquet, C. M. (2011). Comparison of phenotypes produced in response to transient expression of genes encoded by four distinct begomoviruses in *Nicotiana benthamiana* and their correlation with the levels of developmental miRNAs. *Virology Journal*, 8(1), 238. <https://doi.org/10.1186/1743-422x-8-238>
- Anandalakshmi, R., Pruss, G. J., Ge, X., Marathe, R., Mallory, A. C., Smith, T. H., & Vance, V. B. (1998). A viral suppressor of gene silencing in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 95(22), 13079-13084. <https://doi.org/10.1073/pnas.95.22.13079>
- Anderson, P. K., Cunningham, A. A., Patel, N. G., Morales, F. J., Epstein, P. R., & Daszak, P. (2004). Emerging infectious diseases of plants: pathogen pollution, climate change and agrotechnology drivers. *Trends in Ecology & Evolution*, 19(10), 535-544. <https://doi.org/10.1016/j.tree.2004.07.021>
- Anindya, R., & Savithri, H. S. (2004). Potyviral NIa proteinase, a proteinase with novel deoxyribonuclease activity. *Journal of Biological Chemistry*, 279(31), 32159-32169. <https://doi.org/10.1074/jbc.M404135200>
- Anwar, I., Bukhari, H. A., Nahid, N., Rashid, K., Amin, I., Shaheen, S., Hussain, K., & Mansoor, S. (2019). Association of cotton leaf curl Multan betasatellite and Ageratum conyzoides symptomless alphasatellite with tomato leaf curl New Delhi virus in *Luffa cylindrica* in Pakistan. *Australasian Plant Pathology*, 49(1), 25-29. <https://doi.org/10.1007/s13313-019-00668-6>
- Aranda, M. A., & Freitas-Astúa, J. (2017). Ecology and diversity of plant viruses, and epidemiology of plant virus-induced diseases. *Annals of Applied Biology*, 171(1), 1-4. <https://doi.org/10.1111/aab.12361>
- 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 physiology*, 148(1), 436-454. <https://doi.org/10.1104/pp.108.121038>

- Ateka, E. M., Barg, E., Njeru, R. W., Lesemann, D. E., & Vetten, H. J. (2004). Further characterization of 'sweet potato virus 2': a distinct species of the genus Potyvirus. *Archives of Virology*, 149(2), 225-239. <https://doi.org/10.1007/s00705-003-0233-3>
- Ateka, E. M., Barg, E., Njeru, R. W., Thompson, G., & Vetten, H. J. (2006). Biological and molecular variability among geographically diverse isolates of sweet potato virus 2. *Archives of Virology*, 152(3), 479-488. <https://doi.org/10.1007/s00705-006-0879-8>
- Atreya, C. D., & Pirone, T. P. (1993). Mutational analysis of the helper component-proteinase gene of a potyvirus: effects of amino acid substitutions, deletions, and gene replacement on virulence and aphid transmissibility. *Proceedings of the National Academy of Sciences of the United States of America*, 90(24), 11919-11923. <https://doi.org/10.1073/pnas.90.24.11919>
- Atreya, P. L., & López-Moya, J. J. (1995). Mutational analysis of the coat protein N-terminal amino acids involved in potyvirus transmission by aphids. *Journal of General Virology*, 76(2), 265-270. <https://doi.org/10.1099/0022-1317-76-2-265>
- Atreya, P. L., Atreya, C. D., & Pirone, T. P. (1991). Amino acid substitutions in the coat protein result in loss of insect transmissibility of a plant virus. *Proceedings of the National Academy of Sciences*, 88(17), 7887-7891. <https://doi.org/10.1073/pnas.88.17.7887>
- Austin, D. F. (1987). The taxonomy, evolution and genetic diversity of sweet potatoes and related wild species. In *Exploration, Maintenance, and Utilization of Sweet Potato Genetic Resources' Report. of the 1st Sweet Potato Planning Conference*. (pp. 27-59). International Potato Center.
- Balke, I., & Zeltins, A. (2020). Recent advances in the use of plant virus-like particles as vaccines. *Viruses*, 12(3), 270. <https://doi.org/10.3390/v12030270>
- Baltimore, D. (1971). Expression of animal virus genomes. *Bacteriological reviews*, 35(3), 235-241. <https://doi.org/10.1128/br.35.3.235-241.1971>
- Baulcombe, D. (2004). RNA silencing in plants. *Nature*, 431(7006), 356-363. <https://doi.org/10.1038/nature02874>
- Baulcombe, D. C. (2022). The Role of Viruses in Identifying and Analyzing RNA Silencing. *Annual Review of Virology*, 9(1), 353-373. <https://doi.org/10.1146/annurev-virology-091919-064218>
- Becker, N., Rimbaud, L., Chiroleu, F., Reynaud, B., Thébaud, G., & Lett, J. M. (2015). Rapid accumulation and low degradation: key parameters of Tomato yellow leaf curl virus persistence in its insect vector Bemisia tabaci. *Scientific Reports*, 5(1), 17696. <https://doi.org/10.1038/srep17696>
- Bedford, I. D., Briddon, R. W., Brown, J. K., Rosell, R. C., & Markham, P. G. (1994). Geminivirus transmission and biological characterisation of Bemisia tabaci (Gennadius) biotypes from different geographic regions. *Annals of applied biology*, 125(2), 311-325. <https://doi.org/10.1111/j.1744-7348.1994.tb04972.x>
- Bednarek, R., David, M., Fuentes, S., Kreuze, J., & Fei, Z. (2021). Transcriptome analysis provides insights into the responses of sweet potato to sweet potato virus disease (SPVD). *Virus Research*, 295, 198293. <https://doi.org/10.1016/j.virusres.2020.198293>

Bibliography

- Beijerinck, M. W. (1898). Concerning a contagium vivum fluidum as a cause of the spot-disease of Tobacco leaves. *Koninklijke Nederlandsche Akademie van Wetenschappen Proceedings*, 1, 170-176.
- Bennett, A., & Agbandje-McKenna, M. (2020). Geminivirus structure and assembly. *Advances in Virus Research*, 108, 1-32. <https://doi.org/10.1016/bs.aivir.2020.09.005>
- Bernal, J. D., & Fankuchen, I. (1941). X-ray and crystallographic studies of plant virus preparations: I. Introduction and preparation of specimens II. Modes of aggregation of the virus particles. *The Journal of General Physiology*, 25(1), 111. <https://doi.org/10.1085/jgp.25.1.111>
- Bhattacharyya, D., & Chakraborty, S. (2017). Chloroplast: the Trojan horse in plant-virus interaction. *Molecular Plant Pathology*, 19(2), 504-518. <https://doi.org/10.1111/mpp.12533>
- Blackman, R. L., & Eastop, V. F. (2007). *Aphids as Crop Pests*, CABI (H. F. Van Emden & R. Harrington, Eds.). <https://doi.org/10.1079/9780851998190.0000>
- Blanc, S., Ammar, E. D., Garcia-Lampasona, S., Dolja, V. V., Llave, C., Baker, J., & Pirone, T. P. (1998). Mutations in the potyvirus helper component protein: effects on interactions with virions and aphid stylets. *Journal of General Virology*, 79(12), 3119-3122. <https://doi.org/10.1099/0022-1317-79-12-3119>
- Blanc, S., Drucker, M., & Uzest, M. (2014). Localizing viruses in their insect vectors. *Annual review of phytopathology*, 52(1), 403-425. <https://doi.org/10.1146/annurev-phyto-102313-045920>
- Blanc, S., López-Moya, J. J., Wang, R., García-Lampasona, S., Thornbury, D. W., & Pirone, T. P. (1997). A specific interaction between coat protein and helper component correlates with aphid transmission of a potyvirus. *Virology*, 231(1), 141-147. <https://doi.org/10.1006/viro.1997.8521>
- Bonnamy, M., Blanc, S., & Michalakakis, Y. (2023). Replication mechanisms of circular ssDNA plant viruses and their potential implication in viral gene expression regulation. *mBio*, 14(5), e0169223. <https://doi.org/10.1128/mbio.01692-23>
- Bonnet, D. M. V. J., Chaves-Sanjuan, A., Contaldo, N., De Stradis, A., Caliandro, R., Minafra, A., & Geuna, F. (2026). Structural characterization of plum pox virus by cryo-electron microscopy. *Archives of Virology*, 171(1), 11. <https://doi.org/10.1007/s00705-025-06473-5>
- Boulton, M. I. (2008). Construction of infectious clones for DNA viruses: mastreviruses. In *Plant Virology Protocols: From Viral Sequence to Protein Function* (pp. 503-523). Totowa, NJ: Humana Press. https://doi.org/10.1007/978-1-59745-102-4_34
- Boulton, M. I., & Markham, P. G. (1986). The use of squash-blotting to detect plant pathogens in insect vectors. *Developments and applications in virus testing*, 55-69.
- Bragard, C., Caciagli, P., Lemaire, O., López-Moya, J. J., MacFarlane, S., Peters, D., ... & Torrance, L. (2013). Status and prospects of plant virus control through interference with vector transmission. *Annual review of phytopathology*, 51(1), 177-201. <https://doi.org/10.1146/annurev-phyto-082712-102346>

- Briddon, R. W., Brown, J. K., Moriones, E., Stanley, J., Zerbini, M., Zhou, X., & Fauquet, C. M. (2008). Recommendations for the classification and nomenclature of the DNA- β satellites of begomoviruses. *Archives of Virology*, 153(4), 763–781. <https://doi.org/10.1007/s00705-007-0013-6>
- Briddon, R. W., Bull, S. E., & Bedford, I. D. (2006). Occurrence of Sweet potato leaf curl virus in Sicily. *Plant Pathology*, 55(2). <https://doi.org/10.1111/j.1365-3059.2005.01273.x>
- Briddon, R. W., Bull, S. E., Amin, I., Idris, A. M., Mansoor, S., Bedford, I. D., Dhawan, P., Rishi, N., Siwatch, S. S., Salam, A. S., Brown, A. M., Zafar, J. K., & Markham, Y. (2003). Diversity of DNA β : a satellite molecule associated with some monopartite begomoviruses. *Virology*, 312 (1), 106–121. [https://doi.org/10.1016/S0042-6822\(03\)00200-9](https://doi.org/10.1016/S0042-6822(03)00200-9)
- Briddon, R. W., Mansoor, S., Bedford, I. D., Pinner, M. S., Saunders, K., Stanley, J., Zafar, Y., Malik, K. A., & Markham, P. G. (2001). Identification of dna components required for induction of cotton leaf curl disease. *Virology*, 285(2), 234–243. <https://doi.org/10.1006/viro.2001.0949>
- Briddon, Rob W., Bull, S. E., Amin, I., Mansoor, S., Bedford, I. D., Rishi, N., Siwatch, S. S., Zafar, Y., Abdel-Salam, A. M., & Markham, P. G. (2004). Diversity of DNA β : a satellite-like molecule associated with monopartite begomovirus-DNA β complexes. *Virology*, 324(2), 462–474. <https://doi.org/10.1016/j.virol.2004.03.041>
- Briddon, Rob W., Martin, D. P., Roumagnac, P., Navas-Castillo, J., Fiallo-Olivé, E., Moriones, E., Lett, J.-M., Zerbini, F. M., & Varsani, A. (2018). Alphasatellitidae: a new family with two subfamilies for the classification of geminivirus- and nanovirus-associated alphasatellites. *Archives of Virology*, 163(9), 2587–2600. <https://doi.org/10.1007/s00705-018-3854-2>
- Briddon, Robert W., Watts, J., Markham, P. G., & Stanley, J. (1989). The coat protein of beet curly top virus is essential for infectivity. *Virology*, 172(2), 628–633. [https://doi.org/10.1016/0042-6822\(89\)90205-5](https://doi.org/10.1016/0042-6822(89)90205-5)
- Brigneti, G., Voinnet, O., Li, W.-X., Ji, L.-H., Ding, S.-W., & Baulcombe, D. C. (2015). Retraction: 'Viral pathogenicity determinants are suppressors of transgene silencing in *Nicotiana benthamiana*'. *The EMBO Journal*, 34(20), 2595–2595. <https://doi.org/10.15252/embj.201570030>
- Brough, C. L., Sunter, G., Gardiner, W. E., & Bisaro, D. M. (1992). Kinetics of tomato golden mosaic virus DNA replication and coat protein promoter activity in *Nicotiana tabacum* protoplasts. *Virology*, 187(1), 1–9. [https://doi.org/10.1016/0042-6822\(92\)90289-2](https://doi.org/10.1016/0042-6822(92)90289-2)
- Brown, J. K. (1995). The Sweetpotato or Silverleaf Whiteflies: Biotypes of *Bemisia tabaci* or a Species Complex? *Annual Review of Entomology*, 40(1), 511–534. <https://doi.org/10.1146/annurev.ento.40.1.511>
- Brown, J. K., Bird, J., Frohlich, D., Rosell, R. C., Bedford, I. D., & Markham, P. G. (1996). The relevance of variability within the *Bemisia tabaci* species complex to epidemics caused by subgroup III geminiviruses. *Bemisia*, 95.
- Buyel, J. F. (2024). Towards a seamless product and process development workflow for recombinant proteins produced by plant molecular farming. *Biotechnology Advances*, 75, 108403. <https://doi.org/10.1016/j.biotechadv.2024.108403>

Bibliography

- Byers, J. A. (2008). Aphids as Crop Pests. *Crop Science*, 48(3), 1219-1220. <https://doi.org/10.2135/cropsci2008.02.0001br>
- Caciagli, P., Medina Piles, V., Marian, D., Vecchiati, M., Masenga, V., Mason, G., Falcioni, T., & Noris, E. (2009). Virion stability is important for the circulative transmission of *Tomato yellow leaf curl Sardinia virus* by *Bemisia tabaci*, but virion access to salivary glands does not guarantee transmissibility. *Journal of Virology*, 83(11), 5784–5795. <https://doi.org/10.1128/jvi.02267-08>
- Canto, T., Aranda, M. A., & Fereres, A. (2009). Climate change effects on physiology and population processes of hosts and vectors that influence the spread of hemipteran-borne plant viruses. *Global Change Biology*, 15(8), 1884–1894. <https://doi.org/10.1111/j.1365-2486.2008.01820.x>
- Cao, M., Lan, P., Li, F., Abad, J., Zhou, C., & Li, R. (2017). Genome characterization of sweet potato symptomless virus 1: a mastrevirus with an unusual nonanucleotide sequence. *Archives of virology*, 162(9), 2881-2884. <https://doi.org/10.1007/s00705-017-3396-z>
- Carrington, J. C., & Freed, D. D. (1990). Cap-independent enhancement of translation by a plant potyvirus 5' nontranslated region. *Journal of Virology*, 64(4), 1590–1597. <https://doi.org/10.1128/JVI.64.4.1590-1597.1990>
- Carrington, J. C., Cary, S. M., Parks, T. D., & Dougherty, W. G. (1989). A second proteinase encoded by a plant potyvirus genome. *The EMBO journal*, 8(2), 365-370. <https://doi.org/10.1002/j.1460-2075.1989.tb03386.x>
- Casado, C. G., Javier Ortiz, G., Padron, E., Bean, S. J., McKenna, R., Agbandje-McKenna, M., & Boulton, M. I. (2004). Isolation and characterization of subgenomic DNAs encapsidated in “single” T = 1 isometric particles of Maize streak virus. *Virology*, 323(1), 164–171. <https://doi.org/10.1016/j.virol.2004.02.014>
- Chakraborty, S. (2008). Tomato leaf curl viruses from India (Geminiviridae). In B. W. J. Mahy & M. H. V. Van Regenmortel (Eds.), *Encyclopedia of Virology* (pp. 124–133). Elsevier.
- Chase, O., Javed, A., Byrne, M. J., Thuenemann, E. C., Lomonossoff, G. P., Ranson, N. A., & López-Moya, J. J. (2023). CryoEM and stability analysis of virus-like particles of potyvirus and ipomovirus infecting a common host. *Communications Biology*, 6(1), 433. <https://doi.org/10.1038/s42003-023-04799-x>
- Chen, A. Y., Walker, G. P., Carter, D., & Ng, J. C. (2011). A virus capsid component mediates virion retention and transmission by its insect vector. *Proceedings of the National Academy of Sciences*, 108(40), 16777-16782. <https://doi.org/10.1073/pnas.1109384108>
- Cheng, X., & Wang, A. (2017). The Potyvirus silencing suppressor protein VPg mediates degradation of SGS3 via ubiquitination and autophagy pathways. *Journal of Virology*, 91(1). <https://doi.org/10.1128/JVI.01478-16>
- Chiu, C.-W., Li, Y.-R., Lin, C.-Y., Yeh, H.-H., & Liu, M.-J. (2022). Translation initiation landscape profiling reveals hidden open-reading frames required for the pathogenesis of tomato yellow leaf curl Thailand virus. *The Plant Cell*, 34(5), 1804–1821. <https://doi.org/10.1093/plcell/koac019>

- Chiu, M.-H., Chen, I.-H., Baulcombe, D. C., & Tsai, C.-H. (2010). The silencing suppressor P25 of Potato virus X interacts with Argonaute1 and mediates its degradation through the proteasome pathway: PVX silencing suppressor P25 targets AGO1. *Molecular Plant Pathology*, 11(5), 641–649. <https://doi.org/10.1111/j.1364-3703.2010.00634.x>
- Chomicki, G., Schaefer, H., & Renner, S. S. (2020). Origin and domestication of Cucurbitaceae crops: insights from phylogenies, genomics and archaeology. *The New Phytologist*, 226(5), 1240–1255. <https://doi.org/10.1111/nph.16015>
- Chung, B. Y.-W., Miller, W. A., Atkins, J. F., & Firth, A. E. (2008). An overlapping essential gene in the Potyviridae. *Proceedings of the National Academy of Sciences of the United States of America*, 105(15), 5897–5902. <https://doi.org/10.1073/pnas.0800468105>
- Clark, C. A., Davis, J. A., Abad, J. A., Cuellar, W. J., Fuentes, S., Kreuze, J. F., Gibson, R. W., Mukasa, S. B., Tugume, A. K., Tairo, F. D., & Valkonen, J. P. T. (2012). Sweetpotato viruses: 15 years of progress on understanding and managing complex diseases. *Plant Disease*, 96(2), 168–185. <https://doi.org/10.1094/PDIS-07-11-0550>
- Claverie, S., Varsani, A., Hoareau, M., Filloux, D., Roumagnac, P., Martin, D. P., Lefeuvre, P., & Lett, J.-M. (2020). Sorghum mastrevirus-associated alphasatellites: new geminialphasatellites associated with an African streak mastrevirus infecting wild Poaceae plants on Reunion Island. *Archives of Virology*, 165(8), 1925–1928. <https://doi.org/10.1007/s00705-020-04685-5>
- Creager, A. N. H. (2022). Tobacco mosaic virus and the history of molecular biology. *Annual Review of Virology*, 9(1), 39–55. <https://doi.org/10.1146/annurev-virology-100520-014520>
- Crespo, O., Robles, C., Ruiz Rosado, L., & Janssen, D. (2020). Antagonism of Cucumber green mottle mosaic virus against Tomato leaf curl New Delhi virus in zucchini and cucumber. *The Annals of Applied Biology*, 176(2), 147–157. <https://doi.org/10.1111/aab.12535>
- Csorba, T., Kontra, L., & Burgyán, J. (2015). viral silencing suppressors: Tools forged to fine-tune host-pathogen coexistence. *Virology*, 479–480, 85–103. <https://doi.org/10.1016/j.virol.2015.02.028>
- Cuellar, W. J., Galvez, M., Fuentes, S., Tugume, J., & Kreuze, J. (2015). Synergistic interactions of begomoviruses with Sweet potato chlorotic stunt virus (genus *Crinivirus*) in sweet potato (*Ipomoea batatas* L.). *Molecular Plant Pathology*, 16(5), 459–471. <https://doi.org/10.1111/mpp.12200>
- Cuellar, W. J., Kreuze, J. F., Rajamäki, M.-L., Cruzado, K. R., Untiveros, M., & Valkonen, J. P. T. (2009). Elimination of antiviral defense by viral RNase III. *Proceedings of the National Academy of Sciences of the United States of America*, 106(25), 10354–10358. <https://doi.org/10.1073/pnas.0806042106>
- Cuellar, W. J., Tairo, F., Kreuze, J. F., & Valkonen, J. P. T. (2008). Analysis of gene content in sweet potato chlorotic stunt virus RNA1 reveals the presence of the p22 RNA silencing suppressor in only a few isolates: implications for viral evolution and synergism. *The Journal of General Virology*, 89(2), 573–582. <https://doi.org/10.1099/vir.0.83471-0>

Bibliography

- Cuesta, R., Yuste-Calvo, C., Gil-Cartón, D., Sánchez, F., Ponz, F., & Valle, M. (2019). Structure of Turnip mosaic virus and its viral-like particles. *Scientific Reports*, 9(1). <https://doi.org/10.1038/s41598-019-51823-4>
- Cui, H., & Wang, A. (2016). Plum pox virus 6K1 protein is required for viral replication and targets the viral replication complex at the early stage of infection. *Journal of Virology*, 90(10), 5119–5131. <https://doi.org/10.1128/JVI.00024-16>
- Cui, H., & Wang, A. (2019). The biological impact of the hypervariable N-terminal region of potyviral genomes. *Annual Review of Virology*, 6(1), 255–274. <https://doi.org/10.1146/annurev-virology-092818-015843>
- Cui, X., Yaghmaiean, H., Wu, G., Wu, X., Chen, X., Thorn, G., & Wang, A. (2017). The C-terminal region of the Turnip mosaic virus P3 protein is essential for viral infection via targeting P3 to the viral replication complex. *Virology*, 510, 147–155. <https://doi.org/10.1016/j.virol.2017.07.016>
- Czosnek, H., Hariton-Shalev, A., Sobol, I., Gorovits, R., & Ghanim, M. (2017). The incredible journey of begomoviruses in their Whitefly vector. *Viruses*, 9(10), 273. <https://doi.org/10.3390/v9100273>
- da Fonseca Barbosa, L., Yuki, V. A., Marubayashi, J. M., De Marchi, B. R., Perini, F. L., Pavan, M. A., de Barros, D. R., Ghanim, M., Moriones, E., Navas-Castillo, J., & Krause-Sakate, R. (2015). First report of Bemisia tabaci Mediterranean (Q biotype) species in Brazil: Bemisia tabaciQ biotype in Brazil. *Pest Management Science*, 71(4), 501–504. <https://doi.org/10.1002/ps.3909>
- da Veiga Leprevost, F., Grüning, B. A., Alves Aflitos, S., Röst, H. L., Uszkoreit, J., Barsnes, H., Vaudel, M., Moreno, P., Gatto, L., Weber, J., Bai, M., Jimenez, R. C., Sachsenberg, T., Pfeuffer, J., Vera Alvarez, R., Griss, J., Nesvizhskii, A. I., & Perez-Riverol, Y. (2017). BioContainers: an open-source and community-driven framework for software standardization. *Bioinformatics (Oxford, England)*, 33(16), 2580–2582. <https://doi.org/10.1093/bioinformatics/btx192>
- De Barro, P. J., Liu, S.-S., Boykin, L. M., & Dinsdale, A. B. (2011). Bemisia tabaci: A Statement of Species Status. *Annual Review of Entomology*, 56(1), 1–19. <https://doi.org/10.1146/annurev-ento-112408-085504>
- De Barro, P., & Khan, S. (2007). Adult Bemisia tabaci biotype B can induce silverleafing in squash. *Bulletin of Entomological Research*, 97(4), 433–436. <https://doi.org/10.1017/S0007485307005226>
- De Moya-Ruiz, C., Rabadán, P., Juárez, M., & Gómez, P. (2021). Assessment of the current status of potyviruses in watermelon and pumpkin crops in Spain: Epidemiological impact of cultivated plants and mixed infections. *Plants*, 10(1), 138. <https://doi.org/10.3390/plants10010138>
- Di Tommaso, P., Chatzou, M., Floden, E. W., Barja, P. P., Palumbo, E., & Notredame, C. (2017). Nextflow enables reproducible computational workflows. *Nature Biotechnology*, 35(4), 316–319. <https://doi.org/10.1038/nbt.3820>

- Diaz-Pendon, J. A., Li, F., Li, W.-X., & Ding, S.-W. (2007). Suppression of antiviral silencing by cucumber mosaic virus 2b protein in Arabidopsis is associated with drastically reduced accumulation of three classes of viral small interfering RNAs. *The Plant Cell*, 19(6), 2053–2063. <https://doi.org/10.1105/tpc.106.047449>
- Ding, L.-N., Li, Y.-T., Wu, Y.-Z., Li, T., Geng, R., Cao, J., Zhang, W., & Tan, X.-L. (2022). Plant disease resistance-related signaling pathways: Recent progress and future prospects. *International Journal of Molecular Sciences*, 23(24), 16200. <https://doi.org/10.3390/ijms232416200>
- Ding, S.-W. (2010). RNA-based antiviral immunity. *Nature Reviews. Immunology*, 10(9), 632–644. <https://doi.org/10.1038/nri2824>
- Dogra, S. C., Eini, O., Rezaian, M. A., & Randles, J. W. (2009). A novel shaggy-like kinase interacts with the Tomato leaf curl virus pathogenicity determinant C4 protein. *Plant molecular biology*, 71(1), 25-38. <https://doi.org/10.1007/s11103-009-9506-x>
- Dolja, V. V., Kreuze, J. F., & Valkonen, J. P. T. (2006). Comparative and functional genomics of closteroviruses. *Virus Research*, 117(1), 38–51. <https://doi.org/10.1016/j.virusres.2006.02.002>
- Dombrovsky, A., Gollop, N., Chen, S., Chejanovsky, N., & Raccah, B. (2007). In vitro association between the helper component-proteinase of zucchini yellow mosaic virus and cuticle proteins of Myzus persicae. *The Journal of General Virology*, 88(5), 1602–1610. <https://doi.org/10.1099/vir.0.82769-0>
- Domingo-Calap, M. L., Chase, O., Estapé, M., Moreno, A. B., & López-Moya, J. J. (2021). The P1 Protein of Watermelon mosaic virus Compromises the Activity as RNA Silencing Suppressor of the P25 Protein of Cucurbit yellow stunting disorder virus. *Frontiers in Microbiology*, 12, 645530. <https://doi.org/10.3389/fmicb.2021.645530>
- Domingo-Calap, M. L., Moreno, A. B., Díaz Pendón, J. A., Moreno, A., Fereres, A., & López-Moya, J. J. (2020). Assessing the impact on virus transmission and insect vector behavior of a viral mixed infection in melon. *Phytopathology*, 110(1), 174–186. <https://doi.org/10.1094/phyto-04-19-0126-fi>
- 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 of the United States of America*, 94(13), 7088–7093. <https://doi.org/10.1073/pnas.94.13.7088>
- Elena, S. F., & Rodrigo, G. (2012). *Towards an integrated molecular model of plant-virus interactions. Current Opinion in Virology*. 2, 719–724.
- España, M. U., & López-Moya, J. J. (2014). Interference with insect transmission to control plant-pathogenic viruses. In *Plant Virus–Host Interaction* (pp. 125–142). Elsevier. <https://doi.org/10.1016/B978-0-12-411584-2.00006-8>
- Ewels, P. A., Peltzer, A., Fillinger, S., Patel, H., Alneberg, J., Wilm, A., Garcia, M. U., Di Tommaso, P., & Nahnsen, S. (2020). The nf-core framework for community-curated bioinformatics pipelines. *Nature Biotechnology*, 38(3), 276–278. <https://doi.org/10.1038/s41587-020-0439-x>

Bibliography

- Farina, A., Rapisarda, C., Fiallo-Olivé, E., & Navas-Castillo, J. (2023). Tomato Leaf Curl New Delhi Virus Spain Strain Is Not Transmitted by *Trialeurodes vaporariorum* and Is Inefficiently Transmitted by *Bemisia tabaci* Mediterranean between Zucchini and the Wild Cucurbit *Ecballium elaterium*. *Insects*, 14(4), 384. <https://doi.org/10.3390/insects14040384>
- Fauquet, C. M., & Stanley, J. (2003). Geminivirus classification and nomenclature: progress and problems. *The Annals of Applied Biology*, 142(2), 165–189. <https://doi.org/10.1111/j.1744-7348.2003.tb00241.x>
- Feng, M., Zuo, D., Jiang, X., Li, S., Chen, J., Jiang, L., Zhou, X., & Jiang, T. (2018). Identification of Strawberry vein banding virus encoded P6 as an RNA silencing suppressor. *Virology*, 520, 103–110. <https://doi.org/10.1016/j.virol.2018.05.003>
- Fereres, A. (2015). Insect vectors as drivers of plant virus emergence. *Current Opinion in Virology*, 10, 42–46. <https://doi.org/10.1016/j.coviro.2014.12.008>
- Fernández, A., Guo, H. S., Sáenz, P., Simón-Buela, L., Gómez de Cedrón, M., & García, J. A. (1997). The motif V of plum pox potyvirus CI RNA helicase is involved in NTP hydrolysis and is essential for virus RNA replication. *Nucleic Acids Research*, 25(22), 4474–4480. <https://doi.org/10.1093/nar/25.22.4474>
- Fernández-Calvino, L., López-Abella, D., López-Moya, J. J., & Fereres, A. (2006). Comparison of Potato virus Y and Plum pox virus transmission by two aphid species in relation to their probing behavior. *Phytoparasitica*, 34(3), 315–324. <https://doi.org/10.1007/BF02980959>
- Fernández-Calvino, Lourdes, Osorio, S., Hernández, M. L., Hamada, I. B., del Toro, F. J., Donaire, L., Yu, A., Bustos, R., Fernie, A. R., Martínez-Rivas, J. M., & Llave, C. (2014). Virus-induced alterations in primary metabolism modulate susceptibility to *Tobacco rattle virus* in *Arabidopsis*. *Plant Physiology*, 166(4), 1821–1838. <https://doi.org/10.1104/pp.114.250340>
- Fiallo-Olivé, E., & Navas-Castillo, J. (2023). Begomoviruses: what is the secret(s) of their success? *Trends in Plant Science*, 28(6), 715–727. <https://doi.org/10.1016/j.tplants.2023.01.012>
- Fiallo-Olivé, E., & Navas-Castillo, J. (2025). Tissue printing hybridization using digoxigenin-labeled DNA probes for the detection of geminiviruses and associated DNA satellites. In *Methods in Molecular Biology* (pp. 165–172). Springer US. https://doi.org/10.1007/978-1-0716-4454-6_14
- Fiallo-Olivé, E., García-Merenciano, A. C., & Navas-Castillo, J. (2022). Sweet potato symptomless virus 1: First detection in Europe and generation of an infectious clone. *Microorganisms*, 10(9), 1736. <https://doi.org/10.3390/microorganisms10091736>
- Fiallo-Olivé, E., Lett, J.-M., Martin, D. P., Roumagnac, P., Varsani, A., Zerbini, F. M., & Navas-Castillo, J. (2021). ICTV Virus Taxonomy Profile: Geminiviridae 2021: This article is part of the ICTV Virus Taxonomy Profiles collection. *The Journal of General Virology*, 102(12). <https://doi.org/10.1099/jgv.0.001696>

- Fiallo-Olivé, E., Pan, L.-L., Liu, S.-S., & Navas-Castillo, J. (2020). Transmission of begomoviruses and other Whitefly-borne viruses: Dependence on the vector species. *Phytopathology*, 110(1), 10–17. <https://doi.org/10.1094/PHYTO-07-19-0273-FI>
- Fontes, E. P., Gladfelter, H. J., Schaffer, R. L., Petty, I. T., & Hanley-Bowdoin, L. (1994). Geminivirus replication origins have a modular organization. *The Plant Cell*, 6(3), 405–416. <https://doi.org/10.1105/tpc.6.3.405>
- Fortes, I. M., Díaz-Martínez, L., Moriones, E., & Grande-Pérez, A. (2025). Virus–host interactions and genetic exchange in mixed infections of tomato yellow leaf curl virus (TYLCV), tomato leaf curl New Delhi virus (ToLCNDV), and tomato chlorosis virus (ToCV). *Agronomy (Basel, Switzerland)*, 15(5), 1006. <https://doi.org/10.3390/agronomy15051006>
- Fortes, I. M., Sánchez-Campos, S., Fiallo-Olivé, E., Díaz-Pendón, J. A., Navas-Castillo, J., & Moriones, E. (2016). A novel strain of tomato leaf curl New Delhi virus has spread to the Mediterranean basin. *Viruses*, 8(11), E307. <https://doi.org/10.3390/v8110307>
- Franklin, R. E. (1955). Structure of tobacco mosaic virus.
- Franklin, R. E., Klug, A., & Holmes, K. C. (2008). X-ray diffraction studies of the structure and morphology of tobacco mosaic virus. In *Ciba Foundation Symposium - The Nature of Viruses* (pp. 39–55). John Wiley & Sons, Ltd.
- Frischmuth, T., Ringel, M., & Kocher, C. (2001). The size of encapsidated single-stranded DNA determines the multiplicity of African cassava mosaic virus particles. *The Journal of General Virology*, 82(3), 673–676. <https://doi.org/10.1099/0022-1317-82-3-673>
- Fu, Y., Deshoux, M., Cayrol, B., Le Blaye, S., Achard, E., Hudaverdian, S., Cloteau, R., Pichon, E., Strozyk, E., Prunier-Leterme, N., Jousset, E., Sauvion, N., Gaël Thébaud, Le Trionnaire, G., Colella, S., & Uzest, M. (2025). Stylet cuticular gene-directed mutagenesis impairs the pea aphid vector capacity to transmit a plant virus. *PLoS Pathogens*, 21(5), e1013192. <https://doi.org/10.1371/journal.ppat.1013192>
- Fuchs, M., Bar-Joseph, M., Candresse, T., Maree, H. J., Martelli, G. P., Melzer, M. J., Menzel, W., Minafra, A., Sabanadzovic, S., & Report Consortium, I. (2020). ICTV virus taxonomy profile: Closteroviridae. *The Journal of General Virology*, 101(4), 364–365. <https://doi.org/10.1099/jgv.0.001397>
- Fuentes, S., & Salazar, L. F. (2003). First Report of Sweet potato leaf curl virus in Peru. *Plant Disease*, 87(1), 98. <https://doi.org/10.1094/PDIS.2003.87.1.98C>
- Gal-On, A., Antignus, Y., Rosner, A., & Raccach, B. (1992). A zucchini yellow mosaic virus coat protein gene mutation restores aphid transmissibility but has no effect on multiplication. *The Journal of General Virology*, 73(9), 2183–2187. <https://doi.org/10.1099/0022-1317-73-9-2183>
- García-Arenal, F., & Zerbini, F. M. (2019). Life on the edge: Geminiviruses at the interface between crops and wild plant hosts. *Annual Review of Virology*, 6(1), 411–433. <https://doi.org/10.1146/annurev-virology-092818-015536>

Bibliography

- Ghosh, S., Das, N., Das, I., & Maulik, U. (2019). Understanding deep learning techniques for image segmentation. *ACM computing surveys (CSUR)*, 52(4), 1-35. <https://doi.org/10.1145/3329784>
- Gibson, R. W., & Kreuze, J. F. (2015). Degeneration in sweetpotato due to viruses, virus-cleaned planting material and reversion: a review. *Plant Pathology*, 64(1), 1–15. <https://doi.org/10.1111/ppa.12273>
- Gibson, R. W., Mpembe, I., Alicai, T., Carey, E. E., Mwanga, R. O. M., Seal, S. E., & Vetten, H. J. (1998). Symptoms, aetiology and serological analysis of sweet potato virus disease in Uganda. *Plant Pathology*, 47(1), 95–102. <https://doi.org/10.1046/j.1365-3059.1998.00196.x>
- Gilbertson, R. L., Rojas, M. R., Russell, D. R., & Maxwell, D. P. (1991). Use of the asymmetric polymerase chain reaction and DNA sequencing to determine genetic variability of bean golden mosaic geminivirus in the Dominican Republic. *The Journal of General Virology*, 72 (11), 2843–2848. <https://doi.org/10.1099/0022-1317-72-11-2843>
- Gilbertson, R. L., Sudarshana, M., Jiang, H., Rojas, M. R., & Lucas, W. J. (2003). Limitations on geminivirus genome size imposed by plasmodesmata and virus-encoded movement protein: insights into DNA trafficking. *The Plant Cell*, 15(11), 2578–2591. <https://doi.org/10.1105/tpc.015057>
- Giner, A., Lakatos, L., García-Chapa, M., López-Moya, J. J., & Burgyán, J. (2010). Viral protein inhibits RISC activity by argonaute binding through conserved WG/GW motifs. *PLoS Pathogens*, 6(7), e1000996. <https://doi.org/10.1371/journal.ppat.1000996>
- 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. *Proceedings of the National Academy of Sciences of the United States of America*, 105(1), 157–161. <https://doi.org/10.1073/pnas.0709036105>
- Gong, P., Tan, H., Zhao, S., Li, H., Liu, H., Ma, Y., Zang, X., Rong, J., Fu, X., Lozano-Durán, R., Li, F., & Zhou, X. (2021). Geminiviruses encode additional small proteins with specific subcellular localizations and virulence function. *Nature Communications*, 12(1), 4278. <https://doi.org/10.1038/s41467-021-24617-4>
- 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. *Molecular Plant-Microbe Interactions*. 21(8), 1015–1026. <https://doi.org/10.1094/MPMI-21-8-1015>
- Gottlieb, Y., Zchori-Fein, E., Mozes Daube, N., Kontsedalov, S., Skaljac MBrumin, M., Sobol, I., Czosnek, H., Vavre, F., Fleury, F., & Ghanim, M. (2010). The transmission efficiency of tomato yellow leaf curl virus by the whitefly *Bemisia tabaci* is correlated with the presence of a specific symbiotic bacterium species. *Journal of Virology*, 84(18), 9310-9317. <https://doi.org/10.1128/jvi.00423-10>
- Götz, M., Popovski, S., Kollenberg, M., Gorovits, R., Brown, J. K., Cicero, J. M., Czosnek, H., Winter, S., & Ghanim, M. (2012). Implication of *Bemisia tabaci* heat shock protein 70 in Begomovirus-whitefly interactions. *Journal of Virology*, 86(24), 13241–13252. <https://doi.org/10.1128/JVI.00880-12>

- Govier, D. A., & Kassanis, B. (1974). A virus-induced component of plant sap needed when aphids acquire potato virus Y from purified preparations. *Virology*, 61(2), 420-426. [https://doi.org/10.1016/0042-6822\(74\)90278-5](https://doi.org/10.1016/0042-6822(74)90278-5)
- Gray, S. M. (1996). Plant virus proteins involved in natural vector transmission. *Trends in Microbiology*, 4(7), 259–264. [https://doi.org/10.1016/0966-842x\(96\)10040-8](https://doi.org/10.1016/0966-842x(96)10040-8)
- Gray, S., Cilia, M., & Ghanim, M. (2014). Circulative, “nonpropagative” virus transmission: an orchestra of virus-, insect-, and plant-derived instruments. In *Advances in virus research* (Vol. 89, pp. 141-199). Academic Press. <https://doi.org/10.1016/B978-0-12-800172-1.00004-5>
- Gröning, B. R., Hayes, R. J., & Buck, K. W. (1994). Simultaneous regulation of tomato golden mosaic virus coat protein and AL1 gene expression: expression of the AL4 gene may contribute to suppression of the AL1 gene. *The Journal of General Virology*, 75 (4), 721–726. <https://doi.org/10.1099/0022-1317-75-4-721>
- Grüning, B., Dale, R., Sjödin, A., Chapman, B. A., Rowe, J., Tomkins-Tinch, C. H., Valieris, R., Köster, J., & Bioconda Team. (2018). Bioconda: sustainable and comprehensive software distribution for the life sciences. *Nature Methods*, 15(7), 475–476. <https://doi.org/10.1038/s41592-018-0046-7>
- Guo, Z., Li, Y., & Ding, S.-W. (2019). Small RNA-based antimicrobial immunity. *Nature Reviews. Immunology*, 19(1), 31–44. <https://doi.org/10.1038/s41577-018-0071-x>
- Gutiérrez, D. L., Fuentes, S., & Salazar, L. F. (2003). Sweetpotato virus disease (SPVD): Distribution, incidence, and effect on Sweetpotato yield in Peru. *Plant Disease*, 87(3), 297–302. <https://doi.org/10.1094/pdis.2003.87.3.297>
- Gutiérrez, S., Michalakakis, Y., Van Munster, M., & Blanc, S. (2013). Plant feeding by insect vectors can affect life cycle, population genetics and evolution of plant viruses. *Functional Ecology*, 27(3), 610–622. <https://doi.org/10.1111/1365-2435.12070>
- Hahn, S. K. (1979). Effects of viruses (SPVD) on growth and yield of sweet potato. *Experimental Agriculture*, 15(3), 253-256.
- Haldeman-Cahill, R., Daròs, J. A., & Carrington, J. C. (1998). Secondary structures in the capsid protein coding sequence and 3' nontranslated region involved in amplification of the tobacco etch virus genome. *Journal of Virology*, 72(5), 4072–4079. <https://doi.org/10.1128/JVI.72.5.4072-4079.1998>
- Haley, A., Zhan, X., Richardson, K., Head, K., & Morris, B. (1992). Regulation of the activities of African cassava mosaic virus promoters by the AC1, AC2, and AC3 gene products. *Virology*, 188(2), 905–909. [https://doi.org/10.1016/0042-6822\(92\)90551-y](https://doi.org/10.1016/0042-6822(92)90551-y)
- Hanley-Bowdoin, L., Bejarano, E. R., Robertson, D., & Mansoor, S. (2013). Geminiviruses: masters at redirecting and reprogramming plant processes. *Nature Reviews. Microbiology*, 11(11), 777–788. <https://doi.org/10.1038/nrmicro3117>
- Hanssen, I. M., van Esse, H. P., Ballester, A.-R., Hogewoning, S. W., Parra, N. O., Paeleman, A., Lievens, B., Bovy, A. G., & Thomma, B. P. H. J. (2011). Differential tomato transcriptomic

Bibliography

- responses induced by pepino mosaic virus isolates with differential aggressiveness. *Plant Physiology*, 156(1), 301–318. <https://doi.org/10.1104/pp.111.173906>
- Harrison, B. D. (1985). Advances in geminivirus research. *Annual Review of Phytopathology*, 23(1), 55-82. <https://doi.org/10.1146/annurev.py.23.090185.000415>
- Harrison, B. D., Swanson, M. M., & Fargette, D. (2002). Begomovirus coat protein: serology, variation and functions. *Physiological and Molecular Plant Pathology*, 60(5), 257–271. <https://doi.org/10.1006/pmpp.2002.0404>
- Hartitz, M. D., Sunter, G., & Bisaro, D. M. (1997). The tomato golden mosaic virus transactivator (TrAP) is a single-stranded DNA and zincbinding phosphoprotein with an acidic activation domain. *Virology*, 263, 1–14. <https://doi.org/10.1006/viro.1999.9925>
- He, Y. Z., Wang, Y. M., Yin, T. Y., Fiallo-Olivé, E., Liu, Y. Q., Hanley-Bowdoin, L., & Wang, X. W. (2020). A plant DNA virus replicates in the salivary glands of its insect vector via recruitment of host DNA synthesis machinery. *Proceedings of the National Academy of Sciences*, 117(29), 16928-16937. <https://doi.org/10.1073/pnas.1820132117>
- Hehnle, S., Wege, C., & Jeske, H. (2004). Interaction of DNA with the movement proteins of geminiviruses revisited. *Journal of Virology*, 78(14), 7698–7706. <https://doi.org/10.1128/JVI.78.14.7698-7706.2004>
- Hesketh, E. L., Saunders, K., Fisher, C., Potze, J., Stanley, J., Lomonossoff, G. P., & Ranson, N. A. (2018). The 3.3 Å structure of a plant geminivirus using cryo-EM. *Nature Communications*, 9(1). <https://doi.org/10.1038/s41467-018-04793-6>
- Hipp, K., Grimm, C., Jeske, H., & Böttcher, B. (2017). Near-atomic resolution structure of a plant Geminivirus determined by electron cryomicroscopy. *Structure*, 25(8), 1303-1309.e3. <https://doi.org/10.1016/j.str.2017.06.013>
- Hogenhout, S. A., Ammar, E.-D., Whitfield, A. E., & Redinbaugh, M. G. (2008). Insect vector interactions with persistently transmitted viruses. *Annual Review of Phytopathology*, 46(1), 327–359. <https://doi.org/10.1146/annurev.phyto.022508.092135>
- Hong, Y., & Hunt, A. G. (1996). RNA polymerase activity catalyzed by a potyvirus-encoded RNA-dependent RNA polymerase. *Virology*, 226(1), 146-151. <https://doi.org/10.1006/viro.1996.0639>
- Hong, Y., Saunders, K., Hartley, M. R., & Stanley, J. (1996). Resistance to geminivirus infection by virus-induced expression of dianthin in transgenic plants. *Virology*, 220(1), 119–127. <https://doi.org/10.1006/viro.1996.0292>
- <https://ictv.global/report/chapter/geminiviridae/geminiviridae>
- <https://ictv.global/report/chapter/potyviridae>
- <https://www.dpvweb.net/potycleavage/species.html>
- Huet, H., Gal-On, A., Meir, E., Lecoq, H., & Raccach, B. (1994). Mutations in the helper component protease gene of zucchini yellow mosaic virus affect its ability to mediate aphid

- transmissibility. *The Journal of General Virology*, 75(6), 1407–1414. <https://doi.org/10.1099/0022-1317-75-6-1407>
- Huvenne, H., & Smagghe, G. (2010). Mechanisms of dsRNA uptake in insects and potential of RNAi for pest control: a review. *Journal of Insect Physiology*, 56(3), 227–235. <https://doi.org/10.1016/j.jinsphys.2009.10.004>
- Hyodo, K., & Okuno, T. (2014). Host factors used by positive-strand RNA plant viruses for genome replication. *Journal of General Plant Pathology*, 80(2), 123–135. <https://doi.org/10.1007/s10327-014-0505-7>
- Ingham, D. J., Pascal, E., & Lazarowitz, S. G. (1995). Both bipartite geminivirus movement proteins define viral host range, but only BL1 determines viral pathogenicity. *Virology*, 207(1), 191–204. <https://doi.org/10.1006/viro.1995.1066>
- Inoue-Nagata, A. K., Jordan, R., Kreuze, J., Li, F., López-Moya, J. J., Mäkinen, K., Ohshima, K., Wylie, S. J., & ICTV Report Consortium. (2022). ICTV Virus Taxonomy Profile: Potyviridae 2022: This article is part of the ICTV Virus Taxonomy Profiles collection. *The Journal of General Virology*, 103(5). <https://doi.org/10.1099/jgv.0.001738>
- Jayasinghe, W. H., Akhter, M. S., Nakahara, K., & Maruthi, M. N. (2022). Effect of aphid biology and morphology on plant virus transmission. *Pest Management Science*, 78(2), 416–427. <https://doi.org/10.1002/ps.6629>
- Jiang, J., & Laliberté, J.-F. (2011). The genome-linked protein VPg of plant viruses—a protein with many partners. *Current Opinion in Virology*, 1(5), 347–354. <https://doi.org/10.1016/j.coviro.2011.09.010>
- Jiang, T., Hao, T., Chen, W., Li, C., Pang, S., Fu, C., Cheng, J., Zhang, C., Ghorbanpour, M., & Miao, S. (2025). Reprogrammed plant metabolism during viral infections: Mechanisms, pathways and implications. *Molecular Plant Pathology*, 26(2), e70066. <https://doi.org/10.1111/mpp.70066>
- Jin, Y., Zhao, J.-H., & Guo, H.-S. (2021). Recent advances in understanding plant antiviral RNAi and viral suppressors of RNAi. *Current Opinion in Virology*, 46, 65–72. <https://doi.org/10.1016/j.coviro.2020.12.001>
- Johnson, A. C., & Gurr, G. M. (2016). Invertebrate pests and diseases of sweetpotato (*Ipomoea batatas*): a review and identification of research priorities for smallholder production: Invertebrate pests and diseases of sweetpotato smallholder production. *The Annals of Applied Biology*, 168(3), 291–320. <https://doi.org/10.1111/aab.12265>
- Jones, R. A. C. (2021). Global plant virus disease pandemics and epidemics. *Plants*, 10(2), 233. <https://doi.org/10.3390/plants10020233>
- Jones, R. A. C., & Barbetti, M. J. (2012). Influence of climate change on plant disease infections and epidemics caused by viruses and bacteria. *CAB Reviews* 1–33. <https://doi.org/10.1079/pavsnr.20127022>
- Jones, R. A. C., & Naidu, R. A. (2019). Global dimensions of plant virus diseases: Current status and future perspectives. *Annual Review of Virology*, 6(1), 387–409. <https://doi.org/10.1146/annurev-virology-092818-015606>

Bibliography

- Jones, Roger A. C. (2009). Plant virus emergence and evolution: origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. *Virus Research*, 141(2), 113–130. <https://doi.org/10.1016/j.virusres.2008.07.028>
- Juárez, M., Tovar, R., Fiallo-Olivé, E., Aranda, M., Gosálvez, B., & Castillo, P. (2014). First detection of Tomato leaf curl New Delhi virus infecting zucchini in Spain. *Plant Disease* 98. <https://doi.org/10.1094/PDIS-10-131050-PDN>
- Juárez, Miguel, Rabadán, M. P., Martínez, L. D., Tayahi, M., Grande-Pérez, A., & Gómez, P. (2019). Natural hosts and genetic diversity of the emerging tomato leaf curl New Delhi virus in Spain. *Frontiers in Microbiology*, 10(140). <https://doi.org/10.3389/fmicb.2019.00140>
- Kanakala, S., & Ghanim, M. (2016). Implication of the Whitefly Bemisia tabaci Cyclophilin B Protein in the Transmission of Tomato yellow leaf curl virus. *Frontiers in Plant Science*, 7, 1702. <https://doi.org/10.3389/fpls.2016.01702>
- Karyeija, R. F., Kreuze, J. F., Gibson, R. W., & Valkonen, J. P. (2000). Synergistic interactions of a potyvirus and a phloem-limited crinivirus in sweet potato plants. *Virology*, 269(1), 26–36. <https://doi.org/10.1006/viro.1999.0169>
- Kashif, M., Pietilä, S., Artola, K., Jones, R. A. C., Tugume, A. K., Mäkinen, V., & Valkonen, J. P. T. (2012). Detection of viruses in Sweetpotato from Honduras and Guatemala augmented by deep-sequencing of small-RNAs. *Plant Disease*, 96(10), 1430–1437. <https://doi.org/10.1094/PDIS-03-12-0268-RE>
- Kassanis, B. (1939). Intracellular inclusions in virus infected plants. *Annals of Applied Biology*, 26, 705–709. <https://doi.org/10.1111/j.1744-7348.1939.tb06995.x>
- Kasschau, K. D., & Carrington, J. C. (1998). A counterdefensive strategy of plant viruses. *Cell*, 95(4), 461–470. [https://doi.org/10.1016/s0092-8674\(00\)81614-1](https://doi.org/10.1016/s0092-8674(00)81614-1)
- Kenesi, E., López-Moya, J.-J., Orosz, L., Burgyán, J., & Lakatos, L. (2021). Argonaute 2 controls antiviral activity against sweet potato mild mottle virus in Nicotiana benthamiana. *Plants*, 10(5), 867. <https://doi.org/10.3390/plants10050867>
- Kheyr-Pour, A., Bananej, K., Dafalla, G. A., Caciagli, P., Noris, E., Ahoonmanesh, A., Lecoq, H., & Gronenborn, B. (2000). Watermelon chlorotic stunt virus from the Sudan and Iran: sequence comparisons and identification of a whitefly-transmission determinant. *Phytopathology*, 90(6), 629-635. <https://doi.org/10.1094/PHYTO.2000.90.6.629>
- Kiemo, F. W., Salamon, P., Jewehan, A., Tóth, Z., & Szabó, Z. (2022). Detection and elimination of viruses infecting sweet potatoes in Hungary. *Plant Pathology*, 71(4), 1001–1009. <https://doi.org/10.1111/ppa.13519>
- Kiss, Z. A., Medina, V., & Falk, B. W. (2013). Crinivirus replication and host interactions. *Frontiers in Microbiology*, 4, 99. <https://doi.org/10.3389/fmicb.2013.00099>
- Klein, P. G., Klein, R. R., Rodríguez-Cerezo, E., Hunt, A. G., & Shaw, J. G. (1994). Mutational analysis of the tobacco vein mottling virus genome. *Virology*, 204(2), 759–769. <https://doi.org/10.1006/viro.1994.1591>

- Knuhtsen, H., Hiebert, E., & Purcifull, D. E. (1974). Partial purification and some properties of tobacco etch virus induced intranuclear inclusions. *Virology*, 61(1), 200-209. [https://doi.org/10.1016/0042-6822\(74\)90254-2](https://doi.org/10.1016/0042-6822(74)90254-2)
- Kokkinos, C. D., & Clark, C. A. (2006). Interactions Among Sweet potato chlorotic stunt virus and Different Potyviruses and Potyvirus Strains Infecting Sweetpotato in the United States. *Plant Disease*, 90(10), 1347–1352. <https://doi.org/10.1094/pd-90-1347>
- Koonin, E. V., Dolja, V. V., & Krupovic, M. (2015). Origins and evolution of viruses of eukaryotes: The ultimate modularity. *Virology*, 479, 2–25. <https://doi.org/10.1016/j.virol.2015.02.039>
- Koonin, E. V., Dolja, V. V., Krupovic, M., Varsani, A., Wolf, Y. I., Yutin, N., Zerbini, F. M., & Kuhn, J. H. (2020). Global organization and proposed megataxonomy of the virus world. *Microbiology and Molecular Biology Reviews*, 84(2). <https://doi.org/10.1128/mmb.00061-19>
- Koritnik, N., Kežar, A., Kavčič, L., Žnidarič, M. T., Leonardi, A., De, S., Pollari, M., Mäkinen, K., & Podobnik, M. (2026). Species-specific structural adaptation of the potyviral coat protein in virions and virus-like particles. *Communications Biology*, 9(1). <https://doi.org/10.1038/s42003-025-09502-w>
- Kosakovsky, S. L., Posada, D., Gravenor, M. B., Woelk, C. H., & Frost, S. D. W. (2006). GARD: a genetic algorithm for recombination detection. *Bioinformatics* 22(24), 3096–3098. <https://doi.org/10.1093/bioinformatics/btl474>
- Krenz, B., Jeske, H., & Kleinow, T. (2012). The induction of stromule formation by a plant DNA-virus in epidermal leaf tissues suggests a novel intra- and intercellular macromolecular trafficking route. *Frontiers in Plant Science*, 3, 291. <https://doi.org/10.3389/fpls.2012.00291>
- Kreuze, J. F., Karyeija, R. F., Gibson, R. W., & Valkonen, J. P. (2000). Comparisons of coat protein gene sequences show that East African isolates of Sweet potato feathery mottle virus form a genetically distinct group. *Archives of Virology*, 145(3), 567–574. <https://doi.org/10.1007/s007050050047>
- Kreuze, J. F., Savenkov, E. I., & Valkonen, J. P. T. (2002). Complete genome sequence and analyses of the subgenomic RNAs of Sweet potato chlorotic stunt virus reveal several new features for the genus Crinivirus. *Journal of Virology*, 76(18), 9260-9270. <https://doi.org/10.1128/jvi.76.18.9260-9270.2002>
- Kreuze, J. F., Savenkov, E. I., Cuellar, W., Li, X., & Valkonen, J. P. (2005). Viral class 1 RNase III involved in suppression of RNA silencing. *Journal of Virology*, 79(11), 7227-7238. <https://doi.org/10.1128/jvi.79.11.7227-7238.2005>
- Kreuze, J., Cuellar, W. J., & Low, J. W. (2021). Challenge of virus disease threats to ensuring sustained uptake of vitamin-A-rich Sweetpotato in Africa. In *Plant Pathology in the 21st Century* (pp. 73–94). Springer International Publishing. https://doi.org/10.1007/978-3-030-57899-2_5
- Kreuze, Jan F., Perez, A., Gargurevich, M. G., & Cuellar, W. J. (2020). Badnaviruses of sweet potato: Symptomless coinhabitants on a global scale. *Frontiers in Plant Science*, 11, 313. <https://doi.org/10.3389/fpls.2020.00313>

Bibliography

- Kreuze, Jan F., Perez, A., Untiveros, M., Quispe, D., Fuentes, S., Barker, I., & Simon, R. (2009). Complete viral genome sequence and discovery of novel viruses by deep sequencing of small RNAs: a generic method for diagnosis, discovery and sequencing of viruses. *Virology*, 388(1), 1–7. <https://doi.org/10.1016/j.virol.2009.03.024>
- Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S., & Tamura, K. (2024). MEGA12: molecular evolutionary genetic analysis version 12 for adaptive and green computing. *Molecular biology and evolution*, 41(12), msae263. <https://doi.org/10.1093/molbev/msae263>
- Kushwaha, N., Singh, A. K., Basu, S., & Chakraborty, S. (2015). Differential response of diverse solanaceous hosts to tomato leaf curl New Delhi virus infection indicates coordinated action of NBS-LRR and RNAi-mediated host defense. *Archives of Virology*, 160(6), 1499–1509. <https://doi.org/10.1007/s00705-015-2399-x>
- Kusnadi, A. R., Nikolov, Z. L., & Howard, J. A. (1997). Production of recombinant proteins in transgenic plants: practical considerations. *Biotechnology and bioengineering*, 56(5), 473–484. [https://doi.org/10.1002/\(SICI\)1097-0290\(19971205\)56:5<473::AID-BIT1>3.0.CO;2-F](https://doi.org/10.1002/(SICI)1097-0290(19971205)56:5<473::AID-BIT1>3.0.CO;2-F)
- Lakatos, L., Csorba, T., Pantaleo, V., Chapman, E. J., Carrington, J. C., Liu, Y.-P., Dolja, V. V., Calvino, L. F., López-Moya, J. J., & Burgyán, J. (2006). Small RNA binding is a common strategy to suppress RNA silencing by several viral suppressors. *The EMBO Journal*, 25(12), 2768–2780. <https://doi.org/10.1038/sj.emboj.7601164>
- Lakatos, L., Szittyá, G., Silhavy, D., & Burgyán, J. (2004). Molecular mechanism of RNA silencing suppression mediated by p19 protein of tombusviruses. *The EMBO Journal*, 23(4), 876–884. <https://doi.org/10.1038/sj.emboj.7600096>
- Langfelder, P., & Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics*, 9(1), 559. <https://doi.org/10.1186/1471-2105-9-559>
- Larsen, R. C., & Duffus, J. E. (1983). A Simplified Procedure for the Purification of Curly Top Virus and the Isolation. *Phytopathology*, 73, 114–118.
- Laufs, J., Traut, W., Heyraud, F., Matzeit, V., Rogers, S. G., Schell, J., & Gronenborn, B. (1995). In vitro cleavage and joining at the viral origin of replication by the replication initiator protein of tomato yellow leaf curl virus. *Proceedings of the National Academy of Sciences of the United States of America*, 92(9), 3879–3883. <https://doi.org/10.1073/pnas.92.9.3879>
- Lazarowitz, S. G., & Shepherd, R. J. (1992). Geminiviruses: Genome structure and gene function. *Critical Reviews in Plant Sciences*, 11(4), 327–349. <https://doi.org/10.1080/07352689209382350>
- Lecoq, H., & Desbiez, C. (2012). Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture. *Advances in Virus Research*, 84, 67–126. <https://doi.org/10.1016/B978-0-12-394314-9.00003-8>
- Lecoq, H., & Katis, N. (2014). Control of cucurbit viruses. *Advances in Virus Research*, 90, 255–296. <https://doi.org/10.1016/B978-0-12-801246-8.00005-6>

- Lee, D., Lal, N. K., Lin, Z.-J. D., Ma, S., Liu, J., Castro, B., Toruño, T., Dinesh-Kumar, S. P., & Coaker, G. (2020). Regulation of reactive oxygen species during plant immunity through phosphorylation and ubiquitination of RBOHD. *Nature Communications*, 11(1), 1838. <https://doi.org/10.1038/s41467-020-15601-5>
- Legavre, T., Maia, I. G., Casse-Delbart, F., Bernardi, F., & Robaglia, C. (1996). Switches in the mode of transmission select for or against a poorly aphid-transmissible strain of potato virus Y with reduced helper component and virus accumulation. *The Journal of General Virology*, 77(7), 1343–1347. <https://doi.org/10.1099/0022-1317-77-7-1343>
- Li, F., Li, X., Zhao, S., Pan, F., Li, Z., Hao, Y., He, J., Wang, A., Kormelink, R., & Zhou, X. (2025). Antiviral RNA interference in plants: Increasing complexity and integration with other biological processes. *Plant Communications*, 6(10), 101490. <https://doi.org/10.1016/j.xplc.2025.101490>
- Li, F., Xu, D., Abad, J., & Li, R. (2012). Phylogenetic relationships of closely related potyviruses infecting sweet potato determined by genomic characterization of Sweet potato virus G and Sweet potato virus 2. *Virus Genes*, 45(1), 118–125. <https://doi.org/10.1007/s11262-012-0749-2>
- Li, F., Xu, X., Huang, C., Gu, Z., Cao, L., Hu, T., Ding, M., Li, Z., & Zhou, X. (2015). The AC5 protein encoded by Mungbean yellow mosaic India virus is a pathogenicity determinant that suppresses RNA silencing-based antiviral defenses. *The New Phytologist*, 208(2), 555–569. <https://doi.org/10.1111/nph.13473>
- Lico, C., Chen, Q., & Santi, L. (2008). Viral vectors for production of recombinant proteins in plants. *Journal of cellular physiology*, 216(2), 366–377. <https://doi.org/10.1002/jcp.21423>
- Ling, K.-S., Harrison, H. F., Simmons, A. M., Zhang, S. C., & Jackson, D. M. (2011). Experimental host range and natural reservoir of sweet potato leaf curl virus in the United States. *Crop Protection*, 30(8), 1055–1062. <https://doi.org/10.1016/j.cropro.2011.03.009>
- Liu, H., Boulton, M. I., & Davies, J. W. (1997). Maize streak virus coat protein binds single-and double-stranded DNA in vitro. *Journal of general virology*, 78(6), 1265–1270. <https://doi.org/10.1099/0022-1317-78-6-1265>
- Liu, H., Chang, Z., Zhao, S., Gong, P., Zhang, M., Lozano-Durán, R., Yan, H., Zhou, X., & Li, F. (2023). Functional identification of a novel C7 protein of tomato yellow leaf curl virus. *Virology*, 585, 117–126. <https://doi.org/10.1016/j.virol.2023.05.011>
- Livieratos, I. C., Eliasco, E., Müller, G., Olsthoorn, R. C. L., Salazar, L. F., Pleij, C. W. A., & Coutts, R. H. A. (2004). Analysis of the RNA of Potato yellow vein virus: evidence for a tripartite genome and conserved 3'-terminal structures among members of the genus Crinivirus. *The Journal of General Virology*, 85(7), 2065–2075. <https://doi.org/10.1099/vir.0.79910-0>
- Llave, C. (2016). Dynamic cross-talk between host primary metabolism and viruses during infections in plants. *Current Opinion in Virology*, 19, 50–55. <https://doi.org/10.1016/j.coviro.2016.06.013>
- Llave, C., Martínez, B., Díaz-Ruíz, J. R., & López-Abella, D. (2002). Amino acid substitutions within the Cys-rich domain of the tobacco etch potyvirus HC-Pro result in loss of

Bibliography

- transmissibility by aphids. *Archives of Virology*, 147(12), 2365–2375. <https://doi.org/10.1007/s00705-002-0884-5>
- Llave, César, Martínez, B., Díaz-Ruiz, J. R., & López-Abella, D. (1999). Helper component mutations in nonconserved residues associated with aphid transmission efficiency of a pepper isolate of potato virus Y. *Phytopathology*, 89(12), 1176–1181. <https://doi.org/10.1094/phyto.1999.89.12.1176>
- Loebenstein, G. (2012). Viruses in sweetpotato. *Advances in Virus Research*, 84, 325–343. <https://doi.org/10.1016/B978-0-12-394314-9.00009-9>
- Loebenstein, G., & Thottappilly, G. (2013). *Virus and virus-like diseases of major crops in developing countries*. Springer Science & Business Media.
- Loebenstein, G., Thottappilly, G., Fuentes, S., & Cohen, J. (2009). Virus and Phytoplasma Diseases. In *The Sweetpotato* (pp. 105–134). Springer Netherlands. https://doi.org/10.1007/978-1-4020-9475-0_8
- Loebenstein, Gad. (2015). Control of sweet potato virus diseases. *Advances in Virus Research*, 91, pp. 33–45. <https://doi.org/10.1016/bs.aivir.2014.10.005>
- López, C., Ferriol, M., & Picó, M. B. (2015). Mechanical transmission of Tomato leaf curl New Delhi virus to cucurbit germplasm: selection of tolerance sources in Cucumis melo. *Euphytica; Netherlands Journal of Plant Breeding*, 204(3), 679–691. <https://doi.org/10.1007/s10681-015-1371-x>
- Lopez-Gomollon, S., & Baulcombe, D. C. (2022). Roles of RNA silencing in viral and non-viral plant immunity and in the crosstalk between disease resistance systems. *Nature Reviews. Molecular Cell Biology*, 23(10), 645–662. <https://doi.org/10.1038/s41580-022-00496-5>
- López-Moya, J. J., & García, J. A. (2008). Potyviruses. In: *Encyclopedia of Virology* (B. W. J. Mahy & M. H. V. Van Regenmortel, Eds.). Elsevier.
- López-Moya, J. J., & Pirone, T. P. (1998). Charge changes near the N terminus of the coat protein of two potyviruses affect virus movement. *The Journal of General Virology*, 79(1), 161–165. <https://doi.org/10.1099/0022-1317-79-1-161>
- López-Moya, J. J., Wang, R. Y., & Pirone, T. P. (1999). Context of the coat protein DAG motif affects potyvirus transmissibility by aphids. *The Journal of General Virology*, 80(12), 3281–3288. <https://doi.org/10.1099/0022-1317-80-12-3281>
- Louten, J. (2016). Virus structure and classification. In *Essential Human Virology* (pp. 19–29). Elsevier. <https://doi.org/10.1016/B978-0-12-800947-5.00002-8>
- Lozano, G., Trenado, H. P., Fiallo-Olivé, E., Chirinos, D., Geraud-Pouey, F., Briddon, R. W., & Navas-Castillo, J. (2016). Characterization of non-coding DNA satellites associated with sweepoviruses (genus Begomovirus, geminiviridae) – definition of a distinct class of Begomovirus-associated satellites. *Frontiers in Microbiology*, 7. <https://doi.org/10.3389/fmicb.2016.00162>

- Lozano, G., Trenado, H. P., Valverde, R. A., & Navas-Castillo, J. (2009). Novel begomovirus species of recombinant nature in sweet potato (*Ipomoea batatas*) and *Ipomoea indica*: taxonomic and phylogenetic implications. *The Journal of General Virology*, 90(10), 2550–2562. <https://doi.org/10.1099/vir.0.012542-0>
- Lozano-Durán, R. (2024). Viral recognition and evasion in plants. *Annual Review of Plant Biology*, 75(1), 655–677. <https://doi.org/10.1146/annurev-arplant-060223-030224>
- Lozano-Duran, R., Rosas-Díaz, T., Luna, A. P., & Bejarano, E. R. (2011). Identification of host genes involved in geminivirus infection using a reverse genetics approach. *PloS one*, 6(7), e22383. <https://doi.org/10.1371/journal.pone.0022383>
- Lu, J., Du, Z.-X., Kong, J., Chen, L.-N., Qiu, Y.-H., Li, G.-F., Meng, X.-H., & Zhu, S.-F. (2012). Transcriptome analysis of *Nicotiana tabacum* infected by Cucumber mosaic virus during systemic symptom development. *PloS One*, 7(8), e43447. <https://doi.org/10.1371/journal.pone.0043447>
- Lu, Y., Deng, X., Zhu, Q., Wu, D., Zhong, J., Wen, L., & Yu, X. (2023). The dsRNA delivery, targeting and application in pest control. *Agronomy*, 13(3), 714. <https://doi.org/10.3390/agronomy13030714>
- Luan, H., Shine, M. B., Cui, X., Chen, X., Ma, N., Kachroo, P., Zhi, H., & Kachroo, A. (2016). The potyviral P3 protein targets eukaryotic elongation factor 1A to promote the unfolded protein response and viral pathogenesis. *Plant Physiology*, 172(1), 221–234. <https://doi.org/10.1104/pp.16.00505>
- Luan, Y. S., Zhang, J., & An, L. J. (2006). First Report of Sweet potato leaf curl virus in China. *Plant Disease*, 90(8), 1111. <https://doi.org/10.1094/PD-90-1111C>
- Luan, Y. S., Zhang, J., Liu, D. M., & Li, W. L. (2007). Molecular characterization of sweet potato leaf curl virus isolate from China (SPLCV-CN) and its phylogenetic relationship with other members of the Geminiviridae. *Virus Genes*, 35(3), 879–880. <https://doi.org/10.1007/s11262-007-0117-9>
- Maina, S., Barbetti, M. J., Edwards, O. R., de Almeida, L., Ximenes, A., & Jones, R. A. C. (2018). *Sweet potato feathery mottle virus* and *Sweet potato virus C* from East Timorese and Australian Sweetpotato: Biological and Molecular Properties, and Biosecurity Implications. *Plant Disease*, 102(3), 589–599. <https://doi.org/10.1094/pdis-08-17-1156-re>
- Manoussopoulos, I. N., Maiss, E., & Tsagris, M. (2000). Native electrophoresis and Western blot analysis (NEWeB): a method for characterization of different forms of potyvirus particles and similar nucleoprotein complexes in extracts of infected plant tissues. *The Journal of General Virology*, 81(9), 2295–2298. <https://doi.org/10.1099/0022-1317-81-9-2295>
- Mansoor, S., Khan, S. H., Bashir, A., Saeed, M., Zafar, Y., Malik, K. A., Briddon, R., Stanley, J., & Markham, P. G. (1999). Identification of a novel circular single-stranded DNA associated with cotton leaf curl disease in Pakistan. *Virology*, 259(1), 190–199. <https://doi.org/10.1006/viro.1999.9766>
- Mardanova, E. S., Vasyagin, E. A., & Ravin, N. V. (2024). Virus-like particles produced in plants: A promising platform for recombinant vaccine development. *Plants*, 13(24), 3564. <https://doi.org/10.3390/plants13243564>

Bibliography

- Marsian, J., & Lomonossoff, G. P. (2016). Molecular pharming — VLPs made in plants. *Current Opinion in Biotechnology*, 37, 201–206. <https://doi.org/10.1016/j.copbio.2015.12.007>
- Martelli, G. P. (2019). A brief historical account of the family Closteroviridae. In *Citrus Tristeza virus: methods and protocols* (pp. 7-13). New York, NY: Springer New York. https://doi.org/10.1007/978-1-4939-9558-5_2
- Martelli, G. P., & Candresse, T. (2001). Closteroviridae. e L.S. <https://doi.org/10.1038/npg.els.0000747>
- Martelli, G. P., Ghanem-Sabanadzovic, N. A., Agranovsky, A.A., Rwahni, M., A., Dolja, V. V., Dovas, C. I., Fuchs, M., Gugerli, P., Hu, J. S., Jelkmann, W., Katis, N. I., Maliogka, V. I., Melzer, M. J., Menzel, W., Minafra, A., Rott, M. E., Rowhani, A., Sabanadzovic, S., Saldarelli, P. (2012) Taxonomic revision of the family Closteroviridae with special reference to the Grapevine leafroll-associated members of the genus Ampelovirus and the putative species unassigned to the family. *Journal of Plant Pathology*, 7-19. <http://doi.org/10.4454/jpp.fa.2012.022>
- Martínez, F., & Daròs, J.-A. (2014). Tobacco etch virus protein P1 traffics to the nucleolus and associates with the host 60S ribosomal subunits during infection. *Journal of Virology*, 88(18), 10725–10737. <https://doi.org/10.1128/JVI.00928-14>
- Martín-Hernández, A. M., & Picó, B. (2020). Natural resistances to viruses in cucurbits. *Agronomy*, 11(1), 23. <https://doi.org/10.3390/agronomy11010023>
- Mascia, T., & Gallitelli, D. (2016). Synergies and antagonisms in virus interactions. *Plant Science: An International Journal of Experimental Plant Biology*, 252, 176–192. <https://doi.org/10.1016/j.plantsci.2016.07.015>
- Mathur, C., & Savithri, H. S. (2012). Novel ATPase activity of the polyprotein intermediate, Viral Protein genome-linked-Nuclear Inclusion-a protease, of Pepper vein banding potyvirus. *Biochemical and Biophysical Research Communications*, 427(1), 113–118. <https://doi.org/10.1016/j.bbrc.2012.09.020>
- Matsumura, E. E., & Kormelink, R. (2023). Small talk: On the possible role of trans-kingdom small RNAs during plant-virus-vector tritrophic communication. *Plants*, 12(6), 1411. <https://doi.org/10.3390/plants12061411>
- Mavankal, G., & Rhoads, R. E. (1991). In vitro cleavage at or near the N-terminus of the helper component protein in the tobacco vein mottling virus polyprotein. *Virology*, 185(2), 721-731. [https://doi.org/10.1016/0042-6822\(91\)90543-K](https://doi.org/10.1016/0042-6822(91)90543-K)
- Mbanzibwa, D. R., Tian, Y., Mukasa, S. B., & Valkonen, J. P. T. (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. *Journal of Virology*, 83(13), 6934–6940. <https://doi.org/10.1128/JVI.00537-09>
- Mbanzibwa, D. R., Tugume, A. K., Chiunga, E., Mark, D., & Tairo, F. D. (2014). Small RNA deep sequencing-based detection and further evidence of DNA viruses infecting sweetpotato plants in Tanzania. *The Annals of Applied Biology*, 165(3), 329–339. <https://doi.org/10.1111/aab.12136>

- Merits, A., Guo, D., Järvekülg, L., & Saarma, M. (1999). Biochemical and genetic evidence for interactions between potato A potyvirus-encoded proteins P1 and P3 and proteins of the putative replication complex. *Virology*, 263(1), 15–22. <https://doi.org/10.1006/viro.1999.9926>
- Miano, D. W., LaBonte, D. R., Clark, C. A., Valverde, R. A., Hoy, M. W., Hurtt, S., & Li, R. (2006). First report of a begomovirus infecting sweetpotato in Kenya. *Plant Disease*, 90(6), 832–832. <https://doi.org/10.1094/PD-90-0832B>
- Mills-Lujan, K., & Deom, C. M. (2010). Geminivirus C4 protein alters Arabidopsis development. *Protoplasma*, 239(1–4), 95–110. <https://doi.org/10.1007/s00709-009-0086-z>
- Mingot, A., Valli, A., Rodamilans, B., San León, D., Baulcombe, D. C., García, J. A., & López-Moya, J. J. (2016). The P1N-PISPO trans-frame gene of sweet potato feathery mottle Potyvirus is produced during virus infection and functions as an RNA silencing suppressor. *Journal of Virology*, 90(7), 3543–3557. <https://doi.org/10.1128/JVI.02360-15>
- Mnari-Hattab, M., Zammouri, S., Belkadhi, M., Doña, D. B., Ben Nahia, E., & Hajlaoui, M. (2015). First report of tomato leaf curl New Delhi virus infecting cucurbits in Tunisia. *New Disease Report*. <http://dx.doi.org/10.5197/j.2044-0588.2015.031.021>
- Moreno, A. B., & López-Moya, J. J. (2020). When viruses play team sports: Mixed infections in plants. *Phytopathology*, 110(1), 29–48. <https://doi.org/10.1094/phyto-07-19-0250-fi>
- Moreno, A., Tjallingii, W. F., Fernandez-Mata, G., & Fereres, A. (2012). Differences in the mechanism of inoculation between a semi-persistent and a non-persistent aphid-transmitted plant virus. *The Journal of General Virology*, 93(3), 662–667. <https://doi.org/10.1099/vir.0.037887-0>
- Moriones, E., Praveen, S., & Chakraborty, S. (2017). Tomato leaf curl New Delhi virus: An emerging virus complex threatening vegetable and fiber crops. *Viruses*, 9(10), 264. <https://doi.org/10.3390/v9100264>
- Movahed, N., Cabanillas, D. G., Wan, J., Vali, H., Laliberté, J.-F., & Zheng, H. (2019). Turnip mosaic virus components are released into the extracellular space by vesicles in infected leaves. *Plant Physiology*, 180(3), 1375–1388. <https://doi.org/10.1104/pp.19.00381>
- Movahed, N., Patarroyo, C., Sun, J., Vali, H., Laliberté, J.-F., & Zheng, H. (2017). Cylindrical inclusion protein of Turnip mosaic virus serves as a docking point for the intercellular movement of viral replication vesicles. *Plant Physiology*, 175(4), 1732–1744. <https://doi.org/10.1104/pp.17.01484>
- Mubin, M., Amin, I., Amrao, L., Briddon, R. W., & Mansoor, S. (2010). The hypersensitive response induced by the V2 protein of a monopartite begomovirus is countered by the C2 protein. *Molecular Plant Pathology*, 11(2), 245–254. <https://doi.org/10.1111/j.1364-3703.2009.00601.x>
- Mukasa, S. B., Rubaihayo, P. R., & Valkonen, J. P. T. (2006). Interactions between a crinivirus, an ipomovirus and a potyvirus in coinfecting sweetpotato plants. *Plant Pathology*, 55(3), 458–467. <https://doi.org/10.1111/j.1365-3059.2006.01350.x>

Bibliography

- Mukiibi, J. (1977). Effects of mosaic on the yield of sweet potatoes. In *“Proceedings of the 4th International Society for Tropical Root Crops.” International Center of Tropical Agriculture (CIAT)* (pp. 169–170).
- Mwanga, R., Yencho, G. C., & Moyer, J. W. (2002). Diallel analysis of sweet potatoes for resistance to sweet potato virus disease. *Euphytica*, 128, 237–248. <https://doi.org/10.1023/A:1020828421757>
- Navas-Castillo, J., & Fiallo-Olivé, E. (2021). Geminiviruses (Geminiviridae).
- Navas-Castillo, J., López-Moya, J. J., & Aranda, M. A. (2014). Whitefly-transmitted RNA viruses that affect intensive vegetable production. *The Annals of Applied Biology*, 165(2), 155–171. <https://doi.org/10.1111/aab.12147>
- Navas-Castillo, Jesús, Fiallo-Olivé, E., & Sánchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. *Annual Review of Phytopathology*, 49(1), 219–248. <https://doi.org/10.1146/annurev-phyto-072910-095235>
- Nelson, R. S., & Citovsky, V. (2005). Plant viruses. Invaders of cells and pirates of cellular pathways. *Plant Physiology*, 138(4), 1809–1814. <https://doi.org/10.1104/pp.104.900167>
- Ng, J. C. K., & Falk, B. W. (2006). Virus-vector interactions mediating nonpersistent and semipersistent transmission of plant viruses. *Annual Review of Phytopathology*, 44(1), 183–212. <https://doi.org/10.1146/annurev.phyto.44.070505.143325>
- Ng, J. C. K., & Perry, K. L. (2004). Transmission of plant viruses by aphid vectors. *Molecular Plant Pathology*, 5(5), 505–511. <https://doi.org/10.1111/j.1364-3703.2004.00240.x>
- Ngeve, J. M., & Bouwkamp, J. C. (1991). Effects of sweet potato virus disease (SPVD) on the yield of sweet potato genotypes in Cameroon. *Experimental Agriculture*, 27(2), 221–225. <https://doi.org/10.1017/S0014479700018858>
- Nishad, R., Ahmed, T., Rahman, V. J., & Kareem, A. (2020). Modulation of plant defense system in response to microbial interactions. *Frontiers in Microbiology*, 11, 1298. <https://doi.org/10.3389/fmicb.2020.01298>
- Njeru, R. W., Mburu, M. W. K., Cheramgoi, E., Gibson, R. W., Kiburi, Z. M., Obudho, E., & Yobera, D. (2004). Studies on the physiological effects of viruses on sweet potato yield in Kenya. *The Annals of Applied Biology*, 145(1), 71–76. <https://doi.org/10.1111/j.1744-7348.2004.tb00360.x>
- Noris, E., Jupin, I., Accotto, G. P., & Gronenborn, B. (1996). DNA-binding activity of the C2 protein of tomato yellow leaf curl geminivirus. *Virology*, 217(2), 607–612. <https://doi.org/10.1006/viro.1996.0157>
- Noris, E., Vaira, A. M., Caciagli, P., Masenga, V., Gronenborn, B., & Accotto, G. P. (1998). Amino acids in the capsid protein of tomato yellow leaf curl virus that are crucial for systemic infection, particle formation, and insect transmission. *Journal of Virology*, 72(12), 10050–10057. <https://doi.org/10.1128/jvi.72.12.10050-10057.1998>

- Ohnesorge, S., & Bejarano, E. R. (2009). Begomovirus coat protein interacts with a small heat-shock protein of its transmission vector (*Bemisia tabaci*). *Insect Molecular Biology*, 18(6), 693–703. <https://doi.org/10.1111/j.1365-2583.2009.00906.x>
- Okazaki, Y., & Saito, K. (2014). Roles of lipids as signaling molecules and mitigators during stress response in plants. *The Plant Journal: For Cell and Molecular Biology*, 79(4), 584–596. <https://doi.org/10.1111/tpj.12556>
- Olspert, A., Carr, J. P., & Firth, A. E. (2016). Mutational analysis of the Potyviridae transcriptional slippage site utilized for expression of the P3N-PIPO and P1N-PISPO proteins. *Nucleic Acids Research*, 44(16), 7618–7629. <https://doi.org/10.1093/nar/gkw441>
- Ontiveros, I., Diaz-Pendón, J. A., & López-Moya, J. J. (2024). Experimental transmission of plant viruses by aphids or whiteflies. In *Methods in Molecular Biology* (pp. 165–179). Springer US. https://doi.org/10.1007/978-1-0716-3485-1_12
- Ontiveros, I., López-Moya, J. J., & Díaz-Pendón, J. A. (2022). Coinfection of Tomato Plants with *Tomato yellow leaf curl virus* and *Tomato chlorosis virus* Affects the Interaction with Host and Whiteflies. *Phytopathology*, 112(4), 944–952. <https://doi.org/10.1094/phyto-08-21-0341-r>
- Orfanidou, C. G., Efthimiou, K., Katis, N. I., & Maliogka, V. I. (2022). Elucidating the sweet potato virome in Greece with the aid of high-throughput sequencing technology. *Plant Pathology*, 71(9), 1880–1891. <https://doi.org/10.1111/ppa.13630>
- Padidam, M., Beachy, R. N., & Fauquet, C. M. (1995). Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not essential for infectivity. *The Journal of General Virology*, 76(1), 25–35. <https://doi.org/10.1099/0022-1317-76-1-25>
- Pakkianathan, B. C., Kontsedalov, S., Lebedev, G., Mahadav, A., Zeidan, M., Czosnek, H., & Ghanim, M. (2015). Replication of tomato yellow leaf curl virus in its Whitefly vector, *Bemisia tabaci*. *Journal of Virology*, 89(19), 9791–9803. <https://doi.org/10.1128/JVI.00779-15>
- Palanichelvam, K., Kunik, T., Citovsky, V., & Gafni, Y. (1998). The capsid protein of tomato yellow leaf curl virus binds cooperatively to single-stranded DNA. *Journal of General Virology*, 79(11), 2829–2833. <https://doi.org/10.1099/0022-1317-79-11-2829>
- Pan, L.-L., Chen, Q.-F., Zhao, J.-J., Guo, T., Wang, X.-W., Hariton-Shalev, A., Czosnek, H., & Liu, S.-S. (2017). Clathrin-mediated endocytosis is involved in Tomato yellow leaf curl virus transport across the midgut barrier of its whitefly vector. *Virology*, 502, 152–159. <https://doi.org/10.1016/j.virol.2016.12.029>
- Panno, S., Iacono, G., Davino, M., Marchione, S., Zappardo, V., Bella, P., Tomassoli, L., Accotto, G. P., & Davino, S. (2016). First report of tomato leaf curl New Delhi virus affecting zucchini squash in an important horticultural area of southern Italy. *New Disease Reports*. <http://dx.doi.org/10.5197/j.2044-0588.2016.033.006>
- Paprotka, T., Metzler, V., & Jeske, H. (2010). The first DNA 1-like alpha satellites in association with New World begomoviruses in natural infections. *Virology*, 404(2), 148–157. <https://doi.org/10.1016/j.virol.2010.05.003>

Bibliography

- Pasin, F., Daròs, J.-A., & Tzanetakis, I. E. (2022). Proteome expansion in the *Potyviridae* evolutionary radiation. *FEMS Microbiology Reviews*, 46(4). <https://doi.org/10.1093/femsre/fuac011>
- Pasin, F., Shan, H., García, B., Müller, M., San León, D., Ludman, M., Fresno, D. H., Fátýol, K., Munné-Bosch, S., Rodrigo, G., & García, J. A. (2020). Absciscic acid connects phytohormone signaling with RNA metabolic pathways and promotes an antiviral response that is evaded by a self-controlled RNA virus. *Plant Communications*, 1(5), 100099. <https://doi.org/10.1016/j.xplc.2020.100099>
- Pasin, F., Simón-Mateo, C., & García, J. A. (2014). The hypervariable amino-terminus of P1 protease modulates potyviral replication and host defense responses. *PLoS Pathogens*, 10(3), e1003985. <https://doi.org/10.1371/journal.ppat.1003985>
- Patil, B. L., & Dasgupta, I. (2022). Characterization of the functional domains of nuclear shuttle protein (NSP) of Indian cassava mosaic virus using green fluorescent protein as reporter. *Virus Genes*, 58(4), 308–318. <https://doi.org/10.1007/s11262-022-01909-5>
- Peng, Y. H., Kadoury, D., Gal-On, A., Huet, H., Wang, Y., & Raccah, B. (1998). Mutations in the HC-Pro gene of zucchini yellow mosaic potyvirus: effects on aphid transmission and binding to purified virions. *The Journal of General Virology*, 79(4), 897–904. <https://doi.org/10.1099/0022-1317-79-4-897>
- Perez-Egusquiza, Z., Ward, L. I., Clover, G. R. G., & Fletcher, J. D. (2009). Detection of Sweet potato virus 2 in Sweet Potato in New Zealand. *Plant Disease*, 93(4), 427. <https://doi.org/10.1094/PDIS-93-4-0427B>
- Pérez-Rubio, V., Fortes, I., Romero-Rodríguez, B., Arribas-Hernández, L., Moriones, E., & Castillo, A. G. (2025). Complex interactions between genome components of two ToLCNDV isolates from India and Spain determine effective infection in tomato. In *bioRxiv*. <https://doi.org/10.64898/2025.12.11.691045>
- Perring, T. M. (2001). The Bemisia tabaci species complex. *Crop protection*, 20(9), 725-737. [https://doi.org/10.1016/S0261-2194\(01\)00109-0](https://doi.org/10.1016/S0261-2194(01)00109-0)
- Peyret, H., & Lomonossoff, G. P. (2013). The pEAQ vector series: the easy and quick way to produce recombinant proteins in plants. *Plant Molecular Biology*, 83(1–2), 51–58. <https://doi.org/10.1007/s11103-013-0036-1>
- Peyret, H., & Lomonossoff, G. P. (2015). When plant virology met *Agrobacterium*: the rise of the deconstructed clones. *Plant Biotechnology Journal*, 13(8), 1121–1135. <https://doi.org/10.1111/pbi.12412>
- Peyret, H., Brown, J. K. M., & Lomonossoff, G. P. (2019). Improving plant transient expression through the rational design of synthetic 5' and 3' untranslated regions. *Plant Methods*, 15(1). <https://doi.org/10.1186/s13007-019-0494-9>
- Peyret, H., Steele, J. F. C., Jung, J.-W., Thuenemann, E. C., Meshcheriakova, Y., & Lomonossoff, G. P. (2021). Producing vaccines against enveloped viruses in plants: Making the impossible, difficult. *Vaccines*, 9(7), 780. <https://doi.org/10.3390/vaccines9070780>

- Pirone, T. P. (1964). Aphid transmission of a purified stylet-borne virus acquired through a membrane. [https://doi.org/10.1016/S0042-6822\(64\)80015-5](https://doi.org/10.1016/S0042-6822(64)80015-5)
- Pirone, T. P., & Blanc, S. (1996). Helper-dependent vector transmission of plant viruses. *Annual Review of Phytopathology*, 34(1), 227–247. <https://doi.org/10.1146/annurev.phyto.34.1.227>
- Piroux, N., Saunders, K., Page, A., & Stanley, J. (2007). Geminivirus pathogenicity protein C4 interacts with Arabidopsis thaliana shaggy-related protein kinase AtSK η , a component of the brassinosteroid signalling pathway. *Virology*, 362, 428–440. <https://doi.org/10.1016/j.virol.2006.12.034>
- Pogue, G. P., Lindbo, J. A., Garger, S. J., & Fitzmaurice, W. P. (2002). Making an ally from an enemy: plant virology and the new agriculture. *Annual review of Phytopathology*, 40(1), 45-74. <https://doi.org/10.1146/annurev.phyto.40.021102.150133>
- Pollari, M., De, S., Wang, A., & Mäkinen, K. (2020). The potyviral silencing suppressor HCPro recruits and employs host ARGONAUTE1 in pro-viral functions. *PLoS Pathogens*, 16(10), e1008965. <https://doi.org/10.1371/journal.ppat.1008965>
- Poornima Priyadarshini, C. G., Ambika, M. V., Tippeswamy, R., & Savithri, H. S. (2011). Functional characterization of coat protein and V2 involved in cell to cell movement of cotton leaf curl Kokhran virusDabawali. *PLoS ONE*, 6. <https://doi.org/10.1371/journal.pone.0026929>
- Preiss, W., & Jeske, H. (2003). Multitasking in replication is common among geminiviruses. *Journal of Virology*, 77(5), 2972-2980. <https://doi.org/10.1128/jvi.77.5.2972-2980.2003>
- Pruss, G., Ge, X., Shi, X. M., Carrington, J. C., & Bowman Vance, V. (1997). Plant viral synergism: the potyviral genome encodes a broad-range pathogenicity enhancer that transactivates replication of heterologous viruses. *The Plant Cell*, 9(6), 859–868. <https://doi.org/10.1105/tpc.9.6.859>
- Qin, L., Shen, W., Tang, Z., Hu, W., Shangguan, L., Wang, Y., Tuo, D., Li, Z., Miao, W., Valli, A. A., Wang, A., & Cui, H. (2020). A newly identified virus in the family Potyviridae encodes two leader cysteine proteases in tandem that evolved contrasting RNA silencing suppression functions. *Journal of Virology*, 95(1), e01414-20. <https://doi.org/10.1128/JVI.01414-20>
- Qin, S., Ward, B. M., & Lazarowitz, S. G. (1998). The bipartite geminivirus coat protein aids BR1 function in viral movement by affecting the accumulation of viral single-stranded DNA. *Journal of Virology*, 72(11), 9247-9256. <https://doi.org/10.1128/jvi.72.11.9247-9256.1998>
- Qin, Y.-H., Li, X.-C., Zhang, Z.-C., Qiao, Q., Zhang, D.-S., Wang, Y.-J., Tian, Y.-T., & Wang, S. (2016). First Report of Sweet potato badnavirus A in China. *Plant Disease*, 100(4), 865. <https://doi.org/10.1094/pdis-09-15-1081-pdn>
- Raccah, B., & Fereres, A. (2009). Plant Virus Transmission by Insects. In eLS. Wiley. <https://doi.org/10.1002/9780470015902.a0000760.pub2>
- Radouane, N., Ezrari, S., Belabess, Z., Tahiri, A., Tahzima, R., Massart, S., Jijakli, H., Benjelloun, M., & Lahlali, R. (2021). Viruses of cucurbit crops: current status in the Mediterranean

Bibliography

- Region. *Phytopathologia Mediterranea*, 60(3), 493–519. <https://doi.org/10.36253/phyto-12340>
- Rajamäki, M. L., Kelloniemi, J., Alminait, A., Kekarainen, T., Rabenstein, F., & Valkonen, J. P. (2005). A novel insertion site inside the potyvirus P1 cistron allows expression of heterologous proteins and suggests some P1 functions. *Virology*, 342(1), 88-101. <https://doi.org/10.1016/j.virol.2005.07.019>
- Rajamäki, M.-L., Streng, J., & Valkonen, J. P. T. (2014). Silencing suppressor protein VPg of a potyvirus interacts with the plant silencing-related protein SGS3. *Molecular Plant-Microbe Interactions: MPMI*, 27(11), 1199–1210. <https://doi.org/10.1094/MPMI-04-14-0109-R>
- Ramel, F., Birtic, S., Ginies, C., Soubigou-Taconnat, L., Triantaphylidès, C., & Havaux, M. (2012). Carotenoid oxidation products are stress signals that mediate gene responses to singlet oxygen in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 109(14), 5535–5540. <https://doi.org/10.1073/pnas.1115982109>
- Rana, V. S., Popli, S., Saurav, G. K., Raina, H. S., Chaubey, R., Ramamurthy, V. V., & Rajagopal, R. (2016). A Bemisia tabaci midgut protein interacts with begomoviruses and plays a role in virus transmission: Begomovirus-Bemisia tabaci interaction. *Cellular Microbiology*, 18(5), 663–678. <https://doi.org/10.1111/cmi.12538>
- Rana, V. S., Popli, S., Saurav, G. K., Raina, H. S., Jamwal, R., Chaubey, R., Ramamurthy, V. V., Natarajan, K., & Rajagopal, R. (2019). Implication of the Whitefly, Bemisia tabaci, collagen protein in begomoviruses acquisition and transmission. *Phytopathology*, 109(8), 1481–1493. <https://doi.org/10.1094/PHYTO-03-18-0082-R>
- Rana, V. S., Singh, S. T., Priya, N. G., Kumar, J., & Rajagopal, R. (2012). Arsenophonus GroEL interacts with CLCuV and is localized in midgut and salivary gland of whitefly B. tabaci. *PLoS One*, 7(8), e42168. <https://doi.org/10.1371/journal.pone.0042168>
- Restrepo-Hartwig, M. A., & Carrington, J. C. (1994). The tobacco etch potyvirus 6-kilodalton protein is membrane associated and involved in viral replication. *Journal of Virology*, 68(4), 2388–2397. <https://doi.org/10.1128/JVI.68.4.2388-2397.1994>
- Revers, F., & García, J. A. (2015). Molecular biology of potyviruses. In *Advances in Virus Research* (pp. 101–199). Elsevier. <https://doi.org/10.1016/bs.aivir.2014.11.006>
- Robinson, R. W., Robinson, R. W., & Decker-Walters, D. S. (1997). *Cucurbits. Crop production science in horticulture. Cab International* (Vol. 226).
- Rochow, W. F., & Ross, A. F. (1955). Virus multiplication in plants doubly infected by potato viruses X and Y. *Virology*, 1(1), 10–27. [https://doi.org/10.1016/0042-6822\(55\)90003-9](https://doi.org/10.1016/0042-6822(55)90003-9)
- Rodamilans, B., Casillas, A., & García, J. A. (2021). P1 of sweet potato feathery mottle virus shows strong adaptation capacity, replacing P1-HCPro in a chimeric Plum pox virus. *Journal of Virology*, 95(14), e0015021. <https://doi.org/10.1128/JVI.00150-21>
- Rodamilans, B., Valli, A., Mingot, A., San León, D., Baulcombe, D., López-Moya, J. J., & García, J. A. (2015). RNA polymerase slippage as a mechanism for the production of frameshift gene products in plant viruses of the potyviridae family. *Journal of Virology*, 89(13), 6965–6967. <https://doi.org/10.1128/JVI.00337-15>

- Rodamilans, Bernardo, Valli, A., Mingot, A., San León, D., López-Moya, J. J., & García, J. A. (2018). An atypical RNA silencing suppression strategy provides a snapshot of the evolution of sweet potato-infecting potyviruses. *Scientific Reports*, 8(1). <https://doi.org/10.1038/s41598-018-34358-y>
- Rojas, M. R., Hagen, C., Lucas, W. J., & Gilbertson, R. L. (2005). Exploiting chinks in the plant's armor: evolution and emergence of geminiviruses. *Annual Review of Phytopathology*, 43(1), 361–394. <https://doi.org/10.1146/annurev.phyto.43.040204.135939>
- Rojas, M. R., Macedo, M. A., Maliano, M. R., Soto-Aguilar, M., Souza, J. O., Briddon, R. W., Kenyon, L., Rivera Bustamante, R. F., Zerbini, F. M., Adkins, S., Legg, J. P., Kvarnheden, A., Wintermantel, W. M., Sudarshana, M. R., Peterschmitt, M., Lapidot, M., Martin, D. P., Moriones, E., Inoue-Nagata, A. K., & Gilbertson, R. L. (2018). World management of geminiviruses. *Annual Review of Phytopathology*, 56(1), 637–677. <https://doi.org/10.1146/annurev-phyto-080615-100327>
- Rojas, M. R., Zerbini, F. M., Allison, R. F., Gilbertson, R. L., & Lucas, W. J. (1997). Capsid protein and helper component-proteinase function as potyvirus cell-to-cell movement proteins. *Virology*, 237(2), 283–295. <https://doi.org/10.1006/viro.1997.8777>
- Romay, G., Lecoq, H., & Desbiez, C. (2015). Melon chlorotic mosaic virus and associated alphasatellite from Venezuela: genetic variation and sap transmission of a begomovirus–satellite complex. *Plant Pathology*, 64(5), 1224–1234. <https://doi.org/10.1111/ppa.12342>
- Romay, Gustavo, Pitrat, M., Lecoq, H., Wipf-Scheibel, C., Millot, P., Girardot, G., & Desbiez, C. (2019). Resistance against melon chlorotic mosaic virus and Tomato leaf curl New Delhi virus in melon. *Plant Disease*, 103(11), 2913–2919. <https://doi.org/10.1094/PDIS-02-19-0298-RE>
- Rossel, H., & Thottappilly, G. (1987). Complex virus diseases of sweet potato. Exploration, maintenance, and utilization of sweet potato genetic resources. In *Report of First Sweet Potato Planning Conference* (pp. 291–302). International Potato Center.
- Roullier, C., Duputié, A., Wennekes, P., Benoit, L., Fernández Bringas, V. M., Rossel, G., Tay, D., McKey, D., & Lebot, V. (2013). Disentangling the origins of cultivated sweet potato (*Ipomoea batatas* (L.) Lam.). *PloS One*, 8(5), e62707. <https://doi.org/10.1371/journal.pone.0062707>
- Rubino, L., Abrahamian, P., An, W., Aranda, M. A., Ascencio-Ibañez, J. T., Bejerman, N., Blouin, A. G., Candresse, T., Canto, T., Cao, M., Carr, J. P., Cho, W. K., Constable, F., Dasgupta, I., Debat, H., Dietzgen, R. G., Digiaro, M., Donaire, L., Elbeaino, T., ... ICTV Taxonomy Summary Consortium. (2025). Summary of taxonomy changes ratified by the International Committee on Taxonomy of Viruses from the Plant Viruses Subcommittee, 2025. *The Journal of General Virology*, 106(7). <https://doi.org/10.1099/jgv.0.002114>
- Ruiz, L., Simon, A., Velasco, L., & Janssen, D. (2017). Biological characterization of Tomato leaf curl New Delhi virus from Spain. *Plant Pathology*, 66(3), 376–382. <https://doi.org/10.1111/ppa.12587>
- Ruiz, M. L., Simón, A., Velasco, L., García, M. C., & Janssen, D. (2015). First Report of Tomato leaf curl New Delhi virus Infecting Tomato in Spain. *Plant Disease*, 99(6), 894–894. <https://doi.org/10.1094/pdis-10-14-1072-pdn>

Bibliography

- Sáez, C., Martínez, C., Ferriol, M., Manzano, S., Velasco, L., Jamilena, M., López, C., & Picó, B. (2016). Resistance to Tomato leaf curl New Delhi virus in *Cucurbita* spp: Resistance to Tomato leaf curl New Delhi virus in Cucurbitaspp. *The Annals of Applied Biology*, 169(1), 91–105. <https://doi.org/10.1111/aab.12283>
- Saha, S., & Mäkinen, K. (2020). Insights into the functions of eIF4E-binding motif of VPg in potato virus A infection. *Viruses*, 12(2), E197. <https://doi.org/10.3390/v12020197>
- Saijo, Y., & Loo, E. P.-I. (2020). Plant immunity in signal integration between biotic and abiotic stress responses. *The New Phytologist*, 225(1), 87–104. <https://doi.org/10.1111/nph.15989>
- Sainsbury, F., Thuenemann, E. C., & Lomonossoff, G. P. (2009). pEAQ: versatile expression vectors for easy and quick transient expression of heterologous proteins in plants. *Plant Biotechnology Journal*, 7(7), 682–693. <https://doi.org/10.1111/j.1467-7652.2009.00434.x>
- Sakai, J., Mori, M., Morishita, T., Tanaka, M., Hanada, K., Usugi, T., & Nishiguchi, M. (1997). Complete nucleotide sequence and genome organization of sweet potato feathery mottle virus (S strain) genomic RNA: the large coding region of the P1 gene. *Archives of Virology*, 142(8), 1553–1562. <https://doi.org/10.1007/s007050050179>
- Salazar, L. E., & Fuentes, S. (2000). Current knowledge on major virus diseases of sweetpotatoes. In *Pages 14-19 in: Proc. Int. Workshop Sweetpotato Cultivar Decline study*.
- Saldaña, J., Elena, S. F., & Solé, R. V. (2003). Coinfection and superinfection in RNA virus populations: a selection-mutation model. *Mathematical Biosciences*, 183(2), 135–160. [https://doi.org/10.1016/s0025-5564\(03\)00038-5](https://doi.org/10.1016/s0025-5564(03)00038-5)
- Salvador, B., Saénz, P., Yangüez, E., Quiot, J. B., Quiot, L., Delgadillo, M. O., García, J. A., & Simón-Mateo, C. (2008). Host-specific effect of P1 exchange between two potyviruses. *Molecular Plant Pathology*, 9(2), 147–155. <https://doi.org/10.1111/j.1364-3703.2007.00450.x>
- Sánchez-Campos, S., Rodríguez-Negrete, E. A., Cruzado, L., Grande-Pérez, A., Bejarano, E. R., Navas-Castillo, J., & Moriones, E. (2016). Tomato yellow leaf curl virus: No evidence for replication in the insect vector Bemisia tabaci. *Scientific Reports*, 6(1), 30942. <https://doi.org/10.1038/srep30942>
- Santi, L., Huang, Z., & Mason, H. (2006). Virus-like particles production in green plants. *Methods (San Diego, Calif.)*, 40(1), 66–76. <https://doi.org/10.1016/j.ymeth.2006.05.020>
- Sastry, K. S., & A. Zitter, T. (2014). *Plant virus and viroid diseases in the tropics: Volume 2: Epidemiology and management*. Springer Netherlands.
- 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. *Virology*, 264(1), 142–152. <https://doi.org/10.1006/viro.1999.9948>
- 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. *Proceedings of the*

- National Academy of Sciences of the United States of America*, 97(12), 6890–6895. <https://doi.org/10.1073/pnas.97.12.6890>
- Saunders, K., Richardson, J., Lawson, D. M., & Lomonossoff, G. P. (2020). Requirements for the packaging of Geminivirus circular single-stranded DNA: Effect of DNA length and coat protein sequence. *Viruses*, 12(11), 1235. <https://doi.org/10.3390/v12111235>
- Selth, L. A., Dogra, S. C., Rasheed, M. S., Healy, H., Randles, J. W., & Rezaian, M. A. (2005). A NAC domain protein interacts with tomato leaf curl virus replication accessory protein and enhances viral replication. *The Plant Cell*, 17(1), 311–325. <https://doi.org/10.1105/tpc.104.027235>
- Seminis. (2015). Cucurbit Disease Guide-Seminis. A Disease Reference Guide for Cucumber, Melon, Squash, and Watermelon. *Agri Expo*, 66-92.
- Settlage, S. B., See, R. G., & Hanley-Bowdoin, L. (2005). Geminivirus C3 protein: replication enhancement and protein interactions. *Journal of Virology*, 79(15), 9885-9895. <https://doi.org/10.1128/jvi.79.15.9885-9895.2005>
- Sevvana, M., Klose, T., & Rossmann, M. G. (2021). Principles of virus structure. En *Encyclopedia of Virology* (pp. 257–277). Elsevier. <https://doi.org/10.1016/B978-0-12-814515-9.00033-3>
- Shahriari, A. G., Tahmasebi, A., Ghodoum Parizipour, M. H., Soltani, Z., Tahmasebi, A., & Shahid, M. S. (2025). The crucial role of mitochondrial/chloroplast-related genes in viral genome replication and host defense: integrative systems biology analysis in plant-virus interaction. *Frontiers in Microbiology*, 16, 1551123. <https://doi.org/10.3389/fmicb.2025.1551123>
- Shan, H., Pasin, F., Tzanetakis, I. E., Simón-Mateo, C., García, J. A., & Rodamilans, B. (2018). Truncation of a P1 leader proteinase facilitates potyvirus replication in a non-permissive host. *Molecular Plant Pathology*, 19(6), 1504–1510. <https://doi.org/10.1111/mpp.12640>
- Shan, H., Pasin, F., Valli, A., Castillo, C., Rajulu, C., Carbonell, A., Simón-Mateo, C., García, J. A., & Rodamilans, B. (2015). The Potyviridae P1a leader protease contributes to host range specificity. *Virology*, 476, 264–270. <https://doi.org/10.1016/j.virol.2014.12.013>
- Sheffield, F. (1957). Virus diseases of sweet potato in East Africa-Identification of the viruses and their insect vector. *Phytopathology*, 47, 582–590.
- Shi, J., Zhao, L., Yan, B., Zhu, Y., Ma, H., Chen, W., & Ruan, S. (2019). Comparative transcriptome analysis reveals the transcriptional alterations in growth- and development-related genes in sweet potato plants infected and non-infected by SPFMV, SPV2, and SPVG. *International Journal of Molecular Sciences*, 20(5), 1012. <https://doi.org/10.3390/ijms20051012>
- Shukla, D. D., & Ward, C. W. (1989). Structure of potyvirus coat proteins and its application in the taxonomy of the potyvirus group. *Advances in Virus Research*, 36, 273–314. [https://doi.org/10.1016/s0065-3527\(08\)60588-6](https://doi.org/10.1016/s0065-3527(08)60588-6)
- Silhavy, D., Molnár, A., Lucioli, A., Szittya, G., Hornyik, C., Tavazza, M., & Burgyán, J. (2002). A viral protein suppresses RNA silencing and binds silencing-generated, 21- to 25-

Bibliography

- nucleotide double-stranded RNAs. *The EMBO Journal*, 21(12), 3070–3080. <https://doi.org/10.1093/emboj/cdf312>
- Simón-Buela, L., Osaba, L., García, J. A., & López-Moya, J. J. (2000). Preservation of 5'-end integrity of a Potyvirus genomic RNA is not dependent on template specificity. *Virology*, 269(2), 377–382. <https://doi.org/10.1006/viro.2000.0229>
- Simons, J. N. (1976). Aphid Transmission of a nonaphid-transmissible strain of Tobacco etch Virus. <https://doi.org/10.1094/Phyto-66-652>
- Sivalingam, P. N., & Varma, A. (2012). Role of betasatellite in the pathogenesis of a bipartite begomovirus affecting tomato in India. *Archives of Virology*, 157(6), 1081–1092. <https://doi.org/10.1007/s00705-012-1261-7>
- Smith, B. D. (1997). The Initial Domestication of Cucurbita pepo in the Americas 10,000 Years Ago. *Science (New York, N.Y.)*, 276(5314), 932–934. <https://doi.org/10.1126/science.276.5314.932>
- Souto, E. R., Sim, J., Chen, J., Valverde, R. A., & Clark, C. A. (2003). Properties of Strains of Sweet potato feathery mottle virus and Two Newly Recognized Potyviruses Infecting Sweet Potato in the United States. *Plant Disease*, 87(10), 1226–1232. <https://doi.org/10.1094/pdis.2003.87.10.1226>
- Stanley, J., & Townsend, R. (1985). Characterisation of DNA forms associated with cassava latent virus infection. *Nucleic Acids Research*, 13(7), 2189–2206. <https://doi.org/10.1093/nar/13.7.2189>
- Stanley, J., Bisaro, D. M., Briddon, R. W., Brown, J. K., Fauquet, C. M., Harrison, B. D., Rybicki, E., & Stenger, D. C. (2005). Family geminiviridae. *Virus taxonomy: eighth report of the International Committee on Taxonomy of Viruses*, 301–326.
- Steele, J. F. C., Peyret, H., Saunders, K., Castells-Graells, R., Marsian, J., Meshcheriakova, Y., & Lomonosoff, G. P. (2017). Synthetic plant virology for nanobiotechnology and nanomedicine. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology*, 9(4). <https://doi.org/10.1002/wnan.1447>
- Stewart, L. R., Medina, V., Tian, T., Turina, M., Falk, B. W., & Ng, J. C. (2010). A mutation in the Lettuce infectious yellows virus minor coat protein disrupts whitefly transmission but not in planta systemic movement. *Journal of Virology*, 84(23), 12165–12173. <https://doi.org/10.1128/jvi.01192-10>
- Stubbs, G., & Kendall, A. (2012). Helical viruses. *Advances in Experimental Medicine and Biology*, 726, 631–658. https://doi.org/10.1007/978-1-4614-0980-9_28
- Stubbs, G., Warren, S., & Holmes, K. (1977). Structure of RNA and RNA binding site in tobacco mosaic virus from 4-Å map calculated from X-ray fibre diagrams. *Nature*, 267(5608), 216–221. <https://doi.org/10.1038/267216a0>
- Sung, Y. K., & Coutts, R. H. (1996). Potato yellow mosaic geminivirus AC2 protein is a sequence non-specific DNA binding protein. *FEBS letters*, 383(1-2), 51–54. [https://doi.org/10.1016/0014-5793\(96\)00217-7](https://doi.org/10.1016/0014-5793(96)00217-7)

- Sunter, G., & Bisaro, D. M. (1991). Transactivation in a geminivirus: AL2 gene product is needed for coat protein expression. *Virology*, 180(1), 416–419. [https://doi.org/10.1016/0042-6822\(91\)90049-h](https://doi.org/10.1016/0042-6822(91)90049-h)
- Sunter, G., & Bisaro, D. M. (1997). Regulation of a geminivirus coat protein promoter by AL2 protein (TrAP): evidence for activation and derepression mechanisms. *Virology*, 232(2), 269–280. <https://doi.org/10.1006/viro.1997.8549>
- Sunter, Garry, & Bisaro, D. M. (1992). Transactivation of Geminivirus AR1 and BR1 gene expression by the viral AL2 gene product occurs at the level of transcription. *The Plant Cell*, 4(10), 1321. <https://doi.org/10.2307/3869417>
- Susaimuthu, J., Tzanetakis, I. E., Gergerich, R. C., & Martin, R. R. (2008). A member of a new genus in the Potyviridae infects Rubus. *Virus Research*, 131(2), 145–151. <https://doi.org/10.1016/j.virusres.2007.09.001>
- Syller, J. (2005). The roles and mechanisms of helper component proteins encoded by potyviruses and caulimoviruses. *Physiological and Molecular Plant Pathology*, 67(3–5), 119–130. <https://doi.org/10.1016/j.pmpp.2005.12.005>
- Syller, J. (2012). Facilitative and antagonistic interactions between plant viruses in mixed infections. *Molecular Plant Pathology*, 13(2), 204–216. <https://doi.org/10.1111/j.1364-3703.2011.00734.x>
- Syller, J. (2014). Biological and molecular events associated with simultaneous transmission of plant viruses by invertebrate and fungal vectors. *Molecular Plant Pathology*, 15(4), 417–426. <https://doi.org/10.1111/mpp.12101>
- Szabó, E. Z., Manczinger, M., Göblös, A., Kemény, L., & Lakatos, L. (2012). Switching on RNA silencing suppressor activity by restoring Argonaute binding to a viral protein. *Journal of Virology*, 86(15), 8324–8327. <https://doi.org/10.1128/jvi.00627-12>
- Takahashi, Y., & Uyeda, I. (1999). Restoration of the 3' end of Potyvirus RNA derived from poly(A)-deficient infectious cDNA clones. *Virology*, 265(1), 147–152. <https://doi.org/10.1006/viro.1999.0027>
- Tairo, F., Jones, R. A. C., & Valkonen, J. P. T. (2006). Potyvirus complexes in Sweetpotato: Occurrence in Australia, serological and molecular resolution, and analysis of the Sweet potato virus 2 (SPV2) component. *Plant Disease*, 90(9), 1120–1128. <https://doi.org/10.1094/pd-90-1120>
- Tamborindéguy, C., Gray, S., & Jander, G. (2008). Testing the physiological barriers to viral transmission in aphids using microinjection. *Journal of Visualized Experiments: JoVE*, 15, 700. <https://doi.org/10.3791/700>
- Tanaka, Y., Nakamura, S., Kawamukai, M., Koizumi, N., & Nakagawa, T. (2011). Development of a series of gateway binary vectors possessing a tunicamycin resistance gene as a marker for the transformation of *Arabidopsis thaliana*. *Bioscience, Biotechnology, and Biochemistry*, 75(4), 804–807. <https://doi.org/10.1271/bbb.110063>

Bibliography

- Tarun, J. A., Amalin, D., & Balendres, M. A. (2025). Transcriptional, hormonal, and systems-level regulation of defense response in sweet potato against biotic stresses. *Physiological and Molecular Plant Pathology*, 140, 102863. <https://doi.org/10.1016/j.pmpp.2025.102863>
- Tatineni, S., & Hein, G. L. (2023). Plant viruses of agricultural importance: Current and future perspectives of virus disease management strategies. *Phytopathology*, 113(2), 117–141. <https://doi.org/10.1094/phyto-05-22-0167-rvw>
- Tatineni, S., Qu, F., Li, R., Morris, T. J., & French, R. (2012). Triticum mosaic poacevirus enlists P1 rather than HC-Pro to suppress RNA silencing-mediated host defense. *Virology*, 433(1), 104–115. <https://doi.org/10.1016/j.virol.2012.07.016>
- Tena Fernandez, F., González, I., Doblas, P., Rodríguez, C., Sahana, N., Kaur, H., Tenllado, F., Praveen, S., & Canto, T. (2013). The influence of cis-acting P 1 protein and translational elements on the expression of P otato virus Y helper-component proteinase (HCP ro) in heterologous systems and its suppression of silencing activity. *Molecular Plant Pathology*, 14(5), 530-541. <https://doi.org/10.1111/mpp.12025>
- Tian, T., Rubio, L., Yeh, H. H., Crawford, B., & Falk, B. W. (1999). Lettuce infectious yellows virus: in vitro acquisition analysis using partially purified virions and the whitefly Bemisia tabaci. *The Journal of General Virology*, 80(5), 1111–1117. <https://doi.org/10.1099/0022-1317-80-5-1111>
- Tibiri, E. B., Pita, J. S., Tiendrébéogo, F., Bangratz, M., Néya, J. B., Brugidou, C., Somé, K., & Barro, N. (2020). Characterization of virus species associated with sweetpotato virus diseases in Burkina Faso. *Plant Pathology*, 69(6), 1003–1017. <https://doi.org/10.1111/ppa.13190>
- Torralba, B., Blanc, S., & Michalakakis, Y. (2024). Reassortments in single-stranded DNA multipartite viruses: Confronting expectations based on molecular constraints with field observations. *Virus Evolution*, 10(1). <https://doi.org/10.1093/ve/veae010>
- Torrance, L., Andreev, I. A., Gabrenaite-Verhovskaya, R., Cowan, G., Mäkinen, K., & Taliansky, M. E. (2006). An unusual structure at one end of potato potyvirus particles. *Journal of Molecular Biology*, 357(1), 1-8. <https://doi.org/10.1016/j.jmb.2005.12.021>
- Trebicki, P. (2020). Climate change and plant virus epidemiology. *Virus Research*, 286, 198059. <https://doi.org/10.1016/j.virusres.2020.198059>
- Trenado, H. P., Lozano, G., Valverde, R. A., & Navas-Castillo, J. (2007). First Report of Sweet potato virus G and Sweet potato virus 2 Infecting Sweet Potato in Spain. *Plant Disease*, 91(12), 1687. <https://doi.org/10.1094/PDIS-91-12-1687C>
- Trenado, Helena P., Orílio, A. F., Márquez-Martín, B., Moriones, E., & Navas-Castillo, J. (2011). Sweepoviruses cause disease in sweet potato and related Ipomoea spp.: Fulfilling Koch's postulates for a divergent group in the genus Begomovirus. *PloS One*, 6(11), e27329. <https://doi.org/10.1371/journal.pone.0027329>
- Tugume, A. K., Mukasa, S. B., & Valkonen, J. P. T. (2008). Natural Wild Hosts of Sweet potato feathery mottle virus Show Spatial Differences in Virus Incidence and Virus-Like Diseases in Uganda. *Phytopathology*, 98(6), 640–652. <https://doi.org/10.1094/phyto-98-6-0640>

- Tzanetakis, I. E., Martin, R. R., & Wintermantel, W. M. (2013). Epidemiology of criniviruses: an emerging problem in world agriculture. *Frontiers in Microbiology*, 4, 119. <https://doi.org/10.3389/fmicb.2013.00119>
- Úbeda, J. R., Donaire, L., Alcaide, C., & Aranda, M. A. (2026). Pepino mosaic virus: A globally important tomato pathogen and a rising model in molecular virology. *Molecular Plant Pathology*, 27(1). <https://doi.org/10.1111/mpp.70211>
- Untiveros, M., Fuentes, S., & Kreuze, J. (2008). Molecular variability of sweet potato feathery mottle virus and other potyviruses infecting sweet potato in Peru. *Archives of Virology*, 153(3), 473–483. <https://doi.org/10.1007/s00705-007-0019-0>
- Untiveros, M., Fuentes, S., & Salazar, L. F. (2007). Synergistic Interaction of Sweet potato chlorotic stunt virus (Crinivirus) with Carla-, Cucumo-, Ipomo-, and Potyviruses Infecting Sweet Potato. *Plant Disease*, 91(6), 669–676. <https://doi.org/10.1094/PDIS-91-6-0669>
- Untiveros, M., Olsper, A., Artola, K., Firth, A. E., Kreuze, J. F., & Valkonen, J. P. T. (2016). A novel sweet potato potyvirus open reading frame (ORF) is expressed via polymerase slippage and suppresses RNA silencing: A novel coding ORF in sweet potato potyviruses. *Molecular Plant Pathology*, 17(7), 1111–1123. <https://doi.org/10.1111/mpp.12366>
- Untiveros, M., Quispe, D., & Kreuze, J. (2010). Analysis of complete genomic sequences of isolates of the Sweet potato feathery mottle virus strains C and EA: molecular evidence for two distinct potyvirus species and two P1 protein domains. *Archives of Virology*, 155(12), 2059–2063. <https://doi.org/10.1007/s00705-010-0805-y>
- Valli, A. A., Gallo, A., Rodamilans, B., López-Moya, J. J., & García, J. A. (2018). The HCPro from the *Potyviridae* family: an enviable multitasking Helper Component that every virus would like to have. *Molecular Plant Pathology*, 19(3), 744–763. <https://doi.org/10.1111/mpp.12553>
- Valli, A., Dujovny, G., & García, J. A. (2008). Protease activity, self interaction, and small interfering RNA binding of the silencing suppressor P1b from *cucumber vein yellowing ipomovirus*. *Journal of Virology*, 82(2), 974–986. <https://doi.org/10.1128/jvi.01664-07>
- Valli, A., Dujovny, G., & García, J. A. (2008). Protease activity, self interaction, and small interfering RNA binding of the silencing suppressor p1b from *cucumber vein yellowing ipomovirus*. *Journal of Virology*, 82(2), 974–986. <https://doi.org/10.1128/JVI.01664-07>
- Valli, A., Martín-Hernández, A. M., López-Moya, J. J., & García, J. A. (2006). RNA silencing suppression by a second copy of the P1 serine protease of *Cucumber vein yellowing ipomovirus*, a member of the family *Potyviridae* that lacks the cysteine protease HCPro. *Journal of Virology*, 80(20), 10055–10063. <https://doi.org/10.1128/JVI.00985-06>
- Valli, Adrian A., Domingo-Calap, M. L., González de Prádena, A., García, J. A., Cui, H., Desbiez, C., & López-Moya, J. J. (2024). Reconceptualizing transcriptional slippage in plant RNA viruses. *mBio*, 15(10). <https://doi.org/10.1128/mbio.02120-24>
- Valli, Adrián, García, J. A., & López-Moya, J. J. (2015). *Potyviridae*. En *Encyclopedia of Life Sciences* (pp. 1–10). Wiley. <https://doi.org/10.1002/9780470015902.a0000755.pub3>

Bibliography

- Valli, Adrián, García, J. A., & López-Moya, J. J. (2021). Potyviruses (Potyviridae). En *Encyclopedia of Virology* (pp. 631–641). Elsevier.
- Valli, Adrian, López-Moya, J. J., & García, J. A. (2007). Recombination and gene duplication in the evolutionary diversification of P1 proteins in the family Potyviridae. *The Journal of General Virology*, 88(3), 1016–1028. <https://doi.org/10.1099/vir.0.82402-0>
- Valverde, R. A., Clark, C. A., & Valkonen, J. P. (2007). Viruses and virus disease complexes of sweetpotato. *Plant Viruses*, 1(1), 116–126.
- van Engelen, F. A., Molthoff, J. W., Conner, A. J., Nap, J.-P., Pereira, A., & Stiekema, W. J. (1995). pBINPLUS: An improved plant transformation vector based on pBIN19. *Transgenic Research*, 4(4), 288–290. <https://doi.org/10.1007/bf01969123>
- van Wezel, R., Dong, X., Liu, H., Tien, P., Stanley, J., & Hong, Y. (2002). Mutation of Three Cysteine Residues in Tomato yellow leaf curl virus-ChinaC2 Protein Causes Dysfunction in Pathogenesis and Posttranscriptional Gene—Silencing Suppression. *Molecular Plant-Microbe Interactions: MPMI*, 15(3), 203–208. <https://doi.org/10.1094/mpmi.2002.15.3.203>
- Vance, V. B. (1991). Replication of potato virus X RNA is altered in coinfections with potato virus Y. *Virology*, 182(2), 486–494. [https://doi.org/10.1016/0042-6822\(91\)90589-4](https://doi.org/10.1016/0042-6822(91)90589-4)
- Verchot, J., & Carrington, J. C. (1995). Debilitation of plant potyvirus infectivity by P1 proteinase-inactivating mutations and restoration by second-site modifications. *Journal of Virology*, 69(3), 1582–1590. <https://doi.org/10.1128/JVI.69.3.1582-1590.1995>
- Verchot, J., & Carrington, J. C. (1995b). Evidence that the potyvirus P1 proteinase functions in trans as an accessory factor for genome amplification. *Journal of Virology*, 69(6), 3668–3674. <https://doi.org/10.1128/JVI.69.6.3668-3674.1995>
- Verchot, J., Herndon, K. L., & Carrington, J. C. (1992). Mutational analysis of the tobacco etch potyviral 35-kDa proteinase: identification of essential residues and requirements for autoproteolysis. *Virology*, 190(1), 298–306. [https://doi.org/10.1016/0042-6822\(92\)91216-h](https://doi.org/10.1016/0042-6822(92)91216-h)
- Verchot, J., Koonin, E. V., & Carrington, J. C. (1991). The 35-kDa protein from the N-terminus of the potyviral polyprotein functions as a third virus-encoded proteinase. *Virology*, 185(2), 527–535. [https://doi.org/10.1016/0042-6822\(91\)90522-d](https://doi.org/10.1016/0042-6822(91)90522-d)
- Vilsker, M., Moosa, Y., Nooij, S., Fonseca, V., Ghysens, Y., Dumon, K., Pauwels, R., Alcantara, L. C., Vanden Eynden, E., Vandamme, A.-M., Deforche, K., & de Oliveira, T. (2019). Genome Detective: an automated system for virus identification from high-throughput sequencing data. *Bioinformatics*, 35(5), 871–873. <https://doi.org/10.1093/bioinformatics/bty695>
- Vo, T. T. B., Lal, A., Ho, P. T., Troiano, E., Parrella, G., Kil, E.-J., & Lee, S. (2022). Different infectivity of Mediterranean and southern Asian tomato leaf curl New Delhi virus isolates in Cucurbit crops. *Plants*, 11(5), 704. <https://doi.org/10.3390/plants11050704>
- Voinnet, O., & Baulcombe, D. C. (1997). Systemic signalling in gene silencing. *Nature*, 389(6651), 553. <https://doi.org/10.1038/39215>

- Voinnet, O., Lederer, C., & Baulcombe, D. C. (2000). A viral movement protein prevents spread of the gene silencing signal in *Nicotiana benthamiana*. *Cell*, 103(1), 157–167. [https://doi.org/10.1016/s0092-8674\(00\)00095-7](https://doi.org/10.1016/s0092-8674(00)00095-7)
- Voinnet, O., Pinto, Y. M., & Baulcombe, D. C. (1999). Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. *Proceedings of the National Academy of Sciences*, 96(24), 14147–14152. <https://doi.org/10.1073/pnas.96.24.14147>
- Waltermann, A., & Maiss, E. (2006). Detection of 6K1 as a mature protein of 6 kDa in plum pox virus-infected *Nicotiana benthamiana*. *The Journal of General Virology*, 87(8), 2381–2386. <https://doi.org/10.1099/vir.0.81873-0>
- Wang, A. (2021). Cell-to-cell movement of plant viruses via plasmodesmata: a current perspective on potyviruses. *Current Opinion in Virology*, 48, 10–16. <https://doi.org/10.1016/j.coviro.2021.03.002>
- Wang, H., Zhang, C., Dou, Y., Yu, B., Liu, Y., Heng-Moss, T. M., Lu, G., Wachholtz, M., Bradshaw, J. D., Twigg, P., Scully, E., Palmer, N., & Sarath, G. (2017). Insect and plant-derived miRNAs in greenbug (*Schizaphis graminum*) and yellow sugarcane aphid (*Sipha flava*) revealed by deep sequencing. *Gene*, 599, 68–77. <https://doi.org/10.1016/j.gene.2016.11.014>
- Wang, L., Poque, S., & Valkonen, J. P. T. (2019). Phenotyping viral infection in sweetpotato using a high-throughput chlorophyll fluorescence and thermal imaging platform. *Plant Methods*, 15(1). <https://doi.org/10.1186/s13007-019-0501-1>
- Wang, L.-L., Wang, X.-R., Wei, X.-M., Huang, H., Wu, J.-X., Chen, X.-X., Liu, S.-S., & Wang, X.-W. (2016). The autophagy pathway participates in resistance to tomato yellow leaf curl virus infection in whiteflies. *Autophagy*, 12(9), 1560–1574. <https://doi.org/10.1080/15548627.2016.1192749>
- Wang, M., Abad, J., Fuentes, S., & Li, R. (2013). Complete genome sequence of the original Taiwanese isolate of sweet potato latent virus and its relationship to other potyviruses infecting sweet potato. *Archives of virology*, 158(10), 2189–2192. <https://doi.org/10.1007/s00705-013-1705-8>
- Wang, R. Y., & Pirone, T. P. (1999). Purification and characterization of turnip mosaic virus helper component protein. *Phytopathology*, 89(7), 564–567. <https://doi.org/10.1094/PHYTO.1999.89.7.564>
- Wang, X.-W., & Blanc, S. (2021). Insect transmission of plant single-stranded DNA viruses. *Annual Review of Entomology*, 66(1), 389–405. <https://doi.org/10.1146/annurev-ento-060920-094531>
- Wang, Y., Qin, Y., Wang, S., Zhang, D., Tian, Y., Zhao, F., Wang, Y., Lv, H., Qiao, Q., & Zhang, Z. (2021). Species and genetic variability of sweet potato viruses in China. *Phytopathology Research*, 3(1). <https://doi.org/10.1186/s42483-021-00097-8>
- Wang, Z., Wang, Y., Lozano-Duran, R., Hu, T., & Zhou, X. (2022). Identification of a novel C6 protein encoded by tomato leaf curl China virus. *Phytopathology Research*, 4(1). <https://doi.org/10.1186/s42483-022-00151-z>

Bibliography

- War, A. R., Paulraj, M. G., War, M. Y., & Ignacimuthu, S. (2011). Role of salicylic acid in induction of plant defense system in chickpea (*Cicer arietinum* L.). *Plant Signaling & Behavior*, 6(11), 1787–1792. <https://doi.org/10.4161/psb.6.11.17685>
- Wei, K.-J., Jiang, A.-M., Jiang, S., Huang, Y.-J., Jiang, S.-Y., Su, X.-L., Tettey, C. K., Wang, X.-Q., Tang, W., & Cheng, D.-J. (2024). New isolate of sweet potato virus 2 from Ipomoea nil: molecular characterization, codon usage bias, and phylogenetic analysis based on complete genome. *Virology Journal*, 21(1). <https://doi.org/10.1186/s12985-024-02500-0>
- Wei, T., Zhang, C., Hong, J., Xiong, R., Kasschau, K. D., Zhou, X., Carrington, J. C., & Wang, A. (2010). Formation of complexes at plasmodesmata for potyvirus intercellular movement is mediated by the viral protein P3N-PIPO. *PLoS Pathogens*, 6(6), e1000962. <https://doi.org/10.1371/journal.ppat.1000962>
- Weinheimer, I., Boonrod, K., Moser, M., Wassenegger, M., Krczal, G., Butcher, S. J., & Valkonen, J. P. T. (2014). Binding and processing of small dsRNA molecules by the class 1 RNase III protein encoded by sweet potato chlorotic stunt virus. *The Journal of General Virology*, 95(2), 486–495. <https://doi.org/10.1099/vir.0.058693-0>
- Whitfield, A. E., Falk, B. W., & Rotenberg, D. (2015). Insect vector-mediated transmission of plant viruses. *Virology*, 479–480, 278–289. <https://doi.org/10.1016/j.virol.2015.03.026>
- Wilson, C. R. (2014). *Applied plant virology*. CABI.
- Wintermantel, W. M., Cortez, A. A., Anchietia, A. G., Gulati-Sakhujia, A., & Hladky, L. L. (2008). Co-infection by two criniviruses alters accumulation of each virus in a host-specific manner and influences efficiency of virus transmission. *Phytopathology*, 98(12), 1340–1345. <https://doi.org/10.1094/PHYTO-98-12-1340>
- Wu, M., Wei, H., Tan, H., Pan, S., Liu, Q., Bejarano, E. R., & Lozano-Durán, R. (2021). Plant DNA polymerases α and δ mediate replication of geminiviruses. *Nature Communications*, 12(1). <https://doi.org/10.1038/s41467-021-23013-2>
- Wu, S., Kitavi, M., Hamilton, J. P., Buell, C. R., & Fei, Z. (2025). US efforts in Sweetpotato genome sequencing: Advances in the development of reference genomes to facilitate research and breeding of a key food security crop. In *Compendium of Plant Genomes* (pp. 11–17). Springer International Publishing. https://doi.org/10.1007/978-3-031-65003-1_2
- Xia, W.-Q., Liang, Y., Chi, Y., Pan, L.-L., Zhao, J., Liu, S.-S., & Wang, X.-W. (2018). Intracellular trafficking of begomoviruses in the midgut cells of their insect vector. *PLoS Pathogens*, 14(1), e1006866. <https://doi.org/10.1371/journal.ppat.1006866>
- Xu, K., & Nagy, P. D. (2014). Expanding use of multi-origin subcellular membranes by positive-strand RNA viruses during replication. *Current Opinion in Virology*, 9, 119–126. <https://doi.org/10.1016/j.coviro.2014.09.015>
- Xu, X., Zhang, Q., Hong, J., Li, Z., Zhang, X., & Zhou, X. (2019). Cryo-EM structure of a begomovirus geminate particle. *International Journal of Molecular Sciences*, 20(7), 1738. <https://doi.org/10.3390/ijms20071738>

- Xue, Q., Swevers, L., & Taning, C. N. T. (2023). Plant and insect virus-like particles: emerging nanoparticles for agricultural pest management. *Pest Management Science*, 79(9), 2975–2991. <https://doi.org/10.1002/ps.7514>
- Yang LiJun, Y. L., Hidaka, M., Masaki, H., & Uozumi, T. (1998). Detection of potato virus Y P1 protein in infected cells and analysis of its cleavage site. *Bioscience, Biotechnology and Biochemistry*, 62(2), 380–382. <https://doi.org/10.1271/bbb.62.380>
- Yang, X., Guo, W., Li, F., Sunter, G., & Zhou, X. (2019). Geminivirus-associated betasatellites: Exploiting chinks in the antiviral arsenal of plants. *Trends in Plant Science*, 24(6), 519–529. <https://doi.org/10.1016/j.tplants.2019.03.010>
- Yang, X., Li, Y., & Wang, A. (2021). Research advances in potyviruses: From the laboratory bench to the field. *Annual Review of Phytopathology*, 59(1), 1–29. <https://doi.org/10.1146/annurev-phyto-020620-114550>
- Yazdani-Khameneh, S., Aboutorabi, S., Shoori, M., Aghazadeh, A., Jahanshahi, P., Golnaraghi, A., & Maleki, M. (2016). Natural Occurrence of Tomato leaf curl New Delhi virus in Iranian Cucurbit Crops. *The Plant Pathology Journal*, 32(3), 201–208. <https://doi.org/10.5423/PPJ.OA.10.2015.0210>
- Yazdi, H. R. 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(3), 539–545. <https://doi.org/10.1007/s11262-008-0224-2>
- Young, B. A., Stenger, D. C., Qu, F., Morris, T. J., Tatineni, S., & French, R. (2012). Tritimovirus P1 functions as a suppressor of RNA silencing and an enhancer of disease symptoms. *Virus Research*, 163(2), 672–677. <https://doi.org/10.1016/j.virusres.2011.12.019>
- Yu, J., Su, Y., Sun, J., Liu, J., Li, Z., & Zhang, B. (2020). Selection of stable reference genes for gene expression analysis in sweet potato (*Ipomoea batatas* L.). *Molecular and Cellular Probes*, 53(101610), 101610. <https://doi.org/10.1016/j.mcp.2020.101610>
- Yu, N., Christiaens, O., Liu, J., Niu, J., Cappelle, K., Caccia, S., Huvenne, H., & Smagghe, G. (2013). Delivery of dsRNA for RNAi in insects: an overview and future directions: Delivery of dsRNA for RNAi in insects. *Insect Science*, 20(1), 4–14. <https://doi.org/10.1111/j.1744-7917.2012.01534.x>
- Zaidi, S. S.-E.-A., Martin, D. P., Amin, I., Farooq, M., & Mansoor, S. (2017). *Tomato leaf curl New Delhi virus*: a widespread bipartite begomovirus in the territory of monopartite begomoviruses. *Molecular Plant Pathology*, 18(7), 901–911. <https://doi.org/10.1111/mpp.12481>
- Zaidi, S. S.-E.-A., Shafiq, M., Amin, I., Scheffler, B. E., Scheffler, J. A., Briddon, R. W., & Mansoor, S. (2016). Frequent occurrence of Tomato leaf curl New Delhi virus in cotton leaf curl disease affected cotton in Pakistan. *PloS One*, 11(5), e0155520. <https://doi.org/10.1371/journal.pone.0155520>
- Zanardo, L. G., & Carvalho, C. M. (2017). Cowpea mild mottle virus (Carlavirus, Betaflexiviridae): a review. *Tropical Plant Pathology*, 42(6), 417–430. <https://doi.org/10.1007/s40858-017-0168-y>

Bibliography

- Zanardo, Larissa Goulart, de Souza, G. B., & Alves, M. S. (2019). Transcriptomics of plant–virus interactions: a review. *Theoretical and Experimental Plant Physiology*, 31(1), 103–125. <https://doi.org/10.1007/s40626-019-00143-z>
- Zeltins, A. (2013). Construction and characterization of virus-like particles: A review. *Molecular Biotechnology*, 53(1), 92–107. <https://doi.org/10.1007/s12033-012-9598-4>
- Zhang, H., Zhao, X., Cao, X., Khan, L. U., Zhao, R., Wang, H., & Huang, X. (2022). Transmission of Areca palm velarivirus 1 by mealybugs causes yellow leaf disease in betel palm (*Areca catechu*). *Phytopathology*, 112(3), 700–707. <https://doi.org/10.1094/phyto-06-21-0261-r>
- Zhang, J., Dong, J., Xu, Y., & Wu, J. (2012). V2 protein encoded by Tomato yellow leaf curl China virus is an RNA silencing suppressor. *Virus Research*, 163(1), 51–58. <https://doi.org/10.1016/j.virusres.2011.08.009>
- Zhang, K., Lu, H., Wan, C., Tang, D., Zhao, Y., Luo, K., Li, S., & Wang, J. (2020). The spread and transmission of sweet potato virus disease (SPVD) and its effect on the gene expression profile in sweet potato. *Plants*, 9(4), 492. <https://doi.org/10.3390/plants9040492>
- Zhao, J., Zhang, X., Hong, Y., & Liu, Y. (2016). Chloroplast in plant-virus interaction. *Frontiers in Microbiology*, 7, 1565. <https://doi.org/10.3389/fmicb.2016.01565>
- Zhao, Jing, Lei, T., Zhang, X.-J., Yin, T.-Y., Wang, X.-W., & Liu, S.-S. (2020). A vector whitefly endocytic receptor facilitates the entry of begomoviruses into its midgut cells via binding to virion capsid proteins. *PLoS Pathogens*, 16(12), e1009053. <https://doi.org/10.1371/journal.ppat.1009053>
- Zhao, S., Gong, P., Ren, Y., Liu, H., Li, H., Li, F., & Zhou, X. (2022). The novel C5 protein from tomato yellow leaf curl virus is a virulence factor and suppressor of gene silencing. *Stress Biology*, 2(1), 19. <https://doi.org/10.1007/s44154-022-00044-3>
- Zhao, X., Zhao, R., Cao, X., Wang, Y., Wang, H., & Huang, X. (2024). Transmission of areca palm velarivirus 1 (APV1) by *Pseudococcus cryptus*. *Phytopathology Research*, 6(1). <https://doi.org/10.1186/s42483-024-00228-x>
- Zhou, X. (2013). Advances in understanding Begomovirus satellites. *Annual Review of Phytopathology*, 51(1), 357–381. <https://doi.org/10.1146/annurev-phyto-082712-102234>

