Program and Abstracts

November 9 – 13, 2019

Welcome Message

We are pleased to welcome you to the 9th International Geminivirus Symposium and 7th ssDNA Comparative Virology Workshop (IGS 2019), which will be held from November 9th to November 13th, 2019 on the campus of the University of California, Davis, USA.

IGS 2019 will bring together a wide range of scientists from throughout the world who are at the forefront of the study of geminiviruses and other ssDNA viruses. This symposium will provide excellent opportunities for young scientists and graduate students to present their work and to exchange ideas. The program includes sessions ranging from new technologies to historical aspects through keynote talks, short talks and poster sessions. There will be many opportunities for stimulating discussions and interactions.

IGS 2019 will be an opportunity to exchange information, engage in stimulating discussions, connect and collaborate with your fellow researchers from around the world. We are confident that our speakers will provide you with critically relevant and up-to-date information on this active and evolving field of study.

We look forward to welcoming you to the University of California, Davis and hope that you all enjoy this exciting meeting.

Robert L. Gilbertson and William M. Wintermantel

Co-Chairs of the Organizing Committee

Organizing Committee

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Rafael Rivera Bustamante, Cinvestav – Mexico

Supriya Chakraborty, Jawaharlal Nehru University – India

Xueping Zhou, Chinese Academy of Agricultural Sciences – China

Preliminary Agenda

Time	Saturday (11/9)	Sunday (11/10)	Monday (11/11)	Tuesday (11/12)	Wednesday (11/13)
9:00 - 9:05		Introduction	Introduction	Introduction	Introduction
9:05 - 9:10		**Douglas P. Maxwell** *TBD*	**Linda Hanley-Bowdoin** *TBD*	**Shu-Sheng Liu** *"Effects of viruses on phytohormone pathways and plant-insect interactions"*	**Michel Peterschmitt** *"Plant resistance-driven emergence of recombinant begomoviruses"*
9:10 - 9:15					
9:15 - 9:20					
9:20 - 9:25					
9:25- 9:30					
9:30 - 9:35					
9:35 - 9:40		Introduction	**Bjorn Krenz** *"Begomoviral Movement Protein Effects in Human and Plant Cells: Towards New Potential Interaction Partners"*	**Jeremy Di Mattia *** *"Faba bean necrotic stunt virus (Family Nanoviridae, genus Nanovirus) and Alfalfa leaf curl virus (Family Geminiviridae, genus Capulavirus) interactions within co-infected plants and co-transmitting aphid vector"*	**Jesus Aaron Avalos-Calleros *** *"Analysis of a TYLCV natural mutant uncover an element conserved in Old World begomoviruses whose deletion greatly weaken the Rep gene promoter activity"*
9:40 - 9:45		**Stéphane Blanc** *"Gene copy number variation impacts on gene expression in a multipartite virus"*			
9:45 - 9:50					
9:50 - 9:55			Introduction	Introduction	Introduction
9:55 - 10:00			**Elizabeth Fontes** *"An NSP-Interacting Immune Hub: Hijacking Host Transport Functions for vDNA Movement and Suppressing Incompatible Functions"*	**Renate Krause-Sakate** *"Interactions between whitefly species and begomoviruses infecting solanaceas and legumes in Brazil Interactions between Bemisia tabaci and tomato severe rugose virus"*	**Muhammad Tahir** *"Journey towards the end of CLCVs - Friends from Foe"*
10:00 - 10:05					
10:05 - 10:10					
10:10 - 10:15		**James Dale** *"BBTV resistant GM bananas: from the lab to the glasshouse and finally the field"*			**Kehinde Oyeniran *** *"Symptom Evolution Following the Emergence of Maize Streak Virus"*
10:15 - 10:20					
10:20 - 10:25					
10:25- 10:30			**Maria Behnecke *** *"Investigation of nanoviral M-Rep, CP and NSP interaction complexes: BiFC analyses of wt and deletion proteins by confocal microscopy"*	**William Sharpee** *"Evaluating Transmission Dynamics of Cassava Mosaic Begomoviruses (CMB) from Mixed Infections by the Whitefly Vector Bemisia tabaci SSA1-SG1"*	**Simona Kraberger** *"Single-stranded DNA virus diversity associated with honey bees (Apis mellifera) in Arizona"*
10:30 - 10:35					
10:35 - 10:40					
10:40 - 10:55		**Coffee Break**	**Coffee Break**	**Coffee Break**	**Coffee Break**
10:55 - 11:00		Introduction	Introduction	Introduction	Introduction

11:00 - 11:05		**Arvind Varsani** *"Exploration of ssDNA viral sequence space using viral metagenomics"*	**Tatjana Kleinow** *"Phosphorylation of a begomovirus movement protein affects its self-interaction and interplay with the plant host"*	**Alice K. Inoue-Nagata** *"Management of begomovirus disease on tomato in Brazil"*	Student presentation award
11:05 - 11:10					
11:10 - 11:15					
11:15 - 11:20					**Robert L. Gilbertson** *TBD*
11:20 - 11:25					
11:25- 11:30					
11:30 - 11:35		**Sohini Claverie *** *"Diversity and structure of Poaceae-infecting mastreviruses communities on Reunion Island using a viral metagenomics-based approach"*	**Fuh-Jyh Jan** *"A Single Amino Acid Substitution in the Movement Protein Enables Mechanical Transmissibility of a Geminivirus"*	**Veronica Perez-Padilla** *"Revisiting Seed Transmission of the Type Strain of Tomato Yellow Leaf Curl Virus in Tomato Plants"*	
11:35 - 11:40					
11:40 - 11:45					
11:45 - 11:50		Introduction	Introduction	Introduction	
11:50 - 11:55		**Siobain Duffy** *"Improved evolutionary models for ssDNA viruses"*	**Garry Sunter** *"The Geminivirus AC2 protein, a Central Player in the Infection Cycle"*	**Vincent Fondong** *"The fight against geminiviruses in the wake of novel advances in genomics and biotechnology: what lessons from decades-long war on cassava geminiviruses?"*	Next meeting Presentation and voting
11:55 - 12:00					
12:00 - 12:05					
12:05 - 12:10					
12:10 - 12:15					
12:15 - 12:20					
12:20 - 12:25		**Jean-Michel Lett** *"Night at the museum: Contribution of small RNA from historical herbarium specimens in the reconstruction of evolutionary histories of geminiviruses*"	**Kai-Shu Ling** *"Transcriptome Analysis Reveals TYLCV-encoded C4 Protein Interferes a Network of Leaf Developmental Transcription Factors Linking Leaf Upward Cupping Phenotype in Tomato"*	**Tomas A Melgarejo** *"Mechanisms underlying differential virulence of two strains of a New World monopartite tomato-infecting begomovirus"*	
12:25- 12:30					
12:30 - 12:35					
12:35 - 1:35		**Lunch**	**Lunch**	**Lunch**	**Lunch**
1:35 - 1:40		Introduction	Introduction	Introduction	**Field Trip to FPS**
1:40 - 1:45		**Philippe Roumagnac** *"Hidden diversity of endogenous geminiviral sequences across plant genomes and transcriptomes"*	**Supriya Chakraborty** *"New insights into roles of betasatellite in leaf curl disease development"*	**Carl Strausbaugh** *"Management of Beet curly top virus in Sugar Beet"*	
1:45 - 1:50					
1:50 - 1:55					

1:55 - 2:00					
2:00 - 2:05					
2:05 - 2:10					
2:10 - 2:15		**Syed Shan-e-Ali Zaidi** "*CIDER-Seq: An efficient tool to study plant-geminivirus recombinant eccDNAs"*	**Harrold A. van den Burg** *"Protein modifier SUMO recruits E2 conjugating enzyme (SCE1) activity to the Replication initiator protein to allow replication of the Geminivirus TYLCV"*	**Monica Macedo** *"Assessing the effectiveness of a TFP in Brazil based on monitoring the incidence of begomovirus disease and whitefly vector populations in tomato fields in Brazil"*	
2:15 - 2:20					
2:20 - 2:25					
2:25- 2:30		Introduction	Introduction	Introduction	
2:30 - 2:35		**Murilo Zerbini** *"Composition of Begomovirus Populations in Cultivated and Non-Cultivated Hosts Determined by High-Throughput Sequencing"*	**Indranil Dasgupta** *"Developing Tools To Analyze The Begomoviruses And Satellites Associated With Yellow Vein Mosaic And Enation Leaf Curl Diseases Of Okra"*	**Judith Brown** *"Phylogenomic and phylogenetic analyses and climate-niche modeling of global whitefly Bemisia tabaci vector-begomoviruses reflect tight geographical and niche-associated co-diversification"*	
2:35 - 2:40					
2:40 - 2:45					
2:45 - 2:50					
2:50 - 2:55					
2:55 - 3:00					
3:00 - 3:05		**J. Steen Hoyer** *"Rapid Mutation in Experimental Cassava Begomovirus Populations"*	**Yen-Wen Kuo** *"Artificial microRNA strand selection shows a purine-rich preference for geminivirus- and non-virus-based expression vectors in plants"*	**Daniel K. Hasegawa** *"Small RNA profiling of the whitefly, Bemisia tabaci MEAM1 in response to feeding on tomato infected with Tomato yellow leaf curl virus"*	
3:05 - 3:10					
3:10 - 3:15	Registration				
3:15 - 3:30		**Coffee Break**	**Coffee Break**	**Coffee Break**	
3:30 - 3:35		Introduction	Introduction	Introduction	
3:35 - 3:40		**Juliana Osse de Souza *** *"Is the elusive Brazilian curly top virus (BraCTV) a capulavirus?"*	**David Bisaro** *"Regulation of Transcription and Translation: Insights from Geminivirus-Host Interactions"*	**William Wintermantel** *"Utilizing 'omics technologies to enhance studies on virus transmission and control"*	
3:40 - 3:45					
3:45 - 3:50					
3:50 - 3:55		**Faustine Rychebush *** *"Characterization of the Transmission of Alfalfa leaf curl virus, an Aphid-transmitted"*			
3:55 - 4:00					
4:00 - 4:05					
4:05 - 4:10		**Rafaela Fontenele *** *"Novel Divergent Geminivirus Infecting Cactaceae Plants"*	**Cica Urbino** *"Is the assistance of satellites by TYLCV strictly cell autonomous?"*	**Bangya Ma** *"SP2700 - A Novel Antiviral Plant Activator"*	
4:10 - 4:15					
4:15 - 4:20					
4:20 - 4:25		Introduction	Introduction	Introduction	
4:25- 4:30		**Gian Paolo Accotto** *"Can geminiviruses play a lead role in virus-mediated horizontal transfer of chromosomal DNA between plants?"*	**Herve Vandershuren** *"Alteration of geminivirus genome and population driven by CRISPR/Cas9 and RNA interference"*	**Maher Al Rwahnih** *"Recent Ooutbreak of Grapevine red blotch virus in FPS Foundation Vineyard"*	
4:30 - 4:35					
4:35 - 4:40					

4:40 - 4:45					
4:45 - 4:50					
4:50 - 4:55					
4:55 - 5:00		**Fanfang Li** *"Nuclear autophagy degrades a geminivirus nuclear protein to restrict viral infection in Solanaceae plants"*	**Jose T Ascencio-Ibanez** *"Fine Mapping of Broad Geminivirus Resistance in Arabidopsis thaliana"*	**Minor R. Maliano *** *"Subcellular localization and functional analysis of the Grapevine red blotch virus proteins"*	
5:00 - 5:05					
5:05 - 5:10					
5:10 - 5:15		Introduction	Introduction	Introduction	
5:15 - 5:20		**Catherine D Doyle *** *"Two DNA Episomes From the Cassava Genome Alter GeminivirusInfection in the Field and Under Laboratory Conditions"*	**Moshe Lapidot** *"New insights into genetic resistance to TYLCV"*	**Mysore Sudarshana** *"Impact of Grapevine red blotch virus on wine grape production in California"*	
5:20 - 5:25					
5:25- 5:30					
5:30 - 5:35		**Hendrik Reuper *** *"The Role of Stress Granules in Begomoviral Infection"*			
5:35 - 5:40					
5:40 - 5:45					
5:45 - 5:50			**Fenisha Dilipkumar Chahwala *** *"Exploration of defense mechanism involved in solanum lycopersicum by using high throughput RNA-Seq method against begomovirus infection"*	**John Hu** *"Field Evaluation of Transgenic Banana Plants for Resistance to BBTV Infections in Hawaii"*	
5:50 - 5:55					
5:55 - 6:00	**Reception**				
6:00 - 6:05			Dinner	Poster	
6:05 - 6:10					
6:10 - 6:15					
6:15 - 6:20					
6:20 - 6:25	**Opening Ceremony**				
6:25- 6:30					
6:30 - 6:35					
6:35 - 6:40					
6:40 - 6:45	**Richard Michelmore** *TBD*				
6:45 - 6:50					
6:50 - 6:55					
6:55 - 7:00					
7:00 – 8:00	**Welcome Dinner**			**Farewell Dinner**	
8:00 – 9:00			Geminivirus Study Group Workshop		

* Participants for the student presentation award.

Characterization of the Transmission of Alfalfa leaf curl virus, an Aphid-transmitted Geminivirus

Faustine Ryckebusch[1], Martine Granier[1], Jeremy Di Mattia[2], Jean-Louis Zeddam[1], Nicolas Sauvion[2], Michel Peterschmitt[1]

[1]CIRAD, Montpellier, France. [2]INRA, Montpellier, France

Abstract

For decades, whiteflies, leafhoppers and treehoppers were the only known vectors of geminiviruses (family Geminiviridae). Metagenomic analysis revealed four divergent members of this family that are now classified in a new genus named Capulavirus according to their type member Euphorbia Caput medusae latent virus (EcmLV). Among them, Alfalfa leaf curl virus (ALCV) infects mostly Fabaceae and has been shown in our lab to be transmitted by *Aphis craccivora*. As it was the first report of an aphid-transmitted geminivirus, the mode of transmission was characterized. The transmission journey of ALCV starts in the phloem (Vicia faba) where it was shown to be restricted using FISH and EPG. To reach a 50% transmission success it needs at least 10 insects per inoculated plant following an acquisition access period of at least 24 hours. Once the aphid has acquired virus, the viral load remains constant in its body and transmission was still possible up to 11 days post-acquisition. Viral DNA was detected in the midgut and salivary glands which altogether support a circulative persistent mode of transmission involving internalization into insect epithelia mediated by potential receptors. The relatively low transmission efficiency of ALCV may be explained by the non-uniform distribution of ALCV (FISH and EPG) which may hinder its acquisition, and a non-efficient release from the viruliferous aphid into the inoculated tissue as revealed by qPCR. The aphid transmission of ALCV is highly specific because *Aphis tirucallis*, a close relative of *A. craccivora*, undistinguishable with CO1 phylogeny (Coeur d'Acier *et. al.,* 2014), did no transmit ALCV although we identified it as the vector of EcmLV. Thus, we compared the pathways of ALCV between the two aphids to enlight potential transmission barriers in *A. tirucallis* body (qPCR, FISH), and initiate the identification of potential receptors in *A. craccivora* with a Y2H approach.

2

Journey towards the end of CLCVs -Friends from Foe

Muhammad Tahir

Plant Biotechnology Dept., Atta-ur-Rahman School of Applied Biosciences (ASAB), National University of Sciences and Technology (NUST), Islamabad, Pakistan

Abstract

Cotton leaf curl disease (CLCuD) is the major limitation to cotton production in Pakistan. The disease is caused by *Begomoviruses* (family *Geminiviridae*), which contains single stranded, circular DNA genome and is transmitted by a single species of whitefly (*Bemisia tabaci* Gennadius). Approximately 5-6 species of begomoviruses are infecting cotton. Cotton leaf samples showing typical begomovirus like symptoms were collected from distinct locations of Pakistan. Full length begomoviruses, alphasatellite and betasatellite were cloned from the symptomatic samples which showed high identity to Cotton leaf curl Kokhran virus (CLCuKV), Cotton leaf curl Multan Betasatellite and Cotton leaf curl Multan Alphasatellites, respectively. The partial tandem repeat constructs of begomovirus and associated components were developed which were infectious to *Nicotiana benthamiana*. An innovative approach of program cell death was used to reduce cotton infecting begomoviral infection, by targeting the viral genomes using modified cotton leaf curl betasatellite. The *Nicotiana benthamiana* transgenes showed no symptoms compared to control plants. The expression analysis of transgenes' showed high expression and reduced titre of CLCuKV compared to control plants. Further analyses are required to prove the concept of sustainability and durability of the technique compared to RNAi-mediated approaches to control cotton infecting begomoviruses.

3

Nuclear autophagy degrades a geminivirus nuclear protein to restrict viral infection in Solanaceae plants

Fangfang Li, Xueping Zhou, Mingzhen Zhang, Pan Gong, Buwei Cao

Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China

Abstract

Autophagy is an evolutionarily conserved degradation pathway in the cytoplasm and has emerged as a key defense mechanism against invading pathogens. However, there is no evidence showing the autophagic degradation of nuclear proteins or the nuclear autophagy in plants. Here we show that the expression of a geminivirus nuclear protein, C1 of Tomato leaf curl Yunnan virus (TLCYnV) induces autophagy, and ATG8h, as a core autophagy protein, directly interacts with the C1 protein of TLCYnV. The interaction between ATG8h and C1 leads to the translocation of the C1 protein from the nucleus to the cytoplasm and the decreased protein accumulation of C1. The degradation of C1 is blocked by autophagy inhibitors and is compromised when autophagy-related genes (*ATGs*), *ATG8h*, *ATG5,* or *ATG7* are knocked down. The mutation of a potential ATG8 interacting motif (AIM) in C1 abolished its interaction with ATG8h in the cytoplasm and inhibited its autophagy-dependent degradation, and TLCYnV carrying this mutation triggered enhanced symptoms and showed increased viral DNA accumulation in *Solanaceae* plants. Furthermore, silencing of *ATG8h*, *ATG5* or *ATG7* promotes TLCYnV infection in plants. Taken together, these data suggest that the nuclear autophagy contributes to antiviral immunity, and ATG8h acts as an important autophagy protein, which interacts with and degrades a geminivirus nuclear protein through the autophagy pathway.

4

C4 protein encoded by Tomato leaf curl Yunnan virus reverses transcriptional gene silencing through impacting DNA-bound ability of NbDRM2 via interacting with NbDRM2

Xueping Zhou[1,2], Yuzhen Mei[2], Yaqin Wang[2]

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[2]Institute of Biotechnology, Zhejiang University, Hangzhou, China

Abstract

Cytosine DNA methylation is an efficient defense mechanism against geminiviruses, which leads to viral genome methylation and transcriptional gene silencing (TGS). As a counter-defense, geminiviruses encode viral proteins to suppress the viral genome methylation and TGS. However, the molecular mechanism by which viral protein contributes to TGS suppression remains largely unknown. This study finds that C4 protein encoded by *Tomato leaf curl Yunnan virus* (TLCYnV) suppresses viral genome methylation through impairing DNA-bound ability of NbDRM2 by direct interaction with NbDRM2 protein. We show that NbDRM2, a pivotal DNA methyltransferase in methyl cycle, plays an important role in defense against TLCYnV through catalyzing methyl group on specific cytosine sites of viral genome. C4 protein of TLCYnV impacts the viral DNA-bound ability of NbDRM2 and promotes the dissociation of NbDRM2 from viral DNA to interrupt its DNA methyltransferase reaction then inhibits viral genome methylation. Furthermore, we find that a S43A mutation in TLCYnV C4 abolishes its capacity to interact with NbDRM2. Plants infected with TLCYnV carrying C4(S43A) show milder symptoms and lower virus accumulation associated with enhanced viral DNA methylation compared with plants infected by wild-type TLCYnV. Genetic analysis shows that expression of TLCYnV C4 but not NbDRM2-interaction compromised C4 mutant in 16c-TGS *Nicotiana benthamiana* results in the recovery of GFP. This study provides new insights into the molecular mechanism of how a geminiviral protein hijacks NbDRM2 to suppress NbDRM2-mediated viral genome methylation.

5

Development of Agroinfectious Clone of Okra Infecting Begomovirus and Associated Betasatellite

Kanika Gupta, Rashmi Rishishwar, Indranil Dasgupta

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Abstract

Okra (Abelmoschus esculentus) is an important vegetable crop in the tropical and subtropical regions of the world. In India, Yellow Vein Mosaic disease (YVMD) and Okra Enation leaf curl disease (OELCuD) cause huge economic losses in the production of okra. We have earlier reported the association of the begomovirus Mesta Yellow Vein Mosaic Virus (MeYVMV) and the betasatellite Bhendi yellow vein mosaic betasatellite (BYVMB) with YVMD. However, the full length DNA sequence that has been used earlier (Accession No. KJ462074.1) identified as MeYVMV now shows more than 93% identity with sequences identified as Okra Enation leaf curl virus (OELCuV) in the database. To avoid discrepancy in nomenclature, it is proposed to designate this sequence (Accession No. KJ462074.1) as OELCuV. Here, we report the results of our study to test the infectivity of cloned OELCuV and BYVMB (Accession No. KJ462076.1) on okra plants. A partial dimer of the two clones were constructed in the binary vector pCambia2300 and agrobacterium-mediated delivery was done in the shoot apical meristem of the okra plants to introduce the above two clones. After 20 days post-inoculation, we observed accumulation of viral DNA in 25 plants out of 43 inoculated okra plants with symptoms of vein thickening, stem bending, petiole bending, stunting and severe lateral branching similar to those reported for OELCuD. The symptoms were observed only when cloned viral and satellite DNAs were co-inoculated. Accumulation of the viral DNA was observed by PCR using primers designed to amplify only the viral DNA released from the plasmid and by Southern analysis of DNA extracted from newly emerged leaves in okra. The clones and the inoculation procedure can be used for rapid screening for resistance against OELCuV in okra. The development of this technique will help to study the genetic requirements for the pathogenicity of OELCuV and to resolve several outstanding issues regarding the disease etiology.

6

An NSP-Interacting Immune Hub: Hijacking Host Transport Functions for vDNA Movement and Suppressing Incompatible Functions

Marco Aurelio Ferreira[1], Gabriel A.S. Raimundo[1], Laura G.C. Martins[1], Bianca C. Gouveia-Mageste[1], Ruan M. Teixeira[1], Christiane E.M. Duarte[1], Virgílio A.P. Loriato[1], Joao Paulo B. Machado[2], Elizabeth P.B. Fontes[1]

[1]Universidade Federal de Vicosa, Vicosa, MG, Brazil. [2]Universidade Federal de Vicosa, Florestal, MG, Brazil

Abstract

The begomovirus nuclear shuttle protein (NSP) facilitates the intracellular transport of viral DNA from the nucleus to the cytoplasm and acts along with the movement protein (MP) to translocate the viral DNA to adjacent cells. As a facilitator of intra and intercellular transport of viral DNA, NSP is predicted to associate with host proteins from the nucleocytoplasmic translocation machinery, the intracytoplasmic active transport system, and cell-to-cell transport functions. Furthermore, NSP functions as a virulence factor that suppresses antiviral immunity against begomoviruses. In this investigation, we focus on the protein-protein network that converges on NSP with a high degree of centrality and forms an immune hub against begomoviruses. We also describe the compatible host functions hijacked by NSP to promote the nucleocytoplasmic and intracytoplasmic movement of viral DNA. Finally, we discuss the NSP virulence function as a suppressor of the recently described NIK1-mediated antiviral immunity and the cross-talk between antiviral and antibacterial immunity by NIK1. Understanding the NSP-host protein-protein interaction (PPI) network will probably underlie mechanisms for more durable recessive or dominant resistance against begomoviruses.

7

Two DNA Episomes From the Cassava Genome Alter GeminivirusInfection in the Field and Under Laboratory Conditions

Catherine D Doyle, Mary Beth Dallas, Linda Hanley-Bowdoin

North Carolina State University, Raleigh, NC, USA

Abstract

Cassava Mosaic Disease (CMD) is one of the most devastating diseases to cassava, a major root crop in Africa. CMD is caused by a complex of cassava mosaic begomoviruses (CMBs), single-stranded DNA (ssDNA) plant viruses, transmitted by whiteflies (*Bemisia tabaci*). CMBs evolve very rapidly in the field, with viral evolution contributing to disease emergence, spread, and severity. CMD resistance cultivars were deployed in response to the pandemic, but reports of resistance breaking have been observed for some of these cultivars. It has been proposed that two DNA sequences, SEGS-1 and SEGS-2 (sequences enhancing geminivirus symptoms), originating from the cassava genome are responsible in part for breaking CMD resistance and the increased severity of CMD symptoms. The discovery of the SEGS underscores the importance of characterizing CMD and host responses more fully to develop durable disease resistance. SEGS-1 functions with the cassava host and virus to increase disease and break resistance. SEGS-2 is a novel DNA satellite that enhances disease symptoms and is transmitted by whiteflies. SEGS-1 and SEGS-2 have a broader impact on virus and host interactions with other plant hosts and geminiviruses in addition to cassava and CMBs. Previous reports identified that SEGS-1 and SEGS-2 occur as episomes in the field. Recently, we observed the presence of SEGS-1 and SEGS-2 episomes in susceptible, tolerant, and resistant cassava cultivars under laboratory conditions.

8

Phosphorylation of a begomovirus movement protein affects its self-interaction and interplay with the plant host

Tatjana Kleinow, Andrea Happle, Sigrid Kober, Luise Linzmeier, Tina Rehm, Jacques Fritze, Patrick Buchholz, Gabi Kepp, Holger Jeske, Christina Wege

Molecular Biology and Plant Virology, Institute of Biomaterials and biomolecular Systems, Universität Stuttgart, Stuttgart, Germany

Abstract

In the case of bipartite geminiviruses (genus *Begomovirus*), the genome comprises two circular DNAs: DNA-A and DNA-B. The DNA-B component encodes a nuclear shuttle protein (NSP) and a movement protein (MP), which enable systemic spread within host plants and affect pathogenicity collaboratively. Hereby, the MPs mediate multiple functions during intra- and intercellular trafficking, such as binding of viral nucleoprotein complexes, targeting to and modification of plasmodesmata and release of the cargo after cell-to-cell transfer. Previous work discovered for the bipartite begomovirus Abutilon mosaic virus (AbMV) a phosphorylation of MP expressed in bacteria-, yeast- and *Nicotiana benthamiana*. Three phosphorylation sites (Thr-221, Ser-223 and Ser-250) were identified in its C-terminal oligomerization domain by mass spectrometry, suggesting a regulation of MP-MP interactions by posttranslational modification. To examine the influence of these three sites on the self-interaction in more detail, MP mutants were tested for their interaction in yeast by two-hybrid assays, and *in planta* by Förster resonance energy transfer (FRET)-based techniques, respectively. In this study, expression constructs were generated in which the MP gene contains point mutations leading to simultaneous (triple) exchange of Thr-221, Ser-223, and Ser-250 to either uncharged alanine (MP^{AAA}), or phosphorylation-mimicking aspartate residues (MP^{DDD}) in the encoded protein. The triple aspartate version interfered with MP-MP binding. Moreover, the role of Thr-221, Ser-223, and Ser-250 for the viral life cycle within plants was studied by engineered AbMV DNA-B variants in which the MP gene codes either for MP^{AAA} or MP^{DDD}. When co-inoculated with a wild-type DNA-A, both mutated DNA-Bs gave rise to systemic infections in *N. benthamiana*. However, each triple mutation abolished an AbMV-infection in a distinct plant species within the family *Solanaceae* or *Malvaceae*. Systemically infected plants exhibited altered symptom development and viral DNA accumulation. The identification of three phosphorylation sites in AbMV MP, which have an impact on self-interaction, host range, symptom development, and viral DNA accumulation, indicates a regulation of the diverse MP functions by plant-derived posttranslational modification and underscores their importance for host plant-geminivirus interactions.

9

Dynamic, Differential and Time-dependent Subcellular Distribution of Begomoviral Nuclear Shuttle and Movement Proteins

Andrea Happle, Holger Jeske, Tatjana Kleinow

University of Stuttgart, Stuttgart, Germany

Abstract

The circular ssDNA genomes of geminiviruses are replicated in plant cell nuclei and therefore have to overcome two membrane barriers for cell-to-cell movement: nuclear envelope and plasma membrane. For bipartite viruses (*Begomovirus*), their nuclear shuttle proteins (NSP), and movement proteins (MP) accomplish these tasks by way of multiple coordinated functions. It is likely that transport goes along with a regulated and dynamic subcellular distribution of NSP and MP, of which the details need to be elucidated. Consequently, spatial and temporal concertation of MP and NSP fromAbutilon mosaic virus (AbMV) were investigated in *Nicotiana benthamiana*leaf tissues. AbMV transport proteins fused either N- or C-terminally to a fluorescent protein tag were examined by high resolution live cell fluorescence imaging. Singly or co-expressed proteins in the presence or absence of local AbMV infection revealed differential distributions of both MP and NSP. The position of the tag influenced their localization, probably by interference with functional domains. For example, GFP::MP accumulated in a well-defined layer surrounding the nucleus, which was never observed for MP::GFP. Long-term microscopy for up to three days revealed two MP localization patterns: in cortical spots in the cells in early stages of the expression, and in a more homogeneous distribution most prominent at the cell periphery later. Interestingly, AbMV infection inhibited this time-dependent shift in distribution. To uncover cellular structures to which the MP was associated, marker proteins labelling different substructures were co-expressed. Additionally, inhibitors for ER or cytoskeleton integrity were applied. Thereby, a dynamic association of the begomoviral proteins with distinct cellular components, including the ER, was identified for the first time, with strikingly agile MP agglomerates passing along the ER. Heterologous and self-interactions of MP and NSP were dissected by Förster resonance energy transfer (FRET). In summary, the results give new insights into the dynamic distribution of geminiviral transport proteins and hint at their ability to opportunistically exploit plant pathways for transportation.

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Efficiency of Tomato severe rugose virus-infected Soybean, Tomato and Nicandra physaloides as Sources of Inoculum to Tomato Plants

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Abstract

Tomato plants are susceptible to several begomoviruses. In Brazil, the *Tomato severe rugose virus* (ToSRV) is the predominant begomovirus in the main tomato producing regions and causes variable economic damages to the production. ToSRV has a wide host range, including cultivated and weed plants, and is transmitted in a persistent circulative manner by *Bemisia tabaci* MEAM1. The objective of this work was to evaluate the potential of ToSRV-infected soybean (*Glycine max*), tomato (*Solanum lycopersicum*) and the weed *Nicandra physaloides* as sources of inoculum of ToSRV to tomato plants. The experiment was carried out in four field plots, approximately 600 m, distant from each other to avoid possible interference between the different sources of inoculum and the control. First, 200 healthy tomato plants were transplanted in each field plot. Then, 10 ToSRV-infected plants of one species, evaluated as sources of inoculum, was transplanted around the tomato plants. One field plot remained without source of ToSRV inoculum (control). To ensure the presence of the vector, collard plants infested with *B. tabaci* MEAM1 were placed in all four experimental plots. To avoid secondary infection of ToSRV, the tomato plants were treated with soil drench application of the insecticide cyantraniliprole at 15 days intervals. Furthermore, tomato plants exhibiting symptoms of ToSRV infection were immediately eradicated. The evaluation of the incidence of ToSRV in the tomato plants was performed through the observation of symptoms, followed by confirmation of virus infection in some plants by PCR and nucleotide sequencing of some amplicons. Temporal dynamic analysis was performed to confirm the predominance of primary infection in the experimental field plots. The rate of ToSRV-infected plants in the experimental areas where ToSRV-infected plants of *N. physaloides*, tomato and soybean were evaluated as sources of inoculum were 50%, 38.5%, and 8%, respectively. None of the control plants were infected with ToSRV. The disease progress curves best fitted to the monomolecular model, indicating the predominance of primary infections. The results showed that, when in the same proportion, *N. physaloides* and tomato plants have higher potential as sources of ToSRV inoculum in the field than soybean.
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Association of DNA Betasatellite with Tomato Yellow Leaf Curl Virus Affects Tomato Genetic Resistance

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Abstract

Tomato yellow leaf curl virus (TYLCV) a whitefly-transmitted begomovirus, is one of the most severe viruses infecting tomato plants globally. Commercial tomato cultivars resistant to TYLCV are readily available. As *Ty-1* was the first TYLCV-resistant locus identified, and it is dominant in nature (Zamir et al., 1994) many breeding programs utilized it. As a result, today, most commercial tomato hybrids resistant to TYLCV carry *Ty*-1. During November 2016, F_1 hybrid TYLCV-resistant greenhouse tomato plants *cv*. Diagrama, (Nunhems, The Netherlands), grown near the Sea of Galilee, Israel, expressed very severe symptoms of tomato yellow leaf curl disease (TYLCD). Samples were collected from symptomatic tomato plants situated in two different greenhouses. Using TYLCV-specific PCR primers, it was confirmed that these plants are indeed infected with TYLCV. However, due to the severity of disease symptoms expressed by the TYLCV-resistant plants and to the earlier identification of a DNA betasatellite in Jordan (a neighbouring country) (Anfoka et al., 2014), the samples were also tested for the presence of DNA betasatellite using the universal primer pair, B01/B02 (Briddon et al., 2002). It was found that the plants are infected with a DNA betasatellite as well. The betasatellite amplification products were cloned, sequenced and deposited in GenBank (accession number MK456609). Phylogenetic analysis revealed that the closest similarity, with 96% nucleotide identity, was to Cotton leaf curl Gezira betasatellite. Diagrama test plants were inoculated either with TYLCV or with both TYLCV and betasatellite using whiteflies. While TYLCV-infected Diagrama plants developed very minor disease symptoms, the Diagrama plants inoculated with both TYLCV and betasatellite developed severe TYLCD symptoms within 14-21 days. Using a *Ty-1* specific molecular marker (Verlaan et al., 2013) it was found that all the Diagrama plants are carrying the *Ty-1* locus in a heterozygous state. Hence, the association of bettasatellite with TYLCV compromised *Ty-1* resistance.

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Interactions between *Bemisia tabaci* and tomato severe rugose virus

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Abstract

The species *Tomato severe rugose virus* (ToSRV) is transmitted by *Bemisia tabaci* and it is the predominant begomovirus species infecting tomatoes in Brazil. *Bemisia tabaci* is composed by a complex of cryptic species. Among them, Middle East-Asia Minor 1 (MEAM1, formerly known as B biotype) and Mediterranean (MED, formerly known as Q biotype) are the most important worldwide. In Brazil, MEAM1 was first reported in the 90s. Currently, it is the most widespread *B. tabaci* cryptic species in the country as well as the main vector of viruses. However, since its first report in 2014, MED has been spreading in the south and southeast region under greenhouse conditions. It is already known that both species can transmit ToSRV efficiently, but there is no information about the virus-vetor interaction. Then, in order to understand this relationship, we investigated the interactions of ToSRV-infected tomato plants and healthy tomato plants for MEAM1 and MED. Clip-cage with ten whiteflies couples was attached to a leaf, totaling ten clip-cages (ten replicates) per treatment. Whiteflies were left to reproduce for 48 hours in controlled conditions. Then, adults were removed and parameters such as eggs laid, hatchability, adults emerged, survival rate and development time were evaluated. The results showed that, when feeding on ToSRV-infected tomato plants, the number of eggs laid, hatchability and number of adults emerged decreased for both MEAM1 and MED. The *B. tabaci* MED was the most affected: mean number of adults emerged on healthy plants was 52 compared to 6.5 adults on ToSRV-infected tomato plants. Also, MED's life cycle was significantly longer on ToSRV-infected tomato plants than healthy plants, while for MEAM1 it was faster on ToSRV-infected tomato plants, increasing the number of adults emerged during the first three days (over 14 days evaluated). Our results indicate that for MEAM1, the ToSRV left the life cycle faster and might contribute for its secondary transmission in the field. By contrast, despite an efficient vector of ToSRV, MED's life cycle was negatively affected by ToSRV-infected tomato plants.

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Symptom Evolution Following the Emergence of Maize Streak Virus

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Abstract

When a pathogen evolves, it is expected that natural selection will modulate the severity of any pathogen-induced disease traits that either directly or indirectly impact its transmission probability. Accordingly, for many pathogens it has been demonstrated that the basic reproductive numbers are determined by evolutionary trade-offs between the pathogen-induced mortality rate, and the transmission rate. Given that in almost all cases these trade-offs have been demonstrated for pathogens infecting a single host species, it is unclear how they would impact the evolution of broad host-range pathogens that have transmission chains which routinely involve passages through multiple host species. It is also unclear how such trade-offs might impact the damage that pathogens inflict on short-lived cultivated crops; a situation where symptom intensities and host survival rates may have little impact on pathogen fitness. Here we address these unknowns by examining changes over the past ~110 years in the intensity of disease symptoms induced in maize by the broad host-range viral pathogen, maize streak virus (MSV). Specifically, we use the quantified symptom intensities displayed by differentially resistant maize genotypes

infected by cloned MSV isolates to phylogenetically infer the symptom intensities induced by ancestral MSV lineages following the emergence of MSV as a maize pathogen in the mid to late 1800s. Further, we verify the accuracy of these inferences using computationally-predicted ancestral MSV genomes that were then synthesised, made infectious and tested. We find that following the expansion of the MSV host-range to include maize, the intensity of MSV-induced symptom types that are indicative of harm to the host either remained constant (leaf stunting), or decreased (chloroplast destruction). Conversely, an increase was observed in chlorotic leaf areas, a symptom type that is indicative of how successfully MSV colonises the host cell populations upon which its insect transmission vectors. Therefore, despite the complication of MSV having a broad host-range, its apparent adaptation to a crop species with a short life span remains consistent with an evolutionary trade-off between the amount of harm inflicted on infected maize plants, and how effectively the virus positions itself within plants to enable onward transmission.

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Transcriptomic Analysis of Arabidopsis Lines Transgenic for SEGS-1 or SEGS-2

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Abstract

Two DNA sequences, designated SEGS-1 and SEGS-2 (sequencesenhancing geminivirus symptoms), in the cassava genome also occur as episomes in infected plants during Cassava mosaic disease. The SEGS have been associated with increased symptom severity and breaking of geminivirus resistance in cassava and Arabidopsis. We transformed the hypersusceptible Arabidopsis accession (Sei-0) with a single copy of SEGS-1 or SEGS-2, and showed that the transgenic sequences enhance *African cassava mosaic virus*(ACMV) symptoms. RNA-seq data of healthy wild-type and transgenic SEGS1 or SEGS2 plants were generated using the NovasSeq 6000 platform and 23nalysed for differentially expressed genes (DEGs) across the different genotypes. DEGs, which were identified using DESeq2, were called using a fold change of 1.5 and an adjusted p-value of 0.05. The RNA-seq analysis showed that healthy SEGS-2 transgenic and wild-type Sei-0 plants have essentially identical RNA profiles while healthy SEGS-1 transgenic and wild-type Sei-0 plants have 137 DEGs. Comparison of mock and ACMV-inoculated plants identified many DEGs for all three genotypes. These results suggest that while SEGS-1 and SEGS-2 alter host gene expression during the virus infection to enhance symptom severity, SEGS-1 also changes host gene expression in the absence of infection. Gene ontology (GO) enrichment analysis as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathway analysis showed that SEGS-1 DEGs are mainly associated with plant hormonal pathways such as jasmonic acid, brassinosteriod and cytokinin. We are currently performing similar analyses comparing mock-inoculated versus ACMV-infected DEGs in the presence and the absence of the SEGS.

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Faba bean necrotic stunt virus (Family Nanoviridae, genus Nanovirus) and Alfalfa leaf curl virus (Family Geminiviridae, genus Capulavirus) interactions within co-infected plants and co-transmitting aphid vector.

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Abstract

Nanoviruses and geminiviruses are important ssDNA plant viruses with a dramatic impact on vegetable crops. They share ecological and molecular features. Both groups of viruses are restricted to the plant vascular system where they are acquired and then transmitted plant-to-plant by insect vectors through the circulative non-propagative mode. Investigating putative interactions between nanoviruses and geminiviruses is essential to determine the differences or similarities in their transmission process. The *Faba Bean Necrotic Stunt Virus* (FBNSV) is a legume nanovirus vectored by different aphid species including *Aphis craccivora*. The *Alfalfa Leaf Curl Virus* (ALCV) also infects legumes and is so far the only reported geminivirus vectored by an aphid: *Aphis craccivora*. After ingestion with the plant sap, FBNSV and ALCV virions massively accumulate in cells of the aphid anterior midgut (AMG) before passage in the hemolymph and to the salivary glands. Though the precise gut-crossing mechanisms remain elusive, previous experiments have definitely established that FBNSV needs the presence of the virus-encoded Nuclear Shuttle Protein (NSP) for successful aphid-transmission while ALCV uses the capsid strategy and does not require any transmission factor. In co-agroinoculated faba bean, we observed co-infection in a small proportion (approximately 10 %) of the test plants. Transmission tests using these co-infected plants and study of the accumulation and localization of the two viruses in aphid guts indicated that they follow a distinct intracellular route and do not interfere, suggesting for the first time that nanoviruses and capulaviruses may interact differently with their common aphid vector.

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Investigation of nanoviral M-Rep, CP and NSP interaction complexes: BiFC analyses of wt and deletion proteins by confocal microscopy

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Abstract

Nanoviruses (family *Nanoviridae*) are multipartite, single-stranded DNA viruses, infecting predominately legume plants. Nanovirus infections often cause severe symptoms and premature plant death. The transmission is carried out by aphids in a persistent and non-propagative manner. The genome consists of eight circular DNAs, each about 1 kb in size, encoding for a single gene, and encapsidated individually. DNA-R encodes for the master replication initiator protein (M-Rep). The capsid protein (CP) is encoded by DNA-S and forms the virions of each viral DNA. The cell-cycle-link (Clink) protein on DNA-C is involved in the cell cycle regulation in the host cell. NSP (nuclear shuttle protein) functions as transmission component, encoded on DNA-N. These proteins have been investigated in terms of their function, whereas the movement protein (MP) encoded on DNA-M is predicted to be involved in cell to cell movement. Proteins of unknown function are referred to as U1, U2, and U4 and are encoded by DNAs U1, - U2, and U4, respectively. Previous studies have shown that interaction and self-interaction among the nanoviral proteins occur. Intriguingly the interactions between M-Rep & NSP and M-Rep & CP may be of importance in the viral infection cycle. Thus, several deletion mutants of M-Rep, CP and NSP were investigated by bimolecular fluorescence complementation (BiFC) assays to narrow down potential interaction/binding motifs. First results indicate that C-terminal deletion mutants of CP and NSP still have the ability to reconstitute a YFP signal in BiFC assays with M-Rep and NSP, respectively. In addition interaction studies, showing different cellular localizations of wild type and deletion mutants of M-Rep with wild type NSP, will be presented.

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Spatial and Temporal Relationships and Population Dynamics of Geminiviruses at the Cultivated-Wild Plant Host Interface in Cotton-Growing Areas of Pakistan

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Abstract

The emergence of begomoviruses from endemic, wild plant hosts in agricultural ecosystems offers an opportunity to analyze genomic variability and population dynamics associated with outbreaks, epidemics, and their fluxes, and to reconcile virus evolution with epidemiological patterns. Complete viral genome and betasatellite sequences were determined from leaf curl disease-affected cotton and non-cotton host plants collected in Pakistan from 2010-2014, by Illumina sequencing and/or PCR amplification. The spatial and temporal distributions and population dynamics of the resultant sequences were analyzed. The *Cotton leaf curl Kokhran virus*-Burewala (CLCuKoV-Bur) predominated among all viral species detected in symptomatic cotton plants. Unexpectedly, *Cotton leaf curl Multan virus*-Faisalabad (CLCuMuV-Fai), causal virus of the 1990's leaf curl epidemic and thought to have been displaced by CLCuKoV-Bur, was identified in 2 of 16 sites sampled. Previously unreported begomoviruses were identified in 'sentinel plots' harboring 'known' host species of the cotton leaf curl complex (8 or more species or strains, used to attract 'dispersing' virus populations. Also, *Cotton leaf curl Alabad virus* and CLCuKoV-Kokhran, both known to co-occur with CLCuMuV-Fai during the 1990's epidemic, were detected in cotton and 'sentinel' host plants. Based on the analysis, geminiviruses previously not known to infect cotton, including *Chickpea chlorotic dwarf virus*, *Okra enation leaf curl virus*, *Squash leaf curl virus,* and *Tomato leaf curl New Delhi virus,* were identified in symptomatic cotton plants. Also, CLCuMuV was detected in cotton breeding plots in which some of the germplasm was apparently CLCuMV-susceptible. Presence of CLCuMuV in susceptible cotton indicates it has prevailed at low levels, even though (CLCuKoV-Bur) was thought to have displaced in (~2000 onward). This may possibly be due to its broad host range that includes wild and cultivated plant reservoirs. The CLCuM-betasatellite was the most prevalent among betasatellites identified, regardless of plant host or location. Population analysis of begomoviral genome-betasatellites usingTajima's D, Fu, and Li's tests, and SNPs analysis showed diversification of CLCuMuV while CLCuKoV-Bu and CLCuMuV-Ra exhibited genomic and geographic expansion. The sustained diverse mixtures of cotton leaf curl disease-associated begomoviruses infecting cotton and non-cotton hosts, and evidence of ongoing diversification suggest the impending emergence of new variants.

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The Role of Stress Granules in Begomoviral Infection

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Abstract

Stress granules (SGs) are dynamic mRNA-protein-complexes localized in the cytoplasm that rapidly form under stress conditions to stall translation and may disperse when ambient conditions are restored. The formation of SGs is dependent on the RNA-binding ability of the Ras-GAP SH3 domain–binding protein (G3BP). In the mammalian system the C-terminal domain of the viral nonstructural protein 3 (nsP3) of Semliki Forest virus (SFV) forms a complex with the human G3BP (HsG3BP) and sequesters it into viral RNA replication complexes in a manner that inhibits the formation of SGs. The binding domain of nsP3 to HsG3BP was mapped to two tandem 'FGDF' motifs close to the C-terminus of the viral protein. G3BP homologs can be found in plant species and G3BP-binding motifs are also present in different plant viruses. The nuclear shuttle protein (NSP) of different begomoviruses, for example the *Abutilon mosaic virus* (AbMV) and the *Cabbage leaf curl virus* (CbLCuV), harbor a 'FVS(F/Y)'-motif at their C-terminal ends. Only recently the C-terminal end of the NSP of AbMV has been shown to co-localize and interact with AtG3BP2, one of the seven *Arabidopsis thaliana* homologs of HsG3BP. In addition, a mutation of the proclaimed binding motif of AbMV to AVSA leads to a loss of interaction. Here we investigated CbLCuV-infected Arabidopsis T-DNA insertion lines for the different G3BPs (G3BP-KO lines). We monitored CbLCuV-induced phenotype over a period of two to four weeks after inoculation. Furthermore, we analyzed CbLCuV systemic infection in the G3BP-KO lines and determined virus titer by quantitative real-time PCR to see if the absence of single SG proteins has any influence on virus performance. In addition, Col-0 plants were infected with wild-type virus and a mutant in which the 'FVSY'-motif of the NSP was mutated to 'AVSA' to analyze the importance of the G3BP-binding motif in virus infection.

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Begomoviral Movement Protein Effects in Human and Plant Cells: Towards New Potential Interaction Partners

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Abstract

Geminiviral single-stranded circular DNA genomes replicate in nuclei so that the progeny DNA has to cross both the nuclear envelope and the plasmodesmata for systemic spread within plant tissues. For intra- and intercellular transport, two proteins are required: a nuclear shuttle protein (NSP) and a movement protein (MP). New characteristics of ectopically produced Abutilon mosaic virus (AbMV) MP (MP^{AbMV}), either authentically expressed or fused to a yellow fluorescent protein or epitope tags, respectively, were determined by localization studies in mammalian cell lines in comparison to plant cells. Wild-type MP^{AbMV} and the distinct MP^{AbMV}: reporter protein fusions appeared as curled threads throughout mammalian cells. Co-staining with cytoskeleton markers for actin, intermediate filaments, or microtubules identified these threads as re-organized microtubules. These were, however, not stabilized by the viral MP, as demonstrated by nocodazole treatment. The MP of a related bipartite New World begomovirus, Cleome leaf crumple virus (ClLCrV), resulted in the same intensified microtubule bundling, whereas that of a nanovirus did not. The C-terminal section of MP^{AbMV}, i.e., the protein's oligomerization domain, was dispensable for the effect. However, MP expression in plant cells did not affect the microtubules network. Since plant epidermal cells are quiescent whilst mammalian cells are proliferating, the replication-associated protein Rep^{AbMV} protein was then co-expressed with MP^{AbMV} to induce cell progression into S-phase, thereby inducing distinct microtubule bundling without MP recruitment to the newly formed threads. Co-immunoprecipitation of MP^{AbMV} in the presence of Rep^{AbMV}, followed by mass spectrometry identified potential novel MP^{AbMV}-host interaction partners: the peptidyl-prolyl cis-trans isomerase NIMA-interacting 4 (Pin4) and stomatal cytokinesis defective 2 (SCD2) proteins. Possible roles of these putative interaction partners in the begomoviral life cycle and cytoskeletal association modes are presented.

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Can geminiviruses play a lead role in virus-mediated horizontal transfer of chromosomal DNA between plants?

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Abstract

The acquisition of genetic material originating from unrelated species, also known as horizontal gene transfer (HGT), is recognized of primary importance in the evolution of living organisms and their genomes.
While it is clear that eukaryotes repeatedly acquired genes from bacteria, the possible HGT from eukaryote to another eukaryote is only recently under scrutiny, mainly by deep searches in genomic sequences with novel dedicated tools.
Viruses are potential vectors for HGT as they have a high propensity for recombination, their genetic material penetrates host cells, and they are efficiently transmitted between hosts. Based on paleo-virology studies, many examples of potential gene flow between viral and host genomes in various eukaryotic lineages have been proposed, in both “virus-to-host” and “host-to-virus” directions.
While it has been suggested that viruses are HGT vectors between eukaryotes that share the same viral pathogens, to date virus-mediated HGT events have not been examined experimentally or monitored in real time.
We reported spontaneous and surprisingly efficient generation of circular hybrid molecules made of virus and host DNA sequences. These molecules occurred in the form of minicircles during infection of *Beta vulgaris* plants by *Beet curly top Iran virus* (BCTIV), a single-stranded DNA virus belonging to the *Geminiviridae* family. These hybrid minicircles were able to replicate in plants, spreading systemically throughout the plant together with the viral infecting agent BCTIV, and were encapsidated into viral particles.
Importantly, *B. vulgaris* DNA captured in minicircles could be multiplied also in other BCTIV-infected plants, such as *Nicotiana benthamiana* and *Arabidopsis thaliana*. In the tissue of these plants, *B. vulgaris* DNA captured in minicircles could also be transcribed.
Conclusively, we documented in real time the initial steps of a possible path of virus-mediated horizontal transfer of chromosomal DNA between plant species.

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Analysis of a TYLCV natural mutant uncover an element conserved in Old World begomoviruses whose deletion greatly weaken the Rep gene promoter activity

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Abstract

Tomato Yellow Leaf Curl Virus (TYLCV), a monopartite begomovirus native to the Middle Eastern-Mediterranean region, is a major threat to tomato production worldwide. TYLCV was introduced to Mexico circa 1996. In 2011, a variant of TYLCV-IL(Israel strain) was isolated from two plants collected in the locality of Aquismón, San Luis Potosi, Mexico. This variant was found coinfecting with the native begomovirus ToChLPV and with ToSLCV, in *Solanum pimpinellifolium* and in *Solanum lycopersicum* respectively. This variant, designated TYLCV-[SLP], differs from others described to date for two notorious changes in the viral intergenic region: a 29 bp deletion upstream of the conserved “stem-loop" element of the replication origin (Ori), and a 42 bp duplication downstream of the latter element. This duplicated segment includes a Conserved Late Element (CLE) which is responsive to the begomoviral transactivator, TrAP. These natural mutations may have altered the functional properties of the promoters and eventually, the pathogenic characteristics. We functionally analyze the promoters of the genes encoding Rep and CP proteins of TYLCV-[SLP] and the wild type TYLCV-IL (represented by an isolate from Sinaloa, Mexico, TYLCV-[Sin]). The viral promoters were fused to the GUS reporter gene and analyzed by transient expression assays in tobacco protoplasts and in *Nicotiana benthamiana* plants. The results of the protoplast assays revealed that the activity of TYLCV-[SLP] Rep promoter is very weak, ≥15 times lower than the homologous WT promoter. A similar pattern was observed in transitory expression assays *in planta*, although the difference was not as dramatic as in the protoplast experiments: the promoter of TYLCV-[SLP] is ~4 times weaker than its WT counterpart. These observations suggest that the 29-bp deletion in TYLCV-[SLP] contains one or more cis-elements that boost the Rep gene transcription. A comparative analysis revealed the existence of an 18-bp sequence in the former region, conserved in most Old World begomoviruses but absent in all New World begomoviruses. This conserved sequence, which we designate -Activating sequence adjacent to iteron- (Asai), could be a potent regulatory element of Rep gene expression. In contrast, the CP promoters of TYLCV-[SLP] y TYLCV-[Sin] displayed a similar activity in absence of viral factors.

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Phylogenomic and phylogenetic analyses and climate-niche modeling of global whitefly Bemisia tabaci vector-begomoviruses reflect tight geographical and niche-associated co-diversification

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Abstract

The whitefly *Bemisia tabaci* group refers to a number of morphologically undistinguishable biological variants, referred to by some as cryptic species, that are distributed worldwide in tropical and sub-tropical regions, spanning diverse climatic niches. Among these variants, only a few have been associated with agricultural systems, where they have become important pests and vectors of emergent viruses belonging to the genus *Begomovirus* (Geminiviridae). Based on co-cladogenesis and biological studies endemic whitefly variants-begomoviruses are co-evolved. Several variants have been shown to exhibit greater transmission competency compared to non-coevolved pairs. Extents of vector specificity and/or competency are expected to contribute to begomovirus diversification, and several recent anecdotal examples support this hypothesis. As a group, *B. tabaci* exhibit phenotypic plasticity and vary by host range, fecundity, endosymbiont composition, insecticide resistance, plant virus transmission specificity/efficiency, and dispersal 31ehaviour. Although historically members of the group have been referred to as phenotypically distinct races, strains, or biological types, most recently, they have been differentiated based on mtCOI sequence divergence. The shortage of information about gene flow barriers among and between genetic variants of *B. tabaci* is poorly understood, hindering estimates of gene flow, further contributing to the inability to resolve putative cryptic species boundaries. The mitochondrial COI sequence has been informative for pinpointing regions of endemism of genetic variants, but the marker is too saturated to predict species boundaries. Recently a phylogenomic study involving 2184 nuclear orthologs sequenced from globally-representative members of the *B. tabaci* group has resolved at least five distinct species. The nuclear ortholog tree topology mirrors the corresponding major phylogeographical clades delimited by the mtCOI phylogeny. In addition to phenotypic plasticity this cyptic nature has contributed to variable outcomes depending on the potential for porous species boundaries (gene flow) between variants. Tree topologies of begomoviruses and recombinants arising from mixed infections likewise mirror the endemic distributions of their co-evolved *B. tabaci* vector (genetic variants). Results indicate that the newly realized extent of diversity at the nuclear genome level will require a re-consideration, holistically, and with attention to phylogenomics, population genetics, gene flow, phenotypic plasticity, and begomovirus-vector relationships among extant biogeographically-distinct populations.

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Exploration of defense mechanism involved in solanum lycopersicum by using high throughput RNA-Seq method against begomovirus infection.

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Abstract

Solanum lycopersicum (Tomato) have its own economic importance as it is widely acceptable food componant throughout the world. Begomovirus infection make very disastrous impact on production of tomato in fields. There are many efforts going on to beat begomovirus infection around the world. Resistance breakdown for begomovirus in tomato is major concern for development of new varieties through breeding as well as transgenic production. One of the major reason for resistance breakdown is its high mutation and recombination rate of begomovirus. In recent past, H-86 (*Kashi Vishesh*) was known as tolerant variety against begomovirus infection. But, begomovirus has breakdown the tolerence of *Kashi Vishesh.* Nowadays, *Kashi Aman* from India is known tolerant variety against begomovirus infection with having Ty-2 and Ty-3 gene. So, apart from to develop transgenic plants, long term tolerance development should be major concern.

High throughput next generation sequencing technology is the best way to explore the different pathways responsible during stress. Two varieties of tomato (1*. Kashi Aman* 2. *Kashi Vishesh)* were taken to evaluate the infectivity of *Tomato leaf curl joydebpur virus* (KF515609) and *Tomato leaf curl Karnataka betasatelllite* (KF515610). *Kashi Vishesh* showed susceptibility towards virus whereas *Kashi Aman* showed tolerance towards both DNA component of virus. Experimental plant leaves samples were collected at 40dpi. RNA-seq experiment was performed using Ion Torrent S5 platform. Data was collected and Differential expression of gene (DEG) was performed using HiSat2, cufflink, cuffmerge method. More than 100 differentially expressed genes represents significance level of upregulation and down regulation. There is altered expression of prohibitin, cytochrome p450, defensin like proteins, WRKY transcription factor, NAC, alternative oxidase, etc. genes. Expression of nitric oxide synthesis pathway and ethylene responsive genes were also regulated. The present study get insight into the mechanism which can help to not to break down the tolerance against virus infection and improvement of high tolerance against begomovirus infection.

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A Single Amino Acid Substitution in the Movement Protein Enables Mechanical Transmissibility of a Geminivirus

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Abstract

Begomoviruses of the *Geminiviridae* are usually transmitted by whiteflies but rarely by mechanical inoculation. We used tomato leaf curl New Delhi virus (ToLCNDV), a bipartite begomovirus, to address this issue. Most ToLCNDV isolates are not mechanically transmissible to their natural hosts. A closely related ToLCNDV-OM isolate originally identified from a diseased oriental melon plant is mechanically transmissible while a ToLCNDV-CB isolate from a diseased cucumber plant is not. Genetic swapping and pathological tests were performed to identify the molecular determinants involved in mechanical transmission. Various viral infectious clones were constructed and successfully introduced into *Nicotiana benthamiana*, oriental melon, and cucumber plants by *Agrobacterium*-mediated inoculation. Mechanical transmissibility was assessed by direct rub-inoculation with saps prepared from symptomatic *N. benthamiana*. The presence or absence of viral DNA in plants was validated by PCR, Southern blotting and *in-situ* hybridization. The results reveal that the mechanical transmissibility is associated with the movement protein (MP) of DNA-B in ToLCNDV-OM. However, nuclear shuttle protein (NSP) of DNA-B plays no roles in mechanical transmission. Analyses of infectious clones carrying a single amino acid substitution reveal that the 19th glutamate of MP in ToLCNDV-OM is critical for mechanical transmissibility. Substitution of the 19th amino acid from glutamate to glycine in the MP of ToLCNDV-OM abolishes mechanical transmissibility. In contrast, substitution of the 19th amino acid from glycine to glutamate in the MP of ToLCNDV-CB enables mechanical transmission. This is the first identification of a specific geminiviral movement protein as a determinant of mechanical transmissibility.

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Characterization of a new bipartite begomovirus infecting bean in northwestern (NW) Argentina

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Abstract

Beans provide a highly nutritious food, containing protein, fiber, complex carbohydrates, vitamins, and micronutrients. About 400 million people in the tropics eat beans as part of their daily diet. Farmers struggle to satisfy consumer demand, producing around 12 million tons of common beans every year worldwide. In the world 17 begomovirus species have been identified from naturally infecting beans;
while, so far, seven species were identified in Argentina: *Bean golden mosaic virus* (BGMV), *Tomato yellow spot virus* (ToYSV), *Soybean blistering mosaic virus* (SbBMV), *Tomato mottle wrinkle virus* (ToMoWV), *Sida golden mosaic Brazil virus* (SiGMBRV), *Tomato yellow vein streak virus* (ToYVSV) and *Euphorbia mosaic virus* (EuMV). Nucleic acid hybridization probes specific for these begomoviruses were developed, and used to assay field samples. Some of the 34nalysed begomovirus-infected samples did not react with any of the specific probes; so we suspected that other begomovirus species were present. DNA of one of these samples, collected in General Mosconi, Salta province, was amplified by rolling circle amplification (RCA), and cloned. The complete genome of a bipartite begomovirus was full-length sequenced. Analysis of the genome organization and phylogenetic comparisons revealed that the virus is a typical New World begomovirus. Nucleotide comparisons established that the two genomic components, DNA-A (2752 nts) and DNA-B (2522 nts), shared 84% and 74% nucleotide identity with *Pepper leaf roll virus* and *Pavonia yellow mosaic virus*, respectively. Therefore, following species demarcation criteria of the International Committee on Taxonomy of Viruses, this virus isolate belongs to a new begomovirus specie. Bean plants of Alubia cultivar were infected with the new virus through genegun system. The infected plants developed leaf roll and stunting symptoms, similar to those observed in field-infected plants, therefore, the name bean bushy stunt virus is proposed for this new virus.

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A Synergistic Interaction Between Tomato Yellow Leaf Curl Virus and Tomato Mottle Virus and Its Effect on the Plant Host and Vector Transmission

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Abstract

Mixed viral infections in plants affect disease severity, viral diversity, and transmission by insect vectors. Co-infection of two begomoviruses, *Tomato Yellow Leaf Curl Virus* (TYLCV) and *Tomato Mottle Virus* (ToMoV), in tomato leads to a severe phenotype indicative of a facilitative synergistic interaction between the two viruses. TYLCV is a monopartite Old-World Virus and ToMoV is a bipartite New-World virus. Both are transmitted by the silverleaf whitefly (*Bemisia tabaci* MEAM1*)*. This study investigated the role of mixed infections on symptom severity, virus location, and whitefly transmission efficiency.

Tomato seedlings (Lanai) were inoculated with infectious clones of ToMoV, TYLCV, or a mixture of ToMoV and TYLCV. Symptom severity and virus titers of each component were monitored over time. Time points with varying ratios of TYLCV and ToMoV were chosen for whitefly transmission to sucrose sachets, leaf discs, and whole plants to measure differences between single and mixed infections throughout the pathosystem.

Most transmission studies have focused on single infections, and mixed infections have been mainly studied within the plant host. This research offers a unique approach for an emerging threat to food security. As the climate warms, mixed infections are likely to be more common as vector habitats expand beyond tropical and subtropical areas. Understanding the effect of mixed infections on disease severity and transmission is important for disease management.

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Night at the museum: Contribution of small RNA from historical herbarium specimens in the reconstruction of evolutionary histories of geminiviruses

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Abstract

Emerging infectious diseases of plants, almost half of which are caused by viruses, are recognized as a growing threat to global food security. However, little is known about the evolutionary processes and ecological factors that underlie the emergence and success of viruses that have caused past epidemics. With technological advances in the field of ancient DNA and RNA, it is now possible to sequence historical viral genomes, which provides us direct access to the dimension of time in evolutionary studies. Herbarium collections are an enormous source of dated, identified and well-preserved material that can be used to elucidate the emergence and evolutionary history of viral plant pathogens. Geminiviruses are responsible for many of the emerging plant diseases worldwide with a major economic impact on food crops such as cassava, which are a vital source of dietary calories in many sub-Saharan African countries. Their high potential for evolution, with high rates of mutation and recombination, makes such viruses an ideal model for understanding the epidemiological and evolutionary processes associated with viral emergence. Our proof of concept study investigated whether small interfering RNA (siRNA) can be used to reconstruct a complete geminivirus DNA genome from a herbarium sample despite the existence of post-mortem nucleic acid damage. Using a metagenomics approach based on the high-throughput sequencing of siRNA, we obtained a siRNA database from cassava leaf samples presenting typical symptoms of cassava mosaic disease that were collected in 1928 from Madagascar and 1968 from Cameroon, and then stored in the National Museum of Natural History herbarium in Paris. Our preliminary results demonstrate our ability to reconstruct the almost complete sequence of bipartite begomoviruses in particular from a 90-year-old herbarium specimen. These sequences are now used in phylogenetic, comparative genomic and phylogeographic studies to elucidate the emergence and evolutionary history of this important crop pathogen.

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Transcriptome Analysis Reveals TYLCV-encoded C4 Protein Interferes a Network of Leaf Developmental Transcription Factors Linking Leaf Upward Cupping Phenotype in Tomato

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Abstract

Tomato yellow leaf curl virus (TYLCV), a monopartite begomovirus in the family Geminiviridae, causes serious economic losses to tomato crops in the U.S. and around the world. The TYLCV genome contains six open reading frames (ORFs), with V1 and V2 in sense orientation, and C1 to C4 in complementary orientation. The function of V1 and V2 are coat protein and pre-coat. C1 is for virus replication, C2 for trans-activation, and C3 for replication enhancer. However, the C4 protein has not been assigned with definitive functions in TYLCV, although studies in other geminiviruses suggest diverse roles or to some with no known roles. To characterize the C4 function in TYLCV, we generated stable C4 transgenic tomato plants via *Agrobacterium*-mediated transformation. The resulting transgenic tomato plants displayed upward leaf curling and plant stunting phenotypes, similar to the disease symptoms on tomato plants naturally infected by TYLCV. To investigate host genes and pathways in transgenic tomato plants that might be altered by the transgene C4 expression in comparison to those control transgenic plants expressing a GFP protein, we conducted transcriptome profiling analysis that revealed a total of 241 differentially expressed genes (DEGs). Interestingly, expressions of several leaf developmental transcription factors including those in the NAC/NAM, MADS box, LOB, MYB and BZIP families were altered. Existing functions of the stated TFs are for specification of leaflet boundaries, leaf morphogenesis, leaf primordial development, leaf morphogenesis, and for controlling leaf cell number and cell size, which might have led to the leaf curl phenotype in the transgenic C4 plants. Our results provide direct evidence that C4 is a unique virus-encoded protein that causes leaf yellowing and upward cupping phenotype upon TYLCV infection. To conclude, the C4 protein is a virulent factor interfering leaf developmental TFs, which cause the upward cupping of leaves in TYLCV-infected tomato plants.

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Developing Tools To Analyze The Begomoviruses And Satellites Associated With Yellow Vein Mosaic And Enation Leaf Curl Diseases Of Okra

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Abstract

Bhendi yellow vein mosaic disease (BYVMD) was described more than 90 years ago from India and continues to be responsible for low yields in okra/bhendi (*Abelmoschus esculents*). In the past decade, reports of leaf curling, veinal enations, petiole bending and stunting in okra, loosely described as Okra enation leaf curl disease (OELCuD) have also been reported. While BYVMD is caused by the monopartite begomoviral species *Bhendi yellow vein mosaic virus* (BYVMV) and a betasatellite *Bhendi yellow vein mosaic betasatellite* (BYVMB), there is much less clarity on the etiology of OELCuD, although a large number of sequences named *Okra enation leaf curl virus* (OELCuV) have been deposited in databases. Our earlier studies have revealed begomoviral sequences resembling the species *Mesta yellow vein mosaic virus* (MeYVMV), to be associated with BYVMD. Here, to study the distribution of the reported viruses and satellites associated with BYVMD and OELCuD, we first employed PCR-based approaches to amplify such molecules from okra samples displaying such symptoms from around 20 geographically separated locations of southern and western India. Interestingly, we found most samples to be harbouring only OELCuV. In addition, the presence of previously reported satellite molecules did not show any relationship with symptoms in the samples. Subsequently, we analyzed full-length viral DNA sequences, generated using RCA, from some samples. Again, the amplified DNAs showed high sequence identity with OELCuV sequences, irrespective of the symptoms displayed. Next, using cloned DNAs of MeYVMV and BYVMB in partial dimer forms, we obtained symptoms resembling OELCuD in young okra plants within two weeks of agroinoculation. The symptomatic plants showed evidence of accumulation of the inoculated DNAs. The findings and its implications for the managementof BYVMD and OELCuD will be discussed.

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Watermelon chlorotic stunt virus (WmCSV): an Eastern hemisphere begomovirus associated with watermelon disease in Northern Mexico

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Abstract

The genus *Begomovirus* (Family *Geminiviridae*) have been documented as the most serious threat for the global horticultural production. *Watermelon chlorotic stunt virus* (WmCSV) is a bipartite begomovirus that causes severe diseases to cucurbits particularly watermelon across Eastern Mediterranean countries. Watermelon plants exhibiting typical geminivirus symptomatology (leaf curling, yellowing and stunting) and insect vector (*B. tabaci*) infestation were collected from watermelon cultivated field in Sonora Mexico. Using rolling circle amplification (RCA) and full-length genome sequencing of an isolate of WmCSV was obtained. Phylogenetic trees based on multiple sequence alignment of the complete DNA-A and DNA-B sequences of selected begomovirus showed that WmCSV-[MX] sequences segregate most closely with sequences of WmCSV isolate from Jordan. Moreover agrobacterium-mediated virus inoculation was performed in *Solanaceae* (*N. benthamiana* and tomato) and *Cucurbitaceae* (Melon, squash and watermelon) plants fulfilled the Koch's postulates confirming the disease-associated agent. In addition, WmCSV was also detected under mixed infections with SqLCV a New World begomovirus in squash fields. The introduction of WmCSV in a new region could have a negative impact in local agriculture. Therefore, further analysis to explore the interaction between WmCSV, SqLCV and others native begomoviruses are currently developing in important cultivated crops.

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Identification of a novel nanovirus in parsley

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Abstract

Using next generation sequencing to characterize agents associated with a severe stunting disease of parsley from Germany, we identified a hitherto undescribed virus. We sequenced total RNA and rolling circle-amplified DNA from diseased plants. The genome sequence of the virus classifies it as member of the genus *Nanovirus*, yet lacking DNA-U4. In addition to the seven genomic DNAs of the virus, we identified a second DNA-R and seven distinct alphasatellites associated with the disease. We propose the name parsley severe stunt associated virus (PSSaV) for this novel nanovirus.

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SEGS-2 encodes a protein that interacts with two host proteins that impact to the host defense response

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Abstract

Cassava mosaic disease (CMD), a devastating disease of economic importance in Africa, is caused by a complex of begomoviruses collectively designated as cassava mosaic begomoviruses (CMBs). In a previous study, we identified a DNA sequence designated as SEGS-2 that enhances CMD symptoms in cassava. Subsequently, we showed that SEGS-2 enhances *African cassava mosaic virus* (ACMV) symptoms and viral DNA accumulation in a susceptible *Arabidopsis* accession, and enables *Cabbage leaf curl virus* (CaLCuV) to infect a resistant accession. SEGS-2 occurs as a DNA episome in infected plants and in the whitefly vector. The cassava genome also contains over 60 sequences related to SEGS-2. The SEGS-2 episome contains an open reading frame that specifies a 75 amino acid protein, that does not occur in any of the cassava genomic sequences. Mutation of the first ATG of the open reading frame established that its protein product is required for SEGS-2 activity. We identified several putative SEGS-2 host protein partners in a yeast two hybrid screen of an Arabidopsis cDNA library and confirmed the interaction for two of the host factors using bimolecular fluorescent complementation assays in planta. Virus-induced gene silencing experiments indicated that the host proteins may be involved in the host defense response. Our working hypothesis is that SEGS-2 enhances infection by binding to the host proteins and interfering with their ability to contribute to host defenses.

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Novel Divergent Geminivirus Infecting Cactaceae Plants

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Abstract

Cactaceae are native to the new world and have undergone adaptive radiation into diverse morphological shapes and a wide variety of environments. In 1885, the first cacti-infecting virus was described, since then, very little research has been undertaken to explore their associated viral community, with only a few single-stranded RNA viruses having been identified. Here, we investigate the diversity of DNA viruses associated with cacti, using a high-throughput sequencing approach. Cacti samples were collected in Argentina, Bolivia, Brazil, France, Lebanon, Mexico, Spain, Tunisia, and the United States. A novel divergent geminivirus, tentatively named Opuntia virus 1 (OpV1) was identified in a range of cacti species but most commonly in *Opuntia* spp from the United States (Arizona, Utah, New Mexico, Texas, and Colorado) and Mexico. OpV1 genomes range from 2941 to 2962 nucleotides in length and encode a replication-associated protein (Rep), a capsid protein (CP), a replication enhancer protein, a transactivator protein, an AC4 and a movement protein (MP). The OpV1 genome is significantly different from the presently known and classified geminiviruses, sharing <64% genome-wide pairwise identity, indicating that they might represent a new genus within the family. We have isolated and sequenced 79 OpV1 genomes which share >77% genome pairwise identity amongst themselves. Prevalence of OpV1 appears to be highest at the Phoenix Desert Botanical Garden, with the oldest genome being isolated from herbarium samples dating back to 2002 originally from Mexico. Infectious clones with two *in tadem* copies of genomes were generated and agroinfiltrated into *Nicotiana benthamiana* and four

different *Opuntia* spp. The infection was shown to be systemic in *N. benthamiana;* however only one *Opuntia microdasys* thus far has been detected as positive post-infection. No specific symptoms were identified with OpV1 infection in either of the plants. This is the first report of geminivirus infecting cacti and these findings demonstrate the broader diversity of their viral community. These findings highlight the relevance of viral surveys for plant species such as cacti, which are unique and ecologically important to specific ecosystems; such information is essential for conservation, and informed management and cultivation practices.

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Diversity and structure of Poaceae-infecting mastreviruses communities on Reunion Island using a viral metagenomics-based approach

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Abstract

The *Mastrevirus* genus (*Geminiviridae family*) contains circular single-stranded DNA viruses transmitted by leafhoppers (in the Cicadellidae family) to a wide range of either monocotyledonous or dicotyledonous host species. Most of the known monocot-infecting mastreviruses have been identified either in Africa or surrounding islands. This group of mastreviruses have collectively been called the “African streak viruses” (AfSV). Of the 13 AfSV species infecting cultivated and wild *Poaceae* species, six have been identified on Reunion Island. Interestingly, these species were probably introduced at different time and they present with putatively distinct host ranges. Understanding how this virus community operates remains an essential question in the understanding of virus ecology, evolution and emergence. Therefore, to elucidate the diversity, host ranges and structure of mastrevirus communities on Reunion Island, we undertook an extensive survey in a single sampling site of one acre including crop fields, orchards and uncultivated areas. After four sampling campaigns, 2917 samples of 30 cultivated and uncultivated *Poaceae* species were randomly collected, regardless of their health status. Total plant DNA was isolated and circular viral genomes were amplified by a sequence-independent amplification procedure combining rolling circle amplification, a random amplification tagging (RCA-RA) and high-throughput sequencing (Illumina HiSeq). For every sample, mastrevirus reads were classified using phylogenetic placement within species and strains. Mastrevirus species were confirmed by cloning and Sanger sequencing. Besides uncovering previously undescribed mastrevirus species, our results provide an exhaustive view of the mastrevirus-host association network within an agroecosystem. The topology of this network suggests (1) the co-existence of viruses ranging from generalist to specialist and (2) that certain hosts may act as hubs of virus infection and transmission.

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Single-stranded DNA virus diversity associated with honey bees (Apis mellifera) in Arizona

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Abstract

As honey bees (*Apis mellifera*) continue to decline, the need for understanding honey bee behaviour, ecology and potential threats has become increasingly important. Understanding the microbiome associated with these arthropods is essential for honey bee health. Viruses play an important role in honey bee health, and although a great deal is known about the associated RNA viruses, almost nothing is known with regards to DNA viruses. To investigate this further we used a metagenomic enabled approach coupled with traditional molecular techniques to characterize DNA viruses from the hemolymph and brain of nurse and forager worker bees from two honey bee subspecies, Italian (*Apis mellifera linguistica*) and New World Carniolan (*Apis mellifera carnica*) in Arizona, USA. Many new viral species belonging to the families *Genomoviridae* and *Microviridae* were identified, as well as novel circular replication-associated protein encoding single-stranded DNA viruses. Our analyses identified interesting patterns in viral diversity and abundance across worker groups and subspecies. Nurse bees presented as harbouring a higher diversity of identified DNA viruses compared to the foragers. Comparison between subspecies showed a proportionately higher abundance of diverse microviruses in the Italian bees than the New World Carniolan bees. Microviruses infect bacteria and are often host specific, therefore this striking difference may indicate a direct difference in bacterial microbiome composition between the two subspecies.

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Protein modifier SUMO recruits E2 conjugating enzyme (SCE1) activity to the Replication initiator protein to allow replication of the Geminivirus TYLCV

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Abstract

Geminiviruses are small ssDNA viruses that infect a wide range of plants. In order to create a cellular environment favorable for viral replication, geminiviruses manipulate the plant cell cycle. We study the role of the viral Replication initiator protein (Rep, AL1, AC1) during reprograming of the cell cycle and subsequent DNA replication. Research by numerous groups demonstrated that Rep interacts with a plethora of host factors including the SUMO E2 conjugation enzyme 1 (SCE1) and PCNA. We recently reported that Lys residues in the N-terminal half of Rep are required for nuclear localization of Rep from *Tomato Yellow Leaf Curl Virus* (TYLCV) (Maio et al., 2019). Strikingly, the same residues are essential for Rep from *Tomato Golden Mosaic Virus* (TGMV) to interact with SCE1. This interaction with Rep appears to suppress SUMO conjugation at a critical residue for PCNA function (Lys164) (Arroyo-Mateos et al., 2018). I will present data that Rep also interacts directly with the protein modifier SUMO via a novel peptide motif; this motif is widely conserved across Geminivirus genomes. Mutating this SUMO interacting motif (SIM) not only prevents Rep from interacting with SUMO, but it also hinders the interaction with SCE1 while blocking viral replication. Moreover, these three proteins localize together in nuclear bodies, which we previously defined to be sites of SUMO conjugation activity (Mazur et al., 2019). Our data thus suggest that Rep recruits both SCE1 and SUMO to manipulate yet another nuclear function essential for viral DNA replication by stimulating SUMO modification of an unknown protein without altering the global sumoylation profile.

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Monitoring begomovirus diversity on tomatoes in time

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Abstract

A survey was carried out in tomato production fields nearby Brasilia in the central part of Brazil. Tomatoes are produced throughout the year, usually of undetermined growth cultivars. The incidence of begomoviruses is invariably high, and nowadays most of the cultivars are resistant (moderately) to begomovirus infection. This study aimed to determine the diversity of begomoviruses present in this area and its variation in time. The samples were grouped in three periods: (1) 2003-2005; (2) 2009-2011; and (3) 2014-2016; each one formed by 107 to 118 samples. In the first analysis done by RCA-RFLP, a higher diversity of begomoviruses was observed in 2003-2005 than in other groups. A higher diversity of the digestion profiles and mixed infection profiles were seen in the first group. Three pools containing RCA of each individual sample were produced and were sequenced (next generation sequencing - NGS). The major viruses were tomato golden vein virus (TGVV), tomato severe rugose virus (ToSRV), tomato mottle leaf curl virus (ToMoLCV), tomato chlorotic mottle virus (ToCMoV), tomato yellow vein streak virus (ToYVSV), sida micrantha mosaic virus (SiMMV), tomato rugose mosaic virus (ToRMV), tomato apical leaf curl virus (ToALCV) and bean golden mosaic virus (BGMV). Next, PCR was performed using species-specific primers. TGVV, ToCMoV, ToYVSV, ToRMV, and ToALCV were only found in 2003-2005; SiMMV was detected in 2003-2005 and 2009-2011; BGMV in 2014-2016; and ToMoLCV and ToSRV in all three groups. ToSRV and TGVV were detected in almost all samples in 2003-2005 (mixed infection), but TGVV disappeared afterwards. The number of plants infected with ToSRV decreased over the time, while increased for ToMoLCV, suggesting that ToMoLCV became more predominant than ToSRV. During this period the use of *Ty-1* (dominant monogenic gene) containing cultivars increased from 14% to 55%. The whitefly *Bemisia tabaci* MEAM1 species was present in the area during the surveyed years. We concluded that there is a great variation in the begomovirus species composition over time, the overall diversity likely decreased, ToMoLCV and ToSRV are prevalent, and the use of resistant hybrids might be one of the determinants for a shifting of the predominant virus.

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Topical application of dsRNA does not protect tomato plants against begomovirus infection

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Abstract

Plant protection against virus infections can be achieved by the expression of specific dsRNA via transgenic approaches. It is understood that the mechanism of RNA silencing, known as RNA interference (RNAi), is responsible for dsRNA molecules cleavage, incorporation into the RISK complex and further degradation of complementary RNA, for example viral RNA, thus hampering the infection process. More recently, topical application of dsRNA has been attempted as an alternative to transgenic plants in order to avoid the cumbersome steps of plant transformation, selection, and biosafety issues. Here, we tested whether protection against the begomovirus tomato severe rugose virus (ToSRV), the most important begomovirus on tomatoes in Brazil, could be induced in tomato plants following topical application of ToSRV-homologous dsRNA molecules. Initially, pilot assays were performed with a model RNA virus, tomato mosaic virus (ToMV). Robust protection was obtained in tomato plants when ToMV-specific dsRNA molecules were applied 24h before sap inoculation of ToMV, with an average of 60% protection. In order to test if the application of dsRNA against ToSRV would also protect the plants, dsRNA molecules were mechanically applied onto tomato plantlets prior to ToSRV inoculation by using ~30 viruliferous *Bemisia tabaci* MEAM1. In the three independent trials, none of the 34 inoculated plants were protected against ToSRV infection. As expected, all plants without dsRNA application were as well infected, and those plants inoculated with aviruliferous whiteflies or non-inoculated ones were not infected. These results strongly suggest that long dsRNA molecules (~500 bp) do not induce resistance to this particular virus. Hence, a new trial was performed using small RNAs cleaved by the enzyme RNAse III, mimicking the processing by Dicer-like (DCL) proteins of long dsRNAs that naturally occurs in the RNAi pathway. In the two independent experiments, five out of six ToSRV-inoculated plants were infected, indicating that the application of long or small dsRNA molecules does not protect tomato plants against infection by ToSRV. Therefore, although the topical application of dsRNAs strategy has been shown to be useful against different RNA viruses, it may not be true for DNA viruses.

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Monitoring leaf curl disease complexes associated with whitefly-transmitted begomoviruses infecting Papaya in Delhi NCR, India: Results from a long-term survey

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Abstract

Ongoing work initiated in 2004 indicated that cultivated commercial varieties and feral plants of Papaya were susceptible hosts for highly divergent begomoviruses causing leaf curl disease. In addition to DAS-ELISA based serological detection of begomoviruses related to Tomato leaf curl disease, PCR-based screens were used to determine virus incidences in symptomatic and non-symptomatic plants from the region. While mono-partite begomoviruses like *Chilli leaf curl virus* and *Papaya leaf crumple virus* were found to be persistent, *Tomato leaf curl New Delhi virus* was also prevalent among the sampled plants. Several begomoviruses including *Tomato leaf curl Joydebpur virus* were observed to be emerging. Other begomoviruses detected in non-papaya samples, like *Okra leaf curl virus* were host-specific. Symptomatic plants displayed a wide range of leaf curling symptoms. In order to determine the nature of satellite molecules associated with the mono-partite viruses infecting papaya, PCR primers were used to detect beta-satellites in the surveyed samples. Results are discussed in relation to white-fly transmitted begomoviruses and leaf curl disease complexes identified in papayas from the region over a 15 year survey period.

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SEGS-1 Enhances Begomovirus Symptoms and Breaks Resistance in *Arabidopsis Thaliana*

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Abstract

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

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Management of *Beet curly top virus* in Sugar Beet

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Abstract

Beet curly top (CT) is an important yield limiting disease caused by strains of *Beet curly top virus* (BCTV) in arid to semi-arid sugar beet production areas worldwide. Despite efforts to improve resistance in commercial sugar beet cultivars, resistance is still low to intermediate. In 2016, the USDA-ARS sugar beet program in Kimberly, ID released the double haploid sugar beet line KDH13 which contains a high level of resistance to BCTV. However, maintaining resistance to BCTV in sugar beet cultivars is problematic since resistance is multigenic, yield and other disease resistance traits are important to maintain, and several parents are required to generate the three-way hybrid cultivars. Since some resistance to BCTV in sugar beet can be strain specific, the BCTV strains found in western US sugar beet production areas were investigated. The Severe strain of BCTV declined in incidence from 2006 to present day. Insecticides were also investigated to find management options for the beet leafhopper, *Circulifer tenellus* Baker, which vectors the BCTV. Results indicated that the neonicotinoid seed treatments based on clothianidin (sold as Poncho and NipsIt) and thiamethoxam (sold as Cruiser) could significantly reduce CT symptoms for at least 77 days from the time of planting and increase yields by at least 17%. The seed treatments and subsequent CT control not only improved yield variables in the field but could also improve sucrose yields from roots stored under ambient conditions in Idaho. Even without CT pressure in sugar beet fields, the general pest control provided by the seed treatments more than pays for the cost of treatment. Investigations have shown that when control by the seed treatments runs out, the best foliar insecticides to use to extend control are pyrethroids based on esfenvalerate (sold as Asana and Mustang). The foliar insecticides are not effective for season long control but are a cheap way to supplement the control provided by host resistance and the seed treatments. Utilizing a different insecticide chemistry for CT control will also help decreasing the likelihood of resistance building up to the neonicotinoids.

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Field Evaluation of Transgenic Banana Plants for Resistance to BBTV Infections in Hawaii

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Abstract

Banana bunchy top virus (BBTV) causes one of the most devastating virus diseases of bananas in Asia, Africa, and the Pacific, including Hawaii. The recent development of transformation and regeneration systems for banana has made it feasible to develop BBTV-resistant transgenic banana plants. We have transformed the *Musa* x *paradisiaca* cultivar 'Dwarf Brazilian' (AAB) with four different constructs of the *Rep* gene from DNA-1 of BBTV: Construct #1, a single-point mutant *Rep* gene in the sense orientation; Construct #2, the entire *Rep* gene in the antisense orientation; Construct #3, a partial *Rep* gene and stem-loop structure both in the antisense orientation; and Construct #4, the entire *Rep* gene in the sense orientation fused to a partial *Rep* gene and stem-loop structure in the antisense orientation. Embryogenic calli of the 'Dwarf Brazilian' cultivar that were initiated from immature male flowers were used to produce embryogenic cell suspensions (ECS). Cell aggregates from ECS were used as explants for *Agrobacterium*-mediated transformation using the four constructs individually. Embryos formed from transformed ECS following *Agrobacterium* co-cultivation were germinated on media containing antibiotics to select for transformed lines. The resulting 300 lines representing transformants with the four different constructs were evaluated for BBTV resistance by challenging them with viruliferous aphids in the greenhouse. Twenty transgenic lines displayed resistance to BBTV. These lines were confirmed to be independently transformed using Southern blot analyses. A one-acre plot at the Waimanalo Field Station of University of Hawaii on the island of Oahu was used for field-testing of the transgenic, putatively resistant lines that were identified in the greenhouse experiments. These twenty putatively BBTV-resistant banana lines were multiplied *in vitro* and planted out in five separate field trials. Plants in the field were monitored for BBTV symptom development, growth rates, and horticultural characteristics. At the conclusion of each of these field trials, there were plants from a few transgenic lines that had not developed BBTV symptoms. However, none of the transgenic lines displayed both durable resistance and acceptable horticultural characteristics. Currently, CRISPR-Cas constructs that target conserved BBTV sequences are being designed to produce BBTV-resistant transgenic banana plants.

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Effects of Viruses on Phytohormone Pathways and Plant-Insect Interaction

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Abstract

Co-infection of plants by both viruses and insects is common in nature. Yet the dynamics of the tripartite system, in particular the effects of viral infection on plant-insect interactions and the factors involved, remained little studied until very recently. In this article we review the progress in research on these aspects of the tripartite interactions, explore the questions associated with the investigation, and suggest direction for future research on this exiting and likely fruitful field. Virus-induced changes of the tripartite interaction are in many cases attributable to the modulation of phytohormone pathways by viruses. Infection of viruses can affect significantly the synthesis and function of the three well-known phytohormones, i.e., jasmonates, salicylic acid and ethylene as well as plant-insect interactions associated with these phytohormones. The virus-induced changes of the dynamics of these phytohormones and other aspects in the tripartite system are influenced by both intrinsic and external factors such as plant nutrition, secondary metabolites and natural enemies of the vector insects. The effects of viral infection on other phytohormones, such as auxin, gibberellins, cytokinins, brassinosteroids and abscisic acids are yet to be investigated.

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SP2700 - A Novel Antiviral Plant Activator

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Abstract

SP2700 (Trade name: Ninja™) is a biopesticide extracted from soil bacterial fermentation. This compound activates the innate plant defense system and demonstrates antiviral properties. It has been used extensively in plant viral disease management outside of the US. In this study, SP2700's curative antiviral property was examined using three model patho-systems: 1) *Tobacco mosaic virus* (TMV) on tobacco (*Nicotiana tabacum* var. Hicks MR) as a local lesion host, 2) *Tomato bushy stunt virus* (TBSV) on pinto bean (*Phaseolus vulgaris* cv. Pinto) as a local lesion host, 3) TBSV with a green fluorescent protein (GFP) reporter gene on *Nicotiana benthamiana* as a susceptible systemic host. Inoculations with purified TMV virions via a soft brush or TBSV RNA transcripts via rubbing were conducted 1 hour before the application of the product. A half-leaf assay was used for these studies where SP2700 was brushed onto one half of the leaves and water to the other half leaves serving as the control. SP2700 treated half leaves had consistently less TMV local lesions compared to the control with reduction from 76% to 49% on older leaves and 89% to 20% on young leaves based on 4 experiments. A similar effect was observed with TBSV on pinto half leaves. Surprisingly, fewer fluorescent foci were observed in the TBSV-GFP SP2700 treated half leaves despite the total susceptibility of *N. benthamiana* to TBSV systemic infection. This demonstrates that SP2700 is able to decrease viral infections using a non-specific mechanism. In addition to the above greenhouse studies, US field trials conducted from 2016-2019 have also demonstrated SP2700 anti-viral activity in managing *Tomato yellow leaf curl virus* on tomato, *Tomato spotted wilt virus* on tobacco, *Iris yellow spot virus* on onion, *Bean golden mosaic virus* on snap bean, mixed viral infections on squash as well as promise on *Rose rosette virus*. SP2700/Ninja shows great promise as an antiviral compound for US conventional and organic agriculture.

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An Integrative Approach to Transcriptional Co-Regulatory Network Construction and Characterization in *Arabidopsis*

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Abstract

Transcription factors (TFs) play a significant role in gene regulation. They bind to DNA and control the expression of the corresponding gene in a cooperative fashion. While various kinds of experimental data, including ChIP-chip and gene expression studies have been used to identify cooperative TF pairs, our understanding of the interaction among TFs on a genomic scale is still limited. We have developed a novel method to construct the largest co-regulatory network for Arabidopsis by integrating the TF-gene recognized regulation, gene expression and information about TF motifs. Our network of pairwise cooperative TFs is based on two biologically rational assumptions. There is a link between two TFs if i) they have a significantly higher number of common target genes than random expectation ii) there is a high positive correlation between the expressions of the genes. We add an edge between a pair of TFs, denoting a co-regulatory association, if they satisfy these criteria by reasonable threshold. We also investigated the effect of their motif similarity by adding similarity as weight to the edges. For similar motifs, if they are from same family, they are more likely to have no interaction. The development of transcription factor networks will be used to help identify key regulatory elements in geminiviruses, a significant group of plant pathogens. *Tomato golden mosaic virus* (TGMV) and *Spinach curly top virus* (SCTV) transcribe an mRNA from promoter sequences that appear conserved. Understanding the interactions between the virus and host transcription factors to regulate viral promoter activity could provide novel targets for control of these viruses.

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Revisiting Seed Transmission of the Type Strain of Tomato Yellow Leaf Curl Virus in Tomato Plants

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Abstract

The tomato yellow leaf curl disease (TYLCD) is one of the most devastating viral diseases worldwide that causes severe constraint for tomato production areas. It has been generally accepted that most begomoviruses, including the Tomato yellow leaf curl virus (TYLCV) involved in TYLCD, are horizontally transmitted in natural conditions in a circulative persistent manner by the whitefly *Bemisia tabaci*. However, isolates of TYLCV-IL (type strain: Israel) have recently been reported to be vertically transmitted to the progeny plants through tomato seeds. This finding significantly impacts on the production and regulation protocols imposed by the worldwide plant health authorities to control this virus. Due to the repercussion that these data have on seed industry, it seems reasonable to revisit and evaluate the seed transmission of TYLCV before adopting regulatory measures on seed trade.

In the present study replication of TYLCV-IL was detected in tomato and *N. benthamiana* flower reproductive organs, demonstrating a close association of this virus with the seeds during maturation. Additionally, TYLCV DNA was also detected by quantitative PCR in seeds of both plant species naturally or experimentally infected with TYLCV-IL supporting the seed-borne nature of the virus. However, the significant reduction in DNA viral load after surface disinfections of tomato seeds suggests that most of the virus is located externally, as a contaminant of the seed coat. Transmission assays to the offspring, evaluated with two Mediterranean isolates of TYLCV-IL on more than 3000 plants of seven tomato genotypes, revealed no evidence of seed transmission from “surface disinfected” or untreated seeds. Similar results were also obtained for seeds collected from TYLCV-IL-infected *N. benthamiana* plants. The results support that TYLCV-IL can be a seed-borne in tomato or *N. benthamiana* but not seed-transmitted, suggesting that transmission through seeds is not a general property of TYLCV.

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Milk vetch dwarf nanovirus infection and transmission in *Solanaceae family* by *Aphis craccivora*

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Abstract

The milk vetch dwarf virus (MVDV) is an important member of the genus *Nanovirus* and is transmitted by *Aphis craccivora.* MVDV consists of multiple ssDNA components, each approximately 1 kb in length, and two or three alpha-satellite molecules. It mainly infects plants of the legume family, *Fabaceae*. Recently, papaya (*Carica papaya*) collected in Yesan, Korea, displaying symptoms of leaf yellowing and dwarfism were identified as a new host for MVDV. To examine the geographical distribution of MVDV, symptomatic papaya samples were harvested in Vietnam and Taiwan in 2018 along with tomato and pepper samples grown in adjacent papaya fields. After total DNA extraction, PCR was performed using the specific primer sets for segments M, S and alphasatellite C3 of MVDV. Results revealed the presence of MVDV not only in papaya but in pepper and tomato as well which led us to believe infected papaya as transmission origin. This transmission of MVDV from papaya to *Solanaceae* members through an insect vector was reconfirmed in an artificial insect cage. Based on PCR sequence analysis of three MVDV components (M, S and C3), we found that MVDV could be transmitted from infected papaya to pepper and tomato via the insect vector *Aphis craccivora*. Comparative studies based on segment sequences analysis both in natural fields of Vietnam and artificial insect cages in Korea verified that MVDV was transmitted from papaya to the *Solanaceae* members' tomato and pepper. Taken together, these results clearly reveal that MVDV can be infectious in peppers and tomatoes.

Keywords: Milk vetch dwarf virus, *Solanaceae, Fabaceae, Aphis craccivora*

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Hidden diversity of endogenous geminiviral sequences across plant genomes and transcriptomes

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Abstract

Endogenous viral elements (EVE) can be used as 'fossil records' to reveal the genomic features of long extinct virus species. Although numerous known instances exist of single-stranded DNA (ssDNA) genomes becoming stably integrated within the genomes of bacteria and animals, there remain very few examples of such integration events in plants. Most of the EVEs that have been characterized so far belong to family *Caulimoviridae*. However the first plant EVEs to be discovered were geminivirus derived sequences in the nuclear genomes of various *Nicotiana* species. Since then, endogenous geminivirus-like elements (EGV) have also been identified in the genomes of several plants, including yam (several *Dioscorea* species), apple (*Malus domestica*), lettuce (*Lactuca sativa*), cottonwood (*Populus trichocarpa*) and coffee (*Coffea canephora*). We therefore search for evidence of EGVs within 134 plant genome sequences and 797 plant transcriptome sequences. We detected homologues of geminivirus replication-associated protein (*rep*) genes from 17 genomes and 39 transcriptomes from angiosperms. Copy numbers of EGVs within these genomes varied widely with the highest copy numbers, approximately 1000, being found in two varieties of tea (*Camellia sinensis*). Phylogenetic and similarity-based analyses revealed multiple taxonomically novel geminivirus lineages, including two in *Camellia* species which might represent novel genera. We found that some of the *Camellia* and *Dioscorea* EGVs are transcriptionally active, and display evidence of purifying selection, suggesting that expressed geminivirus proteins were, and may still be, functionally active in certain host plants. Collectively our analysis expands the known breadth of past geminivirus diversity, provides a first large-scale view of EGV prevalence, and strengthens support for the hypothesis that EGVs impact the biology of their hosts.

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Artificial microRNA strand selection shows a purine-rich preference for geminivirus- and non-virus-based expression vectors in plants

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Abstract

Artificial microRNA (amiRNA) technology has allowed researchers to direct efficient silencing of specific transcripts using as few as 21 nucleotides (nt). However, not all the artificially designed ~21 nt mature amiRNAs function as expected. We compared a nuclear-replicating DNA viral vector (*Tomato mottle virus*, ToMoV, based), a cytoplasmic-replicating RNA viral vector (*Tobacco mosaic virus*, TMV, based), and a non-viral binary vector to express amiRNAs. All three gave products suggesting amiRNA production in plants when analyzed by small RNA Northern blot analysis. However, small RNA Hi-Seq illumina sequencing showed that the TMV based cytoplasmic-replicating viral vector did not yield authentic amiRNAs compared to results for the other two vectors which yielded specific amiRNAs, and the nuclear-replicating ToMoV-based vector produced the most amiRNAs. Selection of the miRNA guide strand from the mature miRNA duplex has been studied in detail in human and insect systems, but not so much for plants. The ability to express specific amiRNAs in plants, and understanding the factors that determine guide strand selection are needed. Here we used deep sequencing analysis and show that when the structural factors caused by base mismatches in the mature amiRNA duplex were excluded, the nucleotide composition of the mature amiRNA determined the guide strand selection: the strand with excess purines was preferentially selected as the guide strand. Either the 5p or 3p strand of the amiRNA duplexes, whichever contains excess purines, can be selected as the guide strand even though the amiRNAs were expressed in the same cell types and the plants were grown in the same conditions.

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Fine Mapping of Broad Geminivirus Resistance in *Arabidopsis thaliana*

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Abstract

Geminiviruses are the causal agent for a myriad of problems in many crops all around the world, mainly in tropical, subtropical and temperate areas. The search for resistance against these pathogens is necessary since they evolved rapidly, and their vectors are colonizing new areas. We identified an *Arabidopsis thaliana* ecotype that shows lack of response to inoculation with a silencing vector based on Cabbage leaf curl virus (CaLCuV, begomovirus). Once we inoculated the ecotype with the wild type virus, it was clear that there was some form of resistance. Furthermore, we also used a curtovirus (Beet curly top virus) and also showed no symptoms. We then crossed the resistant ecotype (Pla-1) with a susceptible ecotype (Col-0) and inoculated the F_2 generation with CaLCuV. A QTL mapping analysis showed a peak present in chromosome 1, between 8 and 12.5 Mb. Furthermore, recently performed QTL-Seq showed the same peak as previously described but also hinted for a second peak in chromosome 5. Kompetitive allele-specific PCR was used to fine map the trait for CaLCuV and we have found that around 29 genes are possibly involved in the phenotype. We are performing QTL-Seq and RNA Seq analysis to determine the gene (s) responsible for the resistance.

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Composition of Begomovirus Populations in Cultivated and Non-Cultivated Hosts Determined by High-Throughput Sequencing

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Abstract

Tomato-infecting begomoviruses emerged in Brazil in the 1990's following the introduction of *Bemisia tabaci* MEAM1 (previously, *B. tabaci* biotype B). Several lines of evidence suggest that these viruses evolved from indigenous viruses infecting non-cultivated hosts. However, tomato-infecting viruses are only rarely found in non-cultivated hosts, and vice-versa. It is possible that viral populations in a given host are composed primarily of viruses which are better adapted to this host, but also include a very small proportion of viruses which are poorly adapted. Then, after transfer to a different host by the whitefly vector, the composition of the viral population shifts rapidly, with the viruses which are better adapted to the new host becoming predominant. To test this hypothesis, we collected tomato and *Sida* sp. plants, growing next to each other, at two locations (Coimbra and Florestal, both in Minas Gerais state, Brazil), in 2014 and 2018. Viral infection was confirmed by polymerase chain reaction (PCR) using specific primers. Total DNA from one tomato and one *Sida* sp. sample from each location and year were subjected to high-throughput sequencing (HTS). Following a highly stringent set of criteria, reads were mapped to a data set including all New World begomoviruses. The reads were classified as (i) *Tomato severe rugose virus* (ToSRV), (ii) *Sida micrantha mosaic virus* (SiMMV) and (iii) *Sida common mosaic virus* (SiCmMV), when the first three hits were isolates of these species, or (iv) begomovirus, when the first three hits included isolates of different species. For the 2014 samples, >98% of the reads from *Sida* sp. mapped to SiMMV, but 0.01% of the reads mapped to ToSRV. Conversely, >99% of the reads from tomato mapped to ToSRV, with 0.001% mapping to SiMMV. For the 2018 samples, >99% of the *Sida* reads mapped to three *Sida*-infecting viruses, and 0.1% of the reads mapped to ToSRV. These results are consistent with the hypothesis that viral populations in a given host are composed primarily of the virus that is most adapted to this host but also includes a very small proportion of viruses that are less adapted.
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Emergence and Adaptation of Tomato Begomoviruses in Brazil: Assessing Replicative and Transmission Fitness

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Abstract

The prevalence of only a few begomoviruses infecting tomatoes in Brazil is an intriguing fact, on light of the great begomovirus diversity that has been reported in this crop. Most studies that have been performed in an attempt to understand the begomovirus emergence process and consequent epidemics in Brazil have focused on the genetic structure and dispersion patterns of viral populations. Studies addressing the underlying mechanisms leading to emergence and the current patterns of prevalence have not been conducted. Here, we quantified the replicative fitness of two begomoviruses infecting tomato in Brazil, *Tomato severe rugose virus* (ToSRV) and *Tomato yellow spot virus* (ToYSV), in tomato plants and in non-cultivated hosts to which each virus has often been associated, and quantified the transmission efficiency by *B. tabaci* Middle East-Asia Minor 1 (MEAM1) and *B. tabaci* Mediterranean (MED). Interestingly, ToSRV and ToYSV presented similar adaptation levels in tomato. No fitness trade-off across hosts was observed for ToYSV when viral accumulation was evaluated in tomato and in the wild host *L. sibiricus*. In contrast, ToSRV performed better in tomato than in *N. physalloides*. These results reinforce that ToSRV is well adapted to tomato and occasionally spills back to wild hosts, while ToYSV is well adapted to both tomato and *L. sibiricus*. We also compared the replicative fitness of ToSRV and ToYSV during single or mixed infection. Interestingly, while there were no differences in fitness between the two viruses at 14 days after inoculation (dpi), ToYSV had a gain in fitness from 21 to 28 dpi. Furthermore, a negative interference of ToSRV over ToYSV was observed, as ToYSV reached a higher accumulation in single infection than in mixed infection with ToSRV. Together, these results suggest that adaptation to the host does not explain the prevalence of ToSRV over ToYSV in the field. However, while ToSRV was transmitted by *B. tabaci* MEAM1 and MED, ToYSV was not, which could be the reason why this virus is not widespread in the field.

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Aspects of the Association Between Leonurus yellow spot alphasatellite and Bipartite Begomoviruses: Effects on Infection and Transmission by Bemisia tabaci Middle East-Asia Minor 1

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Abstract

The genus *Begomovirus* (family *Geminiviridae*) includes plant viruses with circular, single-stranded DNA (ssDNA) genomes which are transmitted by the whitefly *Bemisia tabaci*. Begomoviruses in the New World can be found in association with alphassatellites, which are circular, ssDNA molecules capable of autonomous replication, but dependent on the helper begomovirus for encapsidation, systemic infection and insect transmission. The impact of the interaction between alphassatellites and begomoviruses is unknown. The objective of this work was to verify the effect of *Leonurus yellow spot alphasatellite* (LeYSA) in the infection of *Tomato yellow spot virus* (ToYSV), *Tomato severe rugose virus* (ToSRV) and *Euphorbia yellow mosaic virus* (EuYMV) in three hosts, *Leonurus sibiricus, Nicotiana benthamiana* and tomato. The plants were inoculated with each virus in the presence or absence of the alphasatellite. Infectivity and symptom development for each begomovirus alone or in the presence of LeYSA were evaluated, and viral DNA accumulation was quantified for each virus and virus-satellite combination. The association of LeYSA with ToYSV was less efficient in tomato than in *L. sibiricus* and *N. benthamiana*, as measured by a lower percentage of plants in which the presence of the alphasatellite was detected. The association between ToSRV and LeYSA was similar in both tomato and *N. benthamiana*. The association between EuYMV and LeYSA in tomato was the least efficient, and in *N. benthamiana* the presence of the alphasatellite was not detected in any of the plants infected with EuYMV. Together, these results indicate distinct levels of interaction between the alphasatellite and different begomoviruses. Quantification of ToYSV and ToSRV DNA-A accumulation indicated that LeYSA does not intefere in the accumulation of these begomoviruses. However, symptoms were more severe in the presence of LeYSA for both viruses and in all hosts. There was a variation in the accumulation of LeYSA relative to the host and the associated begomovirus. Together with previous studies, these results highlight the potential risk of the association between begomoviruses and alphasatellites in both cultivated and non-cultivated plants.
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Evolutionary Dynamics of Bipartite Begomoviruses in the New World Revealed by Complete Genome (DNA-A + DNA-B) Analysis

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Abstract

Several key evolutionary events marked the evolution of geminiviruses, culminating with the emergence of bipartite genomes represented by viruses classified in the genus *Begomovirus*. This genus represents the most abundant group of multipartite viruses, contributing significantly to the observed abundance of multipartite species in the virosphere. Although aspects related to virus-host interactions and evolutionary dynamics have been extensively studied, the bipartite nature of these viruses has been little explored in evolutionary studies. We performed a parallel evolutionary analysis of the DNA-A and DNA-B components of New World begomoviruses. A total of 239 full-length DNA-B sequences obtained in this study, combined with 292 DNA-A and 76 DNA-B sequences retrieved from GenBank, were analyzed. The results indicate that the DNA-A and DNA-B respond differentially to evolutionary processes, with the DNA-B being more permissive to variation and more prone to recombination than the DNA-A. Although a clear geographic segregation was observed for both components, differences in the genetic structure between DNA-A and DNA-B were also observed, with cognate components belonging to distinct genetic clusters. DNA-B coding regions evolve under the same selection pressures than DNA-A coding regions. Together, our results indicate an interplay between reassortment and recombination acting at different levels across distinct subpopulations and components.
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Euphorbia Yellow Mosaic Alphasatellite Interacts With Two Tomato-Infecting Bipartite Begomoviruses And Increases Symptom Severity In Tomato Plants

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Abstract

Begomoviruses (family *Geminiviridae*) are whitefly-transmitted viruses with circular single-stranded DNA genomes that are frequently associated with DNA satellites. Alphasatellites are circular, single stranded DNA molecules, capable of autonomous replication, but dependent on a helper begomovirus for cell-to-cell and systemic movement in the plant and for insect-mediated plant-to-plant transmission. Begomoviruses constitute a serious constraint to crop production worldwide. The presence of *Euphorbia yellow mosaic alphasatellite* (EuYMA) increases the severity of symptoms induced by *Euphorbia yellow mosaic virus* (EuYMV) in *Euphorbia heterophylla*, *Nicotiana benthamiana* and *Arabidopsis thaliana*. The present work investigates whether EuYMA is capable of interacting with two tomato-infecting begomoviruses, *Tomato yellow spot virus* (ToYSV) and *Tomato severe rugose virus* (ToSRV) in tomato and *N. benthamiana*. The plants were biolistically inoculated with infectious clones of each virus and of the alphasatellite in different combinations. Infection was confirmed by PCR with specific primers 21 days after inoculation. Symptoms of ToYSV and ToSRV in the presence or absence of the alphasatellite were evaluated weekly. Interaction between the two begomoviruses and EuYMA was demonstrated by detection of ToYSV, ToSRV and EuYMA in apical leaves of both tomato and *N. benthamiana* plants. This indicates that begomovirus-mediated systemic movement of EuYMA occurs in these hosts. Furthermore, the presence of the alphasatellite increased the severity of symptoms induced by ToYSV and ToSRV in both hosts. However, the association of EuYMA with the two begomoviruses was less efficient in tomato than in *N. benthamiana* (as measured by a lower percentage of plants in which the presence of the alphasatellite was detected). This could be due to difference in DNA accumulation of the satellite in each host, or to less efficient movement of the satellite in tomato compared to *N. benthamiana*. Together, our results suggest that the association of EuYMA with begomoviruses is not species-specific. Further studies are needed to determine the relevance of alphasatellites in natural infections by bipartite begomoviruses.

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Field Infection of *Alpinia purpurata* with Banana Bunchy Top Virus in French Polynesia

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Abstract

Banana bunchy top virus (BBTV) is reported for the first time from French Polynesia (FP) and was shown to be widespread as natural infections in *Alpinia purpurata* (ornamental red ginger, alpinia). Infected alpinia plants were stunted, with shoot proliferation, small chlorotic leaves displaying dark green streaks on minor leaf veins, and reduced flower size. Surveys of alpinia on six islands (Tahiti, Moorea, Raiatea and Tahaa in the Society archipelago, Tubuai in the Austral archipelago, and Nuku Hiva in the Marquesas archipelago) revealed the presence of BBTV in alpinia on Tahiti, Moorea, Raiatea and Tubuai islands. Surveys of banana on Tahiti, Moorea, Tahaa, and Nuku Hiva did not reveal any typical symptoms of banana bunchy top disease though they frequently grow in close proximity to infected alpinia. However, a single infected but symptomless Fe'i banana (*Musa troglodytarum*) was detected on Tahiti. The identity of the virus was established by ELISA and confirmed by PCR and subsequent sequencing. An isolate from Fe'i banana and one from alpinia were examined by high throughput sequencing (HTS) and with confirmation by Sanger sequencing. The isolates were 99.8% identical across the combined sequenced genome components (DNA-R, -M, -N, -C and -S), with only 11 changes over the 5427 nt. Surprisingly DNA-U3 was not detected in either isolate by HTS or by PCR. The sequence of the coding regions of all components clearly placed BBTV-FP in the South Pacific subgroup, though the Common Region-Major was markedly distinct, containing a 17nt insert and for example differing by 20.6% in DNA-R vs South Pacific subgroup average (average intragroup variation 3.7%). Additionally, inter-component recombination was detected, where part of the untranslated region of DNA-N, including the Common Region-Major and Common Region-Stem Loop, from BBTV-FP was inserted into the same position in DNA-S. A 26 nt near identical direct repeat sequence 5' of the CR-M was also observed in DNA-M.

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Tomato yellow leaf curl virus infection in a monocotyledonous weed (*Eleusine indica*)

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Abstract

Tomato yellow leaf curl virus (TYLCV) is one of the most important plant viruses belonging to the genus *Begomovirus* of the family *Geminiviridae*. To identify natural weed hosts that could act as reservoirs of TYLCV, 100 samples were collected at a TYLCV-infected tomato farm in Iksan from 2013 to 2014. The sample weeds were identified as belonging to 40 species from 18 families. TYLCV was detected in 57 samples belonging to 28 species through polymerase chain reaction using root samples including five species (*Eleusine indica*, *Digitaria ciliaris*, *Echinochloa crus-galli*, *Panicum dichotomiflorum* and *Setaria faberi*) from the family Poaceae. Whitefly *Bemisia tabaci*-mediated TYLCV transmission from TYLCV-infected *E. indica* plants to healthy tomatoes was confirmed, and inoculated tomatoes showed typical symptoms, such as leaf curling and yellowing. In addition, TYLCV was detected in leaf and root samples of *E. indica* plants inoculated by both whitefly-mediated transmission using TYLCV-viruliferous whitefly and agro-inoculation using a TYLCV infectious clone. The majority of mastreviruses infect monocotyledonous plants, but few dicotyledonous plants infected with masteviruses have been reported. No exception was reported among begomoviruses known as infecting dicots only. This is the first report of TYLCV as a member of the genus *Begomovirus* infecting monocotyledonous plants.

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CIDER-Seq: An efficient tool to study plant-geminivirus recombinant eccDNAs

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Abstract

Genetic recombination is an important feature of begomoviruses, single stranded DNA virus belonging to family Geminiviridae. Extremely complex and widely distributed recombinations have been observed upon begomovirus infections [1]. Apart from virus-virus recombination, a recent study has revealed spontaneous formation of hybrid DNA minicircles composed of begomovirus and plant genomic DNA sequences [2]. We have recently developed CIDER-Seq (Circular DNA Enrichment sequencing), a pipeline for the unbiased enrichment and highly accurate long-read sequencing of extra-chromosomal circular DNA (eccDNA) molecules [3]. Here we demonstrate the utilization of CIDER-Seq to sequence and characterize recombinant plant-geminivirus eccDNAs. Arabidopsis plants, agroinoculated with cabbage leaf curl virus (CaLCuV), were subjected to modified rolling circle amplification-mediated enrichment of eccDNAs. SMRT (Single Molecule Real Time) libraries were then prepared and sequenced on PacBio Sequel platform. BLAST analysis indicated that almost 80% of the reads contained CaLCuV sequences. Analysis of these reads lead to the identification of 74 Arabidopsis-CaLCuV recombinants, among which 22% (16/74) belonged to CaLCuV DNA-A and 78% (58/74) belonged to CaLCuV DNA-B. The size of recombinants ranged from 624nt to 7,535nt, with an average recombinant size of 3,520nt. Overall 82% (61/74) of recombinant fragments contained chromosomal DNA, while 15% (11/74) and 3% (2/74) had chloroplast and mitochondrial DNA origins, respectively. These host sequences from recombinant fragments did not share significant similarities or patterns, except all being AT rich (~70%) and almost all lacking open reading frames (ORFs). However, in two recombinants we were able to identify complete ORFs coding for Sucrase/ferredoxin-like family protein and DNA double-strand break repair protein. Further experiments are required to characterize the frequency and the role of host-geminivirus recombination events during geminivirus infection.

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AC4 protein, encoded by *Tomato leaf curl Guangdong virus*, is a pathogenic determinant

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Abstract

Tomato leaf curl Guangdong virus (ToLCGuV) is a begomovirus associated with a Tomato yellow leaf curl disease (TYLCD) epidemic in Guangdong province, China. The full length of ToLCGuV is 2744nt encoding 6 ORFs, with AV1 and AV2 in the virion-sense strand and AC1, AC2, AC3, and AC4 in the complementary-sense strand. As the least conservative protein among geminivirus proteins, the function of AC4 during ToLCGuV infection has not been elucidated. In this study, infectious clones of ToLCGuV and a ToLCGuV mutant ($ToLCGuV_{mAC4}$) containing a premature AC4 ORF were constructed. Both ToLCGuV and $ToLCGuV_{mAC4}$ could infect tomato plants by *Agrobacterium*-mediated infiltration. However, $ToLCGuV_{mAC4}$ elicited much milder symptoms compared with ToLCGuV. To further verify the role of AC4 in viral pathogenesis, AC4 was expressed in *Nicotiana benthamiana* by *Potato virus X* (PVX) vector. The results showed that ToLCGuV AC4 enhanced the pathogenicity of PVX and induced severe developmental abnormalities in plants compared with PVX alone or PVX-mAC4. In addition, ToLCGuV AC4 was found to be able to suppress local silencing induced by sense GFP in transgenic *N. benthamiana* line 16c. All these results suggested that while ToLCGuV AC4 is dispensable for viral infectivity, it may be important in viral pathogenesis.

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Passion fruit-infecting Begomoviruses found in Guangxi, China

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Abstract

Passion fruit (Passiflora edulis) native from South America, and now grown worldwide as an edible fruit for the food industry（Zibadi *et al.*, 2004）. Guangxi located in the south of China, being largely subtropical, with suitable climate for passion fruit growing. In recent years, the passion fruit industry expanded rapidly in Guangxi , however, the production of passion fruit seriously endangered by the viral diseases. During 2016-2018, a field survey was conducted for the passion fruit-infecting viruses in Guangxi. 210 samples which showed typical yellowing, crinkle, mottle, vein thickening et al. symptoms on the leaves were collected. Total DNA were extracted following the manufacture's instructions, and PCR were applied for detected the causal begomoviruses using the degenerate primer pair AV494/COPR (Köklü et al., 2006), 30 samples were amplified a 570 bp PCR products, then all the products were purified and cloned in pMD18-T vector, 6-8 positive clones were selected for each sample and then sequenced. all the sequences were blast on NCBI using the blastn tool, and the results showed that the sequences obtained in this study shared highly nucleotide identity with Papaya leaf curl China virus (PaLCCNV) (95% ~99%), Papaya leaf curl Guangdong virus (PaLCGDV)(90%~97%), Tomato leaf curl China virus (ToLCCNV) （90%~97%）, Euphorbia leaf curl virus (EuLCV)（97%~99%）. EuLCV and PaLCGDV was ever reported infecting passion fruit in Taiwan city , and also other begomoviruses reported infecting passion fruit（Cheng *et al.*, 2014；Vaca-Vaca *et al.*, 2017）, but not in Guangxi province, China. All the results in this study indicate that passion fruit is a new host of PaLCCNV and ToLCCNV .

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Geographical Distribution and Diversity of Plantago Lanceolata Latent Virus, a Member of the Capulavirus Genus

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Abstract

The genus *Capulavirus* is a new genus in the family *Geminiviridae* that contains so far four species (*Alfalfa leaf curl virus*, *Euphorbia caput-medusae latent virus*, *French bean severe leaf curl virus* and *Plantago lanceolata latent virus*) that infect both cultivated and non-cultivated plants. Among them, plantago lanceolata latent virus (PlLV) infects ribwort plantain (*Plantago lanceolata* L.), a perennial herb that is native from Eurasia and is now widespread all over the world. PlLV is transmitted by the aphid *Dysaphis plantaginea*, which has a holocyclic lifestyle with two successive host plants: apple and plantain. Mechanical inoculation of sap using carborundum powder or syringe have also proved effective to infect various plants including *P. lanceolata*. Although plants naturally infected with PlLV displayed yellowing symptoms, agroinoculation in controlled conditions of an agroinfectious clone of PlLV in plantain did not reveal evidence of symptoms. In order to better decipher the prevalence and the diversity of PlLV, *P. lanceolata* plant samples were collected from four European countries (Finland, France, Italy and Spain), as well as from Iran and South Africa, and were tested by PCR for the presence of PlLV. While PlLV was initially reported from the Åland Islands (South-West Finland), we show that PlLV is also found from all sampled countries but South Africa. These results suggest that the geographic distribution of PlLV is larger than initially thought and that PlLV prevalence is probably relatively high in the Old World. Six complete genomes of PlLV from four distinct plant populations [Finland (2 genomes), France (1), Italy (1), Spain (1)] were amplified by RCA and/or PCR using a pair of abutting primers from infected samples, cloned and sequenced (Sanger). Partial nucleotidic sequences of the coat protein (*cp*) and replication-associated protein (*rep*) genes of PlLV were also obtained by PCR from samples collected in France (1) and Iran (2). The six PlLV complete genome sequences ranged in size from 2832 to 2834 nt in length and shared 94.1% genome-wide pairwise identity. The characterization of capulaviruses from wild asymptomatic plants such as PlLV is essential to better understanding the ecology and the evolution of geminiviruses.

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Evaluating Transmission Dynamics of Cassava Mosaic Begomoviruses (CMB) from Mixed Infections by the Whitefly Vector *Bemisia tabaci SSA1-SG1*

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Abstract

A pandemic of Cassava mosaic disease (CMD) during the late 1990s and early 2000s was associated with a population outbreak of the whitefly vector *Bemisia tabaci SSA1-SG1* and the recombinant virus East African cassava mosaic virus-Uganda (recombinant between African cassava mosaic virus (ACMV) and East African cassava mosaic virus). This study was undertaken to characterize transmission of ACMV and East African cassava mosaic Cameroon virus (EACMCV) by *B. tabaci SSA1-SG1* from mixed and singly infected cassava plants. qPCR was used to measure titers of both A and B viral DNA components in sucrose sachets on which viruliferous whiteflies fed following an acquisition access period on infected cassava. Successful transmission of the virus was defined as transmission of both A and B components into a sucrose sachet by a single whitefly. Viruliferous *B. tabasi SSA1-SG1* transmitted both ACMV and EACMCV into sucrose sachets. The A and B components of both viruses were transmitted independently. For ACMV, transmission rates of the A component from singly or mixed infected plants was similar, while the B component was transmitted at a higher rate from singly infected than mixed infected plants. For EACMCV, both A and B components were more frequently transmitted from a singly infected plant than a mixed infected plant. Successful transmission (i.e. transmission of both A and B components) occurred at a higher rate from singly than mixed infected plant for both ACMV and EACMCV. Successful transmission of all components of both viruses from mixed infections was rare. EACMCV was transmitted more frequently from mixed infected plants than ACMV. Results from these experiments indicate that transmissions from single infected source plants by *B. tabaci SSA1-SG1* are more frequent than from mixed infections for both ACMV and EACMCV.

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Mechanisms underlying differential virulence of two strains of a New World monopartite tomato-infecting begomovirus

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Abstract

Tomato leaf deformation virus (ToLDeV) is the first native New World begomovirus shown to posses a monopartite genome. Previously, we established that the PA10-3 strain, cloned from a sample collected in 2010, was highly virulent and that infected tomato plants did not undergo recovery, whereas the PA98-1 strain, cloned from a sample collected in 1998, was less virulent and that infected tomato plants underwent recovery. This differential virulence was associated with a recombination event that resulted in a high degree of sequence divergence in the left intergenic region (LIR) and C4 gene. Here, we demonstrate that the LIR is associated with virulence and the disease recovery phenotype in tomato plants. Furthermore, this was independent of the source of the C4 gene, as ectopic expression of this gene from both strains, delivered by two viral vectors with distinct tissue tropism, revealed similar symptoms in *Nicotiana benthamiana* and tomato plants. Furthermore, neither C4 protein suppressed local silencing, whereas both suppressed long-distance silencing. Interchanging the LIR between the PA10-3 and PA98-1 strains, while retaining their cognate Rep reiterated binding sites (iterons) completely reversed the virulence of these strains in *N. benthamiana* and tomato plants, including the recovery phenotype. Interestingly, a higher level of cytosine methylation was detected in the LIR of the PA98-1 strain compared to the PA10-3 strain. The results reveal the complexity of virulence in these recombinant begomoviruses and that it may reflect, in part, differences at the level of transcription.

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Rapid Mutation in Experimental Cassava Begomovirus Populations

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Abstract

High-throughput sequencing has been used to discover novel ssDNA viruses and for surveillance of ssDNA pathogens, but rarely applied to characterization of genetic variation within a laboratory population, as has been done for many RNA viruses. ssDNA viruses show similar rapid, mutation-driven evolutionary dynamics, so sequencing-based experimental approaches should improve our understanding of ssDNA virus diversity. Bipartite cassava begomoviruses are among the most destructive plant viruses. We are measuring evolutionary dynamics in two synergistic whitefly-transmitted begomoviruses that limit cassava production across Africa: African cassava mosaic virus (ACMV) and East African cassava mosaic Cameroon virus (EACMCV). Cassava plants are bombarded with infectious clones (individually and jointly) and infection is allowed to proliferate for at least 14 days. By inoculating with infectious clones we create clonal virus populations descended from known starting sequences, and we can thus detect spontaneous mutations. Virus DNA is enriched with size selection and rolling circle amplification and sequenced using Illumina platforms. Technical replicate libraries are prepared from each DNA sample, allowing systematic quantification of technical variability. We have completed four independent experiments with two different cassava cultivars across multiple vegetative cropping cycles and three temperatures. We have recorded tens of thousands of observations of thousands of distinct spontaneous genetic variants across experiments, covering the vast majority of genome positions. Our data strengthen the hypothesis that ssDNA plant viruses can create diverse populations quickly (within 14 days post-inoculation), underlying their ability to evolve as fast as RNA viruses. Future work will quantify population bottlenecks and selective constraints associated with whitefly transmission between plants vs. transmission via vegetative propagation.

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Is the assistance of satellites by TYLCV strictly cell autonomous?

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Abstract

Begomoviruses are circular single stranded DNA (css) plant viruses with bipartite (A and B) or monopartite (A-like component) genomes. They are sometimes associated with satellites, cssDNA molecules, namely alphasatellites and betasatellites. Like the B component of bipartite begomoviruses, satellites depend on the A or A-like component for their replication (betasatellite) and encapsidation (alphasatellites and betasatellites). Although Tomato yellow leaf curl virus (TYLCV) was only rarely reported with satellites, alphasatellites and betasatellites of various geographic origins are readily assisted by TYLCV in experimental conditions. This result was consistent with the observation that satellite DNA contents were mostly higher than that of TYLCV (Conflon et al., 2018). The ease with which satellites can be assisted with TYLCV was supported further by FISH observations, which showed that the frequency of TYLCV-infected cells that were co-infected with a satellite exceeded 85% for an alphasatellite, and 95% for a betasatellite. Interestingly, a substantial number of cell nuclei were positive only for the satellite, suggesting that the assistance seems to be possible, even with a low amount of TYLCV DNA, and possibly no TYLCV DNA. This later possibility that need to be confirmed with further tests, is according to the “multicellular way of life” theory proposed recently for Faba bean necrotic stunt virus, a multipartite nanovirus with eight separately encapsidated components (Sicard et al.,2019).

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Subcellular localization and functional analysis of the Grapevine red blotch virus proteins

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Abstract

Grapevine red blotch is a recently recognized viral disease of grapevine (*Vitis vinifera*) in California and it has also been reported from other states in the USA, Canada, Mexico, Argentina, Switzerland, South Korea and India. The disease is caused by *Grapevine red blotch virus* (GRBV), a novel geminivirus that is the type species of the genus *Grablovirus* (family *Geminiviridae*). The genome of GRBV encodes six open reading frames (ORFs): three in the viral-sense (V1, V2 and V3) and three in the complementary-sense (C1, C2 and C3). Little is known about the function of the predicted proteins encoded by these genes. Sequence analyses has established that C1 and C2 proteins have aminoacid identity with the RepA and Rep proteins of mastreviruses, respectively, whereas the V1 ORF has been predicted to encode the capsid protein. However, little or no identity with known proteins has been revealed for the predicted V2, V3 and C3 proteins. Here we report a functional analysis of the GRBV proteins based upon: 1) subcellular localization and 2) DNA binding properties. Knowledge of protein localization provides insight into the functions of uncharacterized proteins. Therefore, transient expression of fluorescent-tagged fusion proteins together with different markers of cellular components was used to identify the intracellular localization of these proteins. DNA binding assays were conducted to measure the potential capacity of these proteins to interact with the viral DNA. These results will be discussed in terms of the viral life cycle and disease biology.

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Recombination drives macroevolution of cassava mosaic begomoviruses

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Abstract

Cassava (*Manihot esculenta*) is a staple food crop throughout Africa, and South and Southeast Asia whose production is severely hindered by whitefly-transmitted geminiviruses. The Cassava mosaic disease (CMD) complex is comprised of 11 bipartite begomovirus species exhibiting accelerated rates of evolution, driven by high mutation rates and genetic recombination. Recombination is especially implicated in the emergence of new CMD-causing viral strains; most notably in the emergence of a highly virulent recombinant in the late 1990s that caused severe epidemics through sub-Saharan Africa. While there has been an increase in scientific efforts to understand CMD dynamics and cassava mosaic begomovirus (CMB) evolution, a revised, global-scaled survey of the frequency and patterns of CMB interspecies recombination is currently lacking. We assembled datasets comprised of all publicly available, full-length DNA-A (n=880) and DNA-B (n=369) nucleotide sequences corresponding to the 11 recognized CMB species. Phylogenetic networks and computational analyses using several recombination detection methods revealed extensive recombination in the CMB sequences, and ancestral recombination graphs attempted to order the most significant of these events in time. Results for DNA-A showed unique species-wide recombination events for multiple species (ACMBFV, EACMCV, EACMMV, SLCMV, EACMZV, CMMGV). In contrast, there were no shared recombination events for designated species for the DNA-B. In summary, our results support that recombination is featured prominently in the CMB phylogeny and the prevalence of clade-wide ancestral recombination events in the DNA-A component suggests that recombination is largely driving macroevolutionary patterns of diversity in the CMD complex.

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Novel genomoviruses identified in *Citrus* sp. in Tunisia

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Abstract

CRESS DNA viruses are a heterogenic group of circular replication-associated protein (Rep) encoding single-stranded DNA (ssDNA) viruses that appear to be descended from a common ancestor. The recently characterized family *Genomoviridae* is part of the CRESS DNA virus group. Genomovirus genomes encode a Rep in the virion strand and a capsid protein (CP) on the complementary strand. Interestingly, genomoviruses have been identified from a variety of eukaryotic organisms (fungi, plants, insects, birds, mammals) and environments (sediments, sewage, and wastewater). Currently, the family *Genomoviridae* is divided into nine genera and the cut-off for species demarcation is 78% nucleotide identity. *Sclerotinia gemycircularvirus 1* is the first genomovirus virus identified and cause hypovirulence to a plant pathogenic fungus. Using a high throughput sequencing approach, we identified four novel genomoviruses associated with *Citrus* sp. plants collected in Tunisia, North Africa. Three genomes (CTGmV_1, _2,_3) belong to the same genomovirus species, but one that still needs to be established. These genomes are ~2.2 nts in length and share 62% nucleotide identity with genomoviurs isolate ctcg277 (GB: MK032742, isolated from minnow tissue), an unclassified genomovirus. CTGmV_4 genome is 2,153 nts and shares 57.8% nucleotide identity with giant panda associated gemycircularvirus (GB: MF327560, isolated from pandas faeces). Phylogenetic analysis of the Rep amino acid sequence of the four genomes recovered in this study with genomovirus deposited in GenBank demonstrates they grouped with members of the genus *Gemycircularvirus*. According to the current species demarcation for genomoviruses, in this work, we characterize two new species of genomoviruses isolated from *Citrus* sp.

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Investigating the impacts of Grapevine Red Blotch Virus (GRBV) on grapes through ripening across genotypic and environmental differences

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Abstract

Grapevine Red Blotch Virus (GRBV) has significantly impacted the grape and wine industry since its discovery in 2008. In 2013, research identified GRBV as a single stranded circular DNA virus which is part of the *Geminiviridae* family. This family of viruses contains over 360 species which are grouped into nine different genera, in which GRBV is the only known species in the Grablovirus genera (Zerbini, 2017). Previous studies investigating the impact of GRBV on grape composition, have indicated significant delays in grape ripening. This translates to decreases in sugar accumulation and anthocyanin biosynthesis, which are two main markers that grape growers use to determine ripeness for commercial harvest (Girardello, 2019). Currently, the only treatment available to the industry is complete removal of infected vines, which can cost a vineyard upwards of $60,000/hectare (Ricketts, 2017). Therefore, further research is needed to understand not only the modes of the disease, but also resistant or tolerant genotypes and environmental factors to help negate the impacts of the virus. This project aims to understand the impacts of the virus on grapes through RNA sequencing integrated with metabolomics, hormone abundances, and enzymatic activity. Samples were collected in 2016 and 2017 from Merlot and Cabernet Sauvignon (which was grafted on two different rootstocks 110R and 420A). To determine the progression of the virus during a season, samples were collected at four time points, pre-veraison, veraison (when 50% of the grapes have changed color), post veraison, and harvest (once the total soluble sugars reached 25° Brix) in both years. Differential gene expression between healthy and diseased grapes was analyzed and overlaid with metabolic pathways. Similar to previous findings (Blanco-Ulate, et al. 2017), our data indicate a significant impact on the phenylpropanoid pathway which increased in severity as the season progressed. Genes from infected Merlot grapes were more downregulated than Cabernet Sauvignon irrespective of rootstocks in regard to the phenylpropanoid pathway, terpenoid biosynthesis pathways, and plant-pathogen interactions. This indicates a potential higher viral impact on Merlot than Cabernet Sauvignon. Further findings on metabolite levels will aid in explaining the observed differences in gene expression.

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Use of Terminator 5’ to 3’ riboexonuclease dramatically improves transcript mapping of grapevine red blotch virus by 5’ RLM RACE

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Abstract

Following the manufacturer’s protocol for First Choice 5’ RLM RACE (Invitrogen Cat. AM1708) to map grapevine red blotch virus transcripts yielded sequences resulting from MMLV reverse transcriptase template switching at the 5’ end of virus RNA. The failure of the adapter to ligate to decapped RNA transcripts during the 1 hr incubation at 37°C provided no confidence that the 5’ ends of virus RNA were actually transcript ends. Altering the ligation incubation to 25°C for 2hr followed by 16°C overnight, improved results such that 50% of sequences had the adapter correctly ligated to the 5’ transcript end. Replacing the 5’ RLM RACE calf intestinal phosphatase treatment with Terminator 5’ to 3’ riboexonuclease (Lucigen) treatment prior to adapter ligation and reverse transcription, resulted in the degradation of uncapped RNA sequences, thereby preventing them from serving as templates for reverse transcription template switching at the 5’ ends of RNA that did not have an adapter ligated to the 5’ end. This simplified the identification of multiple transcription start sites in sense and complementary virus transcripts.

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Plant resistance-driven emergence of recombinant begomoviruses

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Abstract

The analysis of plant virus genomes reveals that many were shaped by recombination. However, the history of the emergence dynamics of these recombinants is mostly unknown as well as the underlying evolutionary forces that drove their frequency increase. The pivotal role of recombination in geminivirus evolution is supported by the detection of numerous recombination events in sequence data, and by their high propensity to recombine. These typical features were observed with Tomato yellow leaf curl virus (TYLCV), a tomato begomovirus that was extensively studied because of its global economic importance. TYLCV-IS76 is a recombinant TYLCV detected initially in Morocco. It inherited a 76-nt region of tomato yellow leaf curl Sardinia virus (TYLCSV) starting from the origin of replication (OR) towards the V2 gene. Based on field surveys carried out in Morocco and laboratory analysis, a real time emergence of TYLCV-IS76 has been reconstructed from its generation to the displacement of its parental viruses (1). Its emergence coincided with the deployment of Ty-1 resistant tomato cultivars, and a causal link was demonstrated with various competition tests in which positive selection of TYLCV-IS76 was observed in Ty-1 resistant plants (2). TYLCV-IS141 is a TYLCV recombinant detected in Italy (1, 3, 4, 5). It inherited a 141-nt region of TYLCSV between OR and the initiation codon of the V2 gene. TYLCV-IS141 and TYLCV-IS76 exhibit similar recombination profiles and fitness phenotypes in Ty-1 resistant plants. It was inferred from competition tests carried out with various natural and artificially generated TYLCV-IS76 and TYLCV-IS141 clones, that the fitness phenotype of these recombinants was determined by new beneficial intra genomic interaction rather than by a direct effect of specific mutations. Gene silencing is suspected to be involved in the positive selection of these recombinants because Ty-1 is a RNA dependent RNA polymerase gene.

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Is the elusive Brazilian curly top virus (BraCTV) a capulavirus?

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Abstract

Curly top-like diseases have been described in Brazil since the early 1900s. Because of the symptoms and evidence of transmitted by grafting and leafhopper (genus *Agallia*), but not mechanically, the disease was named the Brazilian curly top (BraCT). Although, it was hypothesized that the causal agent of BraCT was related to the *Beet curly top virus* (BCTV) in the North America, the etiology of BraCT remains unknown. In 2016, tomato plants with curly top-like symptoms (stunting and upcurled leaves with vein purpling) were observed in fields in the Mato Grosso state of Brazil. Samples were brought to the University of California, Davis and PCR tests with degenerate primers for begomoviruses and specific primers for BCTV were negative. We next performed high throughput sequencing on these samples, and the results revealed a putative new geminivirus, most closely related the capulavirus *French bean severe leaf curl virus*. The genome of the putative new geminivirus was amplified by rolling circle amplification (RCA), cloned and sequenced. Sequence comparisons revealed ~90% identity with Tomato-associated geminivirus 1 (ToAGV) from Brazil, and the genome organization was similar to those of capulaviruses, including the V1 gene that encodes the capsid protein (CP). Phylogenetic analysis of the CP amino acid sequence placed this virus in a distinct clade with members of the genera *Topocuvirus*, *Curtovirus* and *Turncurtovirus* and not with members of the genus *Capulavirus*. These results are fully consistent with the virus being leafhopper-transmitted. A dimeric clone was generated in pCAMBIA1300 and agroinoculation used for infectivity studies. By 15 days post agroinoculation, tomato plants developed stunting and upcurled leaves with vein purpling and swelling; symptoms indistinguishable from those observed in the field. PCR tests confirmed infection with the new geminivirus, thereby fulfilling Koch's Postulates. This virus also caused severe symptoms in *Nicotiana benthamiana*, tobacco, common beans (cv. Topcrop) and sugarbeet, revealing a fairly wide host range. Symptoms in sugarbeet resemble those induced by BCTV. Finally, quasi-geminate virions were purified from infected *N. bethamiana* leaves. Taken together, these results suggest that this virus may be the cause of BraCT.

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Assessing the effectiveness of a TFP in Brazil based on monitoring the incidence of begomovirus disease and whitefly vector populations in tomato fields in Brazil

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Abstract

The most prevalent and important viral disease of tomatoes in Brazil is caused by begomoviruses. Due to the high incidence of this disease, typically associated with high population of the whitefly (*Bemisia tabaci*) vector, a mandatory tomato free period (TFP) of ~60 days (December-January) was implemented for processing tomato in Goiás, the largest tomato-producing state in Brazil. A similar TFP is being used in other states, but it is not mandatory. To assess the impact of the TFP on the begomovirus/whitefly pathosystem, the incidence of begomovirus disease was monitored in five production regions (three in Goiás and one each in Minas Gerais and São Paulo) in central Brazil over 2013-2015. Monthly, begomovirus disease incidence was assessed based on symptom evaluation, adult whitefly populations were estimated, and the presence of begomovirus monitored in these whiteflies. In 2013, a medium to high disease incidence was confirmed in all three regions at Goiás state in fields by 60-80 days after transplanting (DAT) following the TFP during all planting periods. In 2014-15, the incidence was high for the first plantings and decreased afterwards. Evaluation done in plants 20-40 DAT suggested the incidence in fields established soon after the TFP was generally low or absent, whereas fields established ~8 weeks after the TFP had higher incidences. Incidence in other states was low. *Tomato severe rugose virus* (ToSRV) was the only begomovirus species detected in the monitored regions. The highest whitefly populations were observed in January to March, coincident with the end period of TFP, and ToSRV was detected in whitefly samples only during the tomato season (February-November). It was concluded that TPF has a positive effect reducing begomovirus incidence in the tomato crop in Brazil. Because TFP coincides with the peak whitefly populations and other non-tomato inoculum sources are present, we expect that the benefit of it is not maximized. Therefore, efforts to increase the efficiency of the TFP should be considered, such as having the TFP during the months with peak whitefly population and delaying the tomato planting season, in addition to the implementation of other control measures in a regional IPM program

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New insights into roles of betasatellite in leaf curl disease development

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Abstract

Satellites are extra viral components that can influence accumulation and pathogenesis of associated helper viruses. Association of helper viruses is the prerequisite for maintenance of satellites. Geminiviruses, the largest group of plant viruses, consist of ssDNA genome and are known to be associated with single stranded satellite DNAs such as alphasatellite, betasatelite and deltasatellite. Among the satellite DNAs, betasatelites has emerged as a serious threat to crop ecosystem of the tropical and sub-tropical region of the world. Betasatellites are ubiquitously associated with leaf curl diseases of vegetable crops such as chillies, tomato, radish, okra etc. Here we discuss betasatellite-chloroplast interaction in the scope of geminivirus pathogenesis. Previously we have demonstrated that *Radish leaf curl betasatellite* (RaLCB) encoded βC1 protein gets localized into the chloroplasts of the infected *Nicotiana benthamiana* plants and causes damages to the oxygen-evolving complex of photosystem II. Further, RaLCB-βC1 protein interacts with the chloroplastic photosynthetic oxygen-evolving protein 23kDa subunit (PsbP). PsbP delays disease development initiated by betasatellite-geminivirus complex and βC1 protein accomplishes counter-defense by physical interaction with PsbP and by interfering with the ability of PsbP to bind geminivirus genome to ensure the establishment of viral pathogenesis.

Although, multitasking roles of βC1 protein in pathogenesis have been elucidated, information about mechanistic role of this protein in DNA accumulation is lacking. The molecular function of βC1 and its mechanism of action remain unexplored due to difficulties in purification of the βC1 protein under native condition. Our results indicate that βC1 protein can hydrolyse ATP and ATP binding interferes with the DNA binding activity of the protein. We have identified key residues that influence these biochemical properties of βC1 protein. The biological implication of ATPase activity of βC1 is negatively correlated with the accumulation of helper begomovirus and betasatellite. Further exploration of ATPase activity of βC1 in modulating host-virus interaction might to understand early events of betasatellite-mediated pathogenesis in a permissive host.

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The Geminivirus AC2 protein, a Central Player in the Infection Cycle.

Garry Sunter

Abstract

Plant viruses of the family *Geminiviridae* cause devastating crop disease worldwide and the development of resistance to this group of devastating plant viruses requires a complete understanding of the complex interactions between virus and host. The geminivirus genome comprises a small, circular single stranded DNA encapsidated within an incomplete icosahedral particle. Once inside a cell the viral DNA undergoes rolling circle replication to generate double-stranded DNA intermediates that serve as template for transcription of viral genes and further rounds of replication. Transcription on the geminiviral DNA template requires intricate interactions with the host, including the use of host RNA polymerase II and a combination of viral and host transcription factors. To ensure the correct temporal regulation of viral gene expression, a typical DNA virus transcription program is utilized, involving repression of the downstream transcription unit that encodes the intermediate *AL2* gene. Repression of the downstream transcription unit is relieved through autoregulation by the AL1 replication protein. In bipartite viruses of the genus *Begomovirus*, the AL2 protein has multiple functions that involves an interaction with at least four host proteins: SnRK1, ADK, PPD2, and rgsCaM. The various outcomes of these interactions include suppression of basal host defenses (SnRK1 and ADK), suppression of RNA silencing (ADK; rgsCaM) and increased expression of primary cytokinin-responsive genes (ADK). These functions are shared with the positional homolog C2 encoded by monopartite viruses in the genus *Curtovirus*. An additional function associated with the AL2 protein of begomoviruses is the ability to transcriptionally activate the two late genes, coat protein (CP) and BR1, which are key proteins critical for plant viral movement and insect transmission. Interestingly, the C2 protein encoded by the monopartite curtoviruses, including *Beet curly top virus* (BCTV) and *Spinach curly top virus* (SCTV), does not have a transcription function, indicating that regulation of the *CP* promoter in SCTV is different to that observed in CaLCuV and TGMV. The mechanism by which AL2 regulates the viral *CP/BR1* promoters is very complex and involves at least one host factor (PPD2). Interestingly, PPD2 is involved in leaf development, which may account for some of the symptoms observed with geminivirus infection. An additional level of *CP* promoter regulation in both begomo-and curtoviruses occurs at the tissue level, with different regulatory circuits functioning in vascular and mesophyll tissue. The ability of begomovirus AL2 and/or curtovirus C2 proteins to interact with various host proteins, either in a transcription-dependent or transcription-independent manner, profoundly impacts the host transcriptome. Together the data collected to date indicates that AL2/C2 is a critical viral protein that plays a central role in coordinating the viral life cycle.

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Gene copy number variation impacts on gene expression in a multipartite virus

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Abstract

Multipartite viruses have a segmented genome and each segment is encapsidated independently. Earlier studies reported that the distinct genome segments reproducibly accumulate at a specific and host-dependent relative frequency, defined as the 'genome formula'. It has been hypothesized that variations of the genome formula could affect gene expression via changes of the viral gene copy number. The multipartite virus model used in this work is a nanovirus, the faba bean necrotic stunt virus (FBNSV), composed of eight DNA genome segments each encoding a single gene. We initiated twenty parallel FBNSV lineages in faba bean plants, transferred them to medicago plants, and monitored both the relative amounts of the DNA segments and those of the corresponding mRNAs in all lineages. Our analyses showed that the genome formula variations directly impact on gene expression. Moreover, when passaging FBNSV from one host species to the other, we observed that the variation of the genome formula allowed the maintenance of similar proportions of the eight viral mRNAs, suggesting that the genome formula adjusts gene expression to a changing environment. Deep-sequencing analysis of FBNSV lineages similarly alternating from faba bean to medicago host plants demonstrated that the genome formula changes result from the flexibility of this viral system. By this we mean that no DNA sequence mutations could be associated to the genome formula host-dependent switch, which can thus be viewed as a plastic phenomenon. Together, our results indicate that the genome formula adapts gene expression of this multipartite virus in a changing environment in a DNA-sequence mutation-independent way.

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NEXT GENERATION SEQUENCING REVEALS TWO GEOGRAPHICALLY DISTINCT BABUVIRUSES CAUSING ABACA BUNCHY TOP IN THE PHILIPPINES

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Abstract

Abaca bunchy top disease in the Philippines is caused by *Banana bunchy top virus* (BBTV) and *Abaca bunchy top virus* (ABTV), both belong to Family *Nanoviridae,* Genus *Babuvirus.* The disease is the most serious problem affecting abaca production in the major growing areas such as Bicol and Eastern Visayas regions located in the islands of Luzon and Visayas respectively, and Davao and Caraga regions in Mindanao. In this study, we have determined by next generation sequencing (NGS), that the two babuviruses causing abaca bunchy top in the country are geographically distinct. Eight virus isolates collected from two locations in the Bicol region (Legazpi and Sorsogon), Leyte province (Abuyog, Baybay, Kananga and Tanuan) in Eastern Visayas, and Davao (Davao City) and Caraga (Surigao del Sur) in Mindanao were analyzed by NGS-IlluminaMiSeq platform using template DNA prepared by rolling circle amplification (RCA). Pre-assembly alignment using Bowtie2 program locally aligned or mapped the short sequence reads of isolates from the Bicol Region to ABTV (Accession No. EF546807.1) while those from Visayas and Mindano to BBTV (KM607655.1) reference genomes in the GenBank. The contigs obtained from sequence reads assembled using Iterative Viral Assembly were BLASTn searched and aligned using MEGA 7.0 to BBTV and ABTV. Pairwise sequence comparison showed that Bicol isolates have 99% overall sequence identity to ABTV, while only 72–73% to BBTV. On the other hand, Eastern Visayas, Davao and Caraga isolates have 98–99% overall identity to BBTV, and 70–72% to ABTV. Following the species demarcation criteria of 75% for nanoviruses, the results revealed that virus detected in Legaspi and Sorsogon in the Bicol region is ABTV while BBTV in the Leyte province of Eastern Visayas, and in Davao City and Surigao del Sur in Mindanao. From this study, it is then important to determine which of the two viruses are prevalent in the abaca growing areas. The result of this study is useful in the development of resistance to abaca bunchy top for the abaca breeding program and in the proper deployment of virus resistant varieties.

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Small RNA profiling of the whitefly, *Bemisia tabaci* MEAM1 in response to feeding on tomato infected with *Tomato yellow leaf curl virus*

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Abstract

The whitefly, *Bemisia tabaci* MEAM1 is a notorious vector of viruses that impact food and fiber production on a global scale. We previously characterized the transcriptomic response of whiteflies that fed on tomato infected with the Begomovirus, *Tomato yellow leaf curl virus* (TYLCV) after 24 h, 48 h, and 72 h. Using the same pools of RNA that were used in these experiments, we sought to characterize changes in whitefly small RNAs using a deep sequencing approach, with a specific focus on piwi-interacting RNAs (piRNAs) and micro-RNAs (miRNAs). Results revealed numerous miRNAs that are specific to whiteflies, while only several miRNAs were differentially expressed in whiteflies that fed on TYLCV-infected tomato, compared to those that fed on healthy tomato. In addition, numerous piRNA clusters were induced and suppressed when whiteflies fed on TYLCV-infected tomato, and interestingly, six protein-coding genes were targeted in the TYLCV treatment. Although piRNAs primarily regulate the activity of transposable elements, this data suggests that they may have additional functions in regulating protein coding genes during whitefly-virus interactions.

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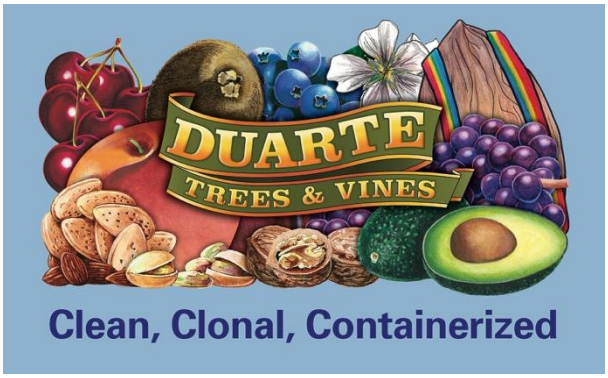

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