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RED NACIONAL DE VIRUS DE PLANTAS

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III Reunión de la Red Nacional de Virología de Plantas

25-27 Mayo 2022

MURCIA

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Celia de Moya · (CEBAS, CSIC)

Manuel Rubio · (CEBAS, CSIC)

Cristina Alcaide · (CEBAS, CSIC)

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MIÉRCOLES · 25 DE MAYO DE 2022

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15:30h Opening meeting

D. Vicente Pallás Benet (Presidente de la Sociedad Española de Fitopatología)
D. Juan José Alcorcón Cabañero (Director CEBAS-CSIC)
D. Alfredo Lacasa Plasencia (Profesor IMIDA Protección Cultivos)
D. Pedro Gómez López (Dr. Patología Vegetal CEBAS-CSIC)

SESSION 1 · VIRUS DETECTION & EPIDEMIOLOGY

Chairpersons: Pedro Gómez & Vicente Pallás

16:00h Plenary talk "Next generation opportunities of high throughput sequencing for plant virus detection and characterization"

· Sébastien Massart · Liège University, Belgium

16:40h Short talks O1-1: "Harnessed plant viruses: towards a universal framework for virus synthetic genomics and high-throughput infection phenotyping"

· Fabio Pasin · IBMCP, CSIC-UPV, University of Padova

17:00h Break (30min)

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· Luis Galipienso · IVIA

O1-3: "Current status of potyviruses in watermelon and pumpkin crops in Spain"

· Celia De Moya-Ruiz · CEBAS-CSIC

O1-4: "Host adaptation and migration fluxes of viruses from melon crops and crop edge weeds"

· Ayoub Maachi · Abiopep SL

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· Israel Pagán · CBGP, UPM-INIA

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· Izán Melero · CSIC-UV

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· Pilar Rabadán · CEBAS-CSIC

O2-3: "Cassava and Ugandan cassava brown streak virus, an attractive case of plant-pathogen coevolution"

· Rafael García López · CNB-CSIC

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· Silvia Ambrós · CSIC-UV

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· Alfonso González de Prádena · CNB-CSIC

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· Ornela Chase · CRAG, CSIC-IRTA-UAB-UB

O3-3: "Transcriptome analyses unveiled differential regulation of AGO and DCL genes by pepino mosaic virus strains"

· Cristina Alcaide · CEBAS-CSIC

O3-4: "RDR6 and Combined Activities of DCL2 and DCL4 Are Involved in RNAi-based Resistance to Potato Viruses induced by Topical Application of dsRNA"

· Francisco Tenllado · CIB-CSIC

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· María López-Martín · COMAV-UPV

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· Rosa Lozano-Durán · CPMB, Univ. Tubingen, Germany

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· Pablo Morales-Martínez · IHSM-UMA-CSIC

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· Araceli G. Castillo · IHSM-UMA-CSIC

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· Laura Medina-Puche · ZMBP, CAS

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· Régis L. Corrêa · CSIC-UV

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· Ana Cristina García-Merenciano · IHSM-UMA- CSIC

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· Mireya Martínez-Pérez · IBMCP, UPV-CSIC

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· Adrián A. Valli · CNB-CSIC

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· Ana Montserrat Martín-Hernández · CRAG, CSIC-IRTA-UAB-UB, IRTA

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· Eduardo Méndez-López · CEBAS-CSIC

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· Miguel A. Aranda · CEBAS-CSIC

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· Enrique Moriones · IHSM-UMA-CSIC

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· Mikel Ojinaga · NEIKER

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· Bernardo Rodamilans · CNB-CSIC

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O5-4: "Carbon dots boost dsRNA delivery in plants and increase local and systemic siRNA production"

· Leonardo Velasco · IFAPA

11:00h Break and Posters (30min)

SESSION 6 · VIRUS-BASED BIOTECHNOLOGY

Chairpersons: Inmaculada Ferriol & Fernando Ponz

11:30h Plenary talk — "Transient expression vectors in plants that are able to self-replicate and move systemically"

· José Antonio Darós · IBMCP, UPV-CSIC

12:10h Short talks — 6-01: "Development of viral vectors based on alfalfa mosaic virus for the silencing of genes of interest in plants"

· David Villar-Álvarez · CSIC-UPV

6-02: "Targeted plant gene silencing based on an asymptomatic viroid"

· Joan Marquez-Molins · CSIC-UV, IBMCP

6-03: "Lessons from icosahedral and flexuous viral structures of whitefly-transmitted members of the genera Torradovirus and Ipomovirus"

· Inmaculada Ferriol · CRAG-UAB-UB-CSIC-IRTA, ICA-CSIC

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· Daniel A. Truchado · CBGP, UPM-INIA/CSIC

13:30h

Review of the meeting
Experience of applicant/members
Student Awards to the best oral communication and best poster

14:00h Farewell Cocktail

ORAL PRESENTATION

#1 SESSION PLENARY TALK

Next generation opportunities of high throughput sequencing for plant virus detection and characterization

Sebastien Massart[1]

1 Laboratory of Plant Pathology, TERRA, Gembloux Agro-Bio Tech, Liège University, Passage des deportes, 2 – 5030 Gembloux - Belgium

The advent of High Throughput Sequencing (HTS) technologies coupled with the bioinformatics analyses of the generated data have deeply impacted the work of plant virologists. Consequently, HTS technologies have deeply modified our ability to detect and study plant viruses from more than a decade. HTS technologies allowed the discovery of hundreds of plant viruses and resolved the aetiology of many uncharacterized plant diseases. Beyond virus discovery application, the presentation will address current technical challenges and ongoing scientific opportunities brought by HTS technologies for plant virus detection and characterization.

The first important challenge is the required improvement of HTS reliability to transform the current "handcraft" situation toward a controlled and reliable "industrial" application, including for official diagnostics. For this purpose, recently written international guidelines for the reliable use of HTS for plant pest detection will be summarized. The presentation will also introduce a new type of control and explain why an "Alien invasion" is required to monitor the contamination burden. The evolution of bioinformatic tools for detecting viruses in sequence datasets has accelerated, in part thanks to SARS-Cov-2 pandemic, and new tools that are available to any researcher will be presented.

Behind technical reliability, new scientific questions can be envisioned. First, the bioinformatics analyses are mainly focused on producing a consensus genome sequence of the detected viruses. This consensus sequence is an artificial construction that might not exist in the sample, hiding the true intra-species diversity and the cloud of viral sequences. So, detecting SNPs and haplotypes in sequencing datasets can also bring valuable information to understand the plant virus evolution in their hosts at plant and national level. Another opportunity corresponds to the current transition of sequencing a single plant to sequencing thousands of plants. Sequencing large number of plants allows for example HTS-based national surveillance program, like SEVIPLANT in Belgium or iCARE in Rwanda, but also to adapt the scientific questions from virus detection to plant virus ecology at ecosystem scale.

Harnessed plant viruses: towards a universal framework for virus synthetic genomics and high-throughput infection phenotyping

Fabio Pasin[1,2,†,*]

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Sequencing advances have led to discovery of an unprecedented number of plant viruses; their biological characterization and role assessment in disease are major bottlenecks that limit epidemiology modeling and design of sound control strategies.

Infectious clone technology is a universal approach that facilitates the study of plant virus biology and genetics [Pasin *et al.*, ***Plant Biotechnol J.*** 2019;17(6):1010-1026. doi: 10.1111/pbi.13084]. We applied principles of minimization and modular design to develop pLX, mini T-DNA vectors (~3 kb) suitable for Agrobacterium-mediated transformation of plants [Pasin *et al.*, ***ACS Synth Biol.*** 2017;6(10):1962-1968. doi: 10.1021/acssynbio.6b00354]. The vectors streamline the development of virus reverse genetic systems, and successfully allowed Agrobacterium-mediated delivery of RNA and DNA viruses to plants. Based on pLX we conceived SynViP, a synthetic genomics framework with plant virome capacity. The framework combines type IIS endonucleases with DNA chemical synthesis for one-step, seamless virus clone assembly [Pasin, ***Biotechnol J.*** 2021;16(5):e2000354. doi: 10.1002/biot.202000354]. SynViP allowed use of a digital template to rescue a genuine plant RNA virus with no biological material requirements.

Metabolite profiling provides comprehensive information to understand plant responses to
biotic and abiotic factors. We optimized a liquid chromatography-mass spectrometry untargeted metabolite profiling and an unsupervised data analysis pipeline for fast, high-throughput phenotyping of plant responses to infection of RNA and DNA viruses.

Finally, we are confident that the proven flexibility and robustness of pLX and SynViP alongside the conceived high-throughput infection phenotyping workflow will facilitate biological characterization of plant viruses as well as engineering and prototyping of next- generation viral vectors.

Southern tomato virus in Spain: incidence and interaction with other viruses

Luis Galipienso[1], Laura Elvira-Gonzalez[1], Ana Alfaro-Fernández[2], María Isabel Font-San-Ambrosio[2], **Luis Rubio**[1].

1 Centro Protección Vegetal y Biotecnología, IVIA, Moncada, Valencia

2 Instituto Agroforestal Mediterráneo, UPV, Valencia

Southern tomato virus (STV), genus *Amalgavirus* and family *Amalgaviridae*, is a persistent virus infecting only tomato and it is worldwide spread. STV is only transmitted by seed with rates up to 80% and infects both coat and embryo hindering seed disinfestation. Field surveys in the Valencian Community and the Canary Islands showed a high incidence of STV. In the Valencian Community, the incidence was higher in tomato commercial varieties than in the local ones (seeds are produced by farmers) and STV was detected in most tomato seedlings. Phylogenetic analysis of worldwide STV isolates showed no correlation between genetic and geographic distances and low genetic diversity. Despite STV being initially associated with some fruit maturation disorders, our studies suggest that the STV infection is asymptomatic. However, STV can modify the expression of some miRNAs, modulating important plant functions with unknown effects. In mixed infections, STV interacts synergically with cucumber mosaic virus (CMV) or pepino mosaic virus (PepMV) increasing the symptom severity induced by CMV or PepMV and increasing CMV accumulation. In triple infections, STV suppresses the antagonist relationship of CMV and PepMV occurring in double infections. The vsiRNAs in STV single-infected tomato plants was scarce, but they increased with the presence of CMV and PepMV. Additionally, the rates of CMV and PepMV vsiRNAs varied depending on the virus combination.

Current status of potyviruses in watermelon and pumpkin crops in Spain

De Moya-Ruiz, C.[1]; Rabadán, M.P.[1]; Juárez, M.[2]; and Gómez, P.[1]

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2 Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández de Elche, Orihuela 03312, Alicante, Spain.

Cucurbits are one of the most important horticultural vegetables in the Mediterranean basin being often threatened by viral diseases. The aim of this study was assess the current status of watermelon mosaic virus (WMV), one of the most prevalent viruses in cucurbit crops, and moroccan watermelon mosaic virus (MWMV) which has recently emerged as a related species. The occurrence of both potyviruses was monitored in apical-leaf samples of watermelon and pumpkin plants, that displayed mosaic symptoms, from three major cucurbit-producing areas in Spain for three consecutive (2018–2020) seasons. Our results revealed that WMV was primarily (53%) found in both cultivated plants, with an unadvertised detection of MWMV. In order to understand about the presence and distribution of both viruses, a set of five-cucurbit plant species were infected with either WMV or MWMV infectious cDNA clones in single and mixed infections, quantifying viral load by RT-qPCR. We found that the viral load varied depending on the plant species and infection type. In single infections, the WMV isolate showed a higher viral load than the MWMV isolate in melon and pumpkin, However, in mixed infections, the WMV isolate was fitter than MWMV isolate in most cucurbit species. These results suggest that the impaired distribution of MWMV in cucurbit crops may be due to the allocation of cultivated plant species, in addition to the high prevalence of WMV.

Host adaptation and migration fluxes of viruses from melon crops and crop edge weeds

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Weeds surrounding crops may act as alternative hosts, playing important epidemiological roles as virus reservoirs and impacting virus evolution. We used high-throughput sequencing (HTS) to identify viruses in Spanish melon crops and plants belonging to three pluriannual weed species, *Ecballium elaterium*, *Malva sylvestris* and *Solanum nigrum*, sampled at the edges of the crops. Melon and E. *elaterium*, both belonging to the family *Cucurbitaceae*, shared three virus species, whereas there was no virus species overlap between melon and the other two weeds. The diversity of cucurbit aphid-borne yellows virus (CABYV) and tomato leaf curl New Delhi virus (ToLCNDV) both in melon and E. *elaterium* was further studied by amplicon sequencing. Phylogenetic and population genetics analyses showed that the CABYV population was structured by host, identifying three sites in the CABYV RNA-dependent RNA polymerase under positive selection, probably reflecting host adaptation. The ToLCNDV population was much less diverse than the CABYV one, likely as a consequence of the relatively recent introduction of ToLCNDV in Spain. In spite of its low diversity, we identified geographical but no host differentiation for ToLCNDV. Potential virus migration fluxes between E. elaterium and melon plants were also analyzed. For CABYV, no evidence of migration between the two hosts populations was found, whereas important fluxes were identified between geographically distant subpopulations for each host. For ToLCNDV, in contrast, evidence of migration between the two hosts was found, pointing towards an important role of E. elaterium as reservoir for ToLCNDV in melon.

Having it all: Is simultaneous optimization of plant virus vertical and horizontal transmission possible?

Israel Pagán

Centro de Biotecnología y Genómica de Plantas UPM-INIA and ETS Ingeniería Agronómica, Alimentaria y de Biosistemas, Universidad Politécnica de Madrid, Madrid 28223, Spain.

Transmission is arguably the most important trait of pathogens as it determined survival and fitness. In accordance, a considerable body of theory aimed at predicting the conditions in which pathogen transmission will be optimized. Applied to plant viruses, most of these works agree in that plant-to-plant horizontal transmission and parent-to-offspring vertical transmission cannot be simultaneously maximized. This is because horizontal transmission is optimal at high multiplication levels, which usually lead to higher virulence (effect on plant progeny production); whereas high vertical transmission rates through seeds require optimal plant reproduction, and therefore lower virulence. However, these models only consider plant defenses via resistance to infection (host ability to reduce pathogen multiplication). We hypothesized that, in plant populations where tolerance (host ability to limit the effect on progeny production at a given virus load) is the predominant defense, both optimal horizontal and vertical transmission are possible because higher virus multiplication can be achieved at low cost in terms of plant fitness. To test this hypothesis, we serially passaged two isolates of turnip mosaic virus (TuMV) in Arabidopsis thaliana populations with different proportions of tolerant genotypes. In the passaged viruses, we analyzed changes in multiplication, virulence, effect on plant lifespan and seed transmission. Results indicated that, after passaging in more tolerant populations, viruses increased the level of multiplication, and reduced virulence and effect on plant lifespan (higher infectious period), which are conditions that favor horizontal transmission. In parallel, these viruses increased seed transmission rate and the absolute number of infected seeds; hence, optimizing vertical transmission. This work shows that vertical and horizontal transmission can be simultaneously optimized, expanding current theoretical framework on the evolution of pathogen transmission mode. It also highlights the need to consider the various strategies of plant defenses to fully understand how plant viruses optimize their transmission efficiency.

Host age-dependent evolution of a plant RNA virus

Izan Melero1, Rubén González[1,2], Francisca de la Iglesia1, Aurelio Gómez-Cadenas[3], Santiago F. Elena[1,4].

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As an organism ages, its patterns of gene expression change and therefore, its metabolism and immunity change, which may result in a different response to pathogen infection. The interplay between host age and pathogen infection has not been thoroughly studied in plants and we lack overall knowledge on how it may affect pathogen evolution. In this work, we used the *Arabidopsis thaliana* - turnip mosaic virus (TuMV) pathosystem to study plant-virus interaction and virus evolution at different developmental stages: vegetative, bolting (transition from vegetative to reproductive) and reproductive growth. We inoculated plants at these three stages with two TuMV strains: one naïve and another well-adapted to A. *thaliana*. For both viral strains, the older the host the faster and more intense the infection was. To study the impact of these differences on virus evolution, we experimentally evolved both viral strains on all three developmental stages. After evolution, we found that viruses evolved on younger hosts experienced a larger increase on disease progression. Sequencing of the evolved viruses' genomes showed that the naïve viruses fixed mutations on the *VPg* cistron, independently of the stage where they were evolved. By contrast, the well-adapted viruses, which already had fixed mutations on *VPg*, showed a different mutation pattern. Remarkably, bolting and flowered hosts selected for mutations on the NIa-Pro cistron. Overall, our study contributes to understand the impact of host age on host-virus interactions and how it conditions the evolution of viruses.

Long-term monitoring and genetic dynamics of cucurbit aphid-borne yellows virus and watermelon mosaic virus in melon and zucchini crops

Pilar Rabadán[1], Miguel Juárez[2] and Pedro Gómez[1]

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2 Escuela Politécnica Superior de Orihuela, Universidad Miguel Hernández de Elche, Orihuela 03312, Alicante, Spain.

Understanding the emergence and prevalence of viral diseases in crops requires a systematic epidemiological surveillance, in addition to examine how ecological and evolutionary process combine to influence viral population dynamics. Here, we monitored extensively the occurrence of six aphid-borne viruses in symptomatic melon and zucchini plants for ten consecutive (2011-2020) cropping seasons in Spain. We found that cucurbit aphid-borne yellows virus (CABYV) and watermelon mosaic virus (WMV) are the most common viruses affecting these crops, with a recurrent high proportion of mixed infections. We hypothesized that mixed infections could impact on the molecular epidemiology, and we then carried out a comprehensive genetic characterization of CABYV and WMV isolates by whole-genome sequencing using the Pacific Biosciences single-molecule real-time (SMRT, PacBio) high-throughput technology. Population genetic analysis showed a fine-scale temporal structure (i.e. contemporary genetic variability of either CABYV or WMV isolates higher than for preceding isolates), without differentiation between melon and zucchini plants, and both under purifying selection. However, codon-based tests revealed 7 codons under positive selection in CABYV populations, and the analysis of molecular variance showed that CABYV genetic variation was in part explained by the significant level of the variance between single and mixed infected samples within years. These results suggest temporally structured populations of CABYV, and might shed light on the ecological role of mixed infections that, along with abiotic factors and agricultural practices, must be considered in determining the prevalence and emergence of the viral diseases.

Cassava and Ugandan cassava brown streak virus, an attractive case of plant-pathogen coevolution

Rafael García López, Irene Gonzalo, Juan Antonio García, Adrián A. Valli

Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología-CSIC, 28049, Madrid, Spain

Unlike the vast majority of potyvirids, Ugandan cassava brown streak virus (UCBSV) encodes a Maf1/ham1-like protein (HAM1), a widespread pyrophosphatase of harmful ITP/XTP non-canonical nucleotides. We reported that HAM1 activity is required for UCBSV to infect cassava (*Euphorbiaceae* family), but not to propagate *in Nicotiana benthamiana (Solanaceae* family). In addition, we demonstrated that HAM1 is linked to the viral RdRP (NIb) during infection, constituting the first described case of a pyrophosphatase-RdRP partnership. The striking high levels of ITP/XTP that we found in cassava suggest that they worked as selection pressure to promote HAM1 viral acquisition. By using a multidisciplinary approach, we are currently investigating the precise molecular mechanisms by which (i) HAM1 helps UCBSV to overcome the antiviral effects of ITP/XTP and (ii) cassava copes with the expected damaging effects of ITP/XTP over endogenous biological processes. For the first, we have successfully produced NIb, NIb-HAM1 and HAM1 in bacteria, and in vitro experiments mimicking viral replication in the presence/absence of ITP/XTP are on their way. For the second, we have searched for features in the host-derived HAM1 that might explain the high concentration of ITP/XTP in cassava. Indeed, we found that HAM1 from *Euphorbiaceae* plants have an extra C-terminal domain that harbours a bona fide nuclear localization signal. This last result allows us to draw a working model in which cassava relocates its HAM1 into the nucleus, giving rise to an ITP/XTP-rich cytoplasm with the consequent harmful effect over cytoplasmic viruses, unless that they express their own HAM1, as UCBSV does.

How virus populations evolve and adapt to host's epicode: the TuMV-Arabidopsis thaliana pathosystem

Silvia Ambrós[1], Santiago F. Elena[1,2]

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In the context of an ongoing project aiming to explore the role of the epigenetic regulation of defense pathways on the evolution of virus populations, we are using as model system the pathosystem formed by the Turnip mosaic potyvirus (TuMV) and Arabidopsis thaliana. We have performed a large-scale evolution experiment passaging 12 times five independent lineages of TuMV in each of a set of five plant genotypes with mutations in key genes affecting different components of the plant epicode pathways (DNA methylation and histone modification). The five epigenetic mutants were dcl2 dcl3 dcl4, ddm1 (DNA methylase involved in heterochromatine stability), ibm1 (histone demethylase), jmj14 (h3k4 histone demethylase), and polV (involved in RNA-directed DNA methylation). After each passage, we evaluated several viral fitness-related traits: the infectivity of the TuMV lineages, the disease progress, the severity of symptoms (virulence), and the viral load. Our results show that TuMV evolved populations have adapted to epigenetically-regulated responses from the host. In ongoing work, viral diversity will be evaluated for all evolved TuMV lineages at different time points by high-throughput sequencing. Next, the plant transcriptomic status (RNA-seq) and methylation patterns (GWBS) of non-infected plants, plants infected with the ancestral TuMV and with the 25 evolved lineages will be characterized to further identify specific genes whose epigenetic regulation has changed as a result of virus adaptation.

#3 SESSION PLENARY TALK

Crosstalk interactions between innate immunity and viral infections in plants

César Llave

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Traditionally, RNA silencing has been regarded as a major antiviral mechanism in plants. Accordingly, genetic defects in the RNA silencing machinery usually correlate with enhanced viral accumulation in infected plants. However, experimental evidence proves that virus-derived, small RNA-mediated cleavage of viral genomes is scarce, and not as efficient as one might anticipate. This suggests that the antiviral effect of RNA silencing relies more on the regulation of host genes essential for the virus to infect, than on the processing of viral RNAs. Over the last few years, growing experimental evidence indicates that plant innate immunity contributes to keep viral infections in check. Innate immunity is widely effective against a vast repertoire of microbial organisms, including bacteria, fungi and oomycetes. Although reverse genetics has revealed a role for several immune components in the antiviral phenotype, extensive research is still necessary to identify novel signaling pathways. Previously in our lab, we learnt that virus infection stimulates the accumulation of salicylic acid (SA), which in turn triggers transcriptional activation of many defense genes, including BAK1-INTERACTING RECEPTOR-LIKE KINASE 1 (BIR1) protein, a plant immune repressor that avoids constitutive activation of the immune response. Genetic defects in BIR1 cause a phenotype of enhanced virus-resistance, which is not dependent of canonical pattern- (PTI) or effector (ETI)-triggered immunity SA-dependent defense pathways. This finding suggests that plant immunity contributes significantly to the antiviral response. Interestingly, virus-responsive BIR1 undergoes extensive epigenetic silencing and post-transcriptional degradation, likely to maintain optimal expression levels. In this talk, we will discuss recent results relative to the mechanistic insights of BIR1 regulation. We found that virus infection promotes accumulation of BIR1-derived small RNAs, likely by interfering with UPF1-dependent non-sense mediated RNA decay. Furthermore, sequence-specific degradation of BIR1 transcripts is compromised in dcl2 dcl4 mutants, indicating that BIR1 is a target of siRNA-mediated RNA silencing. We will also discuss about the biological relevance of BIR1 induction in the context of virus infections. Whereas virus accumulation is not drastically affected by BIR1 overexpression, we found that a modest induction of BIR1 is sufficient to compromise elicitor-induced PTI responses. On the light of our recent data, we will discuss the cross-talk interactions between RNA silencing, plant immunity and virus infection, and how these interactions contribute to maintain the balance between virus proliferation and host integrity.

Plant Virus Genome Is Shaped by Specific Dinucleotide Restrictions That Influence Viral Infection

Alfonso González de Prádena[1], Juan Antonio García1, Adrián A. Valli[1]

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Abstract. The genome of RNA viruses has a stable consensus sequence shaped by different constraints. For instance, animal viruses restrict the presence of CpG and UpA dinucleotides in their genomes to avoid an antiviral mechanism that recognizes/degrades UpA- and CpG-rich RNAs. We have reported that UpA frequency in plant viral genomes is kept at low levels, and that its artificial increase in a segment of plum pox virus produces attenuation. Intriguingly, while antiviral mechanisms restricting UpA- and CpG-rich RNAs are well characterized in animals, their counterparts in plants are unknown. With the aim of uncover those mechanisms exerting UpA restriction in plant viruses, we designed a forward genetic screen based on transgenic Arabidopsis thaliana lines overexpressing, from the same cassette, the standard kanamycin resistance gene and a UpA artificially-enriched variant of the hygromycin resistance gene (*HPT*). We found that most transgenic plants were able to grow in medium with kanamycin, but not with hygromycin. RT-qPCR showed that the accumulation of HPT RNA was much lower in plants carrying the artificially-modified gene than in those transformed with the standard HPT. We have mutagenized seeds from lines carrying the UpA-rich variant of HPT. At the time of writing this abstract we are collecting their progeny. Further screening of mutants on growing media with hygromycin is expected to recover hygromycin-resistant plants as an indication that genes involved in the control of UpA-rich RNAs have been disrupted. If hygromycin- resistant plants were finally found, mapping of mutations causing the reversion of this phenotype would be exciting.

Sophisticated ways to deceive your host: Identifying the RNA silencing suppressor proteins of Sweet potato virus 2

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RNA interference (RNAi) is a key element for the regulation of gene expression in all eukaryotes and constitutes a primary defense mechanism against invasive plant viruses. To counterbalance this barrier, plant viruses encode proteins that repress the host silencing machinery at different steps, hence allowing a successful infection. Our study focused on the identification and characterization of gene products that confer RNA silencing suppressor (RSS) activity in the case of *Sweet potato virus 2* (SPV2, genus *Potyvirus*, family *Potyviridae*). SPV2 infects sweet potato (*Ipomoea batatas*), one of the most important stable food crops worldwide, frequently affected by coinfections between unrelated viruses, leading to highly detrimental yield losses. Therefore, elucidating viral pathogenicity effectors is crucial for the generation of effective control strategies. Different gene products located at viral 5´prime region were tested for RSS activity employing co-agroinfiltration assays with a GFP reporter in *Nicotiana benthamiana* plants. Visual results under UV revealed that different gene products exhibited RSS activity, and that P1 protease expressed in -*cis* or -*trans* enhanced significantly the suppressor activity of other products. Our findings were confirmed by q-RT-PCR and Northern blotting measuring GFP mRNA levels. The RSS capacity of SPV2 proteins were also assessed during viral infection using an infectious full-length clone vector of *Potato virus X* (genus *Potexvirus*, family *Alphaflexiviridae*). These results provide further insights about the variability of molecular determinants used by potyviruses to cope with host defenses and reveal a complex evolutionary scenario in the case of sweet potato potyviruses.

Transcriptome analyses unveiled differential regulation of AGO and DCL genes by pepino mosaic virus strains

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Pepino mosaic virus (PepMV) is an RNA potexvirus that affects tomato crops worldwide. We have described an in planta antagonistic interaction between PepMV isolates of the CH2 and EU strains in which the EU isolate represses the accumulation of the CH2 isolate during mixed infections. To understand the mechanisms underlying this virus-virus interaction, we have carried out transcriptomic analyses of tomato plants singly and mixed-infected with two PepMV isolates of the EU and CH2 strains. We first compared the transcriptomes of singly infected plants, showing that each of the viral strains modulated the host transcriptome differentially. We next compared mixed vs single infections, showing that mixed infections caused transcriptomic alterations similar to those for the sum of single infections at early infection times, but differing clearly at later times post-infection. We also performed a search for host genes potentially involved in the PepMV-EU vs -CH2 antagonism, testing the hypothesis that PepMV-EU, in either single or mixed infections, deregulates host gene expression so that PepMV accumulation gets repressed. Indeed, that seemed to be the case for the genes *AGO1a, DCL2d, AGO2a and DCL2b*, which are involved in the virus RNAi pathway and were upregulated by PepMV-EU but not by PepMV-CH2 at early times post-infection. The pattern of AGO2a expression was validated by RT-qPCR both in tomato and Nicotiana benthamina plants; however, *N. benthamiana ago2* mutant plants, while hyper-susceptible to PepMV, did not show a trend change in the antagonistic interaction between the isolates of the two PepMV strains.

RDR6 and Combined Activities of DCL2 and DCL4 Are Involved in RNAi-based Resistance to Potato Viruses induced by Topical Application of dsRNA

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Exogenous application of double-stranded RNAs (dsRNAs) for inducing virus resistance in plants represents an attractive alternative to transgene-based silencing approaches. Although antiviral resistance conferred by topical application of dsRNA is related to RNA silencing directed against viral infection, the genetic requirements for dsRNA-based vaccination have not been studied. In particular, it is unclear if it employs the same DICER- like and RNA-directed RNA polymerase (RDR) proteins that mediate RNA silencing in response to virus infection. Using *Potato virus X* expressing the green fluorescent protein (PVX-GFP) as a reporter virus together with a suite of RNAi knockdown transgenic lines, here we have shown that RDR6 and the combined activities of DCL2 and DCL4 act to promote efficient resistance to virus infection conferred by topical application of bacterially produced dsRNA in *Nicotiana benthamiana.* In addition, we tested the protective effect of topical application of *Escherichia coli*-encapsulated dsRNA compared to naked dsRNA against single and dual infection by PVX-GFP and *Potato virus* Y. We found that, in our conditions, the effectiveness of E. coli-encapsulated dsRNA to provide RNAi-mediated protection did not differ from that of naked dsRNA. Moreover, deep RNA sequencing analysis revealed that Plant-pathogen interaction (nta04626) and MAPK signaling (nta04016) KEGG pathways, together with resistance protein genes and a number of receptor-like kinase genes were altered by inoculation with dsRNA alone. Our results provide evidence that exogenous dsRNA molecules active innate immunity and are processed by the RNA silencing pathways commonly used by the host in response to virus infection.

Resistance to Zucchini yellow mosaic virus in melon accession IC 274006 is controlled by a recessive gene in chromosome 5

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Plant diseases caused by viruses are one of the most limiting factors in melon (*Cucumis melo*) production. *Zucchini yellow mosaic virus* (ZYMV), a potyvirus transmitted by aphids, is one of the most harmful, as it has a worldwide distribution and affects both yield and fruit quality. Several accessions resistant to ZYMV have been described, but either their genetic control has not been deciphered (IC 274007 and IC 274014) or the provided resistance is isolate dependent (PI414723). The Indian accession IC 274006 confers a high level of resistance to both necrotic and non-necrotic ZYMV isolates. This accession was crossed to the susceptible Spanish landrace `Meló d´Or´ (BGV016451) and their F1 progeny was used to construct three different generations: F_2, $BC_{1\text{-}IC}$ and $B_{C1\text{-}BGV016451}$ (backcrosses to IC 274006 and BGV016451, respectively). These offsprings were inoculated with ZYMV and the segregation for the resistance fitted a monogenic recessive genetic control. In previous works developed by the research group, a candidate resistance region in chromosome 5 was identified by genotyping 50 F2 plants with an existing set of 124 SNPs markers evenly distributed throughout the genome. In the work here presented, this interval was further narrowed by genotyping all the families with 6 high resolution melting markers located within the candidate interval. Progeny tests of 6 F_2 and 2 $B_{C1\text{-}BGV016451}$ selected plants confirmed these results. The SNPs identified in this work will be useful in breeding programs in marker-assisted selection to introgress the resistance to ZYMV in different melon cultivars

#4 SESSION PLENARY TALK

Plant manipulation by geminiviruses

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Viruses, as intracellular parasites, need to subvert the host cell in order to enable viral replication and spread. Due to strict coding limitations, viruses commonly produce a reduced number of proteins; this is the case of geminiviruses, plant DNA viruses that are believed to contain only 4-8 translated open reading frames in their circular single-stranded genome. Strikingly, despite their limited armoury, geminiviruses, like other plant viruses, are able to successfully infect host plants, dramatically altering plant development and physiology and causing devastating diseases to crops worldwide. In our group, we are interested in understanding how geminiviruses manipulate the plant cell and lead to disease, for which we use a combination of approaches, including molecular biology, cell biology, and genetics. Our results have shed light onto the molecular mechanisms underlying the replication of viral DNA, plant anti-viral defence and geminiviral counter-defence, and symptom development, and hint at novel virulence strategies potentially employed by geminiviruses to maximize their coding capacity and their impact on the host cell. We expect that our work will contribute to a deeper understanding of the infection process, which may in turn pave the way to the design of effective and sustainable anti-viral strategies and assist breeding programs to obtain virus-resistant plants.

Unraveling the importance of vesicle trafficking for the movement of geminiviruses

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Tomato yellow leaf curl disease is one of the most important threats to tomato crops worldwide. One of its causal agents, *Tomato yellow leaf curl Sardinian virus* (TYLCSV), is a monopartite member of the genus *Begomovirus* from the family *Geminiviridae*. Due to the few proteins encoded by their viral genome, geminiviruses rely heavily on host cellular machinery and interact with a wide range of plant proteins to complete all processes required for infection, such as viral replication, movement and suppression or evasion of plant defense mechanisms. Therefore, identifying the host proteins involved in viral infection will be an essential step towards understanding the mechanisms underlying this process.

Using a reverse genetic approach our group identified a series of genes involved in vesicle trafficking, which affect geminivirus infection. Four of them are essential as their silencing produce a complete abolishment of TYLCSV infection (-COP, ARF1, CHC1, and CHC2). However, these genes do not affect TYLCSV replication. A series of experiments using confocal microscopy and viral proteins bound to fluorescent markers have been carried out to determine the effect of inhibiting vesicle trafficking over the subcellular localization of TYLCSV's movement proteins. The biological relevance of vesicle trafficking during geminivirus infection will be presented and discussed.

Whole genome, transcriptome, smallRNAome and methylome profiling during tomato- geminivirus interaction

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Geminiviruses constitute the largest family of plant-infecting viruses with small, single- stranded DNA genomes that replicate through double-stranded DNA intermediates. Because of their limited coding capacity, geminiviruses use plant nuclear machinery to amplify their genomes, which are packaged into nucleosomes forming chromatin as multiple circular minichromosomes. Thus, viral minichromosomes must encounter the nuclear pathways that regulate host gene expression and chromatin states. DNA methylation and post-transcriptional gene silencing play critical roles in controlling infection of geminiviruses and this pathogen can counteract these host defense mechanisms and promote its infectivity. Tomato Yellow Leaf Curl Virus (TYLCV) belongs to the Begomovirus genus and is transmitted by the whitefly Bemisia tabaci. With only seven viral proteins, TYLCV must create a proper environment for viral replication, transcription, and propagation. Behind the apparent simplicity of geminiviruses lies a complex network of molecular interactions with their host and their natural vector, which induces a wide variety of transcriptional, post-transcriptional and chromatin changes in the host. To better understand this virus-host interaction at a genetic and epigenetic level we carried out a global approach of the TYLCV-tomato interaction to generate integrated single-base resolution maps by Next-Generation Sequencing of the transcriptome, smallRNAome and methylome of the pathogen and the host. Total RNA and DNA was extracted from tomato–infected plants (three biological replicates) and analysed at 2, 7, 14 and 21-day post-infection (dpi). Analysis of the changes in host transcription during the infection and its correlation with changes in sRNA profiles (microRNA and phasiRNA) and DNA methylation patterns will be presented and discussed.

Molecular determinants underpinning the dynamic dual localization of a viral protein in the plant cell

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A growing body of evidence points at the targeting of several subcellular compartments as a strategy deployed by proteins to achieve multifunctionality. In the case of plant viruses, which have limited coding capacity, this strategy becomes crucial. One example is illustrated by the C4 protein encoded by the geminivirus tomato yellow leaf curl virus (TYLCV). C4 contains two overlapping localization signals, namely an N-myristoylation motif for plasma membrane (PM) tethering and a chloroplast transit peptide (cTP) for chloroplast targeting [1]. PM localization of C4 requires myristoylation; at the PM and plasmodesmata (PD), C4 interacts with the receptor-like kinases BAM1/2 and hinders the cell-to-cell spread of RNAi [1,2]. Following activation of defense, triggered by the presence of the virus-encoded Rep protein during the viral infection, by the bacterial pathogen-associated molecular pattern (PAMP) flg22, or by the plant immunogenic peptide Pep1, the PM-associated C4 is translocated to the chloroplast, where it interacts with the Calcium Sensing Receptor (CAS) and interferes with SA-dependent defense responses. Our results indicate that PM release of C4 requires active phosphorylation, while its chloroplast import depends on a functional protein import TOC/TIC complex [3]. Taking advantage of our ability to trigger C4 PM release in a chloroplast import-deficient background, we have identified new potential interactors of this viral protein during its PM-to-chloroplast relocalization (unpublished data). Our results shed new light onto the molecular and cellular events connecting PM and chloroplasts during plant-geminivirus interactions.

The role of epigenetics in plant immunity priming against viruses

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Once stressed, plants orchestrate epigenetic rearrangements leading to chromatin modifications on specific genes, priming a quicker and more efficient response against subsequent environmental stimuli. Pathogens, on the other hand, can potentially affect the type or intensity of epigenetic responses. Due to their high evolvability and easy experimental manipulation, plant viruses offer an interesting model for studying this kind of interactions. A full-factorial experimental approach was designed with Arabidopsis thaliana and turnip mosaic virus (TuMV) to address whether priming plants with a virus may lead to tolerance to subsequent infections. Higher levels of tolerance to TuMV were observed when plants were previously primed with this virus than when primed with less phylogenetically-related ones. Transcriptome analysis from TuMV-primed and unprimed plants revealed several stress-related transcriptional memory candidate genes. To identify genes directly targeted by epigenetics machinery, a time-course TuMV infection experiment was performed in wild-type and selected DNA methylation and histone modification mutant plants. By comparing transcriptomes from primed plants with the epigenetic mutants and overlapping their locations with the genome occupancy of known epigenetic marks and proteins, arabidopsis' immunity memory genes may be identified. An in-depth analysis of identified candidate primed genes in plants infected with TuMV strains with different fitness may be used as a tool to check if epigenetic routes are also targeted by viruses during adaptation.

Revealing the molecular basis of the transmission of tomato chlorosis virus by Bemisia tabaci and Trialeurodes vaporariorum

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Tomato chlorosis virus (ToCV, *Closteroviidae: Crinivirus*) causes an important emergent viral disease of tomato in addition to infect other cultivated and wild plants. Symptoms caused by ToCV in tomato include interveinal yellowing and thickening of lower leaves that advance towards the upper part of the plant. Criniviruses have a bipartite genome of positive-sense single-stranded RNA. RNA1 contains four open reading frames (ORFs) which encode proteins related to virus replication, and RNA2 contains nine ORFs encoding proteins associated with virus encapsidation, movement and whitefly transmission. Both RNAs are encapsidated separately in flexuous virions with a body-tail (rattlesnake) structure. The body is composed of a single coat protein (CP) whereas the tail is composed of at least four proteins including the minor coat protein (CPm), suggested to be involved in transmission by insect vectors. ToCV is transmitted in a semipersistent manner by whiteflies belonging to two genera: Bemisia tabaci, *Trialeurodes vaporariorum* and *Trialeurodes abutilonea*. In order to know the molecular basis involved in the atypical transmission of ToCV, nine deletion mutants in the CPm gene was constructed using an infectious ToCV RNA2 clone. The viral progeny of the mutant clones was assayed for infectivity in Nicotiana benthamiana and tomato plants as well as for transmission by *B. tabaci* and T. *vaporariorum*. Results obtained to date have revealed a candidate region in the CPm protein that could be involved in the specific transmission of ToCV by *T. vaporariorum,* in agreement with predictions based on in silico analysis of the CPm proteins of criniviruses.

New host-virus interaction pathway: RNA N^6-adenosine methylation

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Methylation of N^6-adenosine (m^{6}A) is a post-transcriptional modification that influences the fate of their RNA targets, mainly through the binding of m^{6}A-recognition proteins (readers). In previous work, we identified the presence of m6A on the RNAs of several plant viruses and found that the relative abundance of m^{6}A in alfalfa mosaic virus (AMV) RNAs regulates viral infectivity. Furthermore, we showed that the demethylase activity of ALKBH9B modulates this viral regulation process, probably via its interaction with the AMV CP. Here we report the upregulation of some m^{6}A machinery genes after AMV infection and analyze the involvement of Arabidopsis m^{6}A readers in the infection cycle of plant viruses. Consistent with the previously observed m6A-dependent antiviral effect, we find that, in Arabidopsis plants, the absence of ECT2/ECT3/ECT5 module of readers promotes the systemic infection of AMV. Furthermore, an ECT2 point mutant specifically defective in m^{6}A recognition loses wild type antiviral activity, suggesting that this effect relies in the capability of this reader to interact with m^{6}A sites. In addition, we performed a state-of-the-art technique, (targets of RNA-binding proteins identified by editing, HyperTRIBE) to corroborate the in vivo interaction between ECT2 and AMV m^{6}A-RNAs.

The mysterious presence of an AlkB domain in the P1 protein of two members of the family Potyviridae

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With more than 200 assigned members in 12 different genera, the family *Potyviridae* is the largest group of plant-infecting RNA viruses. Potyvirids share some features, such as (i) monopartite (except for a few bipartite) and positive sense single-stranded RNA genome, (ii) transmission mediated by vectors, and (iii) picorna-like gene expression strategy. The vast majority of them encode a variable leader serine protease termed P1. Strikingly, the presence of an AlkB domain (ubiquitous among all living organisms) has been detected in the P1 from only two unrelated potyvirids: blackberry virus Y and endive necrotic mosaic virus (ENMV), belonging to the *Brambyvirus* and *Potyvirus* genera, respectively. Having in mind that this domain is also found in proteins of plant viruses from diverse families (i.e. *Alpha-* and *Beta-flexiviridae, Closteroviridae* and *Secoviridae*), and that its role during viral infection is unknown, we decided to put effort into the study of ENMV P1. For that, we have built an infectious cDNA clone of ENMV that, after delivery into endive plants, mimicked a natural ENMV infection. Importantly, deletion of the AlkB domain, or directed point mutation of AlkB catalytic sites, did not abolish ENMV infection, but strongly reduced plant disease and virus accumulation. Given that AlkB-containing proteins usually act to reverse the damage in DNA/RNA due to methylation, and that the presence of m6A marks on viral genomes can impair virus fitness, our current working hypothesis is that P1, via its AlkB domain, alleviates the accumulation of m6A in the ENMV genome.

Insights on the resistance to Cucumber mosaic virus in melon. Localization of the Vacuolar Protein Sorting 41 (CmVPS41) and search for interactors of the MP

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The melon resistant gene *cmv1* encodes a Vacuolar Protein Sorting 41, involved in intracellular trafficking to the vacuole. In the melon lines carrying *cmv1* CMV strains of subgroup II are restricted to the bundle sheath cells and do not enter the phloem, whereas those of subgroup I overcome this restriction. The viral virulence factor that communicates with *cmv1* is the Movement Protein (MP). We have studied the cellular localization of CmVPS41 from PS (susceptible) and SC (resistant) genotypes. They show significant differences in their localization pattern, with structures such as nuclear speckles, membrane dots and transvacuolar bridges that are numerous in CmVPS41PS, but in CmVPS41SC there are much fewer. These CmVPS41 characteristic structures co-localize with the late endosome. Moreover, CmVPS41 from exotic resistant melon accessions are similar to those of SC and the structure that most correlates with susceptibility is the presence of transvacuolar strands. Those patterns do not change in the presence of overexpressed MP. However, during a real infection those patterns change dramatically, with transvacuolar strands disappearing. This suggests that those structures are involved in susceptibility to CMV. We have also searched for interactors of CMV MP by Y2H and found one specific domain of a Niemann's Peak protein C1 (NPC1), a protein that, in animals, is the receptor of Ebolavirus and other Flaviviruses. NPC1 is a transmembrane cholesterol transporter related to VPS41 and to local changes in membrane composition. CRISPR experiments are being carried out to impair both VPS41 and NPC1 in melon.

S-glutathionylation of pepino mosaic virus coat protein: A switch modulating virion formation

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Pepino mosaic virus (PepMV; genus *Potexvirus*, family *Alphaflexiviridae*) is a pandemic virus in tomato crops causing important economic losses worldwide. We have identified a tomato glutathione S-transferase (GSTU) interacting with the PepMV coat protein (CP). *GSTU*-knocked out Micro-Tom plants showed loss of susceptibility to PepMV indicating that GSTU is a proviral factor. A plausible hypothesis explaining the CP-GSTU interaction is that GSTU facilitates CP S-glutathionylation. PepMV CP has only one cysteine (C127) residue conserved among potexviruses, located at the bottom of its RNA-binding pocket. *In vitro* CP glutathionylation assays coupled to SDS-PAGE and mass spectrometry showed that PepMV CP is spontaneously glutathionylated under oxidative conditions. In vivo CP glutathionylation was also observed by SDS-PAGE. Moreover, PepMV in vitro assembly assays revealed that no virus-like particles (VLPs) were assembled when the CP is glutathionylated, suggesting that CP C127 glutathionylation blocks virion formation. The serine-substitution of C127 (C127S) did not impaired virus cell-to-cell movement or encapsidation, but affected virus in planta fitness. Immunosorbent electron microscopy of crude sap from *Nicotiana benthamiana* leaves overexpressing CPC127S showed that it is able to self-assemble into VLPs while no VLPs were observed when wild type CP was overexpressed. We propose that the redox state of the C127 of the PepMV CP is a switch regulating the virion assembly depending on the redox state of its microenvironment during PepMV infection. This is the first time that a glutathionylatable switch is described in plant virus proteins; it is likely that other similar switches exist within the plant virosphere.

#5 SESSION

PLENARY TALK

Loss-of-susceptibility mutants in breeding plant virus resistant crop varieties

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The identification of sources of resistance to plant viruses is traditionally achieved by screening germplasm belonging to the target species and/or close relatives. This approach might be unsuccessful in occasions, as appears to be the case for pepino mosaic virus (PepMV) in tomato. PepMV affects tomato crops worldwide, causing a disease with serious economic repercussions. The existence of potential sources of natural resistance to PepMV has been described, but these seem to be partial, to be controlled by complex genetics and/or to be specific to the viral strain. We have addressed alternative strategies for the development of varieties resistant to PepMV, including the screening of an EMS mutagenized collection of tomato lines, and the CRISPR/Cas targeting of genes encoding potential susceptibility factors identified through molecular approaches. In my talk I will describe our work following these two strategies, which have led to the identification of a handful of tomato genes with both proviral and antiviral functions.

Breaking tomato yellow leaf curl virus resistance in Ty-1 encoding tomato plants associated to mixed infections with the crinivirus tomato chlorosis virus

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The tomato yellow leaf curl disease (TYLCD) severely damage tomato crops worldwide in regions where the *Bemisia tabaci* whitefly (Hemiptera: Aleyrodidae) vector is present. Several viral species of the genus *Begomovirus* (family Geminiviridae) have been associated to TYLCD, with isolates of the *Tomato yellow leaf curl virus* species (TYLCV) being the most widespread. Control of TYLCV damage in tomato is mostly based on the dominant *Ty-1* virus resistance gene resulting into reduced virus accumulation and disease symptoms by enhanced transcriptional gene silencing. However, recent reports suggest the adaptation of TYLCV to *Ty-1*-based resistance. Recombinant TYLCV isolates with a short genetic exchange with isolates of *Tomato yellow leaf curl Sardinia virus* (named as TYLCV-IS76-like isolates) species also associated with TYLCD displace the canonical TYLCV in Ty-1 resistant tomato crops. Higher viral DNA accumulation was shown for TYLCV-IS76-like isolates in *Ty-1* resistant tomatoes. Nevertheless, true resistance breaking was not demonstrated for these isolates as no TYLCD symptomatic infections were reproduced. As Ty-1 resistance breaking resulting in prominent symptoms is claimed by tomato growers, this aspect was investigated further in the current study. Two different TYLCV-IS76-like isolates have been cloned and infectious clones were obtained, which exhibited differential aggressiveness on Ty-1 resistant tomatoes but, again, resistance-breaking was not observed for any of them. Interestingly, our studies demonstrated the involvement of mixed infections with isolates of the B. *tabaci*-transmitted crinivirus (genus *Crinivirus*, family *Closteroviridae*) species *Tomato chlorosis virus* as the cause of TYLCV resistance breaking in Ty-1 resistant tomatoes. Alternatives for an increased robustness of TYLCD genetic resistance in tomato plants are investigated.

Obtaining pepper varieties resistant to Tobamovirus

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The pepper (genus *Capsicum*) is one of the most important horticultural crops worldwide that has been diversified into numerous varieties. Gernika peppers and Ibarra chilli peppers are local ecotypes adapted to the environmental conditions of the Basque Country. Viral infections, among other threats, are responsible for lower yields in crops and lead to significant economic losses. Tobamovirus infection is one of the most common, being one of the predominant the tomato mild green mosaic virus (TMGMV) and tomato mosaic virus (ToMV). Within the genus *Capsicum*, the L genes confer resistance to different Tobamoviruses based on the induction of a hypersensitive response (HR). Due to the susceptibility to this genus of virus, in 2015, a molecular marker-assisted backcrossing (MABC) plant breeding program was launched to introduce the L3 and L4 genes into sensitive local varieties. Genotyping of plants of last generations of the breeding program were assisted with molecular markers linked to these two genes. In this last generation, verification phenotyping by mechanical virus inoculation with pepper mild mottle virus (PMMoV) and analysis with non-radioactive molecular hybridization were performed. As was expected, 100 % of homozygous resistant plants were obtained in the RC4-F4 generation, concluding this genetic breeding program.

Involvement of different plant viruses in the activation of RNA silencing-related genes and the defensive response against Plum pox virus of 'GF305' peach (Prunus persica) grafted with 'Garrigues' almond (P. dulcis)

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Plum pox virus (PPV) causes the serious sharka disease in Prunus trees. Peach [P. persica (L.) Batsch] trees are severely affected by PPV and no definitive source of genetic resistance has been identified. Previous results showed that PPV-resistant 'Garrigues' almond [P. dulcis (Mill.) D.A. Webb] could transfer its resistance to 'GF305' peach through grafting, reducing symptomatology and viral load in PPV-infected plants. A recent study that focused on transcriptomic analyses of peach and almond plants under different conditions of grafting and PPV-inoculation, revealed some genes that could be involved in this effect. In this work, we used the same peach and almond samples, but centered our analyses on small RNAs (sRNAs) expression. Studying massive sequencing data, we identified a specific pattern of sRNAs related to antiviral genes that suggested activation of these genes followed by down regulation to basal levels. Moreover, we found that 'Garrigues' almond plants were infected by different plant viruses that were transferred to peach plants. The large amounts of viral sRNAs found in grafted peaches indicated a strong RNA silencing antiviral response and led us to postulate that these plant viruses could be collaborating in the observed 'Garrigues' effect.

Carbon dots boost dsRNA delivery in plants and increase local and systemic siRNA production

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We have obtained carbon dots (CDs) by a hydrothermal synthesis for developing dsRNA nanocomposites. These CDs were produced using glucose or saccharose as the nucleation source and passivated with branched polyethyleneimines for conferring positive charges. Hydrodynamic analyses and transmission electron microscopy TEM showed that they sized on average 4 and 5 nm, depending on the sugar. The CDs were fluorescent and showed a peak at 468 nm when excited with UV light. Physicochemical characteristics of their surfaces were revealed by X-ray photoelectron spectroscopy (XPS) and Fourier transform infrared spectroscopy (FT-IR). The potential determined that the CDs had positive charges, good electrophoretic mobility and conductivity. Coating of the CDs to dsRNA was quick and efficient. DsRNA naked or coated with the CDs were delivered to leaves of cucumber plants by spraying. Quantitation of the dsRNA that entered the leaves showed that when coated with the CDs 50-fold more dsRNA was detected than when naked dsRNA was applied. Moreover, specific siRNAs derived from the sprayed dsRNAs were 130 times more abundant when the dsRNA was coated with the CDs. Systemic dsRNAs were determined in distal leaves showed a dramatic increase in concentration when delivered as a nanocomposite. Similarly, systemic siRNAs were more abundant in distal leaves when spraying with the CD-dsRNA nanocomposite. Furthermore, FITC-labeled dsRNA was shown to accumulate in the apoplast and increased its entry in the plant when coated. These results indicate that CDs obtained by hydrothermal synthesis are suitable for dsRNA foliar delivery in RNAi plant applications.

Transient expression vector in plants that are able to self-replicate and move systemically

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A plant biotechnologist may think why nobody invented an expression vector that, while keeping genetically compact, can be easily inoculated into a leaf section to autonomously amplify and expand through the whole plant. This plant biotechnologist may also ask that the expression vector was able to counteract the plant RNA silencing machinery that suppresses the expression of the gene of interest. A plant virologist will quickly recognize that these demands match the features of plant viruses. In fact, many attempts have been done to repurpose many different plant viruses into expression vectors during last decades. However, things are not that easy. Viral vectors exhibit cargo limitations and frequently induce symptoms in the host plant. They are also prone to recombination and extra genes are deleted. To take advantage of some properties, others such as systemic movement, are sacrificed. Despite all these limitations, plant virologists have still been able to assemble a wide variety of viral vectors that are useful for many purposes in plant biotechnology and synthetic biology. Production of recombinant proteins or induction of gene silencing for reverse genetics approaches can be considered classics. More applications, however, are emerging nowadays. Viral vectors are used to express transcription factors and biosynthetic enzymes in plant metabolic engineering approaches. Viral vectors are also used to express the CRISPR-Cas reaction components for efficient and heritable plant genome editing. Finally, plant viruses are also being used as scaffolds to produce nanoparticles with unprecedented applications in therapeutics and diagnostics.

Development of viral vectors based on alfalfa mosaic virus for the silencing of genes of interest in plants

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The use of viruses to induce gene silencing (Virus-induced gene silencing, VIGS) is a very promising tool for carrying out genetic functionality studies in plants due to its speed, economy and versatility. VIGS has many advantages over techniques that require the generation of transgenic plants. The development of new VIGS vectors is of particular interest when carrying out genetic studies on plants of agronomic value, especially if they are not susceptible to genetic modification. This work has laid the foundations for the use of alfalfa mosaic virus (AMV) model system as a new VIGS vector. AMV takes the advantage of the particular structure of its movement protein (MP), which is divided in two domains that allow the introduction, between them, of heterologous sequences in the coding region without compromising its functionality. The introduction of lineal (33 nt to 300 nt) but not stem-structures sequences of the Nicotiana tabacum phytoene desaturase (PDS) gene revealed the capacity of the AMV vector to silencing the host gene to a 76% of the wild type accumulation. The observation that the AMV MP belongs to the 30k superfamily or group of 18 viral genera which MPs are structural and functionally related to the 30 kD MP of tobacco mosaic virus, opens the possibility to design VIGS vectors using other viruses assigned to this family.

Targeted plant gene silencing based on an asymptomatic viroid

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Abstract

Gene silencing for functional studies in plants has been largely facilitated by manipulating viral genomes with inserts from selected host genes to trigger virus induced gene silencing (VIGS) against the corresponding mRNAs. However, viral genomes encode multiple proteins and can disrupt plant homeostasis by interfering with endogenous cell mechanisms. To try to circumvent this functional limitation, we have developed a silencing method based on the minimal autonomously-infectious nucleic acids currently known: viroids, which are RNAs with null or residual protein-coding capability. The genome of *Eggplant latent viroid*, an asymptomatic viroid, was manipulated with insertions ranging between 21 to 42 nucleotides. Our results show that, although larger insertions might be tolerated, the maintenance of the secondary structure appears to be critical for viroid-genome stability. Additionally, these modified ELVd molecules are able to induce systemic infection promoting the silencing of target genes in eggplant. Inspired by the design of artificial microRNAs, we have developed a simple, fast, and cost-effective standardized procedure to generate stable insertions into the ELVd genome capable of silencing a specific target gene. Analogously to VIGS, we have termed our approach Viroid Induced Gene Silencing (VdIGS) and demonstrate that is a promising tool for dissecting gene functions in eggplant. Overall, this represents the use of minimal circular replicating RNAs able to spread systemically combined with the production of a tailored sRNA for targeted silencing.

Lessons from icosahedral and flexuous viral structures of whitefly-transmitted members of the genera Torradovirus and Ipomovirus

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Virus-like particles (VLPs) have been used to understand the requirements for virion formation and to solve the structure of many plant viruses. For this purpose, plant transient expression systems have been successfully adopted (e.g.: pEAQ- and pEff-system). We explored the possibility of producing VLPs of two whitefly-transmitted viruses: i) tomato apex necrosis virus (ToANV, genus *Torradovirus*, family *Secoviridae*), which has icosahedral virions and causes infection in tomato crops; and ii) cucumber vein yellowing virus (CVYV, genus *Ipomovirus*, family *Potyviridae*), which has flexuous virions and causes infection in cucurbit crops. The pEAQ-vector system was initially used to produce the capsid protein (CP) of CVYV and the three CPs of ToANV in *Nicotiana benthamiana* plants. Successful expressions were confirmed by Western Blot (WB) with the corresponding specific anti-CP antibodies. However, with this system we were only able to confirm VLP production for ToANV by transmission electron microscopy (TEM). Thus, for CVYV we explored the possibility of using the replicative vector, pEff, which allows assembly of the CP on replicating RNA. The system allowed accumulation of CVYV CP, confirmed by WB, and the production of VLPs, confirmed by TEM. In addition, a high-resolution structure of ToANV VLPs was determined using cryo-electron microscopy (Cryo-EM) and compared with that of another aphid-transmitted member of the same family, broad bean wilt virus 1 (BBWV-1, genus *Fabavirus*, family *Secoviridae*). The novel structural information will be used for biotechnological applications, and to explore the mechanisms of transmission of these three viruses.

Turnip Mosaic virus nanoparticles: a versatile tool in biotechnology

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Turnip Mosaic Virus (TuMV) is a potyvirus of the family *Potyviridae* that causes diseases in different groups of plants, especially cruciferous. Although its role as plant pathogen is of worldwide importance and well-known, we have studied TuMV from a completely different point of view: we took advantage of both its elongated, flexuous form and the structure of its capsid (consisting of approximately 2,000 repetitions of a single capsid protein) to create viral nanoparticles that can be applied in different fields such as theranostics, nanobiomedicine or agriculture. Viral nanoparticles (VNPs) include virions and virus-like particles (VLPs) and have played an important role in biotechnology during the last years. VLPs, unlike virions, are non-infectious because they contain no genetic material. In our group, we have developed TuMV VNPs (both virions and VLPs) through functionalization. This functionalization of TuMV VLPs was carried out via three main ways: chemical conjugation, genetic fusion and the SpyTag/SpyCatcher technology (which consists of a genetic fusion followed by a chemical conjugation). With our work, we want to show how TuMV has a greater relevance than previously thought, beyond being a mere plant pathogen. Moreover, not only have TuMV VNPs already been applied to different fields in science, but they still have a huge potential for the development of novel biotechnological tools in the near future.

POSTER PRESENTATION

Dynamic expression of the Tomato leaf curl New Delhi virus bipartite genome

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Multipartite viral genomes are the most extreme case of genome segmentation, as their genome segments are packed into separate particles all required to complete the infectious cycle. Whitin plant DNA virus, it occurs in the genus *Begomovirus* (family *Geminiviridae*), which includes bipartite species composed by two genomic components (DNA-A and DNA-B), each of approximately 2.7 kb in size. Despite the fact that the accumulation and spread of both DNA components have been described and the functional role of their encoded proteins characterized, a detailed study of how the expression of each genomic particle is modulated through the infection is lacking. The Spanish strain of the begomovirus tomato leaf curl New Delhi virus (ToLCNDV-ES) has represented one of the major constraints to cucurbit crops in the Mediterranean basin for the last ten years. Here, we analyze the whole transcriptional expression of its bipartite genome at 3-, 6- and 12-days post-inoculation in susceptible Piñonet Piel de Sapo melon plants (*Cucumis melo* Ibericus group). Differential accumulation levels for transcripts derived from both DNA-A and DNA-B particles were observed since the earliest stage of the infection, with a higher accumulation of DNA-A transcripts detected at all the analyzed points that increase with the progression of the disease. Understanding the implication of these different expression patterns between both DNA components along the infection could shed light on the ToLCNDV pathogenesis in cucurbits.

Insights into begomovirus-deltasatellite complex diversity: the first deltasatellite infecting legumes

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Legumes play an important nutritional role in the diets of millions of people, mainly in developing countries, but their productivity is seriously affected by a variety of pathogens including viruses. Begomoviruses and associated DNA satellites are involved in pathosystems that include many cultivated and wild dicot plants and the whitefly vector *Bemisia tabaci*. A survey of leguminous plants, both crops and wild species, was conducted in Venezuela to determine the presence of begomoviruses. Molecular analysis identified the presence of bipartite begomoviruses in 37% of the collected plants. Four of the six begomoviruses identified constituted novel species, and two others had not been previously reported in Venezuela. In addition, a novel deltasatellite (cabbage leaf curl deltasatellite, CabLCD) was found to be associated with cabbage leaf curl virus (CabLCV) in several plant species. CabLCD was the first deltasatellite found to infect legumes and the first found in the NewWorld to infect a cultivated plant. Agroinoculation experiments using *Nicotiana benthamiana* plants and infectious viral clones confirmed that CabLCV acts as a helper virus for CabLCD. The begomovirus–deltasatellite complex described here was also found in wild legume plants, suggesting the possible role of these plants in the emergence and establishment of begomoviral diseases in the main legume crops in the region. These results illustrate the increasing complexity faced by researchers and breeders looking to develop control strategies against emerging pathogens, stressing the need for a profound pathological knowledge of unrevealed novel actors like the begomovirus-deltasatellite complexes.

Towards deciphering the sweepovirus-deltasatellite-plant host interactions: expanded natural and experimental helper virus range and effect dependence on virus-host combination

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Sweepoviruses are begomoviruses (genus *Begomovirus*, family *Geminiviridae*) infect sweet potato and other species of the family Convolvulaceae. They group in a phylogenetic cluster separate from the rest of the begomoviruses, seeming to represent one of the earliest points of divergence among the genus. Deltasatellites (genus *Deltasatellite*, family *Tolecusatellitidae*) are small non-coding circular ssDNA satellites associated with begomoviruses, including sweepoviruses. In this study, the genetic diversity of deltasatellites associated with sweepoviruses infecting blue morning glory (*Ipomoea indica*) plants was analyzed by further sampling the populations where the deltasatellite sweet potato leaf curl deltasatellite 1 (SPLCD1) was initially found, expanding the search to other geographical areas in southern continental Spain and the Canary Islands. The sweepoviruses present in the samples coinfected with deltasatellites were also characterized by sequencing in order to define the range of viruses that could act as helper viruses in nature. Additionally, experiments were performed to assess the ability of a number of geminiviruses (the monopartite begomovirus tomato leaf deformation virus, the bipartite begomoviruses Sida golden yellow vein virus and tomato leaf curl New Delhi virus, and the curtovirus beet curly top virus) to transreplicate SPLCD1 in their natural plant hosts or the experimental host *Nicotiana benthamiana*. The results showed that SPLCD1 can be transreplicated by all the geminiviruses assayed in N. *benthamiana* and by tomato leaf curl New Delhi virus in zucchini. The presence of SPLCD1 did not affect the symptomatology caused by the helper viruses, and its effect on viral DNA accumulation depended on the helper virus-host plant combination.

Turnip mosaic virus strain-specific effects on the aphid vector *Myzus persicae*

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Multiple are the works showing how plant viruses can manipulate their vectors in different ways. In fact, viruses can induce changes in their host plants and insect vectors by means of chemical and physiological alterations affecting vector-host plant interactions in order to enhance their own spread. Most of these studies have not considered if such modifications could be dependent of the virus strain or the virus ability to be transmitted by vectors. We report the effect of two different isolates (UK1 and JPN1), representative of two strains (World B and Asian BR) of turnip mosaic virus (Potyvirus, TuMV) on the life-history and settling behaviour of its main aphid vector, *Myzus persicae*. Moreover, a non-aphid transmissible mutant of the UK1 isolate (UK1-NT) was included in the study. *Arabidopsis thaliana* was used as a host plant for all experiments. JPN1 was transmitted by *M.persicae* at a similar rate than UK1 and no relevant differences were found in their aminoacidic sequences encoding for CP and HC-Pro proteins, both involved in TuMV transmission by aphids. The analysis of life-history traits showed a higher intrinsic rate of natural increase (r_m) of *M.persicae* on UK1 and JPN1-infected plants compared with the mock-inoculated plants, being highest for the UK1-infected plants. No differences on the aphid development were found between UK1 and UK1-NT treatments. Finally, aphid settling behaviour experiments under free-choice conditions also showed significant differences between treatments, with a lower number of aphids settled on plants infected with the UK1-NT isolate. The epidemiological implications of these results will be discussed.

Sweet potato symptomless virus 1 in Spain: detection and development of an agroinfectious clone

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Sweet potato (*Ipomoea batatas*), one of the most consumed crop in the world and a staple food for people in many least developed countries, is affected by many viral diseases. In 2017, complete genome sequences of sweet potato symptomless virus 1 (SPSMV-1, genus *Mastrevirus*, family *Geminiviridae*) isolates from seven countries were reported, although a partial genome sequence had been previously identified by deep sequencing of small RNAs in Peru. Currently, SPSMV-1 has been reported infecting sweet potato plants in Brazil, China, Ecuador (Galapagos Islands), Kenya, Korea, Peru, Taiwan, Tanzania, Uruguay, and USA. To assess the presence of this virus in Spain, sweet potato leaf samples collected in Málaga (southern continental Spain) and the Canary Islands of Tenerife and Gran Canaria were analyzed. SPMMV-1 was detected in samples from all the geographical areas studied, as well as in several samples of pathogen-tested *in vitro* plants obtained from a germplasm collection. Sequence analysis of full length genomes of isolates from Málaga, Tenerife and Gran Canaria showed a closely relation between them and with the isolates available from GenBank. Also, an agroinfectious clone was developed and infectivity assays showed that the virus was able to infect *Nicotiana benthamiana*, *Ipomoea indica*, *I setosa* and sweet potato plants. No symptoms were observed in any of the infected plants. To our knowledge this is the first report of the presence of SPSMV-1 in Spain and the first agroinfectious clone developed for this virus.

Identifying host molecular targets of virus infection and adaptation

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The outcome of a virus infection is the result of a complex interplay between the host organism and the viral pathogen, involving a vast array of protein-protein interactions.

With the aim of obtaining a comprehensive view of the interaction network that is established upon infection between host and viral proteins, a high-throughput yeast two-hybrid (HT-Y2H) approach was used for the identification of proteins from *Arabidopsis thaliana* that bind to each of the proteins encoded by the genome of turnip mosaic potyvirus (TuMV). A set of interactors was selected according to criteria of connectivity and differential expression upon infection. When Arabidopsis T-DNA insertion mutants for these genes were challenged with TuMV, significant deviations from the response of the wild-type Col-0 accession were observed.

In addition, experimental evolution of TuMV in a set of Arabidopsis genotypes bearing mutations in either defense-related genes or genes encoding factors required for successful viral infection resulted in the selection of a number of mutations, mostly affecting the viral VPg protein. Among them, mutation D113G is a convergent mutation selected in several lineages evolved in different plant genotypes, whereas mutation R118H was specifically selected in the *jin1* mutant affecting jasmonate signaling. Using the HT-Y2H system, the effect of these mutations on the interaction of VPg with Arabidopsis proteins was analyzed. Interestingly, both mutations severely compromised the interaction of VPg with the translation initiation factor eIF(iso)4E, a key interaction in potyvirus infection.

Identification of virus determinants and mechanisms that regulate Tomato leaf curl New Delhi virus host range

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Tomato leaf curl New Delhi virus (ToLCNDV) is a whitefly-transmitted bipartite begomovirus (genus *Begomovirus*, family *Geminiviridae*), whose genome contain two single-stranded DNA molecules (DNA-A and DNA-B). This virus causes damage to multiple cultivated plant species mainly belonging to the *Solanaceae* and *Cucurbitaceae* families and was limited to Asian countries until 2012, when it was first reported in Spain, causing severe epidemics in cucurbit crops. Our results have demonstrated that isolates spreading throughout the western Mediterranean Basin belong to a novel strain of ToLCNDV (strain ES) and cause severe infections in cucurbit crops but poorly infect tomatoes. However, existence of ToLCNDV isolates that severely affect tomato plants has been reported and infectious clones are available for one of these isolates (India isolate, kindly provided by S. Chakraborty, Jawaharlal Nehru University, New Delhi, India). Our main goal is to identify the viral determinants and to understand the mechanisms associated to the ability of some isolates of ToLCNDV to infect tomato efficiently. This information will help to prevent the threat of ToLCNDV damage in tomato in the Mediterranean Basin. We have agroinoculated tomato cv Moneymaker with infectious clones of ToLCNDV-Spain isolate (ToLCNDV-[ES]; our isolate unable to induce efficient infections in tomato) and ToLCNDV-India isolate (ToLCNDV-[IN], which efficiently infects tomato). The results from local and systemic infections with both viruses and pseudorecombinants between A and B components of the bipartite ToLCNDV-[ES] and ToLNCDV-[IN] will be shown and discussed.

Twenty years of evolution and diversification of digitaria streak virus in Digitaria setigera

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The emergence of new geminivirus species results from their high mutation and recombination rates. In this study, we report the variability and evolution of digitaria streak virus (DSV), a mastrevirus isolated in 1986 from the grass *Digitaria setigera* in the Vanuatu archipelago. Viral DNA was amplified from D. *setigera specimens*, derived from the naturally infected original plant, which were propagated in different laboratories for more than 20 years. From the consensus sequences, the nucleotide substitution rate was estimated. In addition, the intra-host genetic complexity and diversity of DSV populations was characterized. The evolutionary rate of DSV was estimated within the ranges observed in other single-stranded DNA viruses and RNA viruses. Bioinformatic analyses revealed high variability and heterogeneity in DSV populations, which confirmed that mutant spectra are continuously generated and are organized as quasispecies. The analysis of polymorphisms revealed nucleotide substitution biases in viral genomes towards deamination and oxidation of DNA. The differences in variability in each of the genomic regions reflected a modular evolution in the mutant spectra that was not reflected in the consensus sequences. Strikingly, the most variable region of the DSV genome, encoding the movement protein, showed rapid fixation of the mutations, which suggests strong positive selection in this region. Phylogenetic analyses revealed a possible divergence in three genetic lineages from the original Vanuatu DSV isolate.

Differential reaction of sweet pepper plants to infection with tomato chlorosis virus (genus *Crinivirus*, family *Closteroviridae*) is likely depending on the viral variant

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Tomato chlorosis virus (ToCV) is a crinivirus (family *Closteroviridae*) transmitted by whiteflies of the genera *Bemisia* and *Trialeurodes* in a semi-persistent manner that causes significant losses in solanaceous crops including tomato and sweet pepper. Worldwide reports of natural and experimental infection of sweet pepper plants with ToCV are contradictory, raising the question of whether the critical factor determining infection is related to the susceptibility of different cultivars or the nature of virus isolates. In this work, ToCV isolates obtained from different hosts and geographical origins were biologically and molecularly analyzed, transmitted by *B. tabaci* MEAM1 and MED, and the reaction of different sweet pepper cultivars was evaluated under different environmental conditions. Brazilian ToCV isolates from tomato, potato, *Solanum americanum*, and *Physalis angulata* did not infect plants of five sweet pepper cultivars when transmitted by *B. tabaci* MEAM1. Temperatures did not affect the sweet pepper susceptibility to tomato-ToCV isolates from São Paulo, Brazil and Florida (USA). However, ToCV-sweet pepper isolates from Spain and São Paulo (Brazil), were transmitted efficiently to sweet pepper plants by *B. tabaci* MEAM1 and MED. Although the results indicated that ToCV isolates from naturally infected sweet pepper plants seem to be better adapted to plants of this species, phylogenetic analyses based on the complete nucleotide sequences of RNA1 and RNA2 as well as the p22 gene did not reveal significant nucleotide differences. Additional studies are needed to identify intrinsic characteristics of ToCV isolates that favor infection of sweet pepper plants.

Resistance to Cucumber mosaic virus (CMV) in melon Near Introgression Lines (NIL) carrying two or three Quantitative Trait Loci (QTL)

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Cucumber mosaic virus (CMV) can infect more than 1.200 different plant species belonging to *Solanaceae* and *Cucurbitaceae* families. Resistance to CMV has been found in several exotic melon accessions, being one of them the Korean accession "Songwhan Charmi" (SC). A doubled haploid line (DHL) collection was previously developed from a cross between SC as resistant parental and the cultivar Piel de Sapo (PS) as a susceptible parental, in order to study the resistance trait. A Quantitative Trait Loci (QTL) analysis revealed a major QTL, *cmvqw12.1*, located in the linkage group XII. This QTL confers total resistance to subgroup II strain LS. However, for the subgroup I strains M6 and FNY it is necessary but not sufficient. In addition, two more QTLs were described, *cmvqw3.1* located in LGIII and *cmvqw10.1* in LGX. DHLs containing the three QTLs were resistant to M6, whereas DHLs containing two QTLs, being one of them *cmvqw12.1*, were susceptible to M6 strain. In the present work we have developed Near Isogenic Lines (NILs) containing either two or three QTL. These are a NIL with *cmvqw3.1* and *cmvqw12.1*, a NIL with *cmvqw10.1* and *cmvqw12.1* and a NIL with the three QTL. Our aim is to confirm in the NILs the resistance to CMV-M6 observed previously in the DHLs containing two and three QTLs, in order to know whether the results are consistent in these two different genetic backgrounds. From these ILs we are generating mapping populations for both QTLs *cmvqw3.1* and *cmvqw10.1*.

Resistance to cucumber green mottle mosaic virus (CGMMV) in cucumber

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Cucumber cultivation has great economic relevance both nationally and internationally. Cucumber green mottle mosaic virus (CGMMV) is a tobamovirus inducing a disease affecting cucumber crops worldwide and causing large economic losses. Sources of partial genetic resistance to CGMMV have been described, and genes encoding type 1 RNA-dependent RNA polymerases (RDRs) seem to be the most likely candidates to be responsible for the resistance. Specifically, it appears that a *RDR1a/b* gene duplication could be related to CGMMV resistance. In order to confirm if the genetic dosage of *RDR1a/b* correlates with CGMMV resistance, we determined the number of copies and the expression levels of the candidate genes (*RDR1a/b*) in symptomatic and asymptomatic CGMMV infected cucumber plants from different cultivars, as well as viral accumulation. An inverse correlation between CGMMV accumulation and the *RDR1b* duplication was found, and also a positive correlation between duplication and *RDR1b* expression. A second objective was to identify new sources of resistance to CGMMV in cucumber. Six to 8 plants from 300 accessions from the USDA Germplasm Bank were inoculated with CGMMV under controlled conditions; inoculated plants of 8 accessions did not show symptoms after 21 days post-inoculation. Further analysis with these accessions showed that the *RDR1A/1B* gene duplication is common among resistant genotypes, but also pointed toward the existence of at least one source of resistance likely controlled by a different genetics.

Genome-wide analysis of histone repressive modifications induced by *Hop stunt viroid* infection in cucumber.

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The regulation mediated by epigenetic mechanisms, such as DNA methylation and histone modifications has been proposed as a key component of the plant response to pathogens. Constrained by a naked and miniaturized genome (246 - 434 nt), viroids have evolved into versatile molecular entities capable of subverting the host-cell machinery to complete their infectious cycle, but it is currently unknown the genome-wide extent of epigenetic changes under viroid infections. To shed light on this matter, we have attempted the identification of histone repressive marks at a genome-wide level in *Cucumis sativus* plants infected with hop stunt viroid (HSVd) using chromatin immunoprecipitation (ChIP) coupled with next generation sequencing. For this, crosslinked DNA from apical leaves of HSVd-infected cucumber plants at 10-, 17- and 24-days post-inoculation was immunoprecipitated with antibodies specific of the histone H3 (input) and two histone repressive modifications: H3K9me2 and H3K27me3. As expected, profiles observed for each histone modification were different. Modifications in H3K27me3 were predominantly detected in gene bodies while marks in H3K9me2 were mainly found associated to repetitive elements. Host- genome regions enriched in repressive marks in response to HSVd-infection were identified and correlated with differentially methylated regions (DMR) previously identified in viroid-infected plants. Altogether these data offer a temporal overview about the epigenetic reprogramming of histone repressive marks in cucumber genome in response to HSVd infection.

Is eggplant latent viroid located in the chloroplasts of infected cells?

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Viroids are self-replicating, small [250-450 nucleotides (nt)], noncoding, single-stranded circular RNAs infecting higher plants. They are classified into two families: (i) *Pospiviroidae*, in which members replicate and accumulate in the nucleus; and (ii) *Avsunviroidae*, whose members replicate and accumulate in the chloroplasts. *Avsunviroidae* is subdivided into three genera: *Avsunviroid*, *Pelamoviroid* and *Elaviroid*. Avsunviroids and pelamoviroids have already been shown to replicate and accumulate in chloroplasts. Therefore, the objective of this work was to verify, by in situ hybridization with digoxigenin (DIG)-UTP-labelled riboprobes, if eggplant latent viroid (ELVd), the only member of the genus *Elaviroid*, is also associated with this organelle. Leaf pieces from healthy and ELVd-infected eggplant (*Solanum melongena*) 'black beauty' plants were fixed, dehydrated, and embedded in paraffin. Serial sections were collected on polysine-coated slides. DIG-UTP-labelled riboprobes [ELVd (333 nt) and 5S chloroplast rRNA (163 nt)] were obtained via T3 RNA polymerase transcription. Hybridizations were carried out at 54ºC, and DIG-labelled hybrids were detected using anti-DIG antibody conjugated to alkaline phosphatase and colour substrates (BCIP–NBT). In healthy tissues, we observed hybridization signals only for 5S chloroplast rRNAs specific riboprobe under differential interference contrast (DIC) microscope. Hybridization signals with ELVd riboprobe were observed mainly in chloroplasts, of palisade and lacunar parenchyma cells, also in phloem cells, but only from infected tissue sections, reinforcing the notion that ELVd, as well as avsunviroids and pelamoviroids, replicate and accumulate in this organelle. Analysis with nuclear RNA-specific probes will still be performed.

Functional characterization of a tomato transcription factor interacting with the pepino mosaic virus TGB1 protein

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The disease induced by pepino mosaic virus (PepMV; genus *Potexvirus*) in tomato crops causes serious economic losses worldwide. Viruses are obligate parasites that need host proteins to complete their cycles; the identification and study of these proteins is interesting from both applied and fundamental points of view. We used a yeast two-hybrid screening to identify tomato proteins that interact with all five PepMV proteins. Among the identified proteins we focused on IP9, a putative transcription factor of unknown function. IP9 interacts with the PepMV Triple Gene Block 1 (TGB1) protein, a multifunctional protein mainly involved in viral movement and RNA silencing suppression. We employed the CRISPR/Cas9 technology to obtained knock-out mutants for IP9 in tomato cv. MicroTom, and we observed that the edited plants were more resistant to PepMV infection. Further, we used transient expression of IP9 fused to different fluorescent proteins in *Nicotiana benthamiana* and confocal laser scanning microscopy (CLSM) to study its subcellular localization expressed alone, co-expressed with fluorescent-tagged TGB1, or with a PepMV clone tagged with tagRFP. We observed that IP9 and TGB1 colocalized at nuclei of healthy cells, and both relocate to the viral replication complexes (VRCs) in infected cells. We also studied the localization of four IP9 homolog proteins by CLSM and observed that none of them relocate to VRCs during viral infection. Altogether, our results suggest that IP9 acts as a specific proviral factor for PepMV at least in tomato.

Identification of RNA elements involved in protein translation of the *Cucurbit aphid-borne yellows virus* genome

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Viral mRNAs have evolved numerous mechanisms to recruit the host translational machinery, allowing them to compete with host mRNAs and avoid defence mechanisms that act at the level of translation. Thus, while most plant-encoded mRNAs contain a 5´-cap and a poly(A)-tail that act synergistically to stimulate translation, ~80% of known positive-strand RNA plant viruses lack one or both of these features in their genomic and subgenomic RNAs. Some of them contain in their 3´-UTRs RNA elements able to enhance their cap-independent translation (3´-CITEs). In our group we have identified three essential 3´-CITEs in different isolates of Melon necrotic spot virus (MNSV, *Tombusviridae*). We have shown that 3´-CITEs are modular, interchangeable structural elements, since we identified a MNSV isolate that had acquired a 3´-CITE from a virus belonging to a different family, Cucurbit aphid-borne yellows virus (CABYV; *Luteoviridae*). Here we show that CABYV Asiatic and European isolates have two different translational enhancers. Analysis of the secondary structure of these two 3´-CITEs shows that they differ from the 3´-CITEs structures described until now. The activities of these 3´-CITEs are eIF4E-independent, conferring translational competence to RNAs in the absence of this factor. We are currently studying the molecular mechanism of the cap-independent translation enhancement controlled by these 3´-CITEs.

Introduction of the hepatitis delta virus ribozyme increases the efficiency of infectious clones of the crinivirus tomato chlorosis virus

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Tomato chlorosis virus (ToCV) is an emergent plant pathogen causing a yellow leaf disorder in tomato and other solanaceous crops. ToCV is a positive-sense, single stranded (ss)RNA bipartite virus with long and flexuous virions belonging to the genus *Crininivirus* (family *Closteroviridae*). ToCV is phloem-limited, transmissible by whiteflies of the genera *Bemisia* and *Trialeurodes*, and causes symptoms of interveinal chlorosis, bronzing, and necrosis in the leaves of tomato accompanied by a decline in vigor and reduction in fruit yield. The availability of infectious virus clones is a valuable tool for reverse genetic studies that has been long been hampered in the case of closterovirids due to their genome size and complexity. In this work, attempts were made to improve the infectivity of the available agroinfectious cDNA ToCV clones (isolate AT80/99-IC from Spain) by adding the hepatitis delta virus (HDV) ribozyme fused to the 3' end of both genome components, RNA1 and RNA2. The inclusion of the ribozyme generated a viral progeny with RNA1 3' ends more similar to that present in the clone used for agroinoculation. The infectivity of the clones carrying the HDV ribozyme in *Nicotiana benthamiana* plants increased, on average, by 100%. The availability of these clones could form the basis for further approaches to achieve an important goal, the direct agroinfection of tomato plants, the primary host of ToCV, which would facilitate reverse genetic studies, understanding of the virus–vector interactions and breeding programs to incorporate resistance against ToCV in commercial cultivars.

Viroid infection induces a temporal reprograming of plant-defence mechanisms at multiple regulatory levels

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Viroids are circular RNAs of minimal complexity compelled to subvert plant-regulatory networks to accomplish their infectious process. Studies focused on the response to viroid infection have mostly addressed specific regulatory levels and considered a unique infection time. Thus, much remains to be done to understand the temporal evolution and complex nature of viroid-host interactions. Here we present an integrative analysis of the timing and intensity of the genome-wide alterations in cucumber plants infected with *Hop stunt viroid* (HSVd) by integrating differential host transcriptome, sRNAnome and methylome. Our results support that HSVd promotes the redesign of the cucumber regulatory-pathways predominantly affecting specific regulatory layers at different infection-phases. The initial response was characterized by a reconfiguration of the host-transcriptome by differential exon usage, followed by a progressive transcriptional down-regulation modulated by epigenetic changes. Regarding endogenous small RNAs, the alterations were limited and mainly occur at the late stage. Moreover, the silencing of three cucumber transcripts mediated by viroid-derived sRNAs was validated by degradome analysis and reporter assays. The most significant host-alterations were predominantly related to the down-regulation of transcripts involved in plant-defence mechanisms, the restriction of pathogen-movement and the systemic spreading of defence signals. Altogether our data evidence the existence of a dynamic and yet poorly known arms race between the host and the viroid. We expect that these data constituting the first comprehensive map of plant responses to a viroid infection contribute to elucidate the molecular basis of this multifaceted defence and counter-defence layout.

Strategies for the management of virosis in horticultural crops

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The management of virosis in horticulture, both in greenhouses and outdoors, is especially complex when varieties with resistance are not available. The action strategies in the field will depend, basically, on their transmission mechanisms, being fundamental to differentiate those that spread through vector insects from those that spread through mechanisms related to contact, apart from seeds and other systems. In both cases, there are techniques aimed at preventing the virus from entering the crop "primary contamination", while others aim to limit its expansion "secondary contamination". "Clean" plots and planting material with maximum phytosanitary guarantees are always essential starting conditions. In the case of transmission by insects, the primary contaminations will be produced by immigration of viruliferous individuals. Physical barriers, complemented by technological means, can limit the arrival of vectors to the crop, while phytosanitary treatments are hardly going to prevent these transmissions. Joint actions, aimed at reducing the pressure of viruses and their vectors in the region, are especially important. Secondary contamination requires the movement and multiplication of viruliferous insects in the crop and the strategies to limit them imply a "sustainable" control of the vector insect populations and an "adequate" elimination of the affected plants. In virosis transmitted by contact, hygiene and disinfection measures are prioritized at the entrances of the plots. The strategies to limit its expansion, due to secondary contamination, are based on an adequate organization of workers and tasks, on hygiene and disinfection measures, and on the surveillance and elimination of inoculum sources.

The begomovirus Tomato leaf curl New Delhi virus is not seed-transmitted in melon

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Transmission of plant viruses through seed can be one of the major factors contributing to long- distance dispersal through global trade of seeds and can have important ecological consequences for virus dissemination. *Begomoviruses* (genus *Begomovirus*, family *Geminiviridae*), and among them isolates of the species Tomato leaf curl New Delhi virus (ToLCNDV), cause significant yield losses in economically important crops worldwide. These viruses are horizontally transmitted in nature in a circulative and persistent manner by the whitefly *Bemisia tabaci* but in recent years several reports have raised the possibility of vertical transmission through seeds for some members of this genus. We have investigated the possible transmission by melon (*Cucumis melo L.*) seeds of a ToLCNDV isolate of the "Spain" strain, in three different melon cultivars (all susceptible to ToLCNDV). The presence of ToLCNDV in floral tissues and the detection of viral DNA in seeds reveals the seed-borne nature of this virus. However, grow-out studies conducted with the progeny of melon plants germinated from seeds collected from ToLCNDV-infected plants and evaluated at early (1 leaf) or at late (20 leaves) growth stages did not support the vertical transmission of ToLCNDV from seeds to the offspring.

Foliar application of insecticides disrupts feeding behavior of the whitefly *Bemisia tabaci* and the transmission of the crinivirus tomato chlorosis virus in potato plants

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The whiteflies of the *Bemisia tabaci* complex (Hemiptera: Aleyrodidae) are serious agricultural pests that cause severe losses to vegetable, ornamental and fiber crops, mainly as vectors of economically important viruses. Among the most important viruses affecting tomato and other solanaceous crops as potato is tomato chlorosis virus (ToCV) (genus *Crinivirus*, family *Closteroviridae*), which is semi-persistently transmitted by whiteflies of the genera *Bemisia* and *Trialeurodes*. Chemical control is the main method used to manage *B. tabaci* and ToCV; however, this whitefly is resistant to most commercially available insecticides, and some products may not effectively prevent the vector activities associated with virus transmission. The effective management of *B. tabaci* is crucial to reduce the spread of vector-borne diseases and to minimize economic losses. We evaluated the effects of the foliar spraying with the systemic insecticides acetamiprid, flupyradifurone and cyantraniliprole on the probing behavior of non-viruliferous and ToCV-viruliferous B. tabaci MEAM1 and ToCV transmission in potato plants. To evaluate ToCV transmission in greenhouse conditions, viruliferous whiteflies were released on potato plants at different time points after insecticide spraying. The electrical penetration graph (EPG) assay showed that at 3 h after insecticide application, the probing behavior differed, depending mainly on the state of the insects (viruliferous or not), whereas 72 h after application, the probing behavior differed only on plants treated with acetamiprid and flupyradifurone, for both viruliferous and non-viruliferous whiteflies. ToCV transmission was reduced mainly in plants treated with flupyradifurone and acetamiprid, likely as a result of phloem activity disruption.

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The cap-binding protein *eIF4E*, through its interaction with eIF4G, constitutes the core of the eIF4F complex, which plays a key role in the circularisation of mRNAs and their subsequent cap-dependent translation. In addition to its fundamental role in mRNA translation initiation, other functions have been described or suggested for eIF4E in the biology of eukaryotic organisms, including acting as a proviral factor and participating in sexual development. We have used CRISPR/Cas9-mediated genome editing to generate melon *eif4e* knock-out mutant lines. A segregating F2 generation from one of the lines was inoculated with Moroccan watermelon mosaic virus (MWMV); homozygous plants showed virus resistance, while heterozygous and non-mutant plants resulted infected, in agreement with our previous results using RNAi. Interestingly, all homozygous plants of the F2 generation showed also a male-sterility phenotype, with a perfect correlation between the segregation of the male sterility phenotype and the segregation of the *eIF4E* mutation. We next carried out morphological and transcriptomic comparative analysis of melon male floral development to investigate the role of *eIF4E* in the formation of male melon gametes. Our data showed that the sterility phenotype is a post-meiotic and sporophytic phenomenon; in addition, our results strongly suggested that *eIF4E*-specific mRNA translation initiation is a limiting factor for male gametes formation.

I will defend my doctoral thesis just one week before the congress, and I consider that being chosen for an oral presentation at this congress would be to me a great opportunity to close this pre-doctoral stage in the best way.

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Cucumber green mottle mosaic virus (CGMMV) represents a serious threat to cucurbit crops. Since genetic resistance to the virus is not yet available in commercial varieties, we aimed to control this virus by RNAi. Thus, we obtained constructions both for expressing dsRNA in bacteria to treat cucumber plants by topical application and agroinoculation in experiments done in the growth chamber. Greenhouse assays were performed in spring and in summer, when plants were challenged with the virus and differences in several parameters were investigated, including severity of symptoms, dry weight, total height, virus accumulation and virus-derived small interfering RNAs (vsiRNAs). Spraying of plants with dsRNA reduced significatively CGMMV symptoms in the plants in growth chamber tests. Agroinfiltration experiments done under identical conditions were also effective in limiting the progress of CGMMV disease. In a greenhouse assay performed in spring, symptoms were significatively reduced in dsRNA-sprayed plants and the development of the plants improved. Virus titters and vsiRNAs were clearly reduced in dsRNA-treated plants. The effect of protection of the dsRNA was less evident in another greenhouse assay carried out in summer. Besides, we investigated the mobility of long (ds)RNA derived from spraying or agroinfiltrated dsRNA and found that it could be detected in local, close distal and far distal points from the site of application. VsiRNAs were also detected in local and distal points. We also investigated the capacity of specific dsRNAs derived from tomato leaf curl New Delhi virus (ToLCNDV) to limit the disease in zucchini, both by agroinfiltration or direct spraying, but found no protection effect.

Marker-assisted introgression of resistance to Tomato leaf curl Nueva Delhi virus in zucchini

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Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus causing symptoms of curling, vein swelling, and severe mosaic in young leaves, short internodes, and fruit skin roughness. ToLCNDV was first reported affecting zucchini in Murcia (Spain) in 2012. Resistance has not been reported in *Cucurbita pepo*. The *Cucurbita moschata* Spanish landrace AN-CU-45 has been described as resistant to ToLCNDV. In this work, segregating generations derived from the initial cross between two *C. pepo* accessions and AN-CU-45 (F2 generations and backcrosses to *C. pepo* and AN-CU-45) were obtained. The populations were mechanically inoculated with ToLCNDV. Disease assessment was carried out by symptom evaluation and detection of viral titer by qPCR. Results fit a monogenic recessive genetic control of the resistance to ToLCNDV derived from AN-CU-45, similarly to the resistance from the *C. moschata* Indian landrace PI 381814. Moreover, analysis of the segregating generations showed association to the AN-CU-45-derived resistance of the SNPs previously described as linked to the resistance from PI 381814. These SNPs have been used in the breeding program in marker-assisted selection. Currently, the BC2 generation is available.

The search of new virus-resistant sources is a critical step in plant breeding programs. In this work, we have determined a monogenic recessive genetic control of resistance to *Tomato leaf curl New Delhi virus* (ToLCNDV) derived from the *Cucurbita moschata* Spanish landrace AN-CU-45. The same genetic control has been previously reported in the *C. moschata* Indian landrace PI 381814. Moreover, the SNPs previously reported as linked to resistance derived from PI 381814 were also associated with the ToLCNDV resistance from AN-CU-45. In any case, the use of AN-CU-45 facilitates the introgression of the resistance in traditional backgrounds. Currently, we are introgressing AN-CU-45-derived resistance in some susceptible C. pepo accessions, using marker-assisted selection.

Global analysis of transcriptional and post-transcriptional response to tomato leaf curl New Delhi virus infection in resistant and susceptible melon genotypes

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The Spanish strain of tomato leaf curl New Delhi virus (ToLCNDV) has become one of the main viral diseases threatening cucurbit crops in the Mediterranean Basin. In recent studies, we performed a transcriptional analysis of the response to ToLCNDV in resistant (WM-7) and susceptible (Piel de Sapo, PS) melon (*Cucumis melo*) genotypes. This study provided novel insights about the host genes involved in the plant infection response as well as candidate genes linked to the resistance.

To extend this knowledge, here we present an integrative analysis of the timing and intensity of the alterations associated with ncRNA expression and Differential Exons Usage (DEU) in response to ToLCNDV in both susceptible and resistant varieties. Compared with mock-inoculated controls, obtained results support that virus infection induced changes in the accumulation of long ncRNAs transcripts in both analyzed genotypes, although this response was more relevant in the resistant WM-7 plants. Furthermore, a differential trend for DEU in both WM-7 and PS varieties was also observed associated with ToLCNDV infection. Interestingly, significant DEU was identified in WM-7 infected plants affecting transcripts derived from candidate genes linked to ToLCNDV resistance in melon.

Altogether, our results suggest that the resistant response to ToLCNDV infection in melon might comprise multilayered regulatory processes, involving not only transcriptional reprogramming after virus infection but also ncRNA-mediated pathways and DEU.

Differentially expressed genes in melon lines resistant and susceptible to WMV

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Watermelon mosaic virus (WMV) is one of the most damaging viruses affecting cucurbits worldwide. Recessive resistance to WMV has previously been reported in the melon (Cucumis melo L.) African accession TGR-1551. The resistance gene wmv1551 was mapped to a 150 kb region in chromosome 11, where 11 predicted genes were annotated. An RNA-seq analysis was performed to compare the response after inoculation with WMV of the susceptible cultivar Bola de oro (BO) and the resistant RIL-10-3 derived from the cross between TGR-1551 and BO. A differential expression analysis was performed with both DESEQ2 and edgeR methods and a consensus list of 616 differentially expressed genes (DEGs) was obtained. Three DEGs located within the interval of the major QTL were up-regulated in the resistant genotype when compared to the susceptible accession. Those DEGs coded a basic 7S globulin-like protein, a dual specificity protein phosphatase and a mediator of RNA polymerase II transcription subunit, respectively. Other DEGs involved in responses to biotic stresses were also up-regulated in the resistant RIL-10-3, including susceptibility and transcription factors, ubiquitination genes, as well as genes related to hormonal signaling. Moreover, SNPs with a predicted high impact effect over the protein function were located within the coding sequences of most DEGs. The results obtained revealed that the resistance to WMV derived from TGR-1551 entailed a complex transcriptomic remodeling and has provided several resistance candidate genes.

JoinTRV: a pLX-based T-DNA vector system for simultaneous agro-infection of multipartite viruses and heritable CRISPR/Cas9 editing of plant genomes

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Plant viral vectors have been exploited in applications such as metabolic engineering, pharmaceutical production, accelerated breeding, and transient crop reprograming. [1,2]

>20% of plant viruses have two or more genomic segments.[1] *Tobravirus* comprises bipartite members, such as tobacco rattle virus (TRV), that have been engineered as biotechnology tools widely used by plant scientists.[3]

We report herein the use of T-DNA vectors with compatible replication origins for simultaneous delivery of multipartite virus components. We designed JoinTRV, a one- *Agrobacterium*/two-vector system for simultaneous agro-inoculation of TRV RNA1 and RNA2. JoinTRV is based on pLX,[4] mini T-DNA vectors (~3 kb) suitable for *Agrobacterium*- mediated transformation which were used for agro-inoculation of RNA and DNA viruses as well as for development of a synthetic genomics framework with plant virome capacity.[5,6] JoinTRV allowed to launch TRV infection, robust virus-induced gene silencing, recombinant protein production, and CRISPR/Cas9-mediated somatic cell editing with ~90% efficiency. Remarkably, our system allowed heritable, tissue culture-free rescue of mutant progeny from Cas9-transgenic plants inoculated with JoinTRV expressing sgRNAs fused to cell-to-cell mobile sequences.[7]

Finally, JoinTRV and the approach described here expand the virus vector engineering toolbox and simplify plant cell delivery of multi-component viral systems and genome editing reagents.

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