TESIS DOCTORAL
Cristina Sáez Sánchez

MEJORA GENÉTICA DE LA RESISTENCIA AL *tomato leaf curl New Delhi virus* EN CUCURBITÁCEAS

2020

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

COMAV
Instituto de Conservación y Mejora de la Agrodiversidad Valenciana

TESIS DOCTORAL/DOCTORAL THESIS

MEJORA GENÉTICA DE LA RESISTENCIA AL *tomato leaf curl New Delhi virus* EN CUCURBITÁCEAS

BREEDING FOR *tomato leaf curl New Delhi virus* RESISTANCE IN CUCURBITS

Presentada por:

Cristina Sáez Sánchez

Directores:

Dra. María Belén Picó Sirvent

Dr. Carmelo López del Rincón

Dra. María Ferriol Molina

Valencia, septiembre de 2020

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Programa de doctorado en Biotecnología

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

INSTITUTO UNIVERSITARIO DE CONSERVACIÓN Y MEJORA DE LA AGRODIVERSIDAD VALENCIANA (COMAV)

COMAV
Instituto de Conservación y Mejora de la Agrodiversidad Valenciana

TESIS DOCTORAL

MEJORA GENÉTICA DE LA RESISTENCIA AL *tomato leaf curl New Delhi virus* EN CUCURBITÁCEAS

DOCTORAL THESIS

BREEDING FOR RESISTANCE TO *tomato leaf curl New Delhi virus* IN CUCURBITS

Presentada por:

Cristina Sáez Sánchez

Directores:

Dra. María Belén Picó Sirvent
Dr. Carmelo López del Rincón
Dra. María Ferriol Molina

PARA OPTAR AL GRADO DE DOCTOR POR LA
UNIVERSITAT POLITÈCNICA DE VALÈNCIA

Valencia, septiembre de 2020

Dra. María Belén Picó Sirvent y Dr. Carmelo López del Rincón, Catedráticos de la Universitat Politècnica de València y miembros del Instituto de Conservación y Mejora de la Agrodiversidad Valenciana,

Dra. María Ferriol Molina, Profesora titular de la Universitat Politècnica de València y miembro del Instituto Agroforestal Mediterráneo,

CONSIDERAN: que la memoria titulada **"Mejora genética de la resistencia al *tomato leaf curl New Delhi virus* en cucurbitáceas"** que presenta Doña Cristina Sáez Sánchez, para optar al grado de Doctor por la Universitat Politècnica de València, ha sido realizada bajo su dirección en el Instituto Universitario de Conservación y Mejora de la Agrodiversidad Valenciana de la Universitat Politècnica de València, y reúne las condiciones adecuadas para constituir su tesis doctoral, por lo que AUTORIZAN a la interesada para su presentación.

Valencia, septiembre de 2020

Dra. María Belén Picó Sirvent	Dr. Carmelo López del Rincón	Dra. María Ferriol Molina

La Conselleria d'Educació, Investigació, Cultura i Esports (Generalitat Valenciana) y el Fondo Social Europeo (FSECV 2014-2020) han cofinanciado la contratación de la doctoranda como personal investigador de carácter predoctoral (ACIF/2016/188) y dos estancias predoctorales fuera de la Comunitat Valenciana (BEPFI/2017/011 y BEFPI/2018/028). La realización de esta tesis doctoral también se ha realizado en el marco de dos proyectos de investigación del Ministerio de Ciencia, Innovación y Universidades, con cofinanciación de fondos FEDER [Proyectos AGL2017-85563-C2-1-R y RTA2017-00061-C03-03 (INIA)] y del proyecto PROMETEO para grupos de excelencia (2017/078) de la Conselleria d'Educació, Investigació, Cultura i Esports (Generalitat Valenciana).

ÍNDICE

Abreviaturas

ABA: del inglés abscisic acid.
AGO: argonauta.
amiRNAs: del inglés artificial microRNAs.
AmLMV: del inglés *Amaranthus leaf mottle virus.*
ADP: del inglés adenosine diphosphate.
ANOVA: del inglés analysis of variance.
ARC: del inglés adaptor shared by APAF-1, R proteins and CED-4.
ARFs: del inglés auxin responsive factor.
ATG: autofagia.
ATP: del inglés adenosine triphosphate.
AUX: auxinas.
AWMV: del inglés *Algerian watermelon mosaic virus*.
BC: del inglés backcross.
BCTV: del inglés *Beet curly top virus.*
BGMV: del inglés *bean golden mosaic virus*.
bHLH: del inglés basic helix-loop-helix.
BLAST: del inglés basic local alignment search tool.
BnYDV: del inglés *bean yellow disorder virus.*
BPYV: del inglés *beet pseudo yellows virus*.
BRA: del inglés *brassinosteroid.*
BSA: del inglés *bulked segregant analysis.*
BSCTV: del inglés *beet severe curly top virus*
BYV: del inglés *beet yellows virus.*
CABYV: del inglés *cucurbit aphid-borne yellows virus.*
CC: del inglés coiled-coil.
CCYV*:* del inglés *cucurbit chlorotic yellows virus.*
CFMMV: del inglés c*ucumber fruit mottle mosaic virus.*
CGMMV: del inglés *cucumber green mottle mosaic virus*.
ChiLCVD: del inglés *chilli leaf curl virus disease*.
CIM: del inglés composite interval mapping.
CLCuBuV: del inglés *cotton leaf curl Burewala virus*.
CLCuD: del inglés *cotton leaf curl virus disease*.
CLCuMuV: del inglés *cotton leaf curl Multan virus*.
CLCuV: del inglés *cotton leaf curl virus.*
CLSV: del inglés *cucumber leaf spot virus.*
ClYVV: del inglés *clover yellow vein virus.*

CMD: del inglés *cassava mosaic disease.*
CMGs: del inglés *cassava mosaic geminiviruses*.
CMT: cromometilasas.
CMV: del inglés *cucumber mosaic virus*.
CP: del inglés coat protein.
CR: del inglés common region.
CRISPR: del inglés *clustered regularly interspaced short palindromic repeats.*
CRK: del inglés cysteine-rich receptor-kinase.
CTAB: del inglés cetyltrimethyl ammonium bromide.
CuABYV-HN: del inglés *cucumber aphid-borne yellows - Hainan virus.*
CuLCrV: del inglés *cucurbit leaf crumple virus.*
CuMoV: del inglés *cucumber mottle viru*s.
CVYV: del inglés *cucumber vein yellowing virus*.
CYP: del inglés *cyclophilin*.
CYSDV: del inglés *cucurbit yellow stunting disorder.*
DCL: del inglés dicer-like.
DEG: del inglés differentially expressed genes.
DNA: del inglés desoxiribonucleic acid.
DYMD: del inglés *dolichos yellow mosaic virus*.
eIF: del inglés eukaryotic translation initiation factor.
ELISA: del inglés enzyme-linked immunosorbent assay.
EMDV: del inglés *eggplant mottled dwarf virus.*
EPPO: del inglés European and Mediterranean Plant Protection Organization.
ERE: del inglés ethylene-responsive element.
ET: etileno.
ecoTILLING: del inglés ecotype targeting induced local lesions in genomes.
GA: del inglés giberellic acid.
GBS: del inglés genotyping-by-sequencing.
GO: del inglés gene ontology
GRIK: del inglés geminivirus Rep-interacting kinase.
GWAS: del inglés genome-wide association study.
HD-Zip: del inglés Homeodomain-leucine zipper.
HR: del inglés hypersensitive response.
HRM: del inglés High Resolution Melting.
HSP: del inglés heat shock protein.
ICTV: del inglés international committee on taxonomy of viruses.

ILs: del inglés introgression lines.
INDEL: inserción-deleción.
JA: del inglés jasmonic acid.
KGMMV: del inglés *kyuri green mottle mosaic virus.*
KW: Kruskal–Wallis.
LABYV: del inglés *luffa aphid-borne yellows virus.*
LCV-SP: del inglés *lettuce chlorosis virus*
LFA: del inglés lateral flow assay.
LIYV: del inglés *lettuce infectious yellows virus.*
LOD: del inglés likelihood ratio.
LOX: lipoxygenases.
LRR: del inglés leucine-rich repeat.
LTP: del inglés lipid transfer protein.
LYMV: del inglés *legumes yellow mosaic virus.*
M€: millones de euros.
MABYV: *del inglés melon aphid-borne yellows virus*.
MAGIC: del inglés multiparent advanced generation inter-cross populations.
MAPQ: del inglés mapping quality.
MAS: del inglés marker-assisted selection.
MCLCuV: del inglés *melon chlorotic leaf curl virus.*
MCM: del ingés minichromosome maintenance protein complex.
MEAM: del inglés middle east asian minor.
MED: del inglés mediterranean.
MET: del inglés methyltransferase.
miRNA: del inglés microRNA.
MNSV: del inglés *melon necrotic spot virus*
MP: del inglés movement protein.
mRNA: del inglés messenger RNA.
MYMIV: del inglés *mungbean yellow mosaic India virus.*
MYMV: del inglés *mungbean yellow mosaic virus*.
MYSV: del inglés *melon yellow spot virus.*
MWMV: del inglés *moroccan watermelon mosaic virus,*
NBS: del inglés Nucleotide Binding Site.
NGS: del inglés next generation sequencing.
NIK: del inglés NSP-interacting kinase.
NSP: del inglés nuclear shuttle protein.

NW: del inglés new world.
ORF: del inglés open reading frame.
PCR: del inglés polymerase chain reaction.
PABYV: del inglés *pepo aphid-borne yellows virus*
PepGMV: del inglés *pepper golden mosaic virus*.
PepLCV: del inglés *pepper leaf curl virus*.
PepMV: del inglés *pepino mosaic virus.*
PepYLCIV: del inglés *pepper yellow leaf curl Indonesia virus.*
PeVYV: del inglés *pepper vein yellows virus.*
PHYVV: del inglés *pepper huasteco yellow vein virus*.
PMoV: del inglés *parietaria mottle virus.*
PMMoV: del inglés *pepper mild mottle virus.*
PR: del inglés pathogenesis-related.
PRR: del inglés *surface localized pattern recognition receptors*.
PRSV: del inglés *papaya ringspot virus.*
PS: Piñonet Piel de Sapo.
PTGS: del inglés post-transcriptional gene *silencing.*
PVY: del inglés *potato virus* Y.
QTLs: del inglés quantitative trait loci.
RAPD: del inglés random amplified polymorphic DNA.
RDDs: del inglés RNA-Dependent DNA Polymerases.
RdDM: del inglés RNA-directed DNA methylation.
RDRs: del inglés RNA dependent RNA polymerases.
REn: del inglés replication accessory protein.
Rep: del inglés replication initiator protein.
RFLP: del inglés restriction fragment length polymorphism.
RILs: del inglés recombinant inbred lines.
RING: del inglés really interesting new gene.
RISC: del inglés RNA-induced silencing complex.
RLK: del inglés receptor-like kinases.
RNA: del inglés ribonucleic acid.
RNAi: del inglés RNA interference.
RNA-seq: del inglés RNA sequencing.
ROS: del inglés reactive oxygen species.
SA: del inglés salicylic acid.
SABYV: del inglés *suakwa aphid-borne yellows virus.*

SAM: del inglés S-adenosyl methionine.
SBMV: del inglés *southern bean mosaic virus.*
SGS3: del ingles suppressor of gene silencing 3.
siRNA: del inglés small interfering RNA.
SLCV: del inglés *squash leaf curl virus.*
SMLCV: del inglés *squash mild leaf curl virus.*
SMRT: del inglés single-molecule real-time sequencing.
SNF: del inglés sucrose non-fermenting.
SNP: del inglés single-nucleotide polymorphism.
SnRK1: del inglés SNF1-related protein kinase 1.
SPLCV: del inglés *sweet potato leaf curl virus*.
SqVYV: del inglés *squash vein yellowing virus*.
SRAP: del inglés sequence-related amplified polymorphism.
SSR: del inglés simple sequence repeats.
STV: del inglés *southern tomato virus.*
SUMO: del inglés small ubiquitin-like modifier.
SYMMoV: del inglés *squash yellow mild mottle virus.*
TAE: Tris Acetato-EDTA
TBSV: del inglés *tomato bushy stunt virus.*
TF: del inglés transcription factor.
TGS: del inglés transcriptional gene *silencing*.
TICV: del inglés *tomato infectious chlorosis virus.*
TILLING: del inglés targeting induced local lesions in genomes.
TIR: del inglés toll e interleukin receptor.
TMG-1: Taichung Mou Gua.
TMGMV: del inglés *tobacco mild green mosaic virus.*
TMV: del inglés *tobacco mosaic virus.*
ToBRFV: del inglés *tomato brown rugose fruit virus.*
ToCV: del inglés *tomato chlorosis virus.*
ToLCGV: del inglés *tomato leaf curl Gujarat virus*.
ToLCNDV: del inglés *tomato leaf curl New Delhi virus.*
ToLCNDV-ES: aislado español del *tomato leaf curl New Delhi virus.*
ToLCV: del inglés *tomato leaf curl virus.*
ToMV: del inglés *tomato mosaic virus.*
ToTV: del inglés *tomato torrado virus.*
TrAP: del inglés transcriptional activator protein.

TRSV: del inglés *tobacco ringspot virus.*
TSWV: del inglés *tomato spotted wilt virus.*
TuMV: del inglés *turnip mosaic virus.*
TYLCAxV: del inglés *tomato yellow leaf curl Axarquia virus.*
TYLCMaV: del inglés *tomato yellow leaf curl Malaga virus.*
TYLCSV: del inglés *tomato yellow leaf curl Sardinia virus.*
TYLCV: del inglés *tomato yellow leaf curl virus.*
UPS: del inglés ubiquitin-proteasome system.
USDA-ARS: del inglés U.S. Department of Agriculture-Agricultural Research Service.
UTR: del inglés untranslated region.
UV: del inglés ultra-violet.
WBNV: del inglés *watermelon bud necrosis orthotospovirus*.
WGMMV: del inglés *watermelon green mottle mosaic virus.*
WmCSV: del inglés *watermelon chlorotic stunt virus.*
WMV: del inglés *watermelon mosaic virus.*
ZGMMV: del inglés *zucchini green mottle mosaic virus.*
ZTMV: del inglés *zucchini tigre mosaic virus*.
ZYFV: del inglés *zucchini yellow fleck virus.*
ZYFV: del inglés z*ucchini yellow fleck virus.*
ZYMV: del inglés z*ucchini yellow mosaic virus.*

Summary
Resum
Resumen

SUMMARY

The emergence of new viruses in crops of the Southeastern Spain is promoted by different factors. Climatic changes, global commercial networks and the intensive agricultural model stablished in the last years threaten this growing region, the main supplier of vegetables and fruits to the rest of European countries. A new strain of *tomato leaf curl New Delhi virus* (ToLCNDV) is among the most recent emergent viral diseases in this farming area, a bipartite begomovirus naturally transmitted by whiteflies (*Bemisia tabaci*). This strain was first detected in 2012 in the provinces of Murcia and Almeria, from where it propagated to other Mediterranean countries. ToLCNDV generates severe damages in cucurbits, decreasing yields mainly in squash (*Cucurbita pepo*) and melon (*Cucumis melo*) crops. Among control strategies to limit the disease incidence, the use of genetic resistances offers a high level, long term and efficient protection to crops. In this doctoral thesis, a breeding program has been developed to obtain cucurbit varieties with resistance to the Spanish strain of ToLCNDV(-ES).

Although cucurbit germplasm with resistance genes to this virus is poor and localized in few accessions, in this work we have identified for the first-time resistance sources to ToLCNDV in *Cucurbita* spp. and cucumber (*Cucumis sativus*). All resistant accessions here described are landraces or wild types not domesticated or with low levels of genetic manipulation. Moreover, most of these genotypes are originals from India, where ToLCNDV was first described. Likely, the occurrence of co-evolution events took place between wild cucurbits plants and this begomovirus.

Despite not identifying resistance sources in squash, two *Cucurbita moschata* accessions have shown high resistance levels. Both species present intermediate crossability, even so the obtention of interspecific offspring and the partial transference of the resistance was possible. The resistant accessions identified in the works of this thesis and those identified by our group in previous assays, have been characterized to determine the heritage controlling ToLCNDV resistance.

The development of breeding segregating populations and exploiting genetic tools for mapping and cartography, we identified three QTLs controlling resistance to ToLCNDV in melon, one partially dominant with mayor effect in chromosome 11, and two minor modifiers in chromosomes 2 and 12. Epistatic interactions between them have been detected. Following the same strategy, we have also identified a locus with recessive heritage in chromosome 8 of *C. moschata*. However, when we tried to transfer it to *C. pepo* incomplete penetrance occurrence was observed, reflecting the influence of the genetic background in the trait. The genomic regions identified in both species are synthenic and share a common cluster of genes not described previously in cucurbits conferring resistance to other viruses.

The advances in next generation sequencing technologies have offered us a huge quantity of genomic and transcriptomic information. Genotyping by SNPs collections allowed the identification of molecular markers linked to ToLCNDV resistance in melon, pumpkin and squash. These markers suppose a valuable resource in breeding programs, since can be used to assist the transference of the identified resistances to commercial genetic backgrounds.

To further understand the molecular mechanism regulating the resistance, we have performed an RNA sequencing assay (RNAseq), comparing transcriptomes between a resistant and a susceptible accession of *C. melo* in the time course of ToLCNDV infection. The results obtained from the analysis of differential expression levels are compatible whit a complex transcriptional regulation.

Even though the virus is replicated in the resistant melon accession at the beginning of the infection, deregulation of genes involved in hormonal jasmonic signaling, transmembrane transport, photosynthesis and transcription factors reduce the systemic infection in plant. Furthermore, in both resistant and susceptible genotypes, we observed expression changes of genes described in DNA methylation pathway. This suggests that RNA silencing might be the responsible mechanisms of inhibit viral genome transcription, enhancing the resistance response.

RESUM

Els canvis climàtics que succeeixen al nostre planeta, les xarxes comercials que interconnecten els mercats globalment i el model d'agricultura intensiva instaurat durant les últimes dècades, afavoreixen l'emergència de noves virosis en els cultius del sud-est espanyol, principal regió proveïdora de fruites i hortalisses a la resta de països europeus. Entre les virosis emergents més recents en aquesta àrea de cultiu, es troba un nou aïllat del *tomato leaf curl New Delhi virus* (ToLCNDV), un begomovirus de genoma bipartit transmés per mosques blanques (*Bemisia tabaci*). En 2012 es va detectar per primera vegada a les províncies de Múrcia i Almeria, i des d'aleshores s'està propagant per altres països de la conca del Mediterrani, generant greus danys en els cultius de cucurbitàcies, i limitant la producció, principalment, de carabasseta (*Cucurbita pepo*) i meló (*Cucumis melo*). Entre les estratègies per a controlar la malaltia i disminuïr la seua incidència, la utilització de resistències genètiques ofereix un elevat nivell de protecció per als cultius de forma durable i eficient. En aquesta tesi doctoral s'ha desenvolupat un programa de millora genètica enfocat a l'obtenció de varietats de cucurbitàcies resistents a l'aïllat espanyol del ToLCNDV(-ES).

Encara que el germoplasma amb gens de resistència al virus es escàs i està localitzat en poques accesions, en aquest treball hem pogut identificar per primera vegada fonts de resistència al ToLCNDV en el gènere *Cucurbita* i en cogombre (*Cucumis sativus*). Totes les accesions resistents que descrivim pertanyen a tipus silvestres o varietats locals, en què el nivell de domesticació o manipulació genètica és reduït. A més, la major part d'aquestos genotips són originaris de l'Índia, on es va descriure per primera vegada el ToLCNDV. És possible que en aquesta àrea s'haja produït un fenomen de coevolució entre plantes silvestres de cucurbitàcies i aquest begomovirus.

Tot i que no hem identificat fonts de resistència en carabasseta, dos accesions de *Cucurbita moschata* sí que han mostrat un elevat nivell de resistència, i tot i la compatibilitat intermèdia que presenten ambdues espècies, ha sigut possible

l'obtenció de descendència interespecífica i la transferència parcial de la resistència. S'han caracteritzat les accesions resistents identificades als treballs d'aquesta tesi i les identificades pel nostre grup en assajos previs, per tal de determinar el tipus d'herència que regula la resistència al ToLCNDV.

Mitjançant el desenvolupament de poblacions segregants de millora i l'aprofitament de ferramentes genètiques de mapeig i cartografia, hem identificat tres QTLs que controlen la resistència al ToLCNDV en meló, un d'efecte major i herència parcialment dominant en el cromosoma 11 i dos que modifiquen el seu efecte mitjançant interaccions epistàtiques als cromosomes 2 i 12. Seguint aquesta estratègia, també hem identificat un locus de resistència recessiva a ToLCNDV en el cromosoma 8 de C. moschata, encara que la penetració incompleta quan s'intenta transferir a *C. pepo* posa de manifest la influència del fons genètic en el caràcter. Les regions genòmiques involucrades en la resistència de les dos espècies són sintèniques i agrupen un conjunt de gens comuns no descrits prèviament en la resistència a altres virus en cucurbitàcies.

Els avanços en el desenvolupament de les noves tecnologies de seqüenciació massiva han posat al nostre abast una gran quantitat d'informació genòmica i transcriptòmica. Els genotipats mitjançant col·leccions de SNPs han permés identificar marcadors moleculars lligats a la resistència al ToLCNDV en meló, carabassa i carabasseta. Aquests marcadors suposen un valuós recurs en programes de millora, ja que permeten transferir de forma assistida les resistències identificades a fons genètics comercials.

Per tal d'aprofundir en el coneixement dels mecanismes moleculars que donen lloc a la resistència, hem dut a terme un assaig de seqüenciació del RNA (RNA-seq), i així comparar els transcriptomes d'un genotip resistent i altre susceptible de meló durant l'infecció amb ToLCNDV. Els resultats obtinguts en analitzar l'expressió diferencial són compatibles amb el tipus d'herència quantitativa de la resistència i reflecteixen un complex sistema de regulació transcripcional.

Encara que inicialment el virus pot replicar-se en l'accesió resistent de meló, la desregulació de gens implicats en la ruta de senyalització hormonal de l'àcid jasmònic, transportadors transmembrana, fotosíntesi i factors de transcripció, redueix la infecció sistèmica en la planta. A més, hem observat canvis en l'expressió de gens implicats en la metilació del DNA, tant en el genotip resistent com susceptible, el que suggereix que el silenciament gènic mediat per RNA pot estar involucrat en la inhibició de la transcripció del genoma viral, afavorint la resistència.

RESUMEN

Los cambios climáticos que acontecen en nuestro planeta, las redes comerciales que interconectan los mercados globalmente y el modelo de agricultura intensiva instaurado en las últimas décadas, favorecen la emergencia de nuevas virosis en los cultivos del sudeste español, principal región abastecedora de frutas y hortalizas al resto de países europeos. Entre las virosis emergentes más recientes en esta área de cultivo, se encuentra un nuevo aislado del *tomato leaf curl New Delhi virus* (ToLCNDV), un begomovirus de genoma bipartito transmitido por moscas blancas (*Bemisia tabaci*). En 2012 se detectó por primera vez en las provincias de Murcia y Almería, y desde entonces se está propagando por otros países de la cuenca del Mediterráneo, generando graves daños en los cultivos de cucurbitáceas, y limitando la producción, principalmente, de calabacín (*Cucurbita pepo*) y melón (*Cucumis melo*). Entre las estrategias para controlar la enfermedad y disminuir su incidencia, el empleo de resistencias genéticas ofrece un elevado nivel de protección para los cultivos de manera durable y eficiente. En esta tesis doctoral se ha desarrollado un programa de mejora genética enfocado a la obtención de variedades de cucurbitáceas resistentes al aislado español del ToLCNDV(-ES).

Aunque en cucurbitáceas el germoplasma con genes de resistencias al virus es escaso y localizado en pocas accesiones, en este trabajo hemos identificado por primera vez fuentes de resistencia al ToLCNDV en el género *Cucurbita* y en pepino (*Cucumis sativus*). Todas las accesiones resistentes que describimos pertenecen a tipos silvestres o variedades locales, en las que el nivel de domesticación o manipulación genética es reducido. Además, la mayor parte de estos genotipos son originarios de la India, donde se describió por primera vez el ToLCNDV. Es posible que en esta área se haya producido un fenómeno de co-evolución entre plantas silvestres de cucurbitáceas y este begomovirus.

A pesar de que no hemos identificado fuentes de resistencia en calabacín, dos accesiones de *Cucurbita moschata* sí han mostrado un elevado nivel de resistencia, y aunque ambas especies presentan cruzabilidad intermedia, ha sido posible la

obtención de descendencia interespecífica y la transferencia parcial de la resistencia. Las accesiones resistentes identificadas en los trabajos de esta tesis y las identificadas por nuestro grupo en ensayos previos, se han caracterizado para determinar el tipo de herencia que regula la resistencia al ToLCNDV.

Mediante el desarrollo de poblaciones segregantes de mejora y el aprovechamiento de herramientas genéticas de mapeo y cartografía hemos identificado tres QTLs que controlan la resistencia al ToLCNDV en melón, uno de efecto mayor y herencia parcialmente dominante en el cromosoma 11 y dos que modifican su efecto mediante interacciones epistáticas en los cromosomas 2 y 12. Siguiendo esta estrategia, también hemos identificado un *locus* de resistencia recesiva a ToLCNDV en el cromosoma 8 de *C. moschata*, aunque la penetración incompleta cuando se intenta transferir a *C. pepo* pone de manifiesto la influencia del fondo genético en el carácter. Las regiones genómicas involucradas en la resistencia de ambas especies son sinténicas y agrupan un conjunto de genes comunes no descritos previamente en la resistencia a otros virus en cucurbitáceas.

Los avances en el desarrollo de las nuevas tecnologías de secuenciación masiva han puesto a nuestro alcance una gran cantidad de información genómica y transcriptómica. Los genotipados mediante colecciones de SNPs han permitido identificar marcadores moleculares ligados a la resistencia al ToLCNDV en melón, calabaza y calabacín. Estos marcadores suponen un valioso recurso en programas de mejora, ya que permiten transferir de manera asistida las resistencias identificadas a fondos genéticos comerciales.

Para profundizar en el conocimiento de los mecanismos moleculares que dan lugar a la resistencia, hemos llevado a cabo un ensayo de secuenciación del RNA (RNA-seq), para comparar los transcriptomas de un genotipo resistente y otro susceptible de melón durante la infección con ToLCNDV. Los resultados obtenidos al analizar la expresión diferencial son compatibles con el tipo de herencia cuantitativa de la resistencia y reflejan un complejo sistema de regulación transcripcional.

Aunque inicialmente el virus puede replicarse en la accesión resistente de melón, la desregulación de genes implicados en la ruta de señalización hormonal del ácido jasmónico, transportadores transmembrana, fotosíntesis y factores de transcripción, reduce la infección sistémica en la planta. Además, hemos observado cambios en la expresión de genes implicados en la metilación del DNA, tanto en el genotipo resistente como susceptible, lo que sugiere que el silenciamiento génico mediado por RNA puede estar involucrado en la inhibición de la transcripción del genoma viral, favoreciendo la resistencia.

Introducción

1. LA FAMILIA DE LAS CUCURBITÁCEAS

1.1. Importancia económica

Las cucurbitáceas (*Cucurbitaceae*) integran una de las familias botánicas más diversas y destacables en la horticultura por su amplio consumo y cultivo, encontrándose adaptadas a diferentes regiones geográficas de todo el mundo (McCreigh, 2016; Chikh-Ali et al., 2019). Las especies cultivadas de esta familia generan una gran rentabilidad económica a nivel global, que solo superan algunas de las especies de la familia de las solanáceas (García-Mas & Puigdomènech, 2016).

Su gran variabilidad genética en características de fruto y planta, así como su valioso perfil nutricional rico en carbohidratos, fibra, vitaminas, minerales (calcio, hierro, fósforo, etc.) y antioxidantes (licopeno, β-carotenos, etc.) han propiciado un amplio rango de usos culinarios y culturales (Bisognin, 2002; Ozaslan et al., 2006; Ferriol & Picó, 2008; McCreigh, 2016). Entre los cultivos más importantes dentro de esta familia destacan el melón (*Cucumis melo* L.), el pepino (*Cucumis sativus* L.), el calabacín y las calabazas (*Cucurbita* spp.) y la sandía (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), con una producción mundial en 2018 que superaba los 27, 75, 27 y 103 millones de toneladas respectivamente, según la Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAOSTAT, 2018). En la Figura 1 se muestran los diez países que mayores producciones generan de los cuatro principales cultivos de cucurbitáceas.

La cuenca del Mediterráneo es uno de los principales centros de cultivo de estas cuatro especies, donde en 2018 se cultivaron 923.078 ha que generaron una producción de unos 27 millones de toneladas, casi el 30% de la producción mundial si se excluye China, principal productor global de los cuatro cultivos (Figura 1) (FAOSTAT, 2018). En esta región, España es el tercer productor de melón (664.353 t), el segundo de pepino (643.621 t), el primero de calabazas y calabacines (717.645 t) y el cuarto de sandías (1.092.401 t) (FAOSTAT, 2018), siendo el primer exportador mundial en cifras económicas de melón, pepino y sandía (FAOSTAT, 2017).

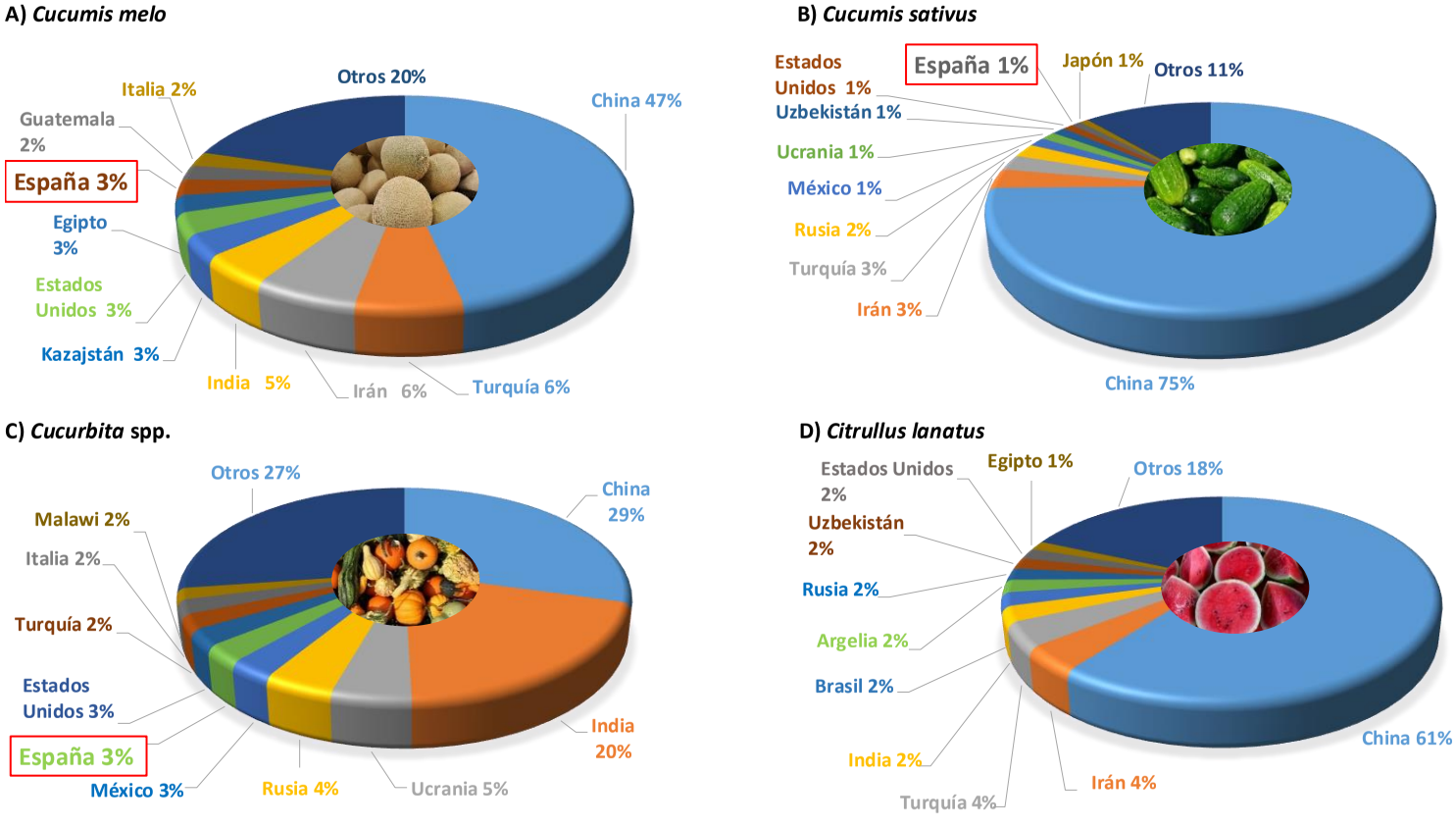

Figura 1. Ranking de los diez principales países productores de melón (A), pepino (B), calabazas y calabacines (C) y sandía (D) en el mundo. Los porcentajes indican la proporción producida por cada país respecto a la producción total mundial. Elaboración propia en base a los datos de FAOSTAT (2018).

En España, la producción de melones al aire libre en cultivo extensivo se concentra en la comunidad de Castilla la Mancha, que produjo casi un tercio del total de la producción nacional en 2017 (MAPA, 2017), mientras que la producción de melón protegido se concentra en la Región de Murcia y la provincia de Almería. Del total de la producción nacional de melón, aproximadamente el 60% se destina a exportación, con destino mayoritario a países de la Unión Europea (MAPA, 2019), siendo las provincias de Murcia y Almería las principales exportadoras nacionales. (Sólo en la provincia de Almería las exportaciones de melón generaron un valor de 55 M€ (Figura 2A).

Respecto a la producción española de pepino y sandía, la mayor parte proviene de cultivo protegido en la provincia de Almería (FAOSTAT, 2017), en la que se produjeron 443.604 t de pepino en la campaña 2017/2018 y 464.581 t de sandía en la campaña 2018/2019 (Observatorio de Precios y Mercados. Junta de Andalucía, 2019). El valor de las exportaciones ascendió a 438 M€ de pepino en la campaña 2017/2018 y 185 M€ de sandía en la campaña 2018/2019 (Figura 2B y D), siendo Alemania, Reino Unido, Francia y Países Bajos los principales mercados de destino.

Las cinco especies del género *Cucurbita* con elevado valor agroecónomico son *C. pepo* L., *C. moschata* Duchense, *C. maxima* Duchense, *C. argyrosperma* Huber y *C. ficifolia* Bouché (Montero-Pau et al., 2016). *Cucurbita pepo* engloba diferentes morfotipos, entre los que destaca el tipo Zucchini o calabacín, y cuyo cultivo se ha impuesto por encima del de las calabazas, produciéndose un 67% más en nuestro país (MAPA, 2017) y siendo muy demandado en países de toda Europa. La mayor parte de la producción en España se realiza en la provincia de Almería, que durante la campaña 2018/2019 exportó 312.468 t, generando un valor de 271 M€ (Observatorio de Precios y Mercados. Junta de Andalucía, 2019) (Figura 2C).

Además de su importancia económica y comercial, la producción de estos cuatro cultivos tiene una elevada incidencia social debido a su capacidad para generar empleo, concentrando una elevada mano de obra dentro del sector agrícola.

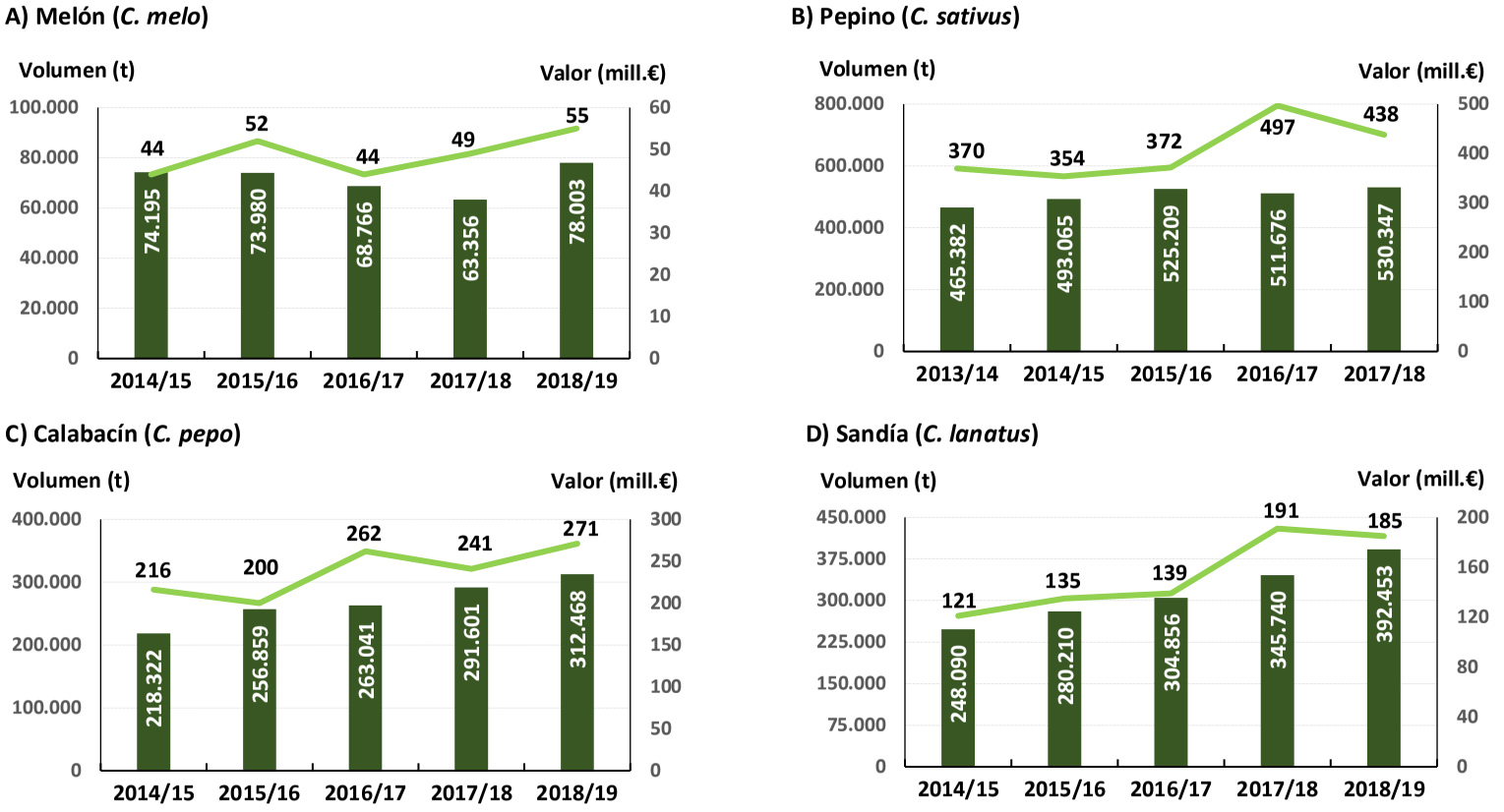

Figura 2. Evolución de las exportaciones de melón, pepino, calabacín y sandía en las últimas campañas procedentes de cultivo protegido en la provincia de Almería. Fuente: observatorio de precios y mercados de la Junta de Andalucía y DATACOMEX 2018/2019.

1.2. Principales limitaciones del cultivo de las cucurbitáceas

La rentabilidad de los cultivos de cucurbitáceas está limitada por diferentes factores bióticos y abióticos, que influyen en la producción, propiedades organolépticas o apariencia de estas hortalizas (Loebenstein & Katis, 2014; Lefebvre et al., 2020). La globalización de la agricultura ha permitido mejorar la calidad y el rendimiento de los cultivos de cucurbitáceas, que se han visto favorecidos por la introducción de nuevos métodos de producción agrícola. Entre ellos cabe destacar el cultivo protegido en invernadero, el uso de túneles de cultivo, la digitalización de las explotaciones agrícolas o el uso de semillas híbridas comerciales, que permiten realizar varios ciclos de cultivo en una misma campaña, aumentar los rendimientos y disminuir los insumos y la superficie cultivada (Pitrat, 2012; Jones & Naidu, 2019). Este intercambio global de técnicas y materiales vegetales, sumado al cambio de las condiciones climáticas del planeta, ha generado nuevos retos en la producción agrícola de las cucurbitáceas (Chakraborty & Newton, 2011; Lecoq & Katis, 2014).

La adaptación a nuevos estreses abióticos, como climas extremos, sequía y suelos deficientes en nutrientes suponen un desafío para la agricultura del futuro, pero, además, el cambio en los ecosistemas y la pérdida de diversidad está favoreciendo la emergencia y diseminación de plagas y enfermedades que afectan a las cucurbitáceas (Khoury et al., 2020).

1.2.1. Plagas

Entre las principales plagas que generan daños en cultivos de cucurbitáceas destacan las arañas rojas (*Tetranychus urticae* Koch y *Oligonychus mexicanus* McGregor y Ortega), los pulgones (*Aphis gossypii* Glover y *Myzus persicae* Sulzer), las moscas blancas (*Bemisia tabaci* Gennadius y *Trialeurodes vaporariorum* Westwood), nemátodos (*Meloidogyne* spp., *Xiphinema americanum* Cobb y *Xiphinema rivesi* Dalmaso) y los trips (*Frankliniella occidentalis* Pergande y *Thrips palmi* Karny), que merman la producción al alimentarse de la planta y además pueden actuar como vectores de transmisión de diferentes virus (Messelink et al., 2020).

1.2.2. Hongos

En términos económicos globales, las enfermedades fúngicas son las que mayores pérdidas generan en las cosechas, seguidas por las de etiología viral (Loebenstein & Katis, 2014). Las principales enfermedades causadas por hongos que limitan el cultivo de las cucurbitáceas están generadas por el oídio [*Podosphaera xanthii* (Castagne) U. Braun y N. Shish. y *Golovinomyces orontii* (Castagne) Heluta], el mildiu [*Pseudoperonospora cubensis* (Berk. & Curt.) Rost], el hongo de la podredumbre del fruto (*Phytophthora capsici* Leonian), didymella [*Didymella bryoniae* (Auersw.) Rehm], colapso [*Monosporascus cannonballus* Pollack & Uecker (*Diatrypaceae*)], mycospharella [*Mycosphaerella citrullina* (C.O. Smith) Grossenbacher], el chancro del tallo [*Macrophomina phaseolina* (Tassi) Goid (*Botryosphariecеae*)] y fusariosis [*Fusarium oxysporum* f. sp. *melonis* (Leach & Currence) W.C. Snyder & H.N. Hansen, *F. oxysporum* f.sp. *niveum* (E.F. Smith) W.C. Snyder & H.N. Hansen, *F. oxysporum* f.sp. *cucumerinum* J.H. Owen y *F. solani* (Mart.) Sacc. f.sp. *cucurbitae* W.C. Snyder and H.N. Hansen] (González et al., 2020a). Entre los hongos del suelo, las fusariosis vasculares en cucurbitáceas generan pérdidas de producción devastadoras (Gómez et al., 2014), al provocar lesiones en la raíz y cuello que pueden llegar a inducir el colapso y muerte de la planta (Egel & Martyn, 2007). Recientemente, se ha detectado una nueva fusariosis del complejo de especies *Fusarium solani* [*Neocosmospora falciformis* (Carrión) L. Lombard] afectando a raíces de melón en el territorio nacional (González et al., 2020b).

El oídio genera una de las enfermedades fúngicas foliares más importantes en cultivos de cucurbitáceas, tanto en invernadero como en campo abierto, al impedir el correcto desarrollo de la planta generando mermas de rendimiento y calidad (Lebeda, et al., 2016). Sin embargo, ya se han descrito resistencias genéticas para esta enfermedad causada por hongos en melón (Oumouloud et al., 2013; Hong et al., 2015; Elkabetz et al., 2016; Li et al., 2017a; Zhu et al., 2018; Howlader et al., 2020), pepino (Dong et al., 2109; Chen et al., 2020), *Cucurbita* spp. (Cohen et al., 2003;

Dhillon et al., 2019; Guo et al., 2019a) y sandía (Kim et al., 2013, 2015; Wechter et al., 2012, 2016; Branham et al., 2017).

1.2.3. Virosis que afectan al cultivo de las cucurbitáceas

Entre los patógenos más importantes que afectan a la agricultura destacan los virus de plantas, tanto por la diversidad de enfermedades que pueden inducir como por la dificultad para combatirlos (Gómez et al., 2009; Rao & Reddy, 2020). Lecoq y Katis (2014) estimaron que del total de cucurbitáceas que se cultivan en el mundo, el 1% estaría sufriendo una infección viral, lo que equivaldría a mil toneladas de partículas víricas anuales en cultivos de cucurbitáceas a nivel global.

Actualmente hay descritas más de 70 especies de virus que infectan cultivos de cucurbitáceas (Lecoq & Katis, 2014). A nivel mundial, los virus más importantes son el virus del mosaico de la sandía (*watermelon mosaic virus*, WMV), el virus de la mancha anular de la papaya (*papaya ringspot virus*, PRSV), el virus del mosaico amarillo del calabacín (z*ucchini yellow mosaic virus*, ZYMV) y el virus del mosaico del pepino (*cucumber mosaic virus*, CMV), que pueden llegar a generar pérdidas completas de cosechas y una elevada disminución del valor comercial de la producción (Pozzi et al., 2020). Otros virus tienen una distribución geográfica más limitada, como el virus de las venas amarillas del calabacín (*squash vein yellowing virus*, SqVYV) o el virus de la mancha amarilla del melón (*melon yellow spot virus*, MYSV), que generan un impacto económico menor (Lecoq & Katis 2014; Chikh-Ali et al., 2019). En la cuenca del Mediterráneo se han identificado hasta 28 virus diferentes infectando cucurbitáceas (Lecoq & Desbiez, 2012).

Los principales transmisores de virus a cucurbitáceas son pulgones (apartado 1.2.3.1) y moscas blancas (apartado 1.2.3.2) (Thakur et al., 2019), y son considerados una grave amenaza por su elevada capacidad destructiva en estos cultivos hortícolas. En algunos casos, otros tipos de transmisión también generan importantes pérdidas económicas en cucurbitáceas. Así, los virus del género *Tobamovirus* (familia *Virgaviridae*) se transmiten por contacto directo o por semilla (Cheng et al., 2019) (Tabla 1), y entre ellos, el virus del mosaico verde jaspeado del pepino (*cucumber*

green mottle mosaic virus, CGMMV) ha dado lugar a graves pérdidas en las cosechas de sandía de diferentes partes del mundo, debido al intercambio de semillas o portainjertos contaminados entre países (Boubourakas et al., 2004). Algunos coleópteros de la familia *Chrysomelidae*, el hongo *Olpidium radicale* Schwartz y Ivimey Cook, nemátodos, trips o saltamontes (*Macrosteles quadrilineatus* Forbes) también son vectores que transmiten virosis a diferentes especies de cucurbitáceas (Freitag, 1956; Moore & McGuire, 1968; Li, et al., 2015; Melgarejo et al., 2010) (Tabla 1).

1.2.3.1. Virus transmitidos por pulgones

Entre los virus transmitidos por áfidos de manera no persistente, los del género *Potyvirus* (familia *Potyviridae*) se encuentran ampliamente distribuidos por todo el mundo y afectan a un diverso número de especies. Al menos 14 especies diferentes de potyvirus se han descrito infectando de manera natural cultivos de cucurbitáceas (Lecoq & Desbiez, 2012; Perotto et al., 2018) (Tabla 2).

Virus como ZYMV, WMV y PRSV generan epidemias devastadoras en los principales cultivos económicos de cucurbitáceas, debido a su alto ratio de diversificación genética (Lecoq et al., 1998; Desbiez et al., 2007; Sevik, 2017; Kaldis et al., 2018). Otro virus ampliamente distribuido es el CMV (género *Cucumovirus*, familia *Bromoviridae*). A principios del siglo XX se describieron por primera vez síntomas de mosaico en plantas de cucurbitáceas causados por CMV (Doolittle, 1916; Jagger, 1916). Actualmente, el virus se ha descrito en todo el mundo, generando pérdidas de cosechas que pueden llegar al 50% si se produce una infección en estadios jóvenes de la planta (Alonso-Prados et al., 1997; Köklü & Yilmaz, 2006, Jacquemond, 2012).

Los pulgones también pueden transmitir virus a cucurbitáceas de manera persistente (Tabla 2), como el CABYV (género *Polerovirus*, familia *Luteoviridae*), que fue descrito por primera vez infectando cultivos de cucurbitáceas al aire libre en Francia, y ocasionando pérdidas del 50% de frutos de pepino comercializable y reducciones del 40% de la producción de melón (Lecoq et al., 1992). Posteriormente,

Tabla 1. Principales virus transmitidos a cucurbitáceas por diferentes vectores, distribución, principales cultivos hospedantes y sintomatología.

Género (Familia)	Virus	Distribución	Hospedantes y sintomatología	
Transmitidos por contacto directo o por semilla				
Tobamovirus (*Virgaviridae*)	Virus del mosaico verde jaspeado del pepino (*cucumber green mottle mosaic virus*, CGMMV)	Mundial	Pepino, melón, sandía, calabacín y calabazas	En hojas moteado, mosaico, lesiones necróticas, abullonado y reducción del desarrollo. Frutos con malformaciones
	Virus del mosaico verde del kyuri (*kyuri green mottle mosaic virus*, KGMMV)	Japón, Corea e Indonesia	Pepino y melón	
	Virus del mosaico moteado de la fruta del pepino (*cucumber fruit mottle mosaic virus*, CFMMV)		Calabacín, sandía, pepino y calabazas	
	Virus del mosaico moteado verde del calabacín (*zucchini green mottle mosaic virus*, ZGMMV)			
	Virus del moteado del pepino (*cucumber mottle virus*, CuMoV)	Japón	Pepino	
	Virus del mosaico verde moteado de la sandía (*watermelon green mottle mosaic virus*, WGMMV)	Taiwán y Norte América	Sandía, calabaza, calabaza blanca (*Benincasa hispida* (Thunb.) Cogn.), calabacín, pepino y balsamina (*Momordica charantia L.*)	
Transmitidos por coleópteros (*Chrysomelidae*) **y semilla**				
Comovirus (*Secoviridae*)	Virus del mosaico de la calabaza (*squash mosaic virus*, SqMV)	Mundial	Melón, pepino, sandía y calabacín. Enanismo, clorosis, de formación de hojas, filimorfismo, manchas junto a los nervios. La piel de los frutos se motea y sufre malformaciones.	

Tabla 1. Continuación

Transmitidos por el hongo *Olpidium radicale* o por semilla			
Carmovirus (*Tombusviridae*)	Virus de las manchas necróticas del melón (*melon necrotic spot virus*, MNSV)	Mundial	Melón, sandía, pepino y calabacín. En hojas se producen lesiones necróticas que pueden generan marchitez o colapso de la planta. La superficie de los frutos se asurca y pueden aparecer manchas necróticas en la pulpa
Aureusvirus (Tombusviridae)	Virus de la mancha de la hoja del pepino (*cucumber leaf spot virus*, CLSV)	Alemania, Gran Bretaña, Jordania, Grecia, Arabia Saudí, Polonia, Bulgaria y España	Pepino. Manchas amarillentas en hojas con el centro necrosado que da lugar a plantas con mal desarrollo
Transmitidos por nemátodos (*Xiphinema americanum*)			
Nepovirus (Secoviridae)	Virus de la mancha anular del tabaco (*tobacco ringspot virus*, TRSV)	Mundial	Pepino. Hojas con mosaico intenso y enanismo de las plantas
	Virus de la mancha anular del tomate (*tomato ringspot virus*, ToRSV)	Mundial	Calabacín. Manchas cloróticas en forma de anillo
Transmitidos por saltamontes (*Macrosteles quadrilineatus* Forbes)			
-	Aster Yellow Mycoplasma	Mundial	Pepino. Hojas cloróticas y enanismo de la planta
Transmitidos por Trips			
Tospovirus (*Bunyaviridae*)	Virus del bronceado del tomate (*tomato spotted wilt virus*, TSWV)	Mundial	Sandía y melón. Hojas cloróticas y necrosis en las venas, con manchas necróticas en forma de anillo.
	Virus de la mancha amarilla del melón (*melon yellow spot virus*, MYSV)	Japón, Tailandia, Taiwán, China, Ecuador y Sudamérica	Sandía, melón, balsamina, pepino, calabaza y lufa. En hojas y frutos aparecen malformaciones, mosaicos, moteados y manchas necróticas.

Fuentes: Melgarejo et al., 2010; Sharma et al., 2016; Supakitthanakorn et al., 2018, CABI, 2020.

la enfermedad se ha descrito en cucurbitáceas a nivel global (Lecoq & Katis, 2014), siendo su presencia frecuente, aunque con baja incidencia en el rendimiento o la calidad de los cultivos (Pitrat, 2012).

1.2.3.2. Virus transmitidos por moscas blancas

Aunque las moscas blancas pueden dañar y debilitar los cultivos de las cucurbitáceas alimentándose por succión del floema de la planta, el principal daño que ocasionan es debido a la transmisión de virus. La mayor parte de virus transmitidos por moscas blancas pertenecen al género *Begomovirus* (familia *Geminiviridae*), y son transmitidos de manera persistente y circulativa (Rosen et al., 2015). En cucurbitáceas, los begomovirus identificados hasta el momento son de genoma bipartito (Idris et al., 2008; Rojas et al., 2018; Luria et al., 2019) y dan lugar a grandes pérdidas económicas tanto en el viejo como en el nuevo mundo, aunque en zonas geográficas separadas entre sí (Castro et al., 2013; Bhatt et al., 2019) (Tabla 3).

Los virus de los géneros *Crinivirus* (familia *Closteroviridae*) e *Ipomovirus* (familia *Potyviridae*) son transmitidos por moscas blancas de manera semipersistente. Desde comienzos del siglo XXI, los crinivirus se han propagado rápidamente y han generado pérdidas en cosechas de cucurbitáceas muy significativas (Abrahamian & Jawdah, 2014). Se han descrito cuatro crinivirus infectando cucurbitáceas (Tabla 3), pero el CYSDV ha sido el más destructivo de estos cultivos en muchas regiones del planeta (Abrahamian & Jawdah, 2014). El virus de la clorosis amarilla de las cucurbitáceas (*cucurbit chlorotic yellows virus*, CCYV) fue identificado por primera vez en 2009 en Japón y provoca daños especialmente en melón y pepino (Gyoutoku et al., 2009). Este virus se ha propagado por Asia, Medio Oriente (Hamed et al., 2011; Abrahamian et al., 2012) y recientemente se ha identificado en California y países Mediterráneos infectando cultivos de melón, pepino y calabacín (Wintermantel et al., 2019; Kheireddine et al., 2020).

Tabla 2. Principales virus transmitidos por pulgones que afectan a cucurbitáceas, distribución, principales cultivos hospedantes y sintomatología.

Género (Familia)	Virus	Distribución	Hospedantes y sintomatología
Transmitidos por áfidos de manera no persistente			
Potyvirus (*Potyviridae*)	Virus del mosaico amarillo del calabacín (*zucchini yellow mosaic virus*, ZYMV)	Mundial	Calabacín, melón, pepino y sandía. Hojas con ampollas, deformaciones, mosaico filimorfismo y enanismo de la planta. Frutos con decoloraciones, deformados, con pulpa endurecida y semillas deformadas
	Virus de la mancha amarilla del calabacín (*zucchini yellow fleck virus*, ZYFV)	Países de la cuenca Mediterránea	Pepino, melón, calabacín, sandía y calabaza. En hojas genera mosaico, punteado amarillo y lesiones necróticas
	Virus del mosaico tigre del calabacín (*zucchini tigre mosaic virus*, ZTMV)	Guadalupe (Francia), China, Florida y Hawai (EE.UU)	Calabacín, melón, balsamina (*Momordica charantia* L.). Hojas con mosaico, ampollas, clorosis y oscurecimiento de las venas
	Virus del mosaico de la sandía (*watermelon mosaic virus*, WMV)	Mundial	Todas las Cucurbitáceas cultivadas, principalmente *C. pepo*, *C. maxima* y *C. moschata*. Hojas deformadas con mosaicos moderados, ampollas y filimorfismo. Acortamiento internodal y poca incidencia en frutos
	Virus del mosaico de la sandía de Marruecos (*moroccan watermelon mosaic virus*, MWMV)	Países de la cuenca Mediterránea, Sudáfrica, Zimbabue, Níger, Camerún, Sudán y República Democrática del Congo	Calabacín, sandía, melón y pepino. En hojas clorosis internervial, mosaico, ampollas, deformaciones, necrosis y filiformismo. Plantas con mal desarrollo y frutos deformes con la superficie abultada
	Virus de la mancha anular de la papaya (*papaya ringspot virus*, PRSV)	Mundial	Calabacín, sandía, pepino y melón. Hojas deformes con mosaico intenso y frutos con pulpa grumosa y manchas anulares en la piel
	Virus del amarilleo de las venas del trébol (*clover yellow vein virus*, ClYVV)	Mundial, donde se cultiva el trébol	Calabacín y calabaza. Moteados amarillos en hojas

Tabla 2. Continuación

Cucumovirus (*Bromoviridae*)	Virus del mosaico del pepino (*cucumber mosaic virus*, CMV)	Mundial	Calabacín, calabaza, melón, pepino y sandía. Mosaico severo en hojas, deformadas y abullonadas. Plantas raquíticas y frutos deformes sin semilla	
Closterovirus (*Closteroviridae*)	Virus del amarilleo de la remolacha (*beet yellows virus*, BYV)	Mundial	Melón. Punteado en hojas, necrosis y amarilleos en nervios junto a clorosis	
Transmitidos por áfidos de manera persistente				
Polerovirus *(Luteoviridae)*	Virus del mosaico de la cabalaza transmitido por áfidos (*cucurbit aphid-borne yellows virus*, CABYV)	Mundial	Melón, sandía, pepino y calabacín	Hojas pequeñas con mosaico, amarilleos, deformación en los bordes y filimorfismo. Los frutos se deforman y asurcan
	Virus del mosaico del pepino transmitido por áfidos (*cucumber aphid-borne yellows - Hainan virus*, CuABYV-HN)	Asia	Pepino	
	Virus del mosaico de la lufa transmitido por áfidos (*luffa aphid-borne yellows virus*, LABYV)	Tailandia y China	Lufa	
	Virus del amarilleo del melón transmitido por áfidos (*melon aphid-borne yellows virus*, MABYV)	China, Filipinas y Tailandia	Sandía, calabaza blanca, calabaza del peregrino (*Lagenaria siceraria* (Molina) Standl.)	
	Virus del amarilleo de suakwa transmitido por áfidos (*suakwa aphid-borne yellows virus*, SABYV)	China, Filipinas, Tailandia, Taiwán y Australia	Calabaza suakwa *(Luffa aegyptiaca* Mill.)	
	Virus del mosaico del calabacín transmitido por áfidos (*pepo aphid-borne yellows virus*, PABYV)	Mali, Sudáfrica, Costa de Marfil, Grecia, Tanzania y Mayotte (Francia)	Calabacín, calabaza, sandía, melón	

Fuentes: Desbiez et al., 2016a; Ali, 2020; CABI, 2020; EPPO, 2020.

Entre los ipomovirus que afectan a cucurbitáceas, destacan el virus de las venas amarillas del pepino (*cucumber vein yellowing virus*, CVYV), que supuso un problema económico durante años (Cohen & Nitzany, 1960; Al-Musa et al., 1985; Louro et al., 2003), y el SqVYV, que llegó a generar pérdidas económicas en la producción de sandía de Florida que ascendían a 60 millones de dólares en la campaña 2004-2005 (Kousik et al., 2012a) (Tabla 3).

1.2.3.3. Infecciones mixtas de virus en cucurbitáceas

Aunque la mayoría de los estudios realizados sobre virus de cucurbitáceas tratan principalmente de infecciones simples, las evidencias muestran que en la naturaleza lo más habitual es encontrar infecciones mixtas (Syller, 2012; Moreno & López-Moya, 2020). La interacción por co-infección de diferentes virus puede agravar el desarrollo de los síntomas y dar lugar a una mayor acumulación viral en la planta (Syller, 2012; Tatineni et al., 2020). En cucurbitáceas se ha estudiado el efecto sinérgico de infecciones mixtas entre virus de diferentes géneros. La interacción entre CMV y dos potyvirus (ZYMV y WMV) se ha caracterizado en melón, calabacín, *C. máxima* Duchesne y sandía, observándose un cambio en los niveles de concentración de estos virus en la planta (Wang et al., 2002; Barbosa et al., 2016; Wang et al., 2019a). Abrahamian et al. (2015) determinaron un incremento en la severidad de síntomas en pepino tras infecciones mixtas de crinivirus y begomovirus. Recientemente, Domingo-Calap et al. (2020) han descrito cómo la infección conjunta de CYSDV y WMV en melón beneficia a ambos virus, favoreciendo y manteniendo su transmisión.

Las infecciones mixtas también favorecen la aparición de nuevos aislados recombinantes (Syller, 2012). Estos sucesos de recombinación e intercambio de material genético se han descrito como el principal factor desencadenante de variabilidad en virus, permitiendo la evolución y adaptación a nuevos hospedantes y la formación de nuevas especies de virus (Xiao et al., 2016; Moriones et al., 2017).

Tabla 3. Principales virus transmitidos por moscas blancas que afectan a cucurbitáceas, distribución, principales cultivos hospedantes y sintomatología.

Género (Familia)	Virus	Distribución	Hospedantes y sintomatología	
Moscas blancas de manera persistente				
Begomovirus (*Geminiviridae*)	Virus de la hoja rizada de la calabaza (*squash leaf curl virus*, SLCuV)	América del Norte, Méjico y cuenca del Mediterráneo	Calabacín, melón, pepino, sandía y calabazas	Mosaicos verdes y amarillos, acucharado y rizado en hojas. Detención del crecimiento de la planta, frutos con la piel rugosa, deformados y con manchas.
	Virus de la hoja arrugada de las cucurbitáceas (*cucurbit leaf crumple virus*, CuLCrV)	América del Norte	Calabacín, melón y sandía	
	Virus de la hoja clorótica rizada del melón (*melon chlorotic leaf curl virus*, MCLCuV)	América del Norte y Méjico	Melón y sandía	
	Virus del moteado amarillo suave de la calabaza (*squash yellow mild mottle virus*, SYMMoV)	América del Norte	Calabaza y sandía	
	Virus del rizado suave de la hoja de la calabaza (*squash mild leaf curl virus*, SMLCV)	América del Norte	Calabazas	
	Virus del enanismo amarillo de la sandía (*watermelon chlorotic stunt virus*, WmCSV)	Oriente Medio	Sandía, melón y calabaza	
	Virus de la hoja rizada del tomate de Nueva Delhi (*tomato leaf curl New Delhi virus*, ToLCNDV)	Subcontinente indio y países de la cuenca del Mediterráneo	Apartado 2.1	
Bigeminivirus (*Geminiviridae*)	Virus de la hoja rizada de las cucurbitáceas (*cucurbit leaf curl virus*, CuLCV)	EE.UU, Méjico, cuenca del Caribe	Calabacín, melón, pepino, sandía, calabazas.	

Tabla 3. Continuación

Género (Familia)	Virus	Distribución	Hospedantes y sintomatología	
Transmisión por moscas blancas de manera semipersistente				
Crinivirus (*Closteroviridae)*	Virus del enanismo amarillo de las cucurbitáceas (*cucurbit yellow stunting disorder*, CYSDV)	España, Egipto, Israel, Jordania, Líbano, Arabia Saudí, Emiratos Árabes, Turquía, EE.UU, Méjico, China, Marruecos y Francia	Melón, pepino, calabaza, calabacín y sandía	Moteado en hojas viejas que avanza a un amarilleamiento intenso en hojas de toda la planta. Plantas con poco desarrollo y frutos pequeños
	Virus de la clorosis amarilla de las cucurbitáceas (*cucurbit chlorotic yellows virus*, CCYV)	Japon, Asia, Medio Oriente, California y países de la cuenca del Mediterráneo	Melón, pepino y calabacín	
	Virus del pseudo amarilleo de la remolacha (*beet pseudo yellows virus*, BPYV)	EE.UU, Costa Rica, Japón, Australia, Países de la cuenca del Mediterráneo	Pepino y melón	
	Virus infeccioso del amarilleo de la lechuga (*lettuce infectious yellows virus*, LIYV)	Méjico y América del Norte	Melón, calabaza, calabacín y sandía	
Ipomovirus (*Potyviridae*)	Virus de las venas amarillas del pepino (*cucumber vein yellowing virus*, CVYV)	Oriente medio y países de la cuenca del Mediterráneo		Hojas con mosaicos y amarilleamiento de las venas. Frutos con amarilleo en piel y endurecimiento de la pulpa
	Virus de las venas amarillas de la calabaza (*squash vein yellowing virus*, SqVYV)	Norte América, cuenca del Caribe, Israel y Sudán	Sandía, calabacín	

Fuentes: Adkins et al., 2007; Melgarejo et al., 2010; Desbiez et al., 2016b; Reingold et al., 2016; Rojas et al., 2018; EPPO, 2020.

En cucurbitáceas, por ejemplo, se ha descrito la aparición de cepas recombinantes de CABYV a partir de infecciones mixtas de la cepa común de CABYV y MABYV (Vafaei & Mahmoodi, 2017). En geminivirus, la recombinación genética en infecciones mixtas se ha descrito como un suceso frecuente, que ha supuesto una eficiente adaptación, propagación y emergencia en nuevos ambientes y a diferentes hospedantes (Davino et al., 2012; Moriones et al., 2017; García Arenal & Zerbini, 2019).

1.2.3.4 Emergencia y control de virus en cucurbitáceas

Las virosis emergentes de cucurbitáceas se han incrementado en los últimos años, por una parte, debido a la rápida diseminación de sus vectores naturales, y por otra, a la mejora en los métodos de diagnóstico que han permitido su identificación (Figura 3) (Lecoq & Desbiez, 2012).

Los insectos son el principal vector de las virosis emergentes, destacando entre ellos las moscas blancas (Fiallo-Olivé et al., 2020). El aumento en las últimas décadas de poblaciones de moscas blancas se ha correlacionado con el desarrollo de resistencia a pesticidas y con el calentamiento global, que favorece las migraciones de los insectos a nuevas zonas cálidas del planeta (Navas-Castillo et al., 2011). La intensificación de la agricultura y la tendencia a la simplificación de los ecosistemas también favorece los brotes de nuevos virus, facilitados por la introducción de virus y plagas exóticas mediante intercambios comerciales de diferentes materiales vegetales (Navas-Castillo et al., 2011; Roossink et al., 2015). Las cucurbitáceas requieren condiciones climáticas específicas para su cultivo, sin embargo, el empleo cada vez más generalizado de semillas híbridas y la prolongación de las estaciones cálidas posibilitan continuar el cultivo durante el año en invernadero o en cultivo extensivo al aire libre, favoreciendo la supervivencia y solape de generaciones de vectores transmisores de virus (Panno et al., 2019; Sagar et al., 2020).

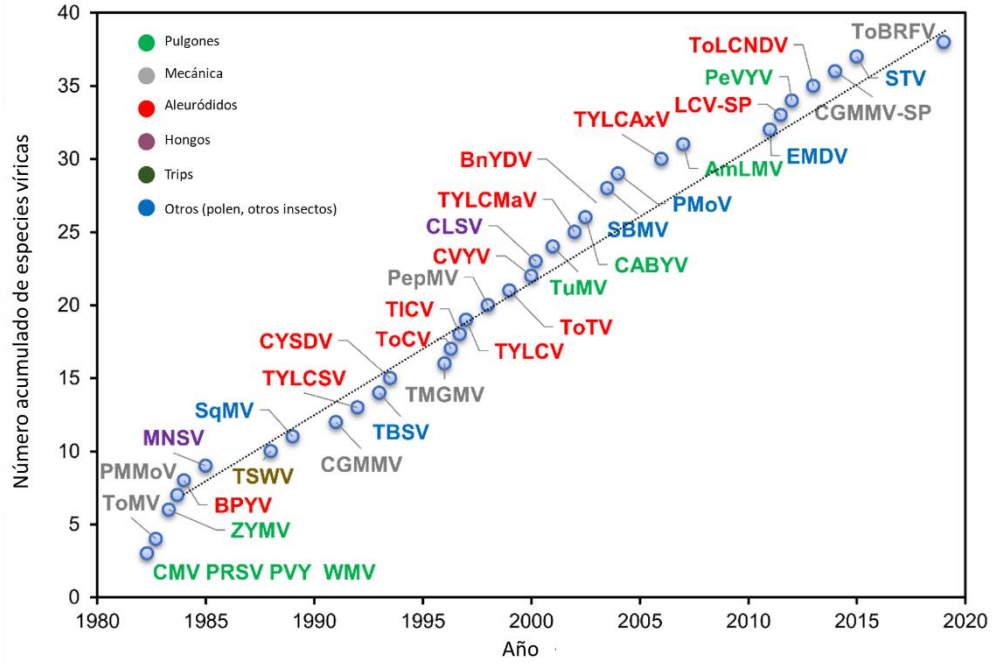

Figura 3. Secuencia temporal de la emergencia de nuevas virosis que afectan a cultivos hortícolas de la región mediterránea española y su modo de transmisión (adaptado de Velasco et al., 2020). El acrónimo de los virus se describe en la sección abreviaturas de esta tesis.

Estos factores hacen que las enfermedades causadas por virus supongan un problema dinámico y en continuo cambio para el sector hortícola de las cucurbitáceas.

Los virus que generan enfermedades en plantas, además de ser patógenos que tienden a variar rápidamente en el tiempo, no se pueden combatir mediante tratamientos químicos o 'viricidas', como es el caso de las plagas, los hongos o las bacterias, lo que dificulta su control (Loebenstein & Katis, 2014; Messelink et al., 2020). El manejo de estas enfermedades se basa principalmente en estrategias de control integrado que incluye medidas preventivas y culturales, dirigidas al control del vector de transmisión (barreras físicas, enemigos naturales, etc.) (Kone et al., 2017), la reducción de la fuente de inóculo, y el uso de resistencias genéticas.

El uso de variedades genéticamente resistentes a virus es la forma de control más sencilla, eficiente, económica y ecológica para combatir las enfermedades víricas en cultivos hortícolas (Lecoq et al., 1998; Gómez et al., 2009; Lecoq & Katis,

2014; Guner et al., 2018a; Zhao et al., 2019; Messelink et al., 2020). A continuación, se describen los principales reservorios de genes de resistencia a virus descritos en los cultivos de las cucurbitáceas más importantes.

1.3. Diversidad genética de las cucurbitáceas para la mejora de resistencia a virus

Entre la gran diversidad de taxones y formas de la familia de las cucurbitáceas, se han identificado fuentes naturales de resistencia a virus, principalmente en especies silvestres y landraces, que han sido utilizadas con éxito en programas de mejora para la obtención de variedades resistentes de interés agronómico (Sugiyama, 2013; Dempewolf et al., 2017; Lefebvre et al., 2020).

1.3.1. Principales fuentes de resistencia y genes identificados en melón

El melón es originario de Asia y su cultivo se inició a partir de al menos dos domesticaciones independientes, una en África y otra en Asia (Sebastian et al., 2010; Endl et al., 2018). La mayoría de las variedades hortícolas de melón consumidas hoy proceden de procesos de domesticación y diversificación en Asia (Endl et al., 2018; Chomicki et al., 2020), en un área que abarca desde la cuenca del Mediterráneo hasta el Lejano Oriente, aunque la India se considera el centro de diversificación más importante ya que es donde mayor variación se ha observado (Dhillon et al., 2007; Luan & Staub, 2008; Blanca et al., 2012; Pitrat, 2012a; Gonzalo et al., 2019). En el ámbito de la mejora genética del melón, fuertes barreras de incompatibilidad sexual interespecífica han impedido el aprovechamiento de la variabilidad genética que existe en especies relacionadas filogenéticamente, como el pepino (*Cucumis sativus*) o especies silvestres (*Cucumis metuliferus* Naudin, *C. anguria* L., *C. myriocarpus* Naudin, etc.), por lo que sólo la variabilidad intraespecífica ha sido empleada en programas de mejora (Pitrat, 2016a).

La mayoría de las resistencias a enfermedades víricas en melón se han descrito en un número reducido de accesiones exóticas o silvestres pertenecientes a los grupos agrestis, momordica, kachri y acidulus procedentes de la India o África, o a los grupos conomon, makuwa y chinensis del Lejano-Oriente (China, Japón y Corea)

(Pitrat, 2012; 2016a; 2016b) (Tabla 4). Algunos genotipos que confieren multirresistencia a diferentes virosis (PI 414723, PI 161375 (Songwhan Charmi), PI 482420 (TGR-1551), PI 313970) se han utilizado ampliamente en la mejora de las variedades comerciales de melón.

1.3.2. Principales fuentes de resistencia y genes identificados en pepino

El pepino es originario del sur del continente asiático probablemente de India, donde se localiza la mayor diversidad de germoplasma silvestre y cultivado (Naegele & Wehner, 2016; Sebastian et al., 2010). Las tres variedades botánicas que forman la especie son *C. sativus* var. *sativus*, que incluye los genotipos de pepino cultivado; *C. sativus* var. *hardwickii* (Royle) Alef, integrada por accesiones silvestres y *C. sativus* var. *Xishuangbannanesis* Qi et Yuan ex Weng, que incluye landraces de la región subtropical del sudoeste de China y áreas colindantes (Weng, 2016).

En *C. sativus* se ha identificado resistencia genética a potyvirus en tres accesiones principalmente: 'Suriman', procedente de América del Sur, 'Taichung Mou Gua' (TMG-1) derivada de un cultivar Taiwanés, y 'Dina-1', una línea de mejora obtenida en Países Bajos (Weng & Wehner 2017; Grumet et al., 2000). Según Grumet et al. (2000) la resistencia a múltiples potyvirus se puede deber a diferentes alelos de un único gen o a un grupo de genes que estarían estrechamente ligados. En otro cultivar tipo japonés también se ha identificado un único gen recesivo que confiere resistencia a ZYMV (Cardoso et al., 2010) (Tabla 5). Por otra parte, se ha identificado resistencia parcial a CVYV en pepino en un cultivar local español (C.sat-10) y en la accesión CE0749, en la que se ha identificado resistencia monogénica parcialmente dominante, localizada en el cromosoma 5 (Picó et al., 2003; Pujol et al., 2019) (Tabla 5).

Tabla 4. Principales genotipos de melón en los que se ha identificado resistencia a virus, genes de resistencia y su regulación.

Accesión	Origen	Grupo	Resistencia	Gen	Regulación	Referencias
PI 414723	India	Momordica	WMV	*Wmv*	Monogénica dominante, de manera parcial o total. Se heredan estrechamente ligados	Pitrat & Lecoq, 1984; McCreight et al., 1992; Gilbert et al., 1994; Ekbic et al., 2010; Mandoulakani et al., 2015; Anagnostou et al., 2000.
			ZYMV	*Zym*		
			ZYMV	*Zym-1*, *Zym-2* y *Zym-3*	Oligogénica. Tres genes dominantes complementarios. Influencia de niveles variables de patogenicidad de los diferentes aislados de ZYMV. Se ha observado que algunos aislados son capaces de superar la resistencia en PI 414723 debido al gen semi-dominante *Flaccida necrosis* (*Fn*), presente en muchas accesiones de melón	Danin-Poleg et al. 1997; Anagnostou et al., 2000; Risser et al., 1981; Pitrat, 2016a
			PRSV, MWMV, AWMV, ZYFV, ZTMV*	*Prv2*	Dominantes y alélicos. *Prv2* da lugar al desarrollo sistémico de lesiones necróticas tras la inoculación. *Prv1* codifica una proteína del tipo NBS-LRR (Nucleotide Binding Site-leucine-rich repeat)	Anagnostou et al., 2000; Brotman et al., 2005
				Prv1		Brotman et al., 2013; Pitrat, 2016a;
PI 161375 (Songwhan Charmi)	Korea	Chinensis	CMV y *Aphys gossypii*, sólo cuando el virus es introducido en la planta por este vector	*Vat*	Dominante	Pitrat et al., 1982; Pitrat & Lecoq, 1980
Mawatauri PI 161375 PI 371795			MNSV, BPYV y KGGMV*			Karchi et al.,1975; Coudriet et al., 1981; Daryono et al., 2005

*KGGMV: virus del moteado verde del kyuri (*kyuri green mottle mosaic virus*), MWMV: virus del mosaico de la sandía de Marruecos (m*oroccan watermelon mosaic virus*), AWMV: el virus del mosaico de la sandía de Argelia (*algerian watermelon mosaic virus*), ZYFV: el virus de la mancha amarilla del calabacín (*zucchini yellow fleck virus*), ZTMV: el virus del mosaico tigre del calabacín (*zucchini tigre mosaic virus*).

Tabla 4. Continuación

PI 161375 (Songwhan Charmi)	Korea	Chinensis	CMV	*cmv1*	Oligogénica recesiva, aunque el gen mayor recesivo (*cmv1*) en el cromosoma 12 confiere resistencia total a algunas cepas del virus	Essafi et al., 2009; Guiu-Aragonés et al., 2014; Pascual et al., 2019
Freeman's Cucumber	Japón	Conomon	CMV		Recesiva multigénica y cuantitativa	Karchi et al., 1975
Yamatouri			CMV	*Creb-2*	Dominante	Daryono et al., 2010
Accesiones de los grupos makuwa y momordica			CMV		No se conoce su regulación	Pitrat, 2016a
Cucumis africanus L.f., *C. anguria, C. zeyheri* Sond. y *C. myriocarpus*			CMV			Catalá et al., 2009
PI 161375 (Songwhan Charmi)			MNSV	*nsv*	Monogénica recesiva, en el cromosoma 12. Caracterizado como un factor de iniciación de la traducción (*Cm-eIf4E*)	Morales et al., 2005; Nieto et al., 2006
PI 124112	India	Momordica	CABYV	*cab-1* y *cab-2*	Dos genes recesivos complementarios	Dogimont et al., 1997
PI 482420 (TGR-1551)	Zimbabwe	Acidulus	WMV		Un gen mayor recesivo en el cromosoma 11 y tres QTLs menores en los cromosomas 4, 5 y 6	Díaz-Pendón et al., 2005; Palomares-Ríus et al., 2011; Pérez-de-Castro et al., 2019
			CYSDV			McCreight et al., 2015
			CABYV		Un gen dominante con influencia de genes modificadores menores	Kassem et al., (2015)
			A. gossypii. y muestra tolerancia a la mosca blanca *Bemisia tabaci*			
PI 313970	India	Acidulus	CYSDV		Con regulación monogénica recesiva	McCreight & Wintermantel, 2011; Wintermantel et al., 2017
PI 313970	India	Acidulus	CABYV, WmCSV, LIYV, y CuLCrV			McCreight et al., 2008

La evaluación de colecciones de germoplasma de pepino para la identificación de resistencia a CYSDV permitió identificar dos accesiones resistentes, controladas por un único gen recesivo (Aguilar et al., 2006). Entre el germoplasma de *C. sativus* procedente de la India también se han identificado dos accesiones con resistencia a CGMMV (Crespo et al., 2018).

Tabla 5. Principales genotipos de pepino en los que se ha identificado resistencia a virus, genes de resistencia y su regulación.

Accesión	**Origen**	**Resistencia**	**Gen**	**Regulación**	**Referencias**
Suriman	América del Sur	WMV	*wmv-1-1*	Recesivo	Wang et al., 1984
Taichung Mou Gua (TMG-1)	Taiwán	Cepa de sandía del PRSV (PRSV-W)	*prsv-1* *Prsv-2*	Alélicos y localizados en el cromosoma 6	Tian et al., 2015
		CABYV			Lecoq et al., 1998; Kabelka & Grumet, 1997; Wang et al., 1984
		CMV			
		WMV	*wmv-2*, *wmv-3*, y *wmv-4*		
		Raza M del MWMV (MWMV-M)		Un gen recesivo	
		ZYFV			
		ZYMV	*zym* $^{TMG-1}$ *zym* $^{Dina-1}$	Alélicos. En el cromosoma 6	Provvidenti, 1987; Kabelka et al., 1997; Amano et al., 2013
Dina-1	Línea de mejora obtenida en Países Bajos				
Kyoto 3 Feet	Japan	WMV	*Wmv*	Dominante	
2245	Línea de introgresión	PRSV		Un gen recesivo	Tian et al., 2015; 2016
		WMV		Un gen recesivo	
		CMV	*cmv6.1*	Un gen mayor recesivo en el cromosoma 6 con influencia cuantitativa	Shi et al., 2018
Chinese Long		CMV	*Cmv*	Monogenico dominante	
C. sativus var. *hardwickii*		CMV		Un gen de resistencia de carácter recesivo	Munshi et al. (2008)

1.3.3. Principales fuentes de resistencia y genes identificados en *Cucurbita* spp.

Las cinco especies del género *Cucurbita* con mayor valor agroeconómico son originarias de América y fueron domesticadas en centros distribuidos por todo el mundo (Paris, 2016). Las principales fuentes de resistencia a virus se han identificado en *C. moschata*, pero también en especies silvestres como *C. ecuadorensis* H. C. Cutler & Whitaker, *C. lundelliana* L.H. Bailey, *C. foetidissima* Kunth, *C. ficifolia* Bouché, *C. ockeechobeensis* (Small) L.H. Bailey, *C. cylindrata* L.H. Bailey *y C. digitata* A. Gray. también con origen americano (Tabla 6).

La especie de mayor valor económico es *C. pepo*, por lo que los programas de mejora se han enfocado a transferir a esta especie las resistencias identificadas en otros taxones mediante cruces interespecíficos. Por ejemplo, aunque la transferencia completa del carácter de resistencia a ZYMV de *C. moschata* a *C. pepo* es problemática (Paris, 2016b), estos genes se han piramidalizado e introducido en el cultivar True French de *C. pepo* (Pachner et al., 2015).

1.3.4. Principales fuentes de resistencia y genes identificados en sandía

La sandía pertenece al género *Citrullus* Schrad. ex Eckl. et Zeyh., compuesto por siete especies: *C. amarus* Schrad., *C. lanatus, C. rehmii* De Winter, *C. mucosospermus* (Fursa) Fursa, *C. colocynthis* Schrad., *C. ecirrhosus* Cogn. y *C. naudinianus* (Sond.) Hook.f. (Chomicki & Renner 2015; Guo et al., 2020a), de entre las que *C. lanatus* incluye la sandía cultivada para consumo (Renner et al., 2014). El cultivo de la sandía se originó al noreste de África (Chomicki & Renner, 2015; Paris, 2015), donde se domesticó. *Citrullus lanatus* ofrece un bajo nivel de diversidad genética respecto a la resistencia a plagas y enfermedades (Ngwepe et al., 2019), pero otras especies del género como *C. colocynthis* y *C. ecirrhosus*, capaces de cruzar con la sandía cultivada, se han descrito como reservorio de genes de resistencia (Levi et al., 2017; Simons et al., 2019). La mayoría de fuentes de resistencia que se han utilizado en la mejora genética de la sandía se ha identificado en la colección del banco de germoplasma de Estados Unidos (USDA-ARS).

Tabla 6. Principales genotipos del género *Cucurbita* spp. en los que se ha identificado resistencia a virus, genes de resistencia y su regulación.

Accesión	Especie	Origen	Resistencia	Gen	Regulación	Referencias
Nigerian Local	*C. moschata*	Nigeria	CMV	*Cmv*	Un gen dominante	
			PRSV	*prv-1*	Un gen recesivo	
			PRSV	*Prv-2*	dominante	McPhail--Medina et al., 2012
			ZYMV	*Zym-0*, *Zym-4*, *zymv-5*	Dos genes dominantes y uno recesivo	Munger & Provvidenti, 1987; Pachner & Lelley, 2004; Pachner et al., 2011
			WMV	*Wmv*	Un gen dominante	Provvidenti, 1986a; Gilbert-Albertini et al., 1993; Brown et al., 2003
Menina	*C. moschata*	Brasil	WMV	*Wmv*	Un gen dominante	Gilbert-Albertini et al., 1993; Brown et al., 2003
			ZYMV	*Zym-1*, *Zym-2* y *Zym-3*	Tres genes dominantes y complementarios	
	C. moschata		SLCV	*Slc-1*, *slc-2*	Un gen dominante y otro recesivo	Montes-García et al., 1998; Vilmorin, 2010
	C. maxima	Brasil	WMV		Tres genes parcialmente dominantes	Maluf et al., 1985, 1997
	C. maxima	Aunstralia	PRSV-W			
	C. ecuadoriensis		ZYMV	zym^{ecu}	Un gen recesivo	Robinson et al., 1988
	C. ecuadoriensis		WMV	WMV^{ecu}		Weeden et al., 1984; Herrington et al., 2001
PI 432441	*C. ecuadoriensis*		MWMV		Dos genes recesivos	Miras et al., 2019
	C. ecuadoriensis		SLCV		Nivel de resistencia variable e influido por el ambiente	McCreight & Kishaba, 1991
	C. lundelliana		SLCV			
	C. foetidissima		SLCV			
	C. ockeechobeensis		SLCV			

En accesiones africanas de *C. lanatus*, *C. amarus*, *C. Colocynthis* y *C. mucosospermus* procedentes de Sudáfrica, Zimbabwe, Botswana y Nigeria, se ha identificado resistencia a PRSV (Strange et al., 2002) y se ha descrito un gen recesivo (*prv*) controlando la resistencia (Guner et al., 2018a). También se ha identificado resistencia a WMV controlada por tres genes (Guner et al., 2018b). Algunas entradas de *C. lanatus* y *C. citroides* procedentes de Zimbabwe se han descrito como resistentes a ZYMV, resistencia conferida por el gen recesivo *zym* (Provvidenti,

1991; Guner, 2004; Guner et al., 2018). Algunas otras accesiones de *C. mucosospermus* originarias de Nigeria también se han descrito con resistencia a ZYMV, pero con dependencia de climas cálidos (Provvidenti, 1986b). Boyhan et al. (1992) también han descrito resistencia a ZYMV en accesiones de *C. colocynthis* y *C. mucosospermus*.

Finalmente, en las especies *C. colocynthis* y *C. lanatus* se han descrito accesiones con resistencia a SqVYV (Kousik et al., 2009; Kousik et al., 2012b).

1.4. Herramientas genéticas y genómicas para la mejora de las cucubitáceas

En la mejora genética de las cucurbitáceas se han desarrollado diferentes estrategias para facilitar el trabajo de selección e introgresión de caracteres agronómicos de interés, como resistencia a enfermedades, calidad de fruto o rendimiento del cultivo (Hao et al., 2020). La combinación de las técnicas de mejora clásicas con las novedosas herramientas moleculares, genómicas y bioinformáticas ha supuesto un avance revolucionario en las últimas décadas (Grumet et al., 2017; Baloglu, 2018), abaratando costes e incrementando la eficiencia de los programas (Montero-Pau et al., 2017).

Actualmente, gracias al desarrollo de las Tecnologías de Secuenciación de Nueva Generación (NGS), se cuenta con la secuencia completa de los genomas de referencia de las principales especies de cucurbitáceas cultivadas (melón, pepino, calabazas y calabacín y sandía) (Zheng et al., 2019). En esta familia, el genoma de pepino fue el primero en completarse (Huang et al., 2009), seguido de los genomas de melón (García-Mas et al., 2012) y sandía (Guo et al., 2013). A pesar de su importancia agrícola, los genomas de las diferentes especies del género *Cucurbita* se han ensamblado recientemente, aunque ya se encuentran disponibles los de *C. pepo* (Montero-Pau et al., 2018), *C. moschata*, *C. maxima* (Sun et al., 2017a) y *C. argyrosperma* (Barrera-Redondo et al., 2019). La continua innovación en las tecnologías de secuenciación ha permitido obtener genomas de referencia cada vez más completos y mejor ensamblados. Este es el caso del genoma de referencia de melón y pepino, que han sido mejorados recientemente gracias a la tecnología de

tercera generación de secuenciación de una sola molécula (SMRT) de Pacific Biosciences (PacBio) (Castanera et al., 2019; Li et al., 2019a).

Estas secuencias genómicas han contribuido al desarrollo y a la ampliación de colecciones de marcadores moleculares, enriqueciendo las poblaciones de mapeo para el análisis genético de diversos caracteres y permitiendo localizar e identificar genes de interés en los cromosomas (Hao et al., 2020). Además, el abaratamiento de los costes que han supuesto las tecnologías NGS ha facilitado la resecuenciación masiva de grandes colecciones de accesiones de cucurbitáceas (por ejemplo, de 1.175 accesiones de melón y 414 de sandía), mejorando el conocimiento de la diversidad global de las diferentes especies (Guo et al., 2019b; Wu et al., 2019; Zhao et al., 2019).

1.4.1. Genotipado mediante marcadores de tipo SNP

Los diferentes tipos de marcadores moleculares utilizados en el genotipado de cucurbitáceas son los marcadores basados en polimorfismos en la longitud de fragmentos de restricción (RFLP), los polimorfismos del DNA amplificados al azar (RAPD), los polimorfismos de un único nucleotido (SNP) o los polimorfismos de secuencias repetitivas cortas (SSR). Entre ellos, los marcadores de tipo SNP tienen la ventaja de que son estables, muy abundantes y se encuentran repartidos por todo el genoma, por lo que facilitan el estudio de la variabilidad al permitir un genotipado de alto rendimiento y su análisis puede realizarse mediante herramientas bioinformáticas (Perkel, 2008; Yeam, 2016; Zhang et al., 2020).

La identificación de nuevos SNPs ligados a caracteres de interés puede realizarse *in vitro*, o *in silico*. La identificación *in vitro* puede ser un proceso laborioso y costoso, debido a que requiere tecnología e instrumentos específicos de laboratorio (Morgil et al., 2020). La identificación *in silico* se basa en el empleo de herramientas bioinformáticas, haciendo uso de los genomas de referencia junto con la resecuenciación de accesiones de interés en mejora (Genome-Wide Association Study, GWAS) (Saïdou et al., 2014; Morgil et al., 2020). Entre las principales tecnologías para abordar esta estrategia se encuentran el genotipado mediante

secuenciación (Genotyping-by-Sequencing, GBS) o el análisis de genotipos segregantes agrupados (Bulked Segregant Analysis, BSA) (Hao et al., 2020). Mediante GBS se realiza una restricción enzimática del genoma, lo que permite reducir su complejidad y generar una librería representativa de su totalidad, identificándose tras la secuenciación y posterior análisis un número muy elevado de SNPs y obteniéndose así el genotipado de accesiones individuales (Elshire et al., 2011; Arbelaez et al., 2015). La técnica de GBS se ha empleado en el estudio de la variabilidad de diferentes especies de cucurbitáceas (Poland & Rife, 2012; Nimmakayala et al., 2016; Branham et al., 2017; Montero-Pau et al., 2017; Gangadhara et al., 2018; Gonzalo et al., 2019), y ha sido un valioso recurso para la identificación de SNPs y posterior genotipado de diferentes accesiones y poblaciones de mapeo en esta tesis doctoral.

La detección de SNPs mediante BSA se realiza agrupando individuos de una progenie con iguales características y comparando su secuencia consenso con la del genoma de referencia, sin necesidad de disponer de un mapa genético (Dou et al., 2018; Song et al., 2018; 2020; Liu et al., 2019b; Pujol et al., 2019).

Cuando se dispone de colecciones de SNPs y se pretende realizar un genotipado a media o baja escala pueden utilizarse metodologías basadas en PCR, como el caso del sistema Agena Bioscience iPLEX ® Gold MassARRAY (Agena Biosciences), que realiza una PCR multiplex seguida de espectrometría de masas para distinguir entre los alelos de los diferentes *loci* (Gabriel et al., 2009), o la técnica de High Resolution Melting (HRM) en la que a partir de una PCR a tiempo real de la región en la que se encuentra el SNP se identifica la variante alélica en función de la fluorescencia emitida y la temperatura de desnaturalización (Bruzzone & Steer, 2015; Taheri et al., 2017; Lee et al., 2018; Park et al., 2018).

1.4.2. Poblaciones de mapeo y análisis de QTLs

La selección asistida por marcadores (MAS) se ha convertido en una herramienta esencial de los programas de mejora para transferir un carácter de interés a una variedad cultivada (Kumar et al., 2006; Hao et al., 2020). La base para llevar

a cabo esta transferencia radica en el estudio genético a partir de los sucesos de recombinación que tienen lugar al cruzar diferentes materiales genéticos (Lv et al., 1994; Kumar, 2006; Kumar & Tiagi, 2018; Feng et al., 2019). La eficiencia de un programa de mejora se ve favorecida por un número adecuado de marcadores repartidos por todo el genoma, pero además por la elección del fondo genético del que se dispone de partida (Hao et al., 2020). Las poblaciones de mejora segregantes permiten mapar caracteres de interés de especies silvestres dentro de fondos genéticos de especies cultivadas (Wilkinson, 2001). Entre ellas se encuentran las poblaciones F_2, retrocruces (BC), líneas recombinantes consanguíneas (RILs), colecciones de líneas de introgresión (ILs) o poblaciones multiparentales (MAGIC).

El genotipado masivo de poblaciones segregantes ha permitido generar mapas genéticos de alta densidad, permitiendo identificar genes candidatos y regiones implicadas en el control de caracteres cuantitativos (Quantitative Trait Loci, QTLs) (Ashkani et al., 2015; Kumar & Tiagi, 2018). El primer mapa genético de *C. pepo*, construido a partir del genotipado mediante SNPs de una población F_2, fue utilizado para mapar QTLs relacionados con caracteres de floración, planta y fruto (Esteras et al., 2012). Este mapa se ha saturado recientemente y se ha mejorado su resolución empleando una población RILs (Montero-Pau et al., 2017) que ha permitido identificar nuevos QTLs implicados en floración temprana, forma, longitud, color de fruto y asociarlos con genes candidatos. También se han construido mapas genéticos de alta densidad en otras especies del género, como en *C. moschata*, permitiendo la identificación de QTLs relacionados con fruto (Zhong et al., 2017).

Para la identificación de QTLs en melón también se han desarrollado numerosos mapas genéticos saturados que han permitido mapar caracteres de interés (Díaz et al., 2011; Gonzalo & Monforte, 2016; Perpiñá et al., 2016; Pereira, 2018; Shang et al., 2020).

En pepino se han mapado cientos de QTLs de importancia agrícola (Wang et al., 2020), utilizando mapas genéticos de alta resolución (Yang et al., 2012; Zhang et al., 2012; Sun et al., 2013; Zhu et al., 2016).

En la búsqueda de resistencia a enfermedades, el análisis de QTLs ha sido muy empleado (Kumar, 2006) y ha supuesto una gran contribución en la mejora de cucurbitáceas para resistencia a hongos, bacterias y virus (Shi et al., 2018; Slomnicka et al., 2018). Entre las cucurbitáceas, los principales QTLs relacionados con la resistencia a virus se han identificado en melón, pepino y sandía. En melón, Guiu-Aragonés et al. (2014) identificaron un gen mayor de resistencia a CMV en el cromosoma 1 (*cmv1*), que requiere la combinación con otros dos QTLs de efecto menor en los cromosomas 3 y 10. La resistencia a CCYV en melón está controlada por un único QTL en el cromosoma 1 (Kawazu et al., 2018).

En pepino la resistencia poligénica al MYSV, está controlada por cuatro QTLs (Sugiyama et al., 2015). En sandía un gen mayor dominante confiere resistencia al ortoposvirus de la necrosis de los brotes de la sandía (*watermelon bud necrosis orthotospovirus*, WBNV), que se ve influido por la regulación de otros genes menores (Nagesh et al., 2018).

1.4.3. Plataformas de genética reversa

Las plataformas de TILLING (Targeting Induced Local Lesions in Genomes) o ecoTILLING (ecotype Targeting Induced Local Lesions in Genomes) también se han utilizado en cucurbitáceas para identificar nuevos alelos mutantes que pueden influir en un carácter agronómico de interés, como la resistencia a enfermedades (Baloglu, 2018). Mediante este tipo de plataformas, Nieto et al. (2007) identificaron una nueva variante alélica en melón que confiere resistencia a MNSV. Mediante TILLING también se ha validado el gen *Vacuolar Protein Sorting 41(CmVPS41*) que confiere resistencia a CMV en un mutante de melón que no desarrollaba la enfermedad (Giner et al., 2017).

1.4.4. Análisis del transcriptoma y expresión diferencial de genes

Además de contar con los genomas de referencia de las principales especies cultivadas de cucurbitáceas también están disponibles los transcriptomas de melón, pepino, sandía y calabacín (Blanca et al., 2011; Montero-Pau et al., 2017; Rhee et al., 2017; Osipowski et al., 2020). Las técnicas de análisis del patrón de expresión

diferencial de transcritos han facilitado la asociación de genes candidatos con determinados caracteres, aumentando el conocimiento de su funcionalidad biológica e implicación en diferentes rutas y procesos celulares en estadios de desarrollo, tejidos o condiciones fisiológicas específicas (Xia et al., 2017; Hao et al., 2020).

En el estudio a gran escala de la expresión diferencial de genes se han abordado dos estrategias principalmente, las plataformas de microarrays y la tecnología de RNAseq. El uso de las plataformas de microarrays, basado en la hibridación del RNA con sondas marcadas con fluoróforos, ofrece un alto rendimiento en el análisis de la expresión génica bajo diferentes ambientes y tratamientos (Baloglu, 2018). Esta técnica se ha utilizado en cucurbitáceas en el estudio del perfil de expresión de la planta tras la infección por virus, por ejemplo, evaluando la inducción génica durante la respuesta resistente de la accesión de *C. melo* TGR-1551 tras la infección con WMV (González-Ibeas et al., 2012), o para estudiar la respuesta transcripcional de genotipos de melón al ser infectados con CMV o con MNSV (Mascarell-Creus et al., 2009; Gómez-Aix et al., 2016).

Sin embargo, la resecuenciación de los transcriptomas mediante RNAseq se ha impuesto a la hora de realizar estudios del patrón de expresión génica en plantas, ya que ofrece la posibilidad de identificar nuevos genes que no habían sido descritos previamente, con mayor precisión y menor coste (Xia et al., 2017). Esta técnica ha tenido una gran repercusión en la mejora genética para la resistencia a virus y en los últimos años se ha comenzado a aplicar a cultivos de cucurbitáceas de diferentes especies, como en el análisis del transcriptoma de sandía tras la infección con CGMMV (Li et al., 2017b; Sun et al., 2019), de pepino tras la infección con CCYV (Sun et al., 2017b), o en la comparación entre los transcriptomas de plantas de pepino con diferente sensibilidad a CMV (Šubr et al., 2020).

La tecnología de RNAseq ha permitido el estudio de la interacción planta-virus, comparando la expresión génica antes y después de la infección, o entre plantas sanas resistentes y susceptibles cuando el patógeno ya se ha identificado, pero además ha permitido la identificación de nuevos virus (Zhan et al., 2019; Wrigh et al., 2020), la

secuenciación completa de sus genomas (Maina et al., 2017) o el estudio de la interacción molecular con su vector de transmisión (Widana Gamage et al., 2018; Kaur et al., 2019).

1.4.5. Ingeniería genética y edición del genoma: transgénesis y CRISPR/Cas9

Una alternativa a la mejora genética clásica para generar resistencia a virus en cucurbitáceas es el uso de las técnicas de transformación y edición genética. La transformación mediante *Agrobacterium thumefaciens* se ha utilizado para transferir genes externos en el fondo genético de cucurbitáceas que confieran resistencia a enfermedades de etiología viral (Yu et al., 2011). Una estrategia habitual es el silenciamiento de genes que el virus necesita para completar el ciclo de infección en la planta. Para ello se diseñan microRNAs artificiales (amiRNAs) que codifican una secuencia no traducible del genoma del virus. La proteína de cubierta de los virus es el transgén más habitual para generar plantas resistentes a virus (Ram et al., 2019). Esta estrategia se ha utilizado en cucurbitáceas para obtener plantas de melón con resistencia a CMV (Yoshioka et al., 1993) y de *C. pepo* resistentes a diferentes potyvirus (Ibaba et al., 2017). La transgénesis en cucurbitáceas también tiene un elevado potencial para generar plantas multirresistentes a diferentes virus, que suelen presentarse en infecciones mixtas (Lecoq et al., 1998).

La edición génica basada en la tecnología CRISPR/Cas9 también se ha utilizado para generar resistencia a virus en cucurbitáceas (Jaganathan et al., 2018). En pepino, la mutación dirigida del gen *4E*(*eIF4E*) confiere inmunidad a CVYV, ZYMV y PRSV (Chandrasekaran et al., 2016). Sin embargo, los trámites legales para comercializar productos editados o modificados genéticamente y las desfavorables convicciones sociales actuales en torno a ellos impiden su aceptación y reducen el valor práctico de estas técnicas.

2. EL VIRUS DE LA HOJA RIZADA DEL TOMATE DE NUEVA DELHI (ToLCNDV)

2.1. Origen y distribución geográfica

El *tomato leaf curl New Delhi virus* (ToLCNDV) o virus de la hoja rizada del tomate de Nueva Delhi, fue descrito por primera vez en 1995 (Srivastava et al., 1995) en cultivos de tomate (*Solanum lycopersicum* L.) en Nueva Delhi (India). Inicialmente fue nombrado como *indian tomato leaf curl virus* (ToLCV-India), pero tras clonar y secuenciar su genoma (Srivastava et al., 1995; Padidam et al., 1995) pasó a llamarse ToLCNDV, acorde a la nomenclatura del Comité Internacional de Taxonomía de Virus (ICTV). Actualmente, el ToLCNDV es una de las enfermedades predominantes en la producción de tomate en la India (Varma et al., 2011; Moriones et al., 2017; Zaidi et al., 2017a), pero además infecta a un extenso rango de especies hospedantes de eudicotiledóneas en más de 15 países del viejo mundo, entre las que se encuentran cultivos de gran importancia económica (Hussain et al., 2005; Zaidi et al., 2017a) (Figura 4). En los primeros años después de su identificación, el virus se detectó en Tailandia, no sólo infectando solanáceas sino también cucurbitáceas como melón, pepino, calabaza de peregrino (*Lagenaria leucantha* Rosby) (Ito et al., 2008) y esponja vegetal (*Luffa cylindrica* (L.) M.Roem.), y en Pakistán en tomate (Zaidi et al., 2017a).

Entre el año 2000 y 2012 el ToLCNDV se extendió por otros países del subcontinente indio y países vecinos del sur de Asia describiéndose en un diverso rango de hospedantes como en tomate, sandía, calabaza, chile (*Capsicum annuum* L.) y balsamina (*Momordica charantia* L.) en Pakistán (Hussain et al., 2000; Mansoor et al., 2000; Hussain et al., 2004; Tahir et al., 2005; Haider et al., 2006), en tomate y melón en Irán (Fazeli et al., 2009; Yazdani-Khameneh et al., 2013), en el cultivar 'Silver light' de melón (C. *melo* grupo makuwa) en Taiwan (Chang et al., 2009), en tomate y balsamina en Bangladesh (Maruthi et al., 2005; Tiwari et al.,

2010), en pepino y chile en Indonesia (Mizutani et al., 2011; Zaidi et al., 2017b) y en calabaza en Filipinas (Phaneendra et al., 2012) (Figura 4).

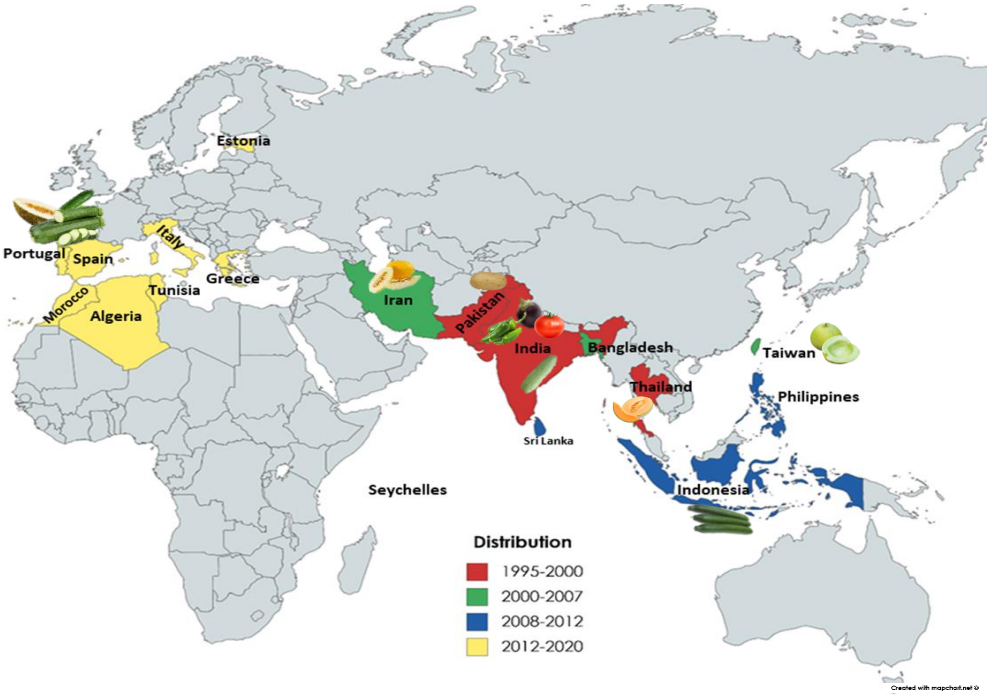

Figura 4. Distribución geográfica y propagación temporal del ToLCNDV en el mundo.

Sólo en India el ToLCNDV se ha detectado en tomate (Gaikwad et al., 2011; Snehi et al., 2016), Lufa (Sohrab et al., 2003), chayote (*Sechium edule* L.) (Mandal et al., 2004), chile (Khan et al., 2005), cáñamo de la India **(***Hibiscus cannabinus* L.) (Raj et al., 2007; Srivastava et al., 2016), papaya (*Carica papaya* L.) (Raj et al., 2008), patata (*Solanum tuberosum* L.) (Usharani et al., 2004; Saha et al., 2014), balsamina (Tiwari et al., 2010), berenjena (*Solanum melongena* L.) (Pratap et al., 2011), zanahoria (*Daucus carota* L.) (Sivalingam et al., 2011), okra (*Abelmoschus esculentus* (L.) moench) (Venkataravanappa et al., 2012), pepino, sandía, calabaza de peregrino (*Lagenaria siceraria* (Molina) Standl.) (Sohrab et al., 2010, Jyothsna et al., 2013; Nagendran et al., 2014; Rajeshwari, & Reddy, 2014), calabacín (Phaneendra et al., 2012), guar (*Cyamopsis tetragonoloba* (L.) Taub.) (Zaffalon et al., 2012), calabaza blanca (*Benincasa hispida* Thunb. Cogn.) (Roy et al., 2013),

adormidera (*Papaver somniferum* L.) (Srivastava et al., 2016) y calabaza hiedra (*Coccinia grandis* (L.) Voigt) (Venkataravanappa et al., 2017).

El rango de hospedantes del ToLCNDV y su distribución geográfica ha seguido incrementándose en los últimos años. Recientemente, en Pakistán se ha identificado infectando patata (Hameed et al., 2017), soja (Jamil et al., 2017) y algodón (*Gossypium hirsutum* L.) (Zaidi et al., 2016) y en Sri Lanka en balsamina, lufa y calabacín (Bandaranayake et al., 2014). En 2018 fue identificado en las islas Saychelles en plantas de tomate (Scussel et al., 2018).

Además de cultivos de interés económico dedicados al consumo alimentario o textil, el ToLCNDV se ha identificado en especies arvenses de interés ornamental o medicinal y en plantas adventicias de la India, Pakistán y Tailandia, habiéndose detectado en *Solanum nigrum* L., algodón de seda (*Calotropis procera* (Aiton) W.T. Aiton) (Zaidi et al., 2017b), *Ageratum* spp. (Shih et al., 2013), jatrofa (*Jatropha* spp.) (Praveen et al., 2008), vinca de Madagascar (*Catharanthus roseus* (L.) G. Don) (Riyaz et al., 2011), *Eclipta prostrata* (L.) L. (Haider et al., 2006), katuk (*Sauropus androgynus* L. Merr.) (Shih et al., 2013), parietaria (*Parietaria officinalis* L.), cerraja (*Sonchus oleraceus* L.) (Janssen et al., 2014), cenizo (*Chenopodium álbum* L. Bosc ex Moq.), correhuela (*Convolvulus arvensis* L.), *Rumex dentatus* L., Santa-María (*Phartenium hysterophorus* L.), *Cestrum nocturnum* L., jazmín de Estrella (*Jasminum multiflorum* L.), calabaza de serpiente (*Trichosanthes cucumerina* L.) (Zahidi et al., 2017b), crosandra (*Crossandra infundibuliformis* (L.)) (Sundararaj et al., 2020), y crisantemo (*Chrysanthemum indicum* L.) (Ashwathappa et al., 2020).

El ToLCNDV estuvo limitado a países asiáticos hasta 2012, cuando se detectó en España por primera vez y se convirtió en una de las enfermedades víricas emergentes más importantes en cultivos de cucurbitáceas en la cuenca del Mediterráneo.

Durante el otoño de 2012 se empezaron a observar síntomas compatibles con una infección por begomovirus en cultivos de calabacín de la región agrícola de Cartagena (Murcia, España). En la primavera de 2013, los mismos síntomas se

observaron en cultivos de Almería (Juárez et al., 2014) y en ese mismo año se identificó al ToLCNDV como el patógeno causante de la enfermedad (Juárez et al., 2013). Desde entonces, el ToLCNDV se ha convertido en la principal enfermedad de etiología viral que afecta a cucurbitáceas en la región (Rodríguez et al., 2019). Durante los años siguientes, la enfermedad se extendió por las principales zonas productoras de cucurbitáceas del país, tanto en la península (Granada, Valencia, Tarragona, Ciudad Real o Extremadura), como en las islas (Baleares y Canarias) (Font-San Ambrosio & Alfaro-Fernández, 2015; Espino de Paz & Otazo Gonzalez, 2018; Espino de Paz et al., 2019), afectando sobre todo al cultivo del calabacín pero también al melón, pepino y calabaza, tanto en cultivo protegido como en explotaciones de cultivo extensivo, provocando graves problemas agroeconómicos en el sector (Font-San Ambrosio & Alfaro-Fernández, 2014; Janssen et al., 2014, Juárez et al., 2014; Ruíz et al., 2015).

La sandía ha sido descrita como hospedante natural del ToLCNDV en España, sin embargo, no se han producido infecciones severas en este cultivo, incluso en regiones en las que la presión de inóculo era alta y otras cucurbitáceas sí resultaban infectadas (Espino de Paz & Otazo Gonzalez, 2018; Juárez et al., 2019).

En 2015, el aislado español del ToLCNDV se detectó también en Túnez infectando melón, pepino y calabacín (Mnari-Hattab *et al*., 2015), en Italia infectando melón, calabazas y calabacín (Panno et al., 2016; Parrella et al., 2018; Trisciuzzi et al., 2018, Panno et al., 2019), en Marruecos y Grecia en cultivos de calabacín (Sifres et al., 2018; Orfanidou et al., 2019), en Argelia en melón, pepino y calabacín (Kheireddine et al., 2019), en la región del Algarve y las Islas Azores (Portugal) (EPPO, 2019a) y en Estonia en plantas de pepino y tomate (EPPO, 2019b). En estas regiones mediterráneas, el ToLCNDV también se ha identificado en *Ecballium elaterium* (L.) A. Rich., *Datura stramonium* L., *Sonchus oleraceus* L. y *S. nigrum*, que podrían actuar como potenciales fuentes de inóculo (Juárez et al., 2019).

El aislado del ToLCNDV descrito en España, denominado ToLCNDV-ES, está mejor adaptado a la infección de cucurbitáceas y muy limitado a la de solanáceas. En los países de la cuenca del Mediterráneo en los que se ha descrito este virus, el calabacín es el cultivo en el que más daños y pérdidas se han registrado (Panno et al., 2018). No obstante, el virus también se ha detectado en plantas de tomate, aunque con baja incidencia y sin generar síntomas que impidan el cultivo (Ruz et al., 2015, 2016; Fortes et al., 2016; Juárez et al., 2019). El aislado ToLCNDV-ES de la cuenca mediterránea también se ha detectado infectando berenjena y pimiento en Italia (Luigi et al., 2019; Parrella et al., 2020). Aunque no se observaron síntomas en los frutos de estas especies, su presencia en estos cultivos supone un reservorio más para la propagación del ToLCNDV y una amenaza para la producción no sólo de las cucurbitáceas sino también de las solanáceas.

2.2. Clasificación, estructura genómica y diversidad genética del ToLCNDV

El ToLCNDV es un virus de cadena sencilla de DNA (grupo II de la Clasificación de Baltimore). De acuerdo a sus características genómicas, vector de transmisión y rango de hospedantes, se clasifica en el género *Begomovirus*, el más extenso dentro de la familia *Geminiviridae*.

Su genoma está formado por dos moléculas circulares de DNA de hebra sencilla, denominadas DNA-A y DNA-B, y ambas son necesarias para que se establezca la infección. Cada fragmento del genoma se encuentra encapsidado en un icosaedro incompleto T=1, con 110 moléculas de proteína de la cápsida que se organizan en 22 capsómeros pentaméricos, y ambos icosaedros se encuentran apareados entre sí (Figura 5). Este tipo de morfología "hermanada" o "geminada" de la cápsida es característica de todos los geminivirus (del latín *gemĭni,* hermanos gemelos) (Lazarowitz, 1992; Brown et al., 2015).

En el caso de la cepa ToLCNDV-ES detectada en los países del Mediterráneo, la longitud del DNA-A y DNA-B es de aproximadamente 2,74 y 2,68 kb, respectivamente (Juárez et al., 2014). Los geminivirus cuentan con pocos genes y

requieren de proteínas del hospedante, como primasas y DNA polimerasas nucleares, para llevar a cabo su replicación. En el genoma del ToLCNDV hay 8 marcos de lectura abierta (open reading frames, ORFs) que codifican las proteínas necesarias para que el virus lleve a cabo su ciclo de replicación e infección (Fondong, 2013).

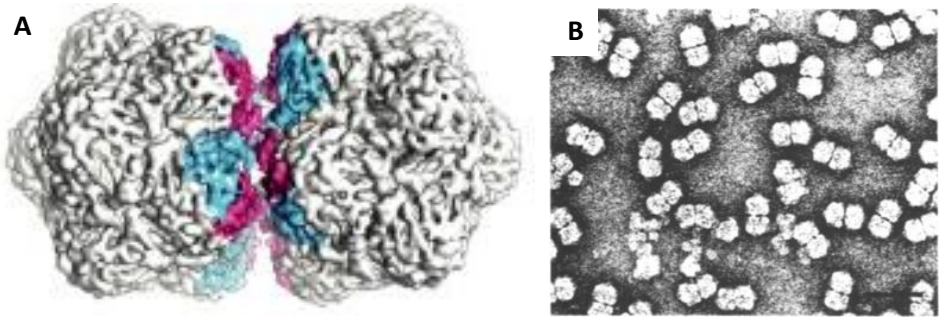

Figura 5. Cápsida característica de los geminivirus. A) Modelo atómico de las 110 subunidaes de la proteína de cubierta de los geminivirus (Hesketh et al., 2018). B) Fotografía al microscopio (Hull, 2002).

La nomenclatura de los ORFs está regulada internacionalmente según el ICTV, y se designan en función del fragmento genómico en el que se localizan (A o B) y de si la proteína que codifican constituye el virión (V) o tiene una función complementaria (C). Los ORFs del DNA-A solapan entre sí, llegando incluso a integrarse unos dentro de otros, dando lugar a la regulación de la expresión y función de las proteínas colindantes (Moriones et al., 2017). Los ORFs del DNA-B, en cambio, no se solapan (Figura 6).

En el fragmento A hay seis ORFs, cuatro que codifican en dirección antisentido de la hebra de DNA del virus (AC1, AC2, AC3, AC4) y dos que codifican la dirección sentido (AV1 y AV2). AC1 codifica la proteína iniciadora de la replicación (Rep), que introduce una mella en la secuencia nucleotídica del virus para iniciar el ciclo de replicación circular del genoma viral (Kushwaha et al., 2017; Rojas et al., 2018). AC2 se expresa al comienzo de la infección y codifica una proteína multifuncional, el activador de la transcripción de proteínas (TrAP), que activa la expresión de genes tardíos como la proteína de la cápsida y los genes codificados en el DNA-B (Cantú-Iris et al., 2019). AC2 también actúa como factor de patogenicidad e interviene en la supresión del silenciamiento por RNA (RNAi) (Voinnet et al.,

1999). AC3 codifica un potenciador de la replicación del DNA viral (REn) (Pradhan et al., 2017). El ORF AC4 se encuentra integrado en el AC1 e interviene en el desarrollo de los síntomas y en la supresión del RNAi (Chellappan et al., 2005; Fondong, 2013; Vinutha et al., 2018). AV1 codifica la proteína de la cápsida (CP), necesaria para la encapsidación del DNA viral y para el reconocimiento por parte del vector de transmisión, y solapa con AV2 que codifica la proteína de pre-cápsida e interviene en la supresión del RNAi a nivel transcripcional y postranscripcional (Wang et al., 2019b; Luna & Lozano-Durán, 2020).

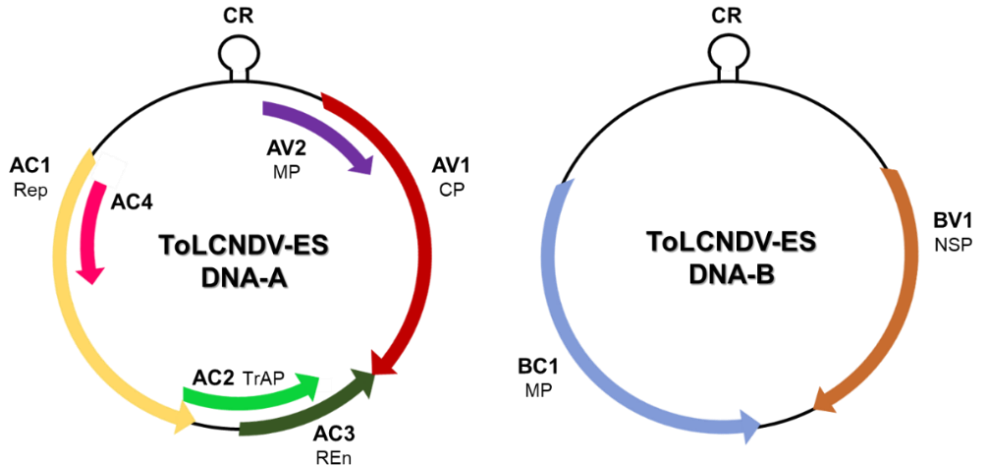

Figura 6. Organización genómica del ToLCNDV-ES. ORFs y dirección en la que codifican las diferentes proteínas en las hebras de DNA A y B. Rep: proteína iniciadora de la replicación, TrAP: activador de la transcripción de proteínas, REn: potenciador de la replicación del DNA viral, CP: proteína de la cápsida, NSP: proteína lanzadera nuclear y MP: proteína de movimiento célula a célula del virus. En la región común (CR) tiene lugar el origen de la replicación.

Las dos ORFs del fragmento B (BC1 en la hebra antisentido del virus y BV1 en la hebra sentido) son necesarias para el desarrollo de los síntomas y el movimiento del virus en la planta. BV1 codifica la proteína lanzadera nuclear (NSP), responsable del transporte del DNA viral entre el citoplasma y el núcleo, y necesaria para que el hospedante reconozca al virus e inicie la respuesta de defensa. BC1 codifica la proteína requerida para el movimiento célula a célula del virus (MP) (Padidam et al., 1996; Hussain et al., 2005; Jeske, 2009; Pratap et al., 2011; Khan et al., 2012; Lee et al., 2020).

En la región intergénica no codificante de ambas partículas genómicas hay una región común (CR) de 163 nt que incluye promotores bidireccionales para activar la expresión de los genes en sentido y antisentido de la hebra de DNA (Cantú-Iris et al., 2019). En la CR hay 30 nt que crean una estructura en forma de horquilla con una secuencia nucleotídica conservada (TAATATTTAC) en la que se localiza el origen de replicación (Padidam et al., 1995; Hanley-Bowdoin et al., 2013; Panno et al., 2019). Mediante el mecanismo de replicación en círculo rodante, el DNA del genoma del virus pasa a ser bicatenario, que es su forma replicativa (Hamley-Bodowin et al., 2013) (Figura 7).

En algunos aislados del ToLCNDV se han descrito betasatélites (Sivalingam et al., 2012; Jyothsna et al., 2013), que son cadenas circulares de DNA de aproximadamente 1,3 kb que se encapsidan por separado y dependen de los segmentos genómicos DNA-A y DNA-B para replicarse y encapsidarse. Codifican una única proteína, βC1, que actúa como un factor de patogenicidad mayor, incrementando la sintomatología por acumulación del DNA viral, suprime el silenciamiento del RNAi y puede favorecer la ampliación del rango de hospedantes (Jyothsna et al., 2013; Zhou et al., 2013; Rojas et al., 2018). El aislado ToLCNDV-ES no presenta betasatélites por lo que los DNAs A y B son suficientes para que se lleve a cabo la infección y para la inducción de síntomas (Ruiz et al., 2015; 2017; Juárez et al., 2019).

Para estudiar la diversidad de los aislados emergentes del ToLCNDV en los países del Mediterráneo se han realizado numerosos análisis genéticos, comparando los genomas identificados en cucurbitáceas, solanáceas y plantas adventicias de esta región entre sí y, además, con las secuencias de los aislados asiáticos. Los aislados de ToLCNDV de la cuenca mediterránea constituyen un grupo genéticamente homogéneo, sin diferencias entre hospedantes, localización geográfica o año, pero distintos de las poblaciones asiáticas del virus (Fortes et al., 2016; Moriones et al., 2017, Zaidi et al., 2017b; Juárez et al., 2019; Panno et al., 2019). Los segmentos genómicos DNA-A y DNA-B de los más de 100 aislados completamente

secuenciados de esta región muestran una homología de secuencia superior al 98,6 y 97,5% respectivamente, mientras que con los aislados asiáticos la homología es, respectivamente, inferior al 93,3 y 82%. Estos aislados se han agrupado en la ya nombrada nueva cepa ToLCNDV-ES (Moriones et al., 2017), con un origen común y una única introducción bastante reciente en el área Mediterránea, que además se ha originado tras varios eventos de recombinación favoreciendo su adaptación a la infección de cucurbitáceas, pero con baja patogenicidad en solanáceas (Fortes et al., 2016; Moriones et al., 2017; Panno et al., 2019).

Juárez et al., (2019) han detectado un nivel de variabilidad genética del virus más alto en *Datura stramonium* que en variedades cultivadas, que se ha incrementado en el transcurso de los años, y que podría manifestar la capacidad evolutiva del ToLCNDV y su potencial amenaza para aumentar su patogenicidad o ampliar la infección de nuevos hospedantes.

Además de esta fuente de variabilidad genética, se ha detectado la cepa ToLCNDV-ES en una infección mixta con el begomovirus del rizado amarillo del tomate (*Tomato yellow leaf curl virus,* TYLCV) en plantas de tomate (Juárez et al., 2019). En Asia se han detectado también infecciones mixtas del ToLCNDV con otros begomovirus bipartitos y con virus de diferentes familias (Sharma et al., 2015; Moriones et al., 2017), lo que ha dado lugar a aislados recombinantes en diferentes hospedantes (Venkataravanappa et al., 2017; Wilisiani et al., 2019; Ashwathappa et al., 2020). Las infecciones mixtas de la cepa ToLCNDV-ES con begomovirus que infectan solanáceas podría dar lugar a la aparición de nuevos aislados recombinantes con la capacidad adaptativa de propagarse también a diferentes especies de esta familia botánica.

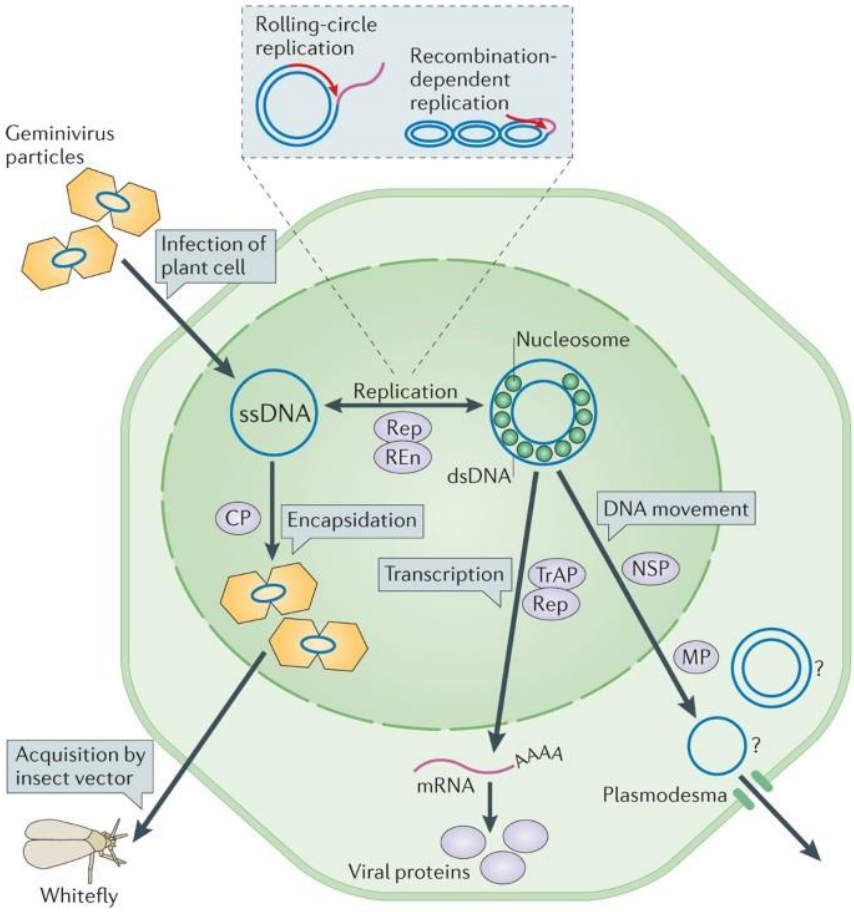

Figura 7. Replicación del DNA viral mediante el mecanismo del círculo rodante y recombinación. A) La mosca blanca inocula el virus en la célula vegetal. B) Tras desencapsidarse, el DNA de cadena sencilla del virus (ssDNA) es transportado al núcleo. C) El ssDNA se convierte a DNA de doble cadena (dsDNA) empleando la maquinaria celular de replicación de la planta; D) se lleva a cabo la replicación del dsDNA mediante el mecanismo del círculo rodante (RCR), al unirse la proteína Rep a la secuencia conservada del origen de replicación y realizar un corte. La actividad helicasa de la proteína Rep facilita la disociación del ADN en esta región, posibilitando la unión de otras proteínas en el extremo 3'-OH del corte, a partir de donde se genera una nueva cadena de DNA complementaria a la hebra molde. Como resultado se obtienen dos moléculas, una dsDNA y otra ssDNA; E) el dsDNA viral se transcribe a mRNAs empleando proteínas nucleares y son transportados al citoplasma para la traducción a proteínas virales; F) las proteínas necesarias para la replicación, transcripción o movimiento de DNA viral son introducidas de nuevo en el núcleo; F) el DNA del virus puede propagarse a otras células adyacentes a través de los canales del plasmodesmo mediante las proteínas de movimiento. La propagación a larga distancia ocurre vía vascular. (Figura tomada íntegramente de Hanley-Bowdoin et al., 2013).

2.3. Transmisión del ToLCNDV

2.3.1. Transmisión por mosca blanca

Los begomovirus son transmitidos de manera natural por las especies que forman el complejo de la mosca blanca del tabaco (*Bemisia tabaci*). El ToLCNDV se ha descrito como un virus limitado al floema de la planta, que se transmite de manera persistente y circulativa por este vector (Fondong, 2013; Rosen et al., 2015; Zaidi et al., 2017b; Rojas et al., 2018, Sagar et al., 2020). Cuando la mosca succiona la savia de una planta infectada para alimentarse, la proteína de la cápsida del virus se une al vector y facilita la transmisión (Sagar et al., 2020). Tras esta interacción, el virus viaja por el canal alimentario del insecto y desde la membrana del intestino medio se filtra a la parte del sistema circulatorio llamada hemocoel encargada de bañar directamente los órganos con la hemolinfa. De esta manera el virus alcanza la glándula salival primaria (Gray et al., 2014; Hasegawa et al., 2018). Cuando la mosca virulífera se alimenta de una planta sana hospedante, inocula el virus y se produce la infección, en un proceso que puede durar 15 minutos (Janssen et al., 2014).

La mosca blanca es capaz de transmitir el virus durante todo su ciclo de vida, de unos 30 días cuando las temperaturas son óptimas (22-25ºC), por lo que la persistencia del ToLCNDV en el vector es de tiempos bastante prolongados (Espino de Paz & Otazo González, 2018). La transmisión transovárica de begomovirus en mosca blanca no se había detectado hasta hace poco, cuando se observó en TYLCV, permitiendo la transmisión del virus durante al menos dos generaciones sin la presencia de fuente de inóculo (Stansly & Naranjo, 2010; Wei et al., 2017; Guo et al., 2019c). La eficacia de transmisión del ToLCNDV-ES en calabacín por una única mosca blanca es del 95% y del 15% en tomate (Janssen et al., 2014).

Entre los tres biotipos de *B. tabaci* que transmiten begomovirus (A o del Nuevo mundo (NW), B o del Este Medio de Asia y Asia menor (MEAM) y Q o Mediterráneo (MED)) (De Barro et al., 2011), hasta el momento se había descrito el biotipo MEAM en India y el haplotipo MED- Q1-en España, como los transmisores del ToLCNDV (Janssen et al., 2017; Maruthi et al., 2007), aunque el haplotipo

MED-Q2 también se ha descrito en Italia como transmisor de la virosis (Bertin et al., 2018). Este biotipo, MED-Q2, es originario de los países Mediterráneos del este, como Israel, Turquía o Chipre, donde ha permanecido confinado desde finales del siglo XX (Bonato et al., 2007). Su expansión por el resto de los países de la cuenca del Mediterráneo se ha visto favorecida por temperaturas muy elevadas y las prácticas de cultivo intensivo (Bertín et al., 2018; Rodríguez et al., 2019), coincidiendo su propagación en esta región con la del ToLCNDV, y suponiendo una posible vía de introducción en el área Mediterránea.

2.3.2. Transmisión mecánica

La transmisión mecánica de los begomovirus no es habitual (Wege & Pohl, 2007), y aproximadamente solo un 5% de los begomovirus que se conocen se pueden transmitir mecánicamente a sus hospedantes (Lee et al., 2020). Sin embargo, se ha demostrado que algunos aislados del ToLCNDV sí pueden ser trasmitidos mecánicamente (Sayed et al., 2013). Usharani et al. (2004) transmitieron mecánicamente un aislado indio de ToLCNDV identificado en patata tanto al hospedante original como a *Nicotiana benthamiana* Donim. Un aislado indio de *Luffa* spp. también se transmitió mecánicamente a esponja vegetal y a *N. benthamiana* (Sohrab et al., 2013). En cambio, el aislado que afecta al melón oriental (grupo makuwa de *C. melo*) en Taiwán puede infectar a plantas de *N. benthamiana,* melón de los grupos conomon y makuwa, pepino, calabaza del peregrino, calabacín y esponja vegetal mediante inoculación mecánica, pero no a otras especies como sandía, *C. moschata*, *C. metuliferus*, o *C. melo* subsp. *melo* var. *reticulatus* (Chang et al., 2010). Además, un aislado de ToLCNDV que infecta pepino tampoco se pudo transmitir mecánicamente a su hospedante natural, a pesar de la similitud que comparten con los genomas de los aislados de patata y melón oriental (Padidam et al., 1995; Samretwanich et al., 2000; Lee et al., 2020).

La cepa ToLCNDV-ES es transmisible mecánicamente en cucurbitáceas (López et al., 2015; Ruíz et al., 2017; Panno et al., 2019). López et al. (2015) transmitieron eficientemente esta cepa a diferentes especies de los géneros *Cucumis*,

Cucurbita y *Citrullus* mediante inoculación mecánica. Panno et al. (2019) confirmaron que la cepa puede trasmitirse mediante inoculación mecánica a *C. pepo*, y *C. melo* de los grupos inodorus y cantalupensis, aunque no pudieron transferirlo a *C. sativus*, *S. lycopersicum*, *S. melongena* y *C. annuum.*

Diferentes estudios han asociado la partícula genómica DNA-B de los begomovirus con la transmisión mecánica (Levy & Czosenk, 2003; Chang et al., 2010; Wege & Pohl, 2007). En un trabajo reciente, Lee et al. (2020) han demostrado que una mutación en el aminoácido 19 del extremo N-terminal de la proteína MP (codificada en el DNA-B) es la responsable de la transmisión mecánica del aislado del ToLCNDV identificado en melón oriental, descartando la implicación en este tipo de transmisión de la proteína de lanzadera nuclear como se había sugerido previamente. Estos autores también destacan que los aislados del ToLCNDV limitados a la propagación por el floema de la planta no pueden ser transmitidos mecánicamente, ya que para que esto ocurra debe producirse una replicación y propagación por el mesófilo tras la inoculación.

Estos resultados contrastan con la capacidad del ToLCNDV-ES de ser transmitido mecánicamente, ya que se ha descrito como una cepa limitada al floema (Ruíz et al., 2017; Manivannan et al., 2019). En cualquier caso, su capacidad de transmisión mecánica es un aspecto de importante valor en los programas de mejora para la búsqueda de fuentes de resistencia. El cribado de grandes colecciones de germoplasma mediante inoculación natural con poblaciones de mosca blanca virulífera o agroinoculación presentan numerosas dificultades, como problemas de reproducibilidad, inoculación selectiva por preferencia del vector, elevado coste y tiempo de ejecución. En cambio, los cribados mediante inoculación mecánica ofrecen reproducibilidad, rapidez, condiciones controladas y bajo coste (Aguilera et al., 2014; Lefebvre et al., 2020).

2.3.3. Transmisión por semilla

La emergencia de nuevas enfermedades de etiología viral se ha asociado al intercambio entre países de semillas que podrían estar infectadas (Hanssen et al.,

2010). En el caso de la transmisión por semilla de begomovirus, este tipo de propagación se había descartado al considerarlos limitados al floema (Manivannan et al., 2019). Sin embargo, en los últimos años se ha detectado transferencia de begomovirus entre generaciones del hospedante a través de las semillas. El primer caso se dio en plantas de boniato (*Ipomoea batatas* (L.) Lam) en las que se comprobó la transmisión por semilla del virus de la hoja rizada del boniato (*Sweet potato leaf curl virus*, SPLCV) (Kim et al., 2015b). Un año después, el TYLCV y el virus del mosaico amarillo del mungo (*mungbean yellow mosaic virus*, MYMV) también se detectaron en plántulas de tomate y judía negra, respectivamente, germinadas a partir de semillas infectadas (Kil et al., 2016; Kothandaraman et al., 2016). La transmisión por semilla del TYLCV también se observó en 2017 en soja blanca (*Glycine max* (L.) Merr.) (Kil et al., 2017) y en 2018 en pimiento dulce (Kil et al., 2018). Otros begomovirus transmitidos por semilla son el virus del mosaico amarillo de los dolichos (*dolichos yellow mosaic virus*, DYMD) en zarandaja (*Lablab purpureus* (L.)) (Suruthi et al., 2018) y el virus del amarilleo de la hoja rizada del pimiento de Indonesia (*pepper yellow leaf curl Indonesia virus*, PepYLCIV) en cultivos de chile (Fadhila et al., 2020). El virus de la cabeza rizada de la remolacha (*Beet curly top virus*, BCTV) es un geminivirus que, aunque no pertenece al género *begomovirus*, también es transmitido por semilla de petunia (Anabestani et al., 2017). Por el contrario, en otros trabajos publicados recientemente, no se ha podido confirmar la transmisión por semilla del TYLCV ni en *N. benthamiana* ni en tomate (Rosas-Díaz et al., 2017; Pérez-Padilla et al., 2019).

En el caso del ToLCNDV, en la India se ha descrito la transmisión por semilla de un aislado en chayote y de otro en balsamina (Sangeetha et al., 2018; Manivannan et al., 2019), y recientemente, también se ha confirmado la transmisión por semilla, de un aislado de ToLCNDV-ES procedente de Italia, en plantas de calabacín (Kil et al., 2020). Esta forma de propagación podría ser otra de las vías mediante las cuales el ToLCNDV se introdujo desde Asia en la región Mediterránea, dos zonas geográficas muy separadas entre sí (Juárez et al., 2019; Kil et al., 2020).

2.3.4. Transmisión mediante injerto

A través del floema también se puede propagar el ToLCNDV al injertar una variedad sana sobre un patrón infectado (Maruthi et al., 2005; Fortes et al., 2016). Akhtar et al. (2019) han desarrollado un método de inoculación del ToLCNDV en germoplasma de tomate mediante chip de injerto, que consiste en realizar un corte en el tallo de la planta a inocular e introducir un pequeño segmento de tallo (chip) procedente de una planta infectada, cubriendo posteriormente la unión con parafilm. El método permitió realizar un cribado de germoplasma de tomate para la búsqueda de resistencia a ToLCNDV de manera rápida y eficiente.

2.4. Métodos de diagnóstico

2.4.1. Evaluación visual de la sintomatología en cucurbitáceas

La sintomatología característica que los begomovirus generan en sus hospedantes incluye principalmente amarilleos, mosaico, rizado y acucharado de las hojas (Sagar et al., 2020). Los síntomas causados por la cepa ToLCNDV-ES en cucurbitáceas son similares a los ocasionados por los aislados asiáticos (Malathi et al., 2017), siendo típico el rizado de las hojas, mosaico intenso, amarilleamiento en hojas, engrosamiento de las venas en hojas jóvenes, acortamiento internodal, enanismo y detención del desarrollo de la planta (Figura 8A) (Panno et al., 2016). Los síntomas más severos se producen en calabacín, generando en la planta un elevado estrés que impide su desarrollo, floración y fructificación (Figura 8A y B), sobre todo si la infección se produce cuando la planta se encuentra en estadíos jóvenes (Font-San Ambrorio & Alfaro-Fernández, 2014; Janssen et al., 2014). Si la infección se produce después del cuajado, en la superficie de los frutos aparecen surcos, deformaciones y manchas, reduciendo el tamaño considerablemente (Figura 8C) (Juárez et al., 2014). En melón aparecen mosaicos en la superficie foliar (Figura 8D) y en los frutos, e incluso los ovarios de las flores, es habitual el rajado longitudinal (Figura 8E y F). Estos síntomas limitan el cultivo y reducen la calidad de los frutos, llegando a impedir su comercialización (Figura 8G).

Figura 8. Síntomas inducidos por ToLCNDV-ES en plantas y frutos de calabacín (*C. pepo*) (A-C) y melón (*C. melo*) (D-G).

En el capítulo I de esta tesis se describe detalladamente la sintomatología que genera el ToLCNDV-ES en las principales especies del género *Cucurbita* y en el capítulo II en pepino.

López et al. (2015) clasificaron la sintomatología del ToLCNDV-ES en cucurbitáceas según la evaluación visual de su severidad. Para ello, crearon una escala numérica compuesta por cinco niveles: ausencia de síntomas (0), síntomas leves (1), síntomas moderados (2), síntomas severos (3) y síntomas muy severos o senescencia de la planta (4). Esta escala permite fenotipar la respuesta a la infección por el virus en colecciones de germoplasma de cucurbitáceas de manera semicuantitativa.

2.4.2. Métodos de identificación molecular

Las principales estrategias para la detección del ToLCNDV se agrupan en técnicas de detección serológicas y técnicas de detección de ácidos nucleicos.

2.4.2.1. Métodos de detección serológica

Para la detección serológica del ToLCNDV se pueden emplear ensayos de flujo lateral (Lateral Flow Assay, LFA) o cualquiera de las diferentes versiones de la técnica ELISA (Enzyme-linked immunosorbent assay). Los dos métodos de ensayo se basan en la reacción antígeno-anticuerpo y para los dos hay disponibles en el mercado kits comerciales. Los dispositivos LFA son tiras reactivas inmunocromatográficas rápidas, de forma que si el antígeno está presente en la muestra (por ejemplo, la proteína de la cápsida viral) aparece una línea de color en la tira reactiva después de 5 minutos. En la técnica ELISA los anticuerpos se unen también al antígeno del virus y la acumulación de sustrato se mide por espectrofotometría, permitiendo realizar una detección semicuantitativa. Es una técnica económica y permite el análisis de un gran número de muestras al mismo tiempo. Sin embargo, presenta baja especificidad, generando riesgo de detección de falsos positivos o negativos. Además, se requieren anticuerpos específicos para cada virus (Alfaro-Fernández et al., 2016; Spadaro et al., 2020).

2.4.2.2. Métodos basados en la hibridación de ácidos nucleicos

Para la detección del ToLCNDV mediante hibridación molecular se han implementado diferentes técnicas, pero en todos los casos lo primero que se requiere es la transferencia de tejido fresco o ácidos nucleicos purificados a una membrana de nylon. La membrana se somete a hibridación con cadenas sencillas de DNA o RNA para aparear específicamente por complementariedad de secuencia. Se pueden sintetizar sondas complementarias de cualquier región del genoma viral (por ejemplo, del gen que codifica la proteína de la cápsida) marcadas radiactivamente, o conjugadas con un fluoróforo o con enzimas que den reacciones colorimétricas o quimioluminiscentes. La intensidad de la señal dependerá de la cantidad de sonda que se una al genoma viral, obteniendo una medida semicuantitativa. Con esta técnica es posible obtener distintos grados de especificidad modificando las condiciones de hibridación o sintetizando las sondas a partir de diferentes zonas genómicas. Además, la técnica es económica, permite procesar un gran número de

muestras, así como llevar a cabo prospecciones de campo para determinar la presencia del ToLCNDV (Alfaro-Fernández et al., 2016; Juárez et al., 2019).

2.4.2.3. Detección mediante PCR y qPCR

La reacción en cadena de la polimerasa (PCR) permite la amplificación mediante primers específicos de DNA de una o varias regiones del genoma del ToLCNDV. Incluso partiendo de pequeñas cantidades de virus, la DNA polimerasa puede generar un elevado número de copias al alternarse ciclos de desnaturalización y renaturalización del DNA. Dependiendo de la modalidad utilizada se puede realizar una detección semicuantitativa del gen o fragmento amplificado mediante electroforesis en gel de agarosa (PCR convencional) (López et al., 2015) o una detección en tiempo real utilizando nucleótidos o sondas marcadas con fluoróforos que permiten la cuantificación absoluta o relativa de la acumulación del ToLCNDV en un tejido infectado (PCR cuantitativa, qPCR) (Simón et al., 2018). La PCR es el método más sensible, específico y versátil para la detección del ToLCNDV, además de ser la técnica más utilizada para su diagnóstico (Islam et al., 2011; Bandaranayake et al., 2014; López et al., 2015).

2.5. Métodos de control

Las medidas para controlar los daños generados por el ToLCNDV-ES se limitan a medidas fitosanitarias, estructurales y de higiene de los cultivos (Junta de Andalucía, 2018; Monserrat & Lacasa, 2014), ya que todavía no se dispone de variedades comerciales con resistencia genética completa al ToLCNDV.

Entre las medidas de carácter fitosanitario, resulta fundamental controlar la propagación del vector de transmisión, ya que evita a la vez la transmisión directa del virus. La mejor estrategia para controlar la mosca blanca es el control integrado, en el que predominen las buenas prácticas culturales (utilización de trampas cromotrópicas, invernaderos estancos, material vegetal de semillero libre de mosca, etc.) junto con el control biológico, combinando ambas con la aplicación, en menor medida, de tratamientos químicos, compatibles con los enemigos naturales (Rodríguez et al., 2019). El control biológico de la mosca blanca mediante *A. swirskii*

en cultivos de calabacín ha permitido reducir la propagación del virus (Téllez et al., 2017), sobre todo si la suelta se realiza al comienzo del cultivo.

Las medidas estructurales previenen la entrada de la mosca blanca en cultivo protegido en invernadero, y engloban la hermeticidad de los invernaderos, la colocación de mantas térmicas cubriendo las plantas en estadio joven, la instalación de mallas anti-insectos en bandas y ventanas y la doble puerta de entrada a las instalaciones.

Por último, para evitar reservorios del virus se debe mantener la higiene en invernaderos y parcelas, retirando y destruyendo inmediatamente restos vegetales y plantas infectadas, así como plantas adventicias en el entorno del cultivo (Monserrat & Lacasa, 2014).

3. MEJORA DE LA RESISTENCIA A GEMINIVIRUS

Una de las principales limitaciones de los cultivos a nivel mundial son las mermas de cosecha que genera la infección por geminivirus (Rojas et al., 2018). La familia *Geminiviridae* está compuesta por diferentes géneros, aunque la mayoría de virus que infectan eudicotiledóneas pertenecen a los géneros *Mastrevirus*, *Begomovirus* y *Curtovirus* (Luna et al., 2019).

De manera natural, la co-evolución entre plantas y geminivirus se ha visto equilibrada por la combinación y alternancia entre las estrategias que desarrollan las plantas para defenderse y los mecanismos y respuestas que generan los virus para evadirlas o sobrepasarlas (Schmitt-Keichinger, 2019; Kumar, 2019a). En la mejora genética de plantas se ha conseguido controlar enfermedades causadas por geminivirus mediante el desarrollo de resistencias genéticas.

3.1. Mecanismos de resistencia a geminivirus

Las principales estrategias vegetales para generar una respuesta de defensa en la infección por geminivirus incluyen mecanismos específicos como la resistencia mediada por genes R, el silenciamiento por RNA o la resistencia recesiva, y otros mecanismos celulares no específicos como la regulación de factores de transcripción,

autofagia o ubiquitinación (Alcaide-Loridan & Jupin, 2012; Sahu et al., 2014a; Kushwaha et al., 2015; Hashimoto et al., 2016; Whitham & Hajimorad, 2016; Haxim et al., 2017; Sharma & Prasad, 2017; Schmitt et al., 2019).

3.1.1. Silenciamiento por RNA

Entre los mecanismos de defensa de las plantas, el silenciamiento mediado por RNA interferente (RNAi) es la principal estrategia para combatir a virus de DNA (Sharma & Prasad, 2017). El silenciamiento génico puede llevarse a cabo a nivel transcripcional (TGS) o post-transcripcinoal (PTGS), en ambos casos se requiere el desarrllo de RNAs interferentes de pequeño tamaño (siRNA) (Verlaan et al., 2013; de Ronde et al., 2014). En el PTGS se produce la degradación mediada por siRNAs por complementariedad de secuencia con la región codificante de los genes del virus, inhibiéndose la traducción de las proteínas. En el TGS los siRNAs son complementarios a la región promotora de los genes del virus, produciéndose la metilación del DNA viral y modificando las histonas y la cromatina, lo que da lugar al bloqueo en la transcripción de los genes del virus (Metilación mediada por RNA, RdDM) (Noris & Catoni, 2020). La ruta RdDM se considera especialmente importante en la defensa de las plantas frente a virus de DNA, incluidos los geminivirus (Wang et al., 2019b). En los últimos años la ruta del TGS se ha propuesto como el mecanismo más importante para combatir la infección por geminivirus, ya que el bloqueo de proteínas implicadas en esta ruta genera plantas hipersusceptibles (Jackel et al., 2016; Noris & Cantoni, 2020; Voorburg et al., 2020).

En la síntesis de los siRNAs y en el proceso de silenciamiento intervienen RNAsas denominadas Dicer-Like (DCL), el complejo de proteínas RISC (RNA-induced silencing complex), RNA polimerasas dependientes de RNA (RDRs), RNA polimerasas dependientes de DNA (RDDs), la familia de proteínas Argonauta (AGO), DNA metiltransferasas, o el gen supresor del silenciamiento 3 (Suppressor of Gene Silencing 3, SGS3) (Sharma & Prasad, 2017; Kong et al., 2020). A nivel post-transcripcional también puede generarse silenciamiento génico de geminivirus mediante miRNAs, que son RNAs no codificantes que se unen al RNA mensajero y

generan su degradación, inhibiendo la traducción (Akbergenov et al., 2006). La expresión de ciertos miRNAs se ha visto alterada en la infección por begomovirus y asociada a la regulación de genes R y factores de transcripción (de Ronde et al., 2014, Zhang et al., 2016; Kundu et al., 2017).

3.1.2. Resistencia mediada por genes R

Cuando un virus infecta la célula vegetal las proteínas codificadas por genes R son capaces de reconocer e interaccionar de manera específica con un factor del virus (factor de avirulencia, *Avr*) (Moffett, 2009; Mushtaq et al., 2018; 2019).

La familia de genes R más extensa que se conoce es la de las NBS-LRR (Dangl & Jones 2001), a la que pertenecen la mayoría de los genes de resistencia a patógenos que se han clonado y caracterizado (Román et al., 2020). Los genes NBS-LRR constan de un dominio C-terminal rico en repeticiones de leucina (LRR), un dominio central de unión a nucleótidos (Nucleotide Binding Site, NBS) y un dominio N-terminal que puede ser de tipo Coiled-coil (CC) o receptor Toll e Interleukin-1 (TIR) (Martin et al., 2003; Marone et al., 2013; Chen et al., 2015). Otros se basan en receptores de la superficie de las membranas e incluyen receptores de tipo kinasas (receptor-like kinases, RLK) y otras proteínas receptoras (Monaghan & Zipfel, 2012; Mushtaq et al., 2018).

La interacción entre virus y genes R puede desencadenar una reacción de incompatibilidad a través de la respuesta hipersensible de la planta (HR), que activa la muerte celular programada e impide la propagación de la infección viral, conteniéndolo en el punto de entrada al tejido vegetal (Schmitt et al., 2019; Heath, 2000).

3.1.3. Resistencia recesiva

La resistencia a geminivirus regulada por genes de herencia recesiva generalmente proceden de una mutación en un gen necesario para que el virus complete su ciclo de replicación. Se trata de resistencia pasiva, no derivada del hospedante, generada por la pérdida de susceptibilidad (Schmitt-Keichinger, 2019).

3.1.4. Factores de transcripción y reprogramación celular

La infección por geminivirus genera una respuesta de defensa sistémica en la planta hospedante, que da lugar a una reprogramación celular e implica cambios en los procesos de transcripción, metabolismo y señalización hormonal (Malathi et al., 2017).

Los factores de transcripción modulan la expresión de genes implicados en la resistencia a patógenos. En estudios de la interacción planta-geminivirus se ha descrito una expresión diferencial en los factores de transcripción NAC, WRKY, bHLH, MYC, MYB y TIFY4B (Chen et al., 2013; Chung & Sunter 2014; Wang et al., 2015; Huang et al., 2016; 2017; Kumar et al., 2019).

Las rutas de señalización hormonal también se ven modificadas cuando se produce la infección por geminivirus (Alazem & Lin, 2015), modificándose la transcripción de ácido salicílico (SA), etileno (ET), ácido jasmónico (JA), brasinoesteroides (BRA), citoquininas, ácido abscísico (ABA), ácido giberélico (GA) y auxinas (AUX) (Bari & Jones; 2009; Miozzi et al., 2014; Rosas-Díaz et al., 2016; Ramesh et al., 2017).

A su vez, la HR activa genes implicados en defensa de la planta entre los que se incluyen modificaciones de la pared celular, metabolitos secundarios como fitoalexinas, especies reactivas de oxígeno, modificadores del flujo de iones Ca^{2+} y proteínas relacionadas con la patogénesis (PR), como es el caso de las proteínas transferentes de lípidos (Van Loon & Van Strien, 1999; Loebenstein, 2009; Sarowar et al., 2009; Carr et al., 2010).

La familia de genes que codifican las proteínas de choque térmico (Heat shock protein, HSP) también se han descrito como factores necesarios para que se produzca la replicación y propagación de geminivirus en las plantas (Czosnek et al., 2013; Gorovits & Czosnek, 2017; Chakraborty & Basak, 2018).

3.1.5. Ubiquitinación y sumoylación

El sistema ubiquitina-proteasoma está implicado en la respuesta inmune de la planta durante la infección por geminivirus, modificando las proteínas virales a nivel post-traduccional (de Ronde et al., 2014; Sahu et al., 2014a; Gnanasekaran et al., 2019; Kumar et al., 2019). Para ello, ubiquitinas y E1, E2 y E3 ubiquitina-ligasas forman un complejo que da lugar a la degradación proteasomal de las proteínas diana. Un caso concreto de ubiquitinacion es la sumoylacion, con péptidos modificadores de pequeñas ubiquitinas que se unen específicamente a enzimas E1, E2 y E3 concretas.

Durante la interacción planta-geminivirus, tanto la ubiquitinacion como la sumoylacion se han descrito como mecanismos de defensa que permiten degradar las proteínas virales evitando la infección (Sánchez-Durán et al., 2011; Verchot et al., 2016). Sin embargo, el complejo ubiquitina-proteasoma también puede ser utilizado por el virus, promoviendo cambios en la maquinaria celular que faciliten su propagación (Alcaide-Loridan & Jupin, 2012; Shen et al., 2016).

3.1.6. Degradación por autofagia

La autofagia es un mecanismo que las plantas utilizan como respuesta a la infección por geminivirus (Haxim et al., 2017; Choi et al., 2018; Su et al., 2020). Al silenciar diferentes factores de autofagia, plantas de *N. benthamiana* aumentaron su susceptibilidad a la infección por geminivirus, mientras que la activación de los mismos factores permitió que las plantas reconocieran la proteína viral βC1 y que fueran resistentes a las infecciones (Haxim et al., 2017).

Las cromometilasas (CMT) están implicadas en la metilación del DNA y contribuyen a la resistencia a geminivirus (Tu et al., 2017). Recientemente, se ha demostrado la implicación de CMTs y modificaciones epigenéticas en las rutas de autofagia (You et al., 2019), implicando factores que regulan la respuesta de defensa durante la infección viral por geminivirus.

3.2. Programas de mejora genética para la resistencia a geminivirus

Los programas de mejora genética para la resistencia a geminivirus se han centrado en aquellos cultivos en los que éstos generan graves pérdidas económicas (Rybicki, 2015), como el TYLCV, el virus de la hoja rizada del algodón (*cotton leaf curl virus*, CLCuV), el complejo de geminivirus causante del mosaico de la yuca (*cassava mosaic geminiviruses*, CMGs), el virus del mosaico dorado de la judía (*bean golden mosaic virus*, BGMV) en leguminosas, o los geminivirus que causan la enfermedad de la hoja rizada del pimiento (*chilli leaf curl virus disease*, ChiLCVD). La herencia de la resistencia a geminivirus se ha estudiado durante años (Lapidot & Friedmann, 2002). Sin embargo, muy pocos genes R se han identificado y clonado, lo que manifiesta la necesidad de estudiar la diversidad genética de los cultivos para identificar resistencia a geminivirus (Kuon et al., 2019). En la tabla 7 se resumen los principales genes y mecanismos implicados en la resistencia a geminivirus, identificados en especies vegetales modelo y cultivadas.

Los genes *Ty* de resistencia a TYLCV (*Ty-1*, *Ty-2*, *Ty-3*, *Ty-4*, *Ty-5* y *Ty-6*) son los más estudiados en la resistencia a geminivirus. Para su introgresión en variedades comerciales se han desarrollado diferentes marcadores moleculares que han permitido la selección asistida en programas de mejora (Jung et al., 2015; Kim et al., 2020). Estos genes se han transferido a diferentes variedades comerciales (Rojas et al., 2018; Sagar et al., 2020), aunque la resistencia conferida individualmente por cada uno puede verse influenciada por las diferentes zonas de cultivo, la temperatura, la virulencia de cada aislado o su capacidad para conferir inmunidad frente a begomovirus de genoma monopartito y fallar en los de bipartito (Vidavski, 2007; Mejía et al., 2010; Rojas et al., 2018; Hutton et al., 2012). La piramidalización de los genes *Ty* es el método más eficiente para lograr resistencia duradera al TYLCV (Vidavski et al., 2008; Prasanna et al., 2015; Hutton et al 2013; Gorovits et al., 2017; Kumar et al., 2019).

Tabla 7. Genes implicados en la resistencia a geminivirus y sus mecanismos de acción.

Gen	Proteína	Especie	Virus	Descripción	Referencias
Ty-1, Ty-3	RNA polimerasa dependiente de RNA del tipo γ (RDRγ)	*Solanum chilense* Dunal	Begomovirus mono (TYLCV) y bipartitos	Alélicos. Amplifican el TGS mediante la metilación del DNA viral	Zamir et al., 1994; Ji et al., 2007; Verlaan et al., 2011, 2013; Butterbach et al., 2014; Caro et al., 2015; Yan et al., 2018; Voorburg et al., 2020
Ty-2	NBS-LRR, *TYNBS1K* Solyc11g069910, Polimerasa de RNA dependiente de DNA de tipo II	*S habrochaites* S. Knapp & D.M Spooner *Solanum habrochaites*	Begomovirus monopartitos (TYLCV) pero no a los bipartitos	Gen dominante Involucrado en RdDM, Su nivel de expresión se reduce en variedades resistentes	Hanson et al., 2006; Barbieri et al., 2010; Prasanna et al., 2015; Yamaguchi et al., 2018 Yang et al., 2014; Kim et al., 2020
Ty-4		*Solanum chilense*	TYLCV	Confiere resistencia con menor eficiencia	Ji et al., 2009; Kadirvel et al., 2013
ty-5	Pelota	*Solanum peruvianum* (L.) Mill.	TYLCV y algunos begomovirus de genoma bipartito (aunque no a todos)	Afecta a la traducción en los ribosomas e impide completar el ciclo de infección viral. Asociado al TF NAC1 y un gen de la familia SUMO (Small Ubiquitin-Like Modifier)	Giordano et al., 2005; Anbinder et al., 2009; Hutton et al., 2012; Kadirvel et al., 2013; Lapidot et al., 2015; Gill et al., 2019
Ty-6		*Solanum chilense*	TYLCV y begomovirus bipartitos	Confiere altos niveles de resistencia, sobre todo en combinación con *Ty-3* o *ty-5*	Hutton et al., 2014; Scott et al., 2015; Gill et al., 2019
SlPerI	Permeasa	*Solanum habrochaites*	TYLCV	Transportador transmembrana	Eybishtz et al., 2009; Sun et al., 2016
LeHT1	Transportador de hexosa	*Solanum habrochaites*	TYLCV	Transportados de azúcares	Eybishtz et al., 2010
SlVSRLip	Lipocalin-like	*S. lycopersicum*	TYLCV	Sintetizado aguas abajo de *LeHT1*	Sade et al., 2012
CMD1, CMD3		*Manihot glaziovii* Muell. Arg	CMD	*CMD1* es recesivo y tiene una posible implicación en la metilación del genoma viral	Akano et al., 2002; Okogbenin et al., 2012; Fondong et al., 2017; Kuria et al., 2017
CMD2		*Manihot esculenta* Crantz	CMD	Monogénico dominante, confiere resistencia de amplio espectro. Genes candidatos propuestos de la familia de las peroxidasas y un gen SGS3 que actúa con las RDR6 y amplifica la señal del RNAi	Akano et al., 2002; Rabbi et al., 2014; Kuon et al., 2019; Mourrain et al., 2000; Maine, 2000; Li et al., 2017c
NbSGS3	Supresor del silenciamiento 3 (SGS3)	*N. benthamiana, S. lycopersicum*	TYLCV	Proteina del union al RNA en PTGS	Li et al., 2017c; Li et al., 2017d; Glick et al., 2008
NIK1	NSP-interacting kinase 1	*A. thaliana, S. lycopersicum, G. max*	Geminivirus	Receptor transmembrana tipo kinasa y LRR-RLK	Santos et al., 2010; Brustolini et al., 2015; Zorzatto et al., 2015

Tabla 7. Continuación

Gen	Proteína	Especie	Virus	Descripción	Referencias
tgr-1		*S. lycopersicum*	ToLCV	Gen recesivo, regula el movimiento del virus en la planta	Bian et al., 2007
gip-1		*Arabidopsis thaliana*	CaLCuV, BCTV, TYLCV	Resistencia recesiva	Reyes et al., 2017
SnRK1	SNF1-related protein kinase 1	*S. lycopersicum*	TYLCV, CaLCuV	Activado por las proteínas GRIK (Geminivirus Rep-interacting kinase) e implicado en la regulación por autofagia y fosforilación de proteínas virales	Baena-González et al., 2007; Shen et al., 2009; 2012; Hulsmans et al., 2016; Soto-Burgos & Bassham, 2017; Li et al., 2020; Su et al., 2020
Control poligénico		*Gossypium arboreum*	CLCuD	Cambios den la expresión de serina/threonina kinasas y NBS-LRR e implicados en la propagación célula a célula	Ullah, 2014; Naqvi et al., 2017; Mushtaq et al., 2018, 2020
Herencia cuantitativa		*Gossypium hirsutum*	CLCuD	Tres QTLs ligados a la resistencia. Involucradas HSP, serina/threonina kinasas y E3 ubiquitina ligasas.	Rahman et al., 2019; Naqvi et al., 2019; Zaidi et al., 2020
Resistencia mono y oligogénica		*Vigna radiata* (L.) R. Wilczek	MYMIV	La resistencia en KMG189 está gobernada por un gen recesivo, ligado a un gen monoxigenasa con contenido en flavina (FMO)	Dhole & Reddy, 2012; Alam et al., 2014; Sai et al., 2017
CYR1	CC-NBS-LRR	*Vigna mungo* (L.) R.Wilczek	MYMIV	Interacciona con la CP del virus	Maiti et al., 2011, 2012; Kundu & Pal, 2012
Resistencia mediada por RNAi		*G. max*	LYMV	siRNAs complementarios a la región intergénica del genoma viral	Yadav et al., 2009
Regulación poligénica		*G. max*	MYMIV	Implicación de genes NBS-LRR, muerte celular programada y resistencia a enfermedades	Yadav & Chattopadhya, 2014; Yadav et al., 2015
CchGLP	Germin-like protein	*C. chinense*	ChiLCV, PHYVV y PepGMV	Favorece la formación de especies reactivas de oxígeno (ROS), implicadas en HR y la muerte celular programada	Barrera-Pacheco et al., 2008; García-Neria & Rivera-Bustamante, 2011; Guevara-Olvera et al., 2012; Mejía-Teniente et al., 2015

CMD: enfermedad del mosaico de la yuca (*cassava mosaic disease*); ToLCV: virus de la hoja rizada del tomate (*tomato leaf curl virus*); CaLCuV: virus de la hoja rizada del repollo (*Cabbage leaf curl virus*); PepLCV: virus de la hoja rizada del pimiento (*pepper leaf curl virus*); CLCuD: virus de la hoja rizada del algodón (*cotton leaf curl virus disease*); MYMIV: virus del mosaico amarillo del frijol negro de India (*mungbean yellow mosaic India virus*); LYMV: virus del mosaico amarillo de las leguminosas *(legumes yellow mosaic virus*); PHYVV: virus Huasteco de las venas amarillas del pimiento (*pepper huasteco yellow vein virus*); PepGMV : virus del mosaico dorado del pimiento (*pepper golden mosaic virus*).

3.3. Mejora genética de la resistencia al ToLCNDV

La amenaza que supone el ToLCNDV para los cultivos de solanáceas y cucurbitáceas en el mundo, y la necesidad de desarrollar estrategias que limiten su infección, ha propiciado el desarrollo de programas de mejora encaminados a la obtención de cultivares con resistencia genética.

3.3.1. Identificación de fuentes de resistencia y caracterización de las mismas

Entre los cultivos de solanáceas se ha identificado resistencia al ToLCNDV en una accesión silvestre de tomate (*Solanum habrochaites*), en la que tres genes dominantes controlan el carácter (Rai et al., 2013). En pimiento y patata se han identificado fuentes de resistencia, aunque su genética no se ha estudiado (Srivastava et al., 2015; Jeevalatha et al., 2017).

En cucurbitáceas se ha estudiado la regulación de la herencia de la resistencia a ToLCNDV en esponja vegetal (*Luffa cylindrica* M. Roem.) y se ha identificado como monogénica dominante (Islam et al., 2010; 2011; Rai et al., 2013). Se ha caracterizado un gen NBS-LRR como gen candidato responsable de la resistencia (Saha et al., 2013).

En *C. melo*, López et al. (2015) identificaron resistencia al ToLCNDV-ES en cinco accesiones procedentes de la India, tres pertenecientes al grupo momordica y dos al grupo kachri. La caracterización de estas fuentes de resistencia forma parte de los objetivos de esta tesis (Capítulo III). La resistencia al ToLCNDV de las accesiones identificadas por López et al. (2015) también ha sido confirmada por Romay et al., (2019), que además han estudiado el control de la genética en la accesión IC-274014, del grupo momordica, identificando dos genes recesivos y uno dominante necesarios para la resistencia.

3.3.2. Resistencia al ToLCNDV conferida por genes *Ty*

La respuesta a la infección del ToLCNDV, tanto de los aislados asiáticos como del aislado ToLCNDV-ES, se ha evaluado en cultivos de tomate portadores de diferentes combinaciones de los genes *Ty*. Kumar et al., (2014) determinaron que el

gen *Ty-2* por sí solo no confiere resistencia a ToLCNDV, y que la mejor estrategia para obtener cultivares resistentes era la piramidalizacion de varios de los genes *Ty*. El alelo *Ty-3*, en homocigosis confiere un alto nivel de resistencia al ToLCNDV en tomate, dando lugar a plantas asintomáticas con baja acumulación viral (Prasanna et al., 2015; Jat et al., 2019; Hussain et al., 2019). La combinación de *Ty-2* con *Ty-3*, da lugar a plantas asintomáticas en campo y también se ha asociado con la resistencia a otros begomovirus bipartitos. *Ty-2* favorece la resistencia de *Ty-3* cuando éste se encuentra en heterocigosis (Prasanna et al., 2015).

Mediante la transmisión del ToLCNDV por chip de injerto en plantas de tomate se observó que el gen *ty-5* daba lugar a plantas resistentes de tomate, al igual que con las combinaciones *Ty-2* con *Ty-3*, *Ty-2* con *ty-5* y *Ty-1/Ty-3* con *Ty-2* (Akhtar et al., 2019).

Finalmente, Fortes et al., (2016) evaluaron la respuesta a la infección por ToLCNDV-ES en híbridos comerciales F_1 de tomate con el gen *Ty-1*, que sólo mostraron síntomas muy leves a la infección, considerándose que este gen también confiere resistencia al aislado detectado en la cuenca mediterránea.

3.3.3. Análisis de la expresión diferencial de genes tras la infección con ToLCNDV

El estrés que genera la infección por ToLCNDV en la planta da lugar a un reajuste transcripcional que ocasiona la desregulación de diferentes genes del hospedante. Para llevar a cabo la caracterización funcional de genes implicados en defensa de la planta frente al ToLCNDV, se han realizado estudios y análisis comparativos del nivel de transcritos durante la infección. En la tabla 8 se muestran los principales genes de defensa frente ToLCNDV identificados en trabajos de análisis de control genético y estudios comparativos a nivel transcriptómico.

Tabla 8. Principales genes implicados en la respuesta de defensa frente al ToLCNDV en diferentes especies hospedantes.

Gen	Cultivo	Descripción	Referencias
SlRPT4	Tomate	Gen de la subunidad proteasomal 26S RPT4a (complejo UPS). En un cultivar resistente dio lugar a la HR y muerte celular programada. Su silenciamiento propició el desarrollo de síntomas. La proteína que codifica se une a la estructura en horquilla del DNA viral, interfiriendo en la transcripción de los componentes genómicos y evitando la propagación del virus.	Sahu et al., 2016
SlDEAD35	Tomate	DEAD-box RNA helicasa, su inducción favorece la resistencia	Sahu et al., 2010; Pandey et al., 2019, 2020
NBS-LRR y proteínas transferentes de lípidos (LTP)	Pimiento	Inducción en genotipos resistentes	Kushwaha et al., 2015
NBS-LRR	Patata	Sobreexpresión en un cultivar resistente	Jeevalatha et al., 2017
HSP y HD-Zip	Patata	La inducción en genotipos susceptibles facilita la multiplicación del virus e incrementa la severidad de la infección	
AUX, ET, SA	Patata	En cultivares resistentes y susceptibles se indujeron genes codificantes de auxinas, etileno y ácido salicílico	
NAC	Tomate	Sobreexpresión de dos factores de transcripción NAC vinculados a la membrana durante la infección con ToLCNDV	Bhattacharjee et al., 2017
CmNAC y CmARP4	Melón	En el genotipo susceptible resultaron muy inducidos durante la infección. La proteína relacionada con la actina *CmARP4*, está implicado en el transporte y replicación del virus y *CmNAC* en el incremento en la acumulación del DNA viral	Román et al., 2019
SlTRNI	Tomate	TORNADO1. Implicación en la expansión celular y en la formación de venas. Su expresión está regulada por el factor de transcripción *SlWRKY16*	Mandal et al., 2015
SlWRKY16	Tomate	Inducción en respuesta a la activación de la ruta del SA en condiciones de estrés. Tanto *SlTRN*I como *SlWRKY16* se encuentran muy inducidos durante la infección por ToLCNDV, sugiriendo su implicación en el desarrollo de síntomas severos	
SlARM18	Tomate	proteína ARMADILLO con dominios repetitivos. Su inducción favorece la resistencia al virus. Asociada con dominios U-box y mecanismos de ubiquitinación.	Sahu et al., 2010; Mandal et al., 2018

3.3.4. Silenciamiento génico por RNA del ToLCNDV

El silenciamiento génico como respuesta antiviral también se ha observado en la infección por ToLCNDV en diferentes hospedantes. Sahu et al. (2010) identificaron un nivel más elevado de siRNAs en el cultivar resistente a ToLCNDV de tomate en comparación con el susceptible, relacionándolo con una carga viral reducida y baja infectividad. La tolerancia a ToLCNDV también se ha relacionado con un elevado nivel de siRNAs en *C. annuum*, asociándolo con la expresión diferencial de genes implicados en el silenciamiento post-transcripcional (RDR6, AGO1 y SGS3) (Kushwaha et al., 2015).

El silenciamiento génico a nivel transcripcional se ha descrito como una estrategia de defensa contra ToLCNDV en plantas resistentes de tomate (Sahu et al., 2014b). La metilación de las citosinas del DNA del virus, tanto en regiones codificantes como no codificantes, se observó en plantas tolerantes. Además, algunos genes implicados en la ruta RdDM, como metiltransferasas, DCL y cromometilasas (CMT3) también se sobrexpresaron en el cultivar tolerante.

La expresión de microRNAs durante la infección con ToLCNDV lleva asociada una inducción de síntomas en tomate (Naqvi et al., 2010; 2011b; Pradhan et al., 2015). La inducción de miR159, miR319, y miR172 en tomate y pimiento dio lugar a plantas con síntomas típicos de rizado en hojas. miR168 y miR162 estuvieron asociados a DCL1 y AGO1, respectivamente. Durante la infección por ToLCNDV en tomate, estos miRNAs actúan sobre factores de transcripción como TCP, MYB, NAC, homeodominios de leucina (Homeodomain-leucine zipper, HD-Zip), y factores implicados en la respuesta de auxinas (Auxin responsive factor, ARFs), generando la desregulación del desarrollo vegetal y provocando síntomas como el acucharado de las hojas (Naqvi et al., 2010).

Objetivos

El virus de la hoja rizada del tomate de Nueva Delhi (*tomato leaf curl New Delhi virus*, ToLCNDV) se ha convertido en una de las principales amenazas para el cultivo de cucurbitáceas en los países de la cuenca del Mediterráneo. La estrategia más prometedora para controlar la incidencia de esta enfermedad es el uso de germoplasma con resistencia genética.

El objetivo global de esta tesis doctoral ha sido el desarrollo de un programa de mejora genética para la identificación y caracterización de fuentes de resistencia al ToLCNDV en melón, calabacín y pepino, los tres cultivos que han sufrido daños más severos en el área Mediterránea. Para lograr este fin, se han abordado tres sub-objetivos que enmarcan los cinco capítulos en los que se estructura esta tesis:

Objetivo 1: Identificar fuentes de resistencia al ToLCNDV en pepino, calabacín y especies relacionadas del género *Cucurbita*, aprovechando la variación genética natural en cucurbitáceas.

Objetivo 2: Caracterizar el control genético de la resistencia en las accesiones identificadas, incluyendo las descritas previamente en melón, mapear las regiones genómicas implicadas y desarrollar marcadores moleculares ligados a la resistencia.

Objetivo 3: Analizar molecular y funcionalmente las resistencias para la identificación de genes candidatos.

Capítulo I

CAPÍTULO I

Resistance to *Tomato leaf curl New Delhi virus* in *Cucurbita* spp.

Cristina Sáez, Cecilia Martínez, María Ferriol, Susana Manzano, Leonardo Velasco, Manuel Jamilena, Carmelo López & Belén Picó

Annals of Applied Biology, 2016, 169: 91-105

ABSTRACT

Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite begomovirus (family *Geminiviridae*) first reported in India and its neighbouring countries. ToLCNDV severely affects zucchini crop (*Cucurbita pepo*) in the main production areas of Southern Spain since 2012. This emerging begomovirus is a serious threat to this and other cucurbit crops. Breeding resistant cultivars is the most promising method for disease control, but requires the identification of sources of resistance in the *Cucurbita* genus. In this work, we screened for ToLCNDV resistance a large collection of *Cucurbita* spp. accessions, including landraces and commercial cultivars of the main cultivated species, *C. pepo*, *Cucurbita moschata* and *Cucurbita maxima* and wild species. The screening was performed using mechanical and whitefly inoculation. The level of resistance was assessed by scoring symptom severity and by measuring the virus content with quantitative polymerase chain reaction in selected genotypes. Diversity in the response was observed within and among species. Severe symptoms and high viral amounts were found at 30 days after mechanical and whitefly inoculation in *C. pepo*, in all accessions belonging to the Zucchini morphotype and to other morphotypes of both subspecies, *pepo* and *ovifera*, and even in the wild relative *Cucurbita fraterna* . *C. maxima* was also highly susceptible. This species showed characteristic symptoms of leaf decay and intense yellowing, different from those of mosaic, curling and internode shortening found in *C. pepo*. The only species showing resistance was *C. moschata*. Four accessions were symptomless or had some plants with only mild symptoms after three independent rounds of mechanical inoculation with different inoculum sources. Two of them also remained symptomless after virus inoculation with viruliferous whiteflies. ToLCNDV was detected in these asymptomatic accessions at 15 and 30 days post inoculation, but viral amounts were much lower than those found in susceptible genotypes, suggesting a high level of resistance. The symptoms in the susceptible accessions of this species were also different, with a characteristic leaf mottling, evolving to a severe mosaic. The newly identified *C. moschata* resistant accessions are good candidates for breeding programmes to avoid the damage caused by ToLCNDV.

INTRODUCTION

Tomato leaf curl New Delhi virus (ToLCNDV) is a member of the genus *Begomovirus* (family *Geminiviridae*), with a bipartite genome, comprised of two circular single-stranded DNA molecules of approximately 2.7 kb each (designated as DNA-A and DNA-B), which are encapsidated in geminate particles. Both DNA-A- and DNA-B-encoded transcripts are required for infection and symptom development in host plants, although the DNA-A component is capable of autonomous replication inside the host (Papidam et al., 1995; Fauquet et al., 2008; Ito et al., 2008). ToLCNDV is transmitted in a persistent manner by the whiteflies of the Bemisia tabaci sibling species group (Chang et al., 2010; Islam et al., 2010; Khan et al., 2012; Jyothsna et al., 2013).

ToLCNDV was first reported on tomato (*Solanum lycopersicum* L.) in India (Papidam et al., 1995; Srivastava et al., 1995). Later, it was found in neighbouring countries on several hosts, particularly vegetable species of the *Cucurbitaceae* and *Solanaceae* families (Chang et al., 2010; Pratap et al., 2011; Khan et al., 2012; Jyothsna et al., 2013; Bandaranayake et al., 2014). During the last decade, its host range has increased and the virus has invaded new countries, arriving into Europe. A severe outbreak of ToLCNDV occurred in greenhouse and field-grown zucchini and melon crops in the main production area of Southern Spain in 2012–2013 (Juárez et al., 2014). Since then, this virus has been causing a great impact with catastrophic losses in this horticultural region, and is considered a serious threat to these and other cucurbit crops in the Mediterranean area.

Spain is one of the main world producers of zucchini and melon (FAOSTAT, 2015), and the first exporting country in Europe. The production of these crops has been severely affected by a number of viruses, particularly RNA viruses transmitted by aphids (Ferriol and Picó, 2008; Paris, 2008). However, apart from the typical New World begomovirus *Squash leaf curl virus* (SLCV), begomovirus association with zucchini and melon has been so far unknown in this region (Lecoq & Desbiez, 2012).

In many regions of the world, control strategies for begomovirus diseases focus on vector management. Several approaches including insecticide applications and physical barriers are used for reducing establishment of whitefly populations. In addition, cultural practices such as virus-free transplants, crop-free periods, weed management and roguing of infected plants are suggested for managing whiteflies (Seal et al., 2006; Lecoq & Desbiez, 2012; Janssen et al., 2014). However, these vector management strategies are not always fully effective. The complex epidemiological factors associated with these diseases, such as broad host range, accelerated rates of virus and vector evolution and the migratory behaviour of whiteflies hinder the development of effective long-term management strategies (Snehi et al., 2015). Therefore, breeding resistant cultivars is an essential element of a sustainable approach to manage the diseases caused by begomoviruses.

Since ToLCNDV was first detected affecting tomato and other solanaceous crops (Naqvi et al., 2010; Sahu et al., 2012; Rai et al., 2013; Ruíz et al., 2015), resistance studies are more advanced in this family (Kushwaha et al., 2015), and the search for resistance in cucurbits has not been a primary goal in the affected countries. Nonetheless, resistance screenings have been reported in sponge gourd (*Luffa cylindrica* M. Roem.), a popular cucurbit vegetable in India severely affected by this virus (Islam et al., 2010, 2011). Although most ToLCNDV isolates are naturally transmitted only by whiteflies, some of them have been shown to be mechanically (sap) transmitted to different hosts (Samretwanich et al., 2000; Usharani et al., 2004; Chang et al., 2010; Sohrab et al., 2013), including the new Spanish isolates. In a previous work, we developed a protocol for mechanical inoculation using a ToLCNDV isolate from Almeria, in southern Spain. Using this protocol, we demonstrated that this isolate has a wide host range, as it was successfully transmitted to 4 genera and 13 species of the *Cucurbitaceae* family, including the main crop species, such as cucumber, watermelon, melon, squash and zuc-chini, as well as crop-related exotic germplasm. The availability of this highly efficient method for mechanical transmission facilitated the identification of

resistance in Indian melons (López et al., 2015). This resistance is now being used to develop resistant melon cultivars.

There is an urgent need of developing resistant cultivars in zucchini. Losses in this crop are being especially devastating (Alfaro & Font, 2014; Janssen et al., 2014). The mechanical transmission method developed by López et al. (2015) also allowed a preliminary study of the response of the main species of the genus *Cucurbita*. In general, the susceptibility of *Cucurbita pepo* L. was much higher than that of the other cultivated species of the genus, especially *Cucurbita moschata* Duchesne and *Cucurbita maxima* Duchesne. This preliminary assay suggested a differential response of the species in the genus that needs to be further characterised and confirmed under natural infection with the vector to be useful in the development of resistant cultivars. Here, we report the screening of a collection of 110 *Cucurbita* accessions selected to represent the variability in the genus with both mechanical transmission, using different virus sources and whitely inoculation. The identification of two *C. moschata* accessions highly resistant to both mechanical and whitefly inoculation, which remained symptomless and showed a reduced viral accumulation, provides the first sources for breeding ToLCNDV-resistant *Cucurbita* cultivars.

MATERIALS AND METHODS

Plant material

A total of 110 *Cucurbita* accessions were first screened in two assays, one in climatic chamber using mechanical inoculation with a ToLCNDV isolate from affected fields in Almeria, and the second during spring–summer season in Almeria under greenhouse conditions with viruliferous whiteflies (Tables 1 and 2). The *Cucurbita* collection represents the three main cultivated species of the genus, *C. pepo* [64 accessions of subsp. *pepo* and 9 of subsp. *ovifera* (L.) D.S. Decker (= *texana* var. *ovifera*), and 2 F1 hybrids (subsp. *pepo* × *pepo* and subsp. *pepo* × *ovifera*)], *C. maxima* (14), and *C. moschata* (14), as well as six acces-sions of four wild types (two of *Cucurbita fraterna* L.H. Bailey, two of *Cucurbita okeechobeensis*

L.H. Bailey subsp. *martinezii* (L.H. Bailey) T.W. Walters & D.S. Decker, one of *Cucurbita lundelliana* L.H. Bailey, and one of *Cucurbita foetidissima* Kunth), and one of the cultivated *Cucurbita ficifolia* Bouché. All these accessions were selected from a collection of around 600 entries maintained at the germplasm bank of the Institute for the Conservation and Breeding of Agricultural Biodiversity (COMAV). Some of them were collected by the COMAV team and others originated from exchanges with other germplasm banks (mainly USDA-NPGS and CATIE). This selection aimed to represent the variability of the full collection. In *C. pepo*, the two subspecies (*pepo* and *ovifera*) and the main morphotypes within each subspecies (subsp. p*epo*: pumpkin, vegetable marrow, zucchini, cocozelle; subsp. *ovifera*: acorn, scallop, crookneck; and ornamental gourds) were represented. In *C. maxima* and *C. moschata*, accessions from the centre of origin and from secondary centres of diversity were included.

A selection of accessions having all or most of the plants with no or mild symptoms at the end of both screening assays (mechanical and whitefly inoculation) was assayed again to confirm their response. In this second experiment, we used mechanical inoculation in a climatic chamber with two inocula (the same inoculum from infected fields used previously and a new one obtained from an infectious clone as described below) to confirm the resistance of the selected accessions and to validate the use of the infectious clone in resistance screenings.

Virus sources for mechanical inoculation

ToLCNDV-infected zucchini plants from Almeria were the original source of inoculum for mechanical inoculation as described in López et al. (2015). The virus was transmitted to zucchini seedlings of the susceptible accession MU-CU-16 by virus-free whiteflies. Leaf extracts from these zucchini plants were collected 15 days after whitefly transmission and used as virus source for the first screening assay with the whole *Cucurbita* collection and for the second assay to confirm the response of some selected genotypes.

In this second assay, an additional virus source was used in mechanical transmissions, derived from a ToLCNDV-infectious clone. Dimeric clones of the DNA-A and DNA-B of a ToLCNDV isolate, from an infected zucchini plant in Almeria, were generated using rolling circle amplification and cloned into the binary vector pBINPLUS (Engelen et al., 1995). The clones were fully sequenced and showed 99% nucleotide identity with the sequence of the Spanish ToLCNDV isolate (KF749224 and KF749225; Juárez et al., 2014). Clones pBIN2TOA4R and pBIN2TOB14R with the complete dimers for DNA-A and DNA-B, respectively, were used separately for the transformation of *Agrobacterium tumefaciens* LBA4404. Two cultures of *A. tumefaciens*, each transformed with infectious clones pBIN2TOA4R or pBIN2TOB14R and grown in the selective media containing 25 μgmL−1 rifampicin and 50 μgmL−1 kanamycin, were sedimented by centrifugation, adjusted to 0.5 OD600, induced for 2 h at 28 ℃ and infiltrated by injection into petioles of MU-CU-16 zucchini plants. Fifteen days after agroinoculation, leaf extracts from plants showing ToLCNDV symptoms were used as virus source for mechanical inoculation in the second screening assay performed to confirm the response of some selected accessions.

Mechanical inoculation

With either inoculum from field or derived from the infectious clone, mechanical inoculation of ToLCNDV was performed as described in López et al. (2015). Briefly, 1 g of infected zucchini leaf tissue was ground in inoculation buffer in a 1:4 (*w:v*) proportion. The resultant homogenate was used for inoculation of one cotyledon and one fully expanded leaf of each plant, previously dusted with carborundum (600 mesh), by gently rubbing with cotton-bud sticks soaked with the crude homogenate.

For the mechanical inoculation, 10 plants per accession were inoculated in the first screening with the whole *Cucurbita* collection, and five plants per accession were inoculated with each of the two inocula (field and infectious clone) in the second assay performed to confirm the response of some selected accessions. In both

assays, two additional plants per accession were mock-inoculated with buffer and carborundum, or not inoculated to be used as negative controls. Seeds were disinfected by soaking them in 5% sodium hypochlorite for 3 min. Subsequently, they were kept in Petri dishes at 37°C for 48 h, and seedlings were transplanted to pots in a climatic chamber with controlled environmental conditions of 25/18°C day/night temperature, 60/95% day/night relative humidity and a 16–8 h light/dark photoperiod. Seedlings at the three true-leaf stage were mechanically inoculated.

Whitefly inoculation

Seedlings at the three–four true-leaf stage of the *C. pepo* susceptible cultivar Sinatra were transplanted into the greenhouse on 15 March. Infected adult plants of the same cultivar with clear symptoms of ToLCNDV, coming from an infected field in Almeria, were transplanted 37 days later to establish a population of viruliferous whiteflies in the greenhouse. Polymerase chain reaction (PCR) analysis was used, before transplanting, to confirm that the adult plants contained ToLCNDV, but not *Cucumber vein yellowing virus* (Picó et al., 2005) or *Cucumber yellowing stunting disorder virus*, two other local viruses transmitted by whiteflies. Once whiteflies were established in the plants of the first transplanting and these started to show symptoms of ToLCNDV, seedlings of the different *Cucurbita* accessions to be evaluated were distributed in the greenhouse and kept in nursery trays till the end of the assay. Two replications of six plants each were evaluated for each accession. For the first replication, the infection started on 30 April and for the second on 13 May. In both cases the assay was concluded 35 days later.

Symptoms evaluation and virus detection by polymerase chain reaction

In the first screening with the whole *Cucurbita* collection using mechanical inoculation, plants were kept in a climatic chamber, and every plant was evaluated for ToLCNDV symptoms at 30 days post inoculation (dpi). Symptoms were assessed visually, using a scale from 0 (absence of symptoms) to 4 (very severe symptoms or dead plant) detailed in López et al. (2015). The same conditions were used in the second assay, performed to confirm the response of some selected accessions, but

symptoms were scored at 15 and 30 dpi. In this assay, the presence of the virus was analysed at 15 and 30 dpi using a PCR reaction designed to detect the presence of both viral components. Total DNA from apical leaves was extracted using the CTAB method (Doyle & Doyle, 1990). DNA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) and diluted to a final concentration of 50 $ng \cdot \mu L^{-1}$. One microlitre aliquots of total DNA (50 ng) were used as templates in PCR reactions of 25 μL, containing 1 U of Taq DNA poly-merase (Biotools, Madrid, Spain), 1 μM of two different primer pairs (To-A1F/To-A1R and To-B1F/To-B1R) and 0.2 mM dNTPs in 75 mM Tris–HCl (pH 9.0), 2 mM $MgCl_2$, 50 mM KCl and 20 mM $(NH_4)_2SO_4$. The two primer pairs were derived from the Spanish isolate Murcia 11.1, one from the DNA-A, accession number KF749225, (To-A1F 5′-GGGTTGTGAAGGCCCTTGTAAGGTGC-3′, positions 476–501, and To-A1R 5′-AGTACAGGCCATATACAACATTAATGC-3′, positions 954–979), and the other from the DNA-B, accession number KF749228,(To-B1F 5′-GAAACACAAGAGGGCTCGGA-3′, positions 637–656, and To-B1R 5′-GCTCCACTATCAAAGGGCGT-3′, positions 1294–1313). Cycling conditions con-sisted of incubation at 94 °C for 5 min and 35 cycles of 95 °C for 30 s, 55 °C for 45 s, and 72 °C for 45 s, with a final extension of 10 min at 72 °C. The resulting PCR products of 504 and 677 bp in length were analysed by electrophoresis in 1.5% agarose gels in TAE buffer.

A quantitative polymerase chain reaction (qPCR) assay was also performed in selected samples to estimate virus titer in the most resistant accessions. Three biological samples (plants per genotype) were analysed at 15 and 30 dpi. Amplifications were done with primers designed from the DNA-A: ToLCNDVF1 (5′-AATGCCGACTACACCAAGCA-3′, positions 1145–1169) and ToLCNDVR1 (5′-GGATCGAGAAGAGA GTGGCG-3′, positions 1399–1418), producing a fragment of 274 bp. The qPCR was performed in a Rotorgene thermocycler (Qiagen, Hilden, Germany). The reaction mix contained 7.5 μL of iTaq Universal SYBR Green supermix (2×) (BIORAD, Hercules, CA, USA), 1 μM of each primer and 1.5 μL of total DNA. Cycling conditions consisted of incubation at 95 °C for 5 min and

40 cycles of 95 ℃ for 5s and60∘C for 30 s. Three technical replications were performed per sample. Relative accumulation of ToLCNDV in the plants was calculated by the comparative Ct (cycle threshold) method, using the gene *CpACS27A* from *C. pepo* as an internal standard (ACS27FWDRACE 5′-CCACTTGGTGCCACAATCCAACGG-3′ and ACS27RE VRACE 5′-GCCTATCCAAAGACCTCGGCCTTCCC-3′). Firstly, we demonstrated that the efficiency of amplification for each amplicon was roughly equivalent, regardless of the amount of template cDNA. The relative accumulation of the virus to a calibrator sample was calculated using the formula $2^{-\Delta\Delta Ct}$, where ΔΔCt is the difference between the ΔCt of each sample and the ΔCt of the calibrator sample.

Symptoms after whitefly transmission in the greenhouse assay with the whole *Cucurbita* collection were scored using the same scale, at 21, 28 and 35 dpi (days after the introduction of plants in the greenhouse with the viruliferous whitefly population). Quantitative polymerase chain reaction was also used to estimate virus titer in a selected set of the accessions analysed in the greenhouse. Two biological replications (two pools of six plants) were analysed per genotype at 28 dpi, using the same procedure described above.

Data analysis

Resistance was evaluated as the host response to virus infection estimated from symptom severity and in some selected genotypes from viral titre. In the first screening assay, the percentage of symptomatic plants and the mean and range of symptom scores were calculated for each genotype after mechanical and whitefly inoculation. The mean and range of symptom scores were also calculated, along with the percentage of PCR positive plants, in the second assay performed to confirm resistance of selected genotypes. The viral titer was estimated by qPCR in some selected plants representing different responses.

RESULTS

Response of *Cucurbita* spp to mechanical and whitefly-mediated ToLCNDV transmission

A total of 75 accessions of *C. pepo* were assayed. Most of them were highly susceptible to the mechanical transmission of ToLCNDV, developing severe to very severe symptoms at 30 dpi (mean symptom score 3.6, ranging from 2.3 to 4) (Table 1). The observed symptoms included upward and downward curling and severe mosaic of young leaves and short internodes (Fig. 1A). Natural infection revealed differences in infection progress among the *C. pepo* accessions at 21 dpi (Table 1). However, all of them had very severe symptoms at the end of the assay (mean symptom score of 1.2 and 3.9 at 21 and 35 dpi, respectively).

The accessions assayed represented the main morphotypes of the two subspecies of *C. pepo* (*pepo* and *ovifera*), and the response to mechanical transmission of ToLCNDV was similar in all of them (Table 1). All the accessions of the pumpkin and vegetable marrow morphotypes, from diverse origins, were highly susceptible (mean symptom score 3.5 and 3.7, respectively, at 30 dpi). The Spanish and Italian accessions of the two more modern morphotypes of subsp. *pepo*, zucchini and cocozelle were also found to be highly susceptible (mean symptom score 3.4 and 3.5, respectively). Results indicated that accessions of the subspecies *ovifera*, both edible and ornamental types, were as susceptible as those of subsp. *pepo* (mean symptom score 3.8). The two Mexican accessions of the wild species *C. fraterna* were as highly susceptible to ToLCNDV as the cultivated genotypes (mean symptom score 3.8). Finally, the two F1 hybrids (subsp. *pepo* × *pepo* and subsp. *pepo* × *ovifera*) assayed as a part of a breeding program for developing new *Cucurbita* rootstocks were also susceptible. The early response to whitefly inoculation was less severe in wild *C. fraterna* (Figure 2). Differences in plant vigour may partly account for these differences as the less vigourous genotypes, belonging to wild *C. fraterna*, might have a delayed expression of virus symptoms (Table 1). However, in all cases a very severe infection was observed at the end of the greenhouse assay.

Table 1. Response of *Cucurbita pepo* accessions to mechanical and whitefly inoculation with ToLCNDV.

			Mechanical inoculation[b]		Whitefly transmission [c]						
			Symptoms		Symptomatic plants (%)		Symptoms				Plant vigour
	Species/ subspecies/ morphotype		30 dpi		21 dpi	35 dpi	21 dpi		35 dpi		28 dpi
Origin		Accession [a]	Mean	Range			Mean	Range	Mean	Range	Mean
Cucurbita pepo											
Subsp. pepo											
Guatemala	Pumpkin	CATIE-11368	3.6	3–4	20	100	0.75	0–2	4.0	4–4	2.0
Turkey	Pumpkin	PI-169462	3.0	2–4	0	100	0.0	0–0	4.0	4–4	2.5
Turkey	Pumpkin	PI-204698	3.3	2–4	0	100	0.0	0–0	4.0	4–4	1.0
Turkey	Pumpkin	PI-171628	4.0	4–4	100	100	2.2	1–3	4.0	4–4	2.4
Italy	Pumpkin	PU-TON	3.6	3–4	100	100	2.3	1–4	4.0	4–4	2.0
Italy	Pumpkin	PU-TOP	3.4	3–4	60	100	0.60	0–1	4.0	4–4	1.0
Spain (Guadalajara)	Pumpkin	359	4.0	4–4	60	100	1.0	0–3	4.0	4–4	1.0
Spain (Cuenca)	Pumpkin	1012	3.0	1–4	80	100	0.80	0–1	4.0	4–4	2.0
Spain (Cuenca)	Pumpkin	1086	3.7	3–4	80	100	1.4	0–3	4.0	4–4	1.2
Spain (Huelva)	Pumpkin	AN-CU-83	4.0	4–4	40	100	0.80	0–3	4.0	4–4	2.8
Spain (Canary Islands)	Pumpkin	CA-CU-43	4.0	4–4	50	100	1.5	0–4	4.0	4–4	1.8
Spain (Canary Islands)	Pumpkin	CA-CU-46	4.0	4–4	100	100	3.8	3–4	4.0	4–4	2.0
Spain (Canary Islands)	Pumpkin	CA-CU-48	2.3	1–4	75	100	1.8	0–4	4.0	4–4	1.0
Spain (Canary Islands)	Pumpkin	CA-CU-57	3.0	2–4	100	100	2.5	1–4	4.0	4–4	2.5
Spain (Canary Islands)	Pumpkin	CA-CU-59	4.0	4–4	25	100	0.25	0–1	4.0	4–4	2.5
Spain (Canary Islands)	Pumpkin	CA-CU-110	3.4	3–4	50	100	2.0	0–4	4.0	4–4	2.5
Spain (Canary Islands)	Pumpkin	CA-CU-192	4.0	4–4	100	100	4.0	4–4	4.0	4–4	1.0
Spain (Canary Islands)	Pumpkin	CA-CU-21	3.0	1–4	100	100	2.5	1–4	4.0	4–4	1.0
Hungary	Pumpkin	IVIA-506	2.4	1–4	100	100	2.5	1–3	4.0	4–4	2.5
Morocco	V. Marrow	AFR-CU-12	4.0	4–4	100	100	2.3	1–4	4.0	4–4	2.5
Morocco	V. Marrow	AFR-CU-8	4.0	4–4	80	100	2.2	0–3	4.0	4–4	2.5
Morocco	V. Marrow	AFR-CU-15	3.2	3–4	100	100	2.5	1–3	4.0	4–4	2.5
Morocco	V. Marrow	AFR-CU-17	4.0	4–4	75	100	1.5	0–3	4.0	4–4	1.5
Morocco	V. Marrow	AFR-CU-22	4.0	4–4	0	100	0.0	0–0	4.0	4–4	2.4
Spain (Guadalajara)	V. Marrow	942	4.0	4–4	40	100	1.0	0–3	4.0	4–4	1.0
Spain (Guadalajara)	V. Marrow	949	4.0	4–4	80	100	1.4	0–3	4.0	4–4	2.8
Spain (Huesca)	V. Marrow	A-CU-12	4.0	4–4	40	100	0.40	0–1	4.0	4–4	2.4
Spain (Almería)	V. Marrow	AN-CU-113	3.0	2–4	100	100	3.7	3–4	4.0	4–4	2.0
Spain (Cádiz)	V. Marrow	AN-CU-27	4.0	4–4	100	100	4.0	4–4	4.0	4–4	2.0
Spain (Segovia)	V. Marrow	CL-CU-19	3.0	1–4	50	100	0.50	0–1	4.0	4–4	1.3
Spain (Valladolid)	V. Marrow	CL-CU-21	3.2	1–4	50	100	1.5	0–3	4.0	4–4	2.0
Spain (Cuenca)	V. Marrow	CM-CU-32	3.9	3–4	50	100	1.0	0–3	4.0	4–4	2.0
Spain (Cuenca)	V. Marrow	CM-CU-47	3.6	3–4	33	100	0.30	0–1	4.0	4–4	2.0
Spain (Valencia)	V. Marrow	V-CU-10	4.0	4–4	50	100	2.0	0–4	3.3	1–4	2.3
Spain (Alicante)	V. Marrow	V-CU-32	3.3	3–4	100	100	1.0	1–1	4.0	4–4	3.0
Spain (Canary Islands)	V. Marrow	CA-CU-79	4.0	4–4	50	100	2.0	0–4	4.0	4–4	2.5
Spain (Canary Islands)	V. Marrow	CA-CU-82	4.0	4–4	67	100	0.70	0–1	4.0	4–4	2.3
Spain (Canary Islands)	V. Marrow	CA-CU-83	3.8	3–4	100	100	2.5	1–3	4.0	4–4	2.8
Spain (Canary Islands)	V. Marrow	CA-CU-84	4.0	4–4	50	100	0.50	0–1	4.0	4–4	2.3
Spain (Canary Islands)	V. Marrow	CA-CU-113	3.4	2–4	100	100	4.0	4–4	4.0	4–4	1.5
Spain (Valencia)	V. Marrow	IVIA-032	4.0	4–4	50	100	0.50	0–1	4.0	4–4	0.80
Spain (Teruel)	V. Marrow	A-CU-2	3.3	2–4	75	100	1.5	0–3	4.0	4–4	2.3
Spain (Tarragona)	V. Marrow	C-CU-3	3.5	3–4	75	100	2.5	0–4	4.0	4–4	2.5
Ecuador	Zucchini	ECU-227	3.7	2–4	50	100	1.3	0–4	4.0	4–4	2.0
Italy	Zucchini	ZU-NVM	2.7	2–4	60	100	0.60	0–1	4.0	4–4	1.8
Spain (Guadalajara)	Zucchini	435	4.0	4–4	60	100	0.80	0–1	4.0	4–4	1.0
Spain (Huesca)	Zucchini	A-CU-13	2.6	2–4	0	100	0.0	0–0	4.0	4–4	1.8
Spain (Caceres)	Zucchini	E-CU-10	4.0	4–4	67	100	0.70	0–3	4.0	4–4	2.0

Table 1. Continued.

Origin	Species/ subspecies/ morphotype	Accession [a]	Mechanical inoculation[b] Symptoms 30 dpi Mean	Range	Whitefly transmission [c] Symptomatic plants (%) 21 dpi	35 dpi	Symptoms 21 dpi Mean	Range	35 dpi Mean	Range	Plant vigour 28 dpi Mean
Spain (Caceres)	Zucchini	E-CU-27	4.0	4–4	100	100	1.7	1–3	4.0	4–4	2.8
Spain (Murcia)	Zucchini	MU-CU-20	3.1	3-4	75	100	1.75	0–4	4.0	4–4	3.0
Spain (Murcia)	Zucchini	MU-CU-16	4.0	4–4	50	100	0.50	0–1	4.0	4–4	2.3
Spain (Córdoba)	Cocozelle	AN-CU-75	3.7	2–4	60	100	1.8	0–4	4.0	4–4	1.0
Spain (Barcelona)	Cocozelle	C-CU-9	4.0	4–4	60	100	1.4	0–3	4.0	4–4	1.6
Spain (Castellón)	Cocozelle	PAS-15834	3.4	2–3	75	100	2.5	0–4	4.0	4–4	1.5
Spain (Castellón)	Cocozelle	PASCUAL-40	3.0	3–3	50	100	1.5	0–4	4.0	4–4	2.8
Spain (Alicante)	Cocozelle	V-CU-185	3.6	3–4	100	100	4.0	4–4	4.0	4–4	2.0
Spain (Valencia)	Cocozelle	V-CU-74	3.6	3–4	100	100	2.3	1–3	4.0	4–4	2.3
Italy	Cocozelle	CO-DBT	4.0	4–4	100	100	2.8	1–4	4.0	4–4	2.0
Italy	Cocozelle	CO-LBS	2.6	1–4	67	100	2.3	0–4	4.0	4–4	2.0
Italy	Cocozelle	CO-LUF	2.5	1–4	100	100	2.3	1–3	4.0	4–4	2.0
Italy	Cocozelle	CO-ROM	4.0	4–4	40	100	1.0	0–3	4.0	4–4	2.0
Italy	Cocozelle	CO-SPQ	4.0	4–4	100	100	1.7	1–3	4.0	4–4	1.0
Italy	Cocozelle	CO-VAL	4.0	4–4	100	100	3.5	1–4	4.0	4–4	3.0
Greece	Cocozelle	Grecia-6	3.6	2–4	40	100	0.90	0–4	4.0	4–4	2.8
Subsp. ovifera											
USA	Crookneck	NSL-5206	3.8	2–4	100	100	3.3	3–4	4.0	4–4	1.0
USA	Crookneck	NSL-5227	4.0	4–4	50	100	1.3	0–4	4.0	4–4	1.8
USA	Crookneck	PI-106681	3.3	3–4	25	100	0.25	0–1	4.0	4–4	1.5
USA	Crookneck	USA-CU-2	4.0	4–4	100	100	2.8	1–4	4.0	4–4	1.8
USA	Acorn	PI-615111	4.0	4–4	60	100	1.6	0–3	4.0	4–4	1.8
USA	Acorn	PI-518687	3.4	2–4	25	100	0.30	0–1	4.0	4–4	1.3
Spain (Valencia)	Scallop	V-CU-196	4.0	4–4	100	100	1.0	1–1	4.0	4–4	2.0
Spain (Valencia)	Ornamental	V-CU-81	3.7	3–4	20	100	0.40	0–1	4.0	4–4	1.4
Spain (Valencia)	Ornamental	IMA-569	4.0	4–4	25	100	1.0	0–4	4.0	4–4	2.8
F1	Hybrid	TFxPI-171628	4.0	4–4	50	100	1.5	0–4	4.0	4–4	2.0
F1	Hybrid	TFx V-CU-196	4.0	4–4	50	100	2.0	0–4	4.0	4–4	2.3
Cucurbita fraterna											
Mexico		PI-614701	3.6	3–4	0	100	0.0	0–0	3.0	3–3	0.0
Mexico		PI-532354	4.0	4–4	100	100	1.0	1–1	4.0	4–4	1.0

ToLCNDV, *Tomato leaf curl New Delhi virus*.

[a]Most of the assayed accessions are from the germplasm collection of the Institute for Conservation and Breeding of theAgrodiversity, Spain (COMAV-UPV). PI and NSL genotypes were kindly provided by U.S. Deptartment of Agriculture National Plant Germplasm System and CATIE by the Centro Agronómico Tropical de Investigación y Enseñanza, Costa Rica.

[b]Mean and range of symptoms scored in 10 plants per genotype mechanically inoculated with a ToLCNDV isolate originally identified in infected zucchini plants from Almeria, according to the following scale: 0, absence of symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms; 4, very severe symptoms or plant death (López *et al*., 2015).

[c]Percentage of plants showing symptoms of ToLCNDV after whitefly transmission in the greenhouse experiment. Mean and range of symptoms were scored in 12 plants per genotype (two sets of six plants). Mean vigour of the plants of each genotype was scored from 0 (weak plants) to 4 (highly vigourous).

Cucurbita maxima accessions, mainly from America and Africa, were susceptible to ToLCNDV (mean symptom score 3.1, ranging from 2.2 to 4), in general with less severe symptoms than the *C. pepo* accessions (Table 2). However, although whitefly inoculation caused a delayed and less severe infection at the beginning of the assay in *C. maxima* in comparison with *C. pepo* , at the end of the

assay both species gave similar results with 100% symptomatic plants and very severe symptoms (Figure 2). This species showed characteristic symptoms of leaf decay and intense yellowing, different from those found in *C. pepo* (Fig. 1B).

The only accession assayed of *C. ficifolia* was highly susceptible to the infection (mean symptom score 4). The wild species assayed showed variable responses to mechanical inoculation (mean symptom scores from 0 to 4) (Table 2), but were all highly susceptible after whitefly inoculation.

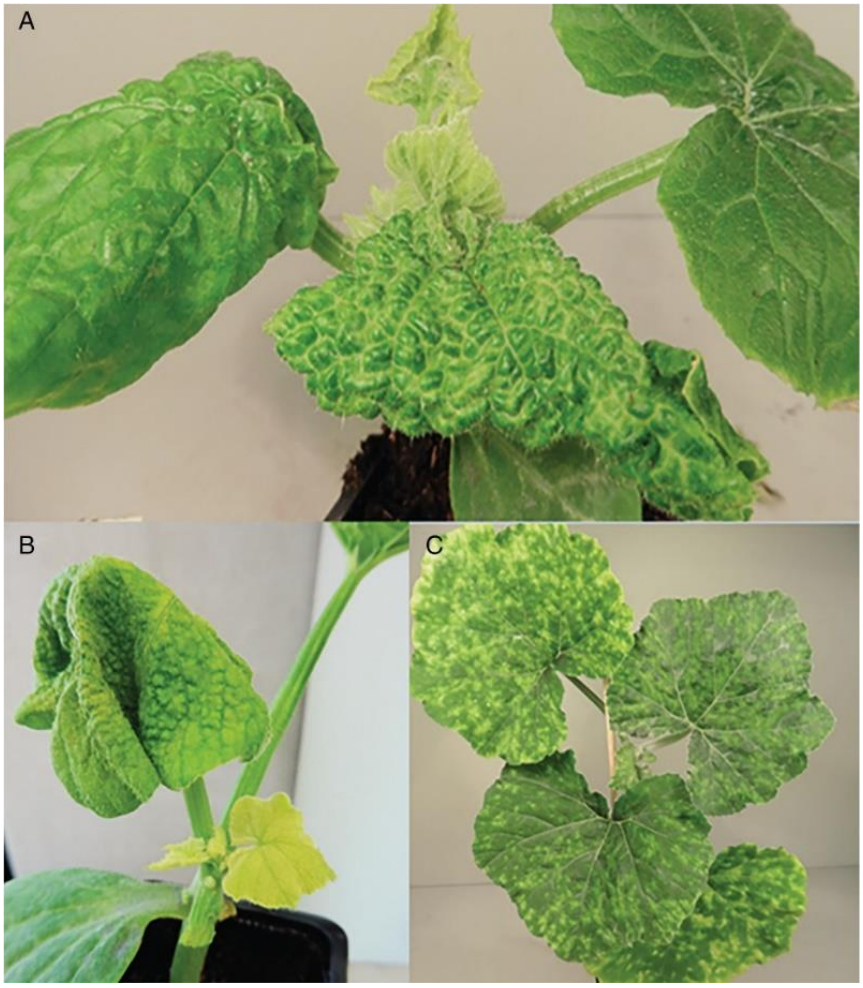

Figure 1. Symptoms of *Tomato leaf curl New Delhi virus* in *Cucurbita* species. (A) Typical symptoms of curling and severe mosaic of young leaves and short internodes observed in *Cucurbita pepo* (scored as 4, severe symptoms). (B) Characteristic symptoms of leaf decay and intense yellowing found in most accessions of *Cucurbita maxima*. (C) Symptoms of leaf mottling evolving to severe mosaic in the susceptible accessions of *Cucurbita moschata*.

Table 2. Response of *Cucurbita* spp. accessions to mechanical and whitefly inoculation with ToLCNDV.

Origin	Species/ subespecies/ morphotype	Accession[c]	Mechanical inoculation[a]		Whitefly transmission [b]						
			Symptoms		Symptomatic plants (%)		Symptoms				Plant vigour
			30dpi		21dpi	35dpi	21dpi		35dpi		28dpi
			Mean	Range			Mean	Range	Mean	Range	Mean
C. maxima											
Argentina		SUD-CU-6	3.6	2-4	25	100	1.0	0-4	4.0	4-4	3.0
Argentina		MAX-306	2.6	1-4	0	100	0.0	0-0	4.0	4-4	2.0
Argentina		BGV-15415	4.0	4-4	0	100	0.0	0-0	4.0	4-4	2.8
Bolivia		PI-543227	2.2	1-4	50	100	0.69	0-1	4.0	4-4	2.0
Chile		VAV-3202	3.4	1-4	100	100	2.0	1-3	4.0	4-4	2.1
Colombia		CATIE-9824	2.6	0-4	50	100	1.0	0-3	4.0	4-4	2.8
Peru		VAV-4273	2.8	1-4	0	100	0.0	0-0	4.0	4-4	2.2
Morocco		AFR-CU-1	3.0	2-4	0	100	0.0	0-0	4.0	4-4	3.2
Morocco		AFR-CU-8	3.4	3-4	0	100	0.0	0-0	4.0	4-4	2.0
Morocco		AFR-CU-18	3.6	2-4	0	100	0.0	0-0	4.0	4-4	2.0
Angola		AFR-CU-38	3.8	2-4	0	100	0.0	0-0	4.0	4-4	2.1
Angola		AFR-CU-73	2.3	1-4	20	100	0.0	0-1	4.0	4-4	2.1
African Republic		VAV-2422	3.1	2-4	0	100	0.0	0-0	4.0	4-4	2.0
Spain (Jaen)		AN-CU-59	3.0	1-4	20	100	1.0	0-4	4.0	4-4	2.8
C. moschata											
Costa Rica		PI-369346	2.5	1-4	0	100	0.0	0-0	4.0	4-4	1.0
Cuba		SUD-CU-8	3.6	3-4	20	80	0.30	0-1	3.2	0-4	1.0
Ecuador		ECU-46	1.7	0-4	0	100	0.0	0-0	4.0	4-4	2.3
Guatemala		PI-264551	2.6	2-4	20	75	0.30	0-1	3.0	0-4	1.0
Dom. Republic		SUD-CU-13	2.8	2-4	0	100	0.0	0-0	3.0	1-4	1.0
USA		PI-604506	0.70	0-1	0	0	0.0	0-0	0.0	0-0	2.0
Canada		PI-550689	1.2	1-4	0	50	0.0	0-0	1.5	0-3	1.2
Zimbawe		PI-482527	4.0	4-4	0	100	0.0	0-0	2.7	0-4	1.3
Nigeria		Nig.Local	0.60	0-1	60	40	0.80	0-1	1.7	0-4	2.0
Spain (Canary Islands)		CA-CU-26	1.3	0-2	0	100	0.0	0-0	4.0	4-4	3.0
Spain (Jaen)		AN-CU-45	1.4	0-3	0	60	0.0	0-0	2.4	0-4	1.6
Spain (Valencia)		IVIA-205	1.8	1-3	0	100	0.0	0-0	4.0	4-4	2.0
Japan		KUROKAWA	0.40	0-1	0	75	0.0	0-0	1.8	0-4	1.0
India		PI-381814	0.20	0-1	0	0	0.0	0-0	0.0	0-0	2.0
Other species											
Peru	***C. ficifolia***	GRIF9448	4.0	4-4	100	100	3.0	3-3	4.0	4-4	1.0
Belize	***C. lundeliana***	PI-438542	0.60	0-2	0	100	0.0	0-0	4.0	4-4	0.5
Mexico	***C. foetidisima***	PI-442197	3.0	2-4	0	100	0.0	0-0	3.8	3-4	1.0
Mexico	***C. okeechobeensis***	PI-532363	0.0	0-0	100	100	1.0	1-1	4.0	4-4	1.0
Mexico	***C. okeechobeensis***	PI-512105	2.0	1-4	60	100	0.80	0-1	4.0	4-4	0.8

ToLCNDV, Tomato leaf curl New Delhi virus.

[a]Most of the assayed accessions are from the germplasm collection of the Institute for Conservation and Breeding of the Agrodiversity, Spain (COMAV-UPV). PI and NSL genotypes were kindly provided by U.S. Department of Agriculture National Plant Germplasm System and CATIE by the Centro Agronómico Tropical de Investigación y Enseñanza, Costa Rica.

[b]Mean and range of symptoms scored in 10 plants per genotype mechanically inoculated with a ToLCNDV isolate originally identified in infected zucchini plants from Almeria, according to the following scale: 0, absence of symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms; 4, very severe symptoms or plant death (López *et al.*, 2015).

[c]Percentage of plants showing symptoms of ToLCNDV after whitefly transmission in the greenhouse experiment. Mean and range of symptoms were scored in 12 plants per genotype (two sets of six plants). Mean vigour of the plants of each genotype was scored from 0 (weak plants) to 4 (highly vigourous).

Cucurbita moschata showed less severe symptoms than the other species (Table 2). Most of the accessions assayed had mean scores of symptoms from mild to moderate after mechanical inoculation (mean symptom score 1.7, ranging from 0.2 to 4). Whitefly inoculation resulted in a significantly delayed infection with variable symptoms, from mild to very severe symptoms at the end of the assay (Figure 2). Susceptible accessions developed severe symptoms with characteristic leaf mottling evolving to severe mosaic, but without the leaf curling and the internodes shortening found in *C. pepo* (Fig. 1C). Four accessions displayed interesting results after mechanical inoculation: PI 604506 (the cultivar Cheese Large) from the USA, PI 381814 from India, Nigerian Local from Nigeria, and Kurokawa from Japan. All had mean symptom scores below 1, with all plants ranging from mild to no symptoms. The Indian and American accessions (PI 381814 and PI 604506) also remained symptomless after whitefly inoculation, whereas Nigerian Local and Kurokawa showed some plants with severe symptoms under greenhouse conditions (Table 2).

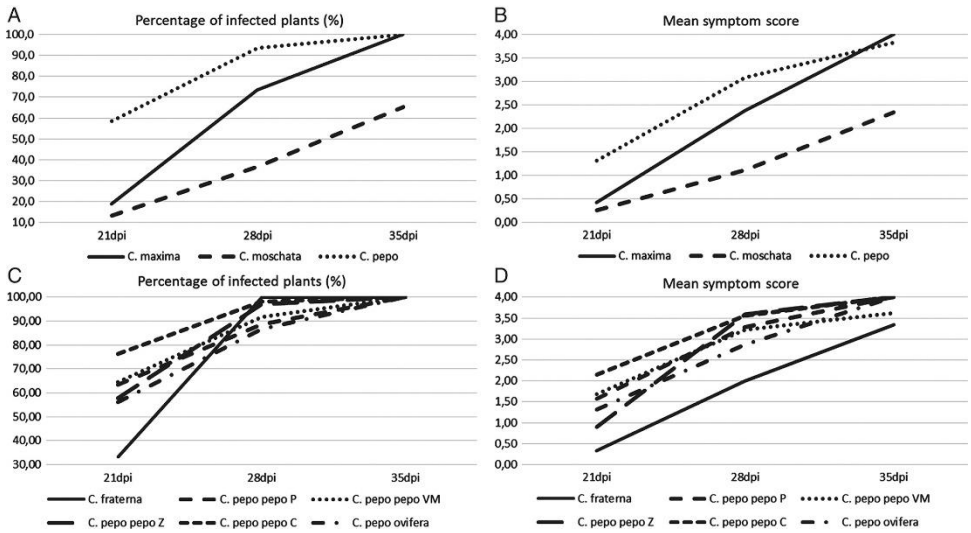

Figure 2. Time course response of accessions belonging to the three main cultivated *Cucurbita* species (*Cucurbita pepo* , *Cucurbita moschata* and *Cucurbita maxima*) to whitefly inoculation with Tomato leaf curl New Delhi virus, measured as percentage of symptomatic plants (A) and mean symptom score (B) (on a scale from 0, symptomless, to 4, very severe symptoms). Means of 12 plants (two sets of six plants) are shown. Detailed information of the different *C. pepo* subsp. *pepo* morphotypes is included (P: Pumpkin; VM: Vegetable Marrow; Z: Zucchini; C: Cocozelle) and is compared with the subsp. *ovifera* (C) and the wild relative *Cucurbita fraterna* (D).

The accumulation of ToLCNDV was evaluated in two pools, each of six plants, in nine selected *C. moschata* genotypes, representing a range of responses after whitefly inoculation (Figure 3). These were the four accessions having scores below one after the mechanical inoculation (PI 604506, PI 381814, Nigerian Local and Kurokawa), two additional accessions that remained with moderate symptoms after both mechanical and whitefly inoculation (PI 550689 and AN-CU-45), and three accessions that had moderate symptoms after mechanical inoculation, but severe at the end of the whitefly assay (PI 264551, IVIA 205 and PI 369346). Five highly susceptible accessions, three *C. pepo* and two *C. maxima*, were used as controls. The *C. pepo* and *C. maxima* susceptible controls showed the highest accumulation of the virus, which was similar in both species (Figure 3). ToLCNDV was also detected in the *C. moschata* accessions. The accessions with severe symptoms at the end of the assay had viral titres between 15 and 1.5 times lower than the other species. *Cucurbita moschata* accessions showing no or mild to moderate symptoms (PI 604506, PI 381814, Nigerian Local, Kurokawa and AN-CU-45) displayed the lowest viral titres.

Confirmation of ToLCNDV resistance in *Cucurbita moschata*

The response of the resistant *C. moschata* genotypes was confirmed in a second screening assay, along with some selected *C. pepo* and *C. maxima* accessions that showed different symptom levels in the first assay. Both the inoculum derived from zucchini field-infected plants used before and the new one coming from the ToLCNDV-infectious clone were used. Results of these inoculations are shown in Table 3 and confirmed the previously obtained results. All accessions showed similar results, and no differences were found in the evolution of symptoms between the two inocula sources, thus confirming the utility of the infectious clone for resistance screenings.

All the assayed plants were sampled for ToLCNDV detection by PCR. Both DNA components were detected in most plants of all genotypes, confirming the viral infection, even in symptomless plants (Table 3). Highly severe infections were

confirmed in *C. pepo*, *C. fraterna* and *C. ficifolia*. The highly susceptible plants of these species had symptoms evolving from moderate to severe.

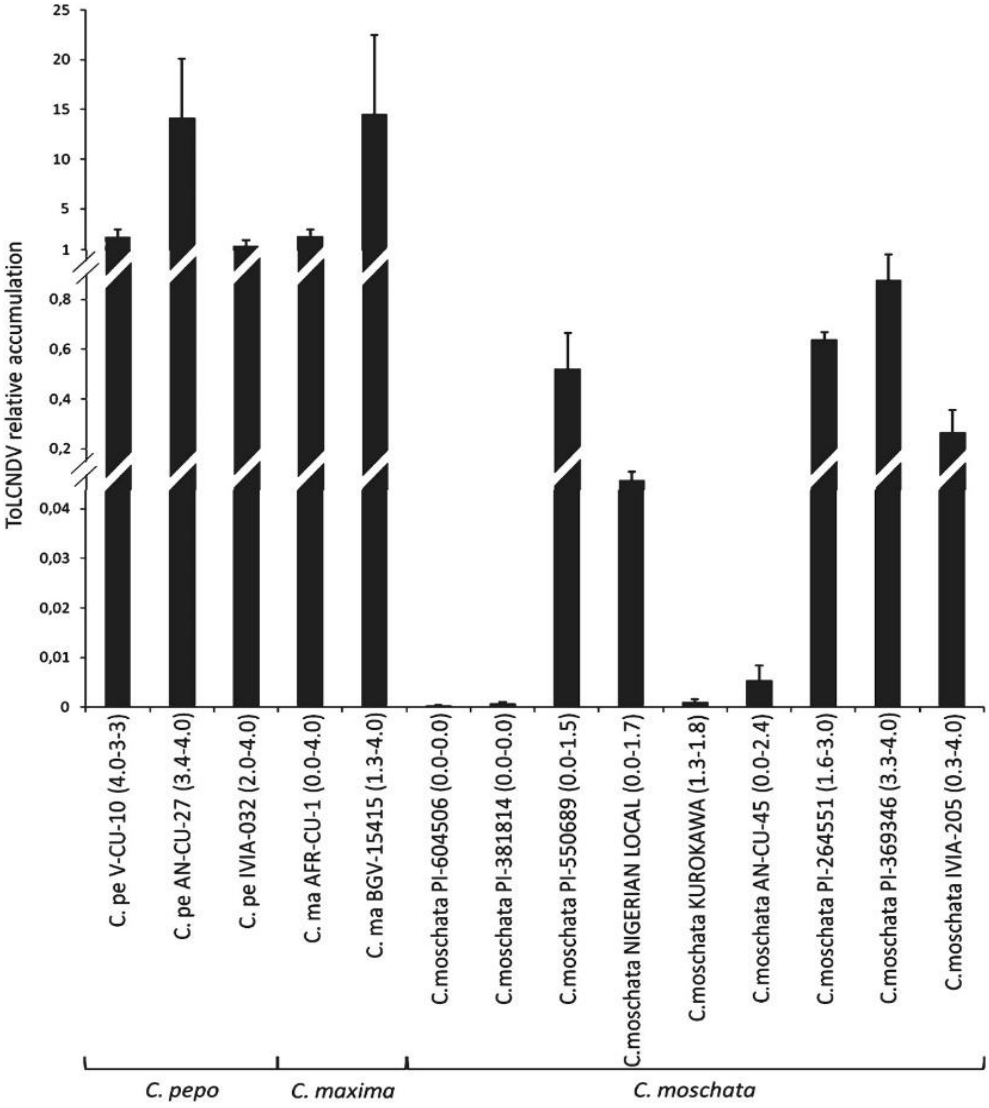

Figure 3. Relative quantification of Tomato leaf curl New Delhi virus (ToLCNDV) in the apex of infected plants at 28 dpi by quantitative polymerase chain reaction. The measurements were performed in several genotypes of *Cucurbita pepo*, *Cucurbita maxima* and *Cucurbita moschata* with different responses to ToLCNDV after whitefly inoculation. Two pools of six plants each were independently amplified and three technical replications were done on each pool (mean and standard errors are included in the figure). The mean symptom scores at 28 and 35 dpi after whitefly inoculation are shown in parentheses after the accession's name.

Table 3. Confirmation of the response of selected *Cucurbita* genotypes to mechanical inoculation with two inoculum sources of ToLCNDV.

Species/Accession	Inoculum from field infected plants				Inoculum from agroinoculated plants			
	15dpi		30dpi		15dpi		30dpi	
	Mean [a]	% PCR positive plants [b]	Mean (range)	% PCR positive plants	Mean	% PCR positive plants	Mean (range)	% PCR positive plants
C. maxima								
CATIE-9824	1.8	60	2.4 (0-4)	100	1.0	60	1.6 (0-4)	80
PI-543227	1.6	100	2.2 (1-4)	100	1.8	80	3.2 (1-4)	80
AFR-CU-1	2.0	100	2.6 (1-4)	100	1.8	100	3.0 (2-4)	80
VAV-4273	1.3	80	2.7 (1-4)	100	3.2	80	3.2 (2-4)	100
AFR-CU-18	2.4	100	3.0 (2-4)	100	2.7	100	4.0 (4-4)	100
AFR-CU-38	3.6	100	3.8 (3-4)	100	2.4	80	3.4 (2-4)	80
C. moschata								
PI-381814	0.0	100	0.20 (0-1)	100	0.0	80	0.0 (0-0)	80
PI-604506	0.80	100	0.60 (0-1)	100	0.0	80	0.0 (0-0)	80
NIGERIAN LOCAL	0.60	60	0.60 (0-1)	80	0.0	80	0.0 (0-0)	80
KUROKAWA	0.25	80	0.20 (0-1)	100	0.0	40	0.0 (0-0)	60
AN-CU-45	0.60	100	0.80 (0-1)	100	0.20	60	1.4 (0-3)	100
IVIA-205	1.5	80	1.5 (0-4)	100	2.0	60	2.0 (0-4)	80
PI-550689	0.80	100	1.2 (0-4)	100	0.50	20	2.4 (0-4)	60
PI-369346	2.3	100	2.6 (1-4)	100	1.6	80	1.7 (0-4)	60
ECU-46	0.30	60	1.7 (1-4)	100	1.0	100	2.7(3-4)	100
PI-482527	3.0	100	4.0 (4-4)	100	4.0	100	4.0 (4-4)	100
SUD-CU-8	2.7	100	3.2 (2-4)	100	1.6	100	3.0 (2-4)	100
C. pepo								
IVIA-506	2.7	100	2.7 (0-4)	100	2.0	100	3.0 (0-4)	100
CM-CU-37	2.6	100	3.6 (2-4)	100	2.5	100	3.5 (2-4)	100
CATIE-11368	3.6	100	3.8 (3-4)	100	3.0	100	4.0 (4-4)	100
359	4.0	100	4.0 (4-4)	100	4.0	100	4.0 (4-4)	100
C. fraterna								
PI-614701	3.8	100	4.0 (4-4)	100	1.8	100	4.0 (4-4)	100
C. ficifolia								
GRIFF-9448	4.0	100	4.0 (4-4)	100	4.0	100	4.0 (4-4)	100
C. lundeliana								
PI-438542	0.20	75	0.40 (0-2)	100	0.80	40	0.20 (0-1)	60
C. foetidissima								
PI-442197	2.0	100	3.0 (2-4)	100	2.8	60	2.0 (1-4)	100
C. okeechobeensis								
PI-512105	1.2	100	1.7 (1-4)	100	1.0	80	1.5 (1-4)	100

PCR, polymerase chain reaction; ToLCNDV, *Tomato leaf curl New Delhi virus*.
[a]Mean and range of symptoms scored in five plants per genotype mechanically inoculated with field inoculum (ToLCNDV isolate originally identified in infected zucchini plants from Almeria) and with an infectious clone (a ToLCNDV isolate from Almeria, cloned into *Agrobacterium* and transmitted to susceptible zucchini plants), according to the following scale: 0, absence of symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms; 4, very severe symptoms or plant death.
[b]Percentage of plants that were PCR positive using standard PCR.

Similarly to the first screening assay, symptoms were initially less severe in the selected *C. maxima* accessions, but evolved to very severe in most accessions. A moderate infection was found in *C. lundelliana* and *C. okeechobeensis*, with lower symptom scores than in *C. foetidissima*. The four accessions of *C. moschata* selected previously remained with symptom scores below 1.0 after the two independent inoculations. ToLCNDV was detected in plants of these accessions. Positive results with standard PCR indicated that ToLCNDV is present even in the symptomless accessions. qPCR performed with four selected accessions gave similar results to those obtained after whitefly inoculation. The two resistant *C. moschata* (PI 604506, PI 381814) had very low viral titers, and the susceptible *C. moschata* accession PI 482527 had viral load five times lower than that found in the susceptible accession of *C. pepo* used as control (Figure 4).

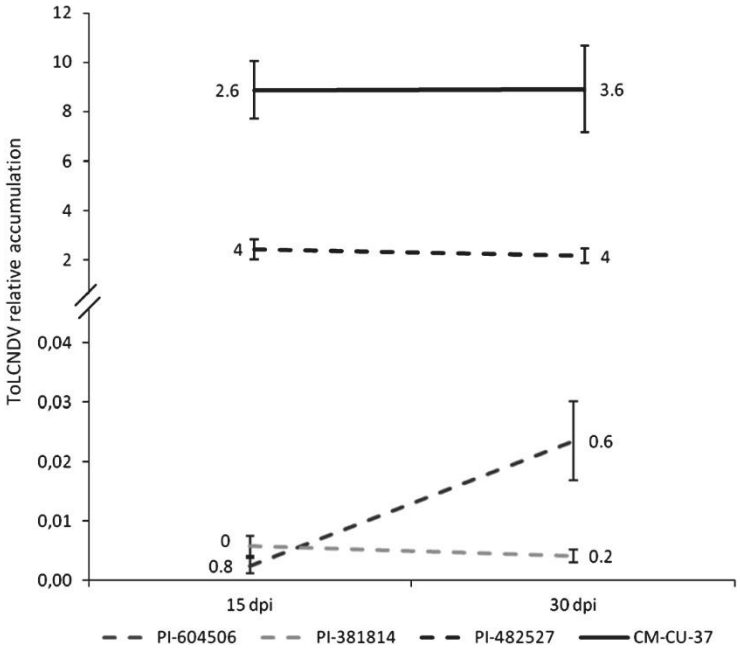

Figure 4. Relative quantification of Tomato leaf curl New Delhi virus in the apex of infected plants at 15 and 30 dpi (mechanical inoculation using field inoculum) by quantitative polymerase chain reaction. The measurements were performed in the two resistant accessions of *Cucurbita moschata*, in one accession of the same species with severe symptoms at the end of the assay, and in one *Cucurbita pepo* susceptible control. Three plants per accession were analysed and three technical replications were done on each sample (mean and standard errors are included in the figure). The mean symptom scores at 15 and 30 dpi after mechanical inoculation are shown.

DISCUSSION

Begomoviruses had not been a main problem of cucurbits in Europe until recently (Lecoq and Desbiez, 2012). However, the increasing and severe impact of ToLCNDV in zucchini fields in Southern Spain (Alfaro & Font, 2014; Janssen *et al.* , 2014) points to this virus as the most serious threat of this crop in the Mediterranean region, the main suppliers of vegetables to Europe.

Our results confirm the high susceptibility of the species *C. pepo* to both mechanical and whitefly transmission of ToLCNDV. The knowledge of the genetic diversity of the species (Formisano *et al.* , 2012; Gong *et al.* , 2012; Esteras *et al.* , 2013) allowed us to select a set of accessions representing most of the main morphotypes of the two subspecies of *C. pepo* . These accessions included the most ancient and rustic morphotype of the subsp. *pepo* , the Pumpkin morphotype (Ferriol *et al.* , 2003; Paris *et al.* , 2003; Ferriol *et al.* , 2007), but also landraces belonging to the vegetable marrow morphotype, developed in Europe after European contact with America and still appreciated in Mediterranean countries (Paris & Brown, 2005), and the more modern cocozelle and zucchini morphotypes, developed in Italy in the last century (Gong *et al.* , 2012). Despite the diversity of the collection, no total or partial resistance or useful tolerance were identified in this subspecies. Similar susceptibility was found in the American accessions representative of the *ovifera* subspecies and in *C. fraterna* , which is supposed to be one of the wild ancestors of *C. pepo* (Gong *et al.*, 2012). The high susceptibility observed in the whole range of diversity of these species evidence that this virus is a major threat to the cultivation of zucchini.

Despite the high susceptibility of *C. pepo*, the genus *Cucurbita* is highly variable and this variability can be exploited to identify sources of resistance to ToLCNDV in other species. The species *C. maxima* , represented by accessions from its centre of origin, Argentina and surrounding countries, and from secondary centres of diversification in Africa (Ferriol *et al.* , 2003), showed a delayed infection compared with *C. pepo* . However, disease symptoms evolved from moderate to

very severe as infection progressed in most of the assayed accessions, mainly after whitefly infection. This response is therefore not useful for developing resistant cultivars.

The best results were found in *C. moschata*. This species also displayed the best response in the preliminary screening that we performed to study the host range of the Spanish isolate of ToLCNDV by mechanical transmission (López *et al.* , 2015). *Cucurbita moschata* originates from the lowlands of Central America, but within the *Cucurbita* genus is one of the species that became most spread worldwide after European contact (Ferriol *et al.* , 2003). Nowadays, it is not a major crop, but a staple grown as local landraces in many developing countries of Asia, Africa and the Americas. These local landraces represent a reservoir of genes of interest already used for *C. pepo* breeding (Paris, 2008). In fact, although the assayed collection included accessions from the centre of origin, the accessions with the best responses, remaining nearly symptomless after all inoculations assays were the Large Cheese improved cultivar from the USA (PI 605406, Burpee Company) and the Indian landrace PI 381814. Similarly, resistance to ToLCNDV in melon was found in Indian accessions (López *et al.*, 2015), which can be related with the co-evolution of host and pathogen in this area, in which ToLCNDV was detected for the first time infecting cucurbits many years ago.

The plants of these two accessions of *C. moschata* remained symptomless and with a very low virus titre after whitefly inoculation. As the whitefly inoculation was not performed using clip cages in individual plants, vector non-preference or antibiosis mechanisms might account for this resistant behaviour. However, the response of these accessions (no or mild symptoms and low viral load) after three rounds of mechanical inoculation, with both field and clone inocula, support the existence of high levels of resistance to the virus.

Finding virus resistance in *C. moschata* is not unexpected as this species has been often used as a source of virus resistance in the *Cucurbita* genus. For example, Nigerian Local is one of the multi-resistant accessions used for *C. pepo* breeding

(Brown *et al.* , 2003), with reported resistance to Zucchini yellow mosaic virus, Watermelon mosaic virus, Papaya ringspot virus W (*Potyvirus* , family *Potyviridae*) and Cucumber mosaic virus (*Cucumovirus* , family *Bromoviridae*). Some of these resistance genes have been used for breeding *C. moschata* and *C. pepo* . In fact, most of the resistance genes of *C. pepo* have been introduced in this species through inter-specific crosses. Also, this species includes several sources of moderate resistance to the begomovirus SLCV in field tests (McCreight & Kishaba, 1991), whereas *C. pepo, C. fraterna* and *C. maxima* are highly susceptible.

The wild species *C. ecuadorensis* , *C. lundelliana* , *C. foetidissima* and *C. ockeechobeensis* are potential sources of resistance to SLCV, although they show different behaviour under greenhouse and field tests (McCreight & Kishaba, 1991). With ToLCNDV, the most promising species after mechanical inoculation were *C. lundelliana* and *C. ockeechobeensis*. However, both developed severe infections after whitefly inoculation. Differences in the response of these wild species after mechanical and whitefly transmission could be due to difficulties in the mechanical inoculation and to the poor adaptation of these species to growth in a climatic chamber.

The lack of clear resistance within the wild *Cucurbita* species enhances the importance of the new selected *C. moschata* accessions, which are good candidates for breeding programmes to avoid damage caused by ToLCNDV as they are partially crossable to *C. pepo* (Whitaker & Robinson, 1986). We are now crossing them to susceptible *C. moschata* and to *C. pepo*, to construct segregant populations for inheritance studies and to introgress the resistance into zucchini. The validation of the use of inoculum derived from the infectious clones for resistance screenings will facilitate its use in further genetic studies with segregant populations.

REFERENCES

Alfaro Fernández A.O., Font San Ambrosio M.I. (2014) Sintomatología del virus del rizado del tomate de Nueva Delhi (*Tomato leaf curl New Delhi virus*, ToLCNDV) en los cultivos españoles. *Phytoma España*, 257, 36– 40.

Bandaranayake W.M.E.K., Wickramarachchi W.A.R.T., Wickramasinghe H.A.M., Rajapakshe R.G.A.S., Dissanayake D.M.K.K. (2014) Molecular detection and characterization of begomoviruses associated with cucurbitaceae vegetables in Sri Lanka. *Journal of the National Science Foundation of Sri Lanka*, 42, 265– 271.

Brown R.N., Bolanos-Herrera A., Myers J.R., Jahn M.M. (2003) Inheritance of resistance to four cucurbit viruses in *Cucurbita moschata*. *Euphytica*, 129, 253– 258.

Chang H.-H., Ku H.-M., Tsai W.-S., Chien R.-C., Jan F.-J. (2010) Identification and characterization of a mechanical transmissible begomovirus causing leaf curl on oriental melon. *European Journal of Plant Pathology*, 127, 219– 228.

Doyle J.J., Doyle J.L. (1990) Isolation of plant DNA from fresh tissue. *Focus*, 12, 13– 15.

Engelen F.A. van, Molthoff J.W., Conner A.J., Nap J.-P., Pereira A., Stiekema W.J. (1995) pBINPLUS: an improved plant transformation vector based in pBIN19. *Transgenic Research*, 4, 288– 290.

Esteras C., Formisano G., Roig C., Díaz A., Blanca J., Garcia-Mas J., Gómez-Guillamón M.L., López-Sesé A.I., Lázaro A., Monforte A.J., Picó B. (2013) SNP genotyping in melons: genetic variation, population structure, and linkage disequilibrium. *Theoretical and Applied Genetics*, 126, 1285– 1303.

FAOSTAT (2015) Food and Agriculture Organization of the United Nations. Statistics Division. URL http://faostat3.fao.org/home/S. Accessed on 10 09 15.

Fauquet C.M., Briddon R.W., Brown J.K., Moriones E., Stanley J., Zerbini M., Zhou X. (2008) Geminivirus strain demarcation and nomenclature. *Archives of Virology*, 153, 783– 821.

Ferriol M., Picó B. (2008) Pumpkin and winter squash. In Handbook of Plant Breeding. Vegetables I, pp. 317– 349. Eds J. Prohens and F. Nuez. Heidelberg: Springer.

Ferriol M., Picó B., Nuez F. (2003) Genetic diversity of a germplasm collection of *Cucurbita pepo* using SRAP and AFLP markers. *Theoretical and Applied Genetics*, 107, 271– 282.

Ferriol M., Picó B., Nuez F. (2007) Genetic diversity of *Cucurbita spp*. in the Canary Islands: a bridge between America and Europe. In Plant Genetic Resources of Geographical and Other Islands. Conservation, Evaluation and Use for Plant Breeding. Cap 1. The Importance of Islands for the Conservation of Plant Genetic Resources, pp. 25– 32. Sassari, Italy: CNR-ISPAAM. Ed S. Bullita. Proceedings of the XVII Eucarpia Genetic Resources Section Meeting, Castelsardo, Italy.

Formisano G., Roig C., Esteras C., Ercolano M.R., Nuez F., Monforte A.J., Picó M.B. (2012) Genetic diversity of Spanish *Cucurbita pepo* landraces: an unexploited resource for summer squash breeding. *Genetic Resources and Crop Evolution*, 59, 1169– 1184.

Gong L., Paris H.S., Nee M.H., Stift G., Pachner M., Vollmann J., Lelley T. (2012) Genetic relationships and evolution in *Cucurbita pepo* (pumpkin, squash, gourd) as revealed by simple sequence repeat polymorphisms. *Theoretical and Applied Genetics*, 124, 875– 891.

Islam S., Munshi A.D., Mandal B., Behera T.K., Kumar R. (2010) Genetics of resistance in *Luffa cylindrica* Roem. against *tomato leaf curl New Delhi virus*. *Euphytica*, 174, 83– 89.

Islam S., Munshi A.D., Verma M., Arya L., Mandal B., Behera T.K., Kumar R., Lal S.K. (2011) Screening of *Luffa cylindrica* Roem. for resistance against *Tomato leaf curl New Delhi virus* , inheritance of resistance, and identification of SRAP markers linked to the single dominant resistance gene. *Journal of Horticultural Science and Biotechnology*, 86, 661– 667.

Ito T., Sharma P., Kittipakorn K., Ikegami M. (2008) Complete nucleotide sequence of a new isolate of tomato leaf curl New Delhi virus infecting cucumber, bottle gourd and muskmelon in Thailand. *Archives of Virology*, 153, 611– 613.

Janssen D., Ruíz L., Simón A., García C. (2014) Control en invernadero del virus de Nueva Delhi de la hoja rizada del tomate. *Vida Rural*, 378, 32– 36.

Juárez M., Tovar R., Fiallo-Olivé E., Aranda M.A., Gosálvez B., Castillo P., Moriones E., Navas-Castillo J. (2014) First detection of *Tomato leaf curl New Delhi virus* infecting Zucchini in Spain. *Plant Disease*, 98, 857– 858.

Jyothsna P., Haq Q.M.I., Singh P., Sumiya K.V., Praveen S., Rawat R., Briddon R.W., Malathi V.G. (2013) Infection of tomato leaf curl New Delhi virus (ToLCNDV), a bipartite begomovirus with betasatellites, results in enhanced level of helper virus components and antagonistic interaction between DNA B and betasatellites. *Applied Microbiology and Biotechnology*, 97, 5457– 5471.

Khan M.S., Ji S.H., Chun S.C. (2012) Begomoviruses and their emerging threats in South Korea: a review. *Plant Pathology Journal*, 28, 123– 136.

Kushwaha N., Singh A.K., Basu S., Chakraborty S. (2015) Differential response of diverse solanaceous hosts to tomato leaf curl New Delhi virus infection indicates coordinated action of NBS-LRR and RNAi-mediated host defense. *Archives of Virology*, 160, 1499– 1509.

Lecoq H., Desbiez C. (2012) Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture. In Viruses and Virus Diseases of Vegetables in the Mediterranean Basin. Advances in Virus Research. Volume 84, pp. 67– 126. Eds G. Loebenstein and H. Lecoq. San Diego, CA, USA: Elsevier.

López C., Ferriol M., Picó M.B. (2015) Mechanical transmission of *Tomato leaf curl New Delhi virus* to cucurbit germplasm: selection of tolerance sources in *Cucumis melo*. *Euphytica*, 204, 279– 691.

McCreight J.D., Kishaba A.N. (1991) Reaction of cucurbit species to squash leaf curl virus and sweetpotato whitefly. *Journal of the American Society for Horticultural Science*, 116, 137– 141.

Naqvi A.R., Haq Q.M.R., Mukherjee S.K. (2010) MicroRNA profiling of *tomato leaf curl new delhi virus* (tolcndv) infected tomato leaves indicates that deregulation of mir159/319 and mir172 might be linked with leaf curl disease. *Virology Journal*, 7, 281.

Papidam M., Beachy R.N., Fauquet C.M. (1995) Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not essential for infectivity. *Journal of General Virology*, 76, 25– 35.

Paris H.S. (2008) Summer squash. In Handbook of Plant Breeding. Vegetables I, pp. 351–379. Eds J. Prohens and F. Nuez. Heidelberg: Springer.

Paris H.S., Brown R.N. (2005) The genes of pumpkin and squash. *HortScience*, 40, 1620–1630.

Paris H.S., Yonash N., Portnoy V., Mozes-Daube N., Tzuri G., Katzir N. (2003) Assessment of genetic relationships in *Cucurbita pepo* (Cucurbitaceae) using DNA markers. *Theoretical and Applied Genetics*, 106, 971– 978.

Picó B., Sifres A., Nuez F. (2005) Quantitative detection of cucumber vein yellowing virus in susceptible and partially resistant plants using real-time PCR. *Journal of Virological Methods*, 128, 14– 20.

Pratap D., Kashikar A.R., Mukherjee S.K. (2011) Molecular characterization and infectivity of a *Tomato leaf curl New Delhi virus* variant associated with newly emerging yellow mosaic disease of eggplant in India. *Virology Journal*, 8, 305.

Rai N.K., Sahu P.P., Gupta S., Reddy M.K., Ravishankar K.V., Singh M., Sadashiva A.T., Prasad M. (2013) Identification and validation of an ISSR marker linked to Tomato leaf curl New Delhi virus resistant gene in a core set of tomato accessions. *Vegetable Science*, 40, 1– 6.

Ruíz M.L., Simón A., Velasco L., García M.C., Janssen D. (2015) First report of *Tomato leaf curl New Delhi* virus infecting tomato in Spain. *Plant Disease*, 99, 894.

Sahu P.P., Rai N.K., Puranik S., Roy A., Khan M., Prasad M. (2012) Dynamics of defense related components in two contrasting genotypes of tomato upon infection with *Tomato Leaf Curl New Delhi Virus* . *Molecular Biotechnology*, 52, 140– 150.

Samretwanich K., Chiemsombat P., Kittipakorn K., Ikegami M. (2000) Tomato leaf curl geminivirus associated with cucumber yellow leaf disease in Thailand. *Journal of Phytopathology*, 148, 615– 617.

Seal S.E., Jeger M.J., Van den Bosch F. (2006) Begomovirus evolution and disease management. *Advances in Virus Research*, 67, 297– 316.

Snehi S.K., Raj S.K., Prasad V., Singh V. (2015) Recent research findings related to management strategies of begomoviruses. *Journal of Plant Pathology and Microbiology*, 6, 273.

Sohrab S.S., Karim S., Varma A., Abuzenadah A.M., Chaudhary A.G., Damanhouri G.A., Mandal B. (2013) Characterization of *Tomato Leaf Curl New Delhi Virus* infecting cucurbits: evidence for sap transmission in a host specific manner. *African Journal of Biotechnology*, 12, 5000– 5009.

Srivastava K.M., Hallan V., Raizada R.K., Chandra G., Singh B.P., Sane P.V. (1995) Molecular cloning of Indian tomato leaf curl virus genome following a simple method of concentrating the supercoiled replicative form of viral DNA. *Journal of Virological Methods*, 51, 297– 304.

Usharani K.S., Surendranath B., Paul-Khurana S.M., Garg I.D., Malathi V.G. (2004) Potato leaf curl—a new disease of potato in northern India caused by a strain of *Tomato leaf curl New Delhi virus* . *Plant Pathology*, 53, 235.

Whitaker T.W., Robinson R.W. (1986) Squash breeding. In Breeding Vegetable Crops, pp. 209– 242. Ed M.J. Bassett. Westport, CT, USA: AVI.

Capítulo II

CAPÍTULO II

Resistance to *tomato leaf curl New Delhi virus* in *Cucumis sativus*

Cristina Sáez, Laura G. M. Ambrosio, José Vicente Valcárcel, María José Díez, Belén Picó & Carmelo López

European Journal of Plant Pathology, under revision

ABSTRACT

Tomato leaf curl New Delhi virus (ToLCNDV) is a severe threat for cucurbits production worldwide. Resistance has been reported in sponge gourd (*Luffa cylindrica*), melon (*Cucumis melo*) and pumpkin (*Cucurbita moschata*), but at present there are no resistance to ToLCNDV in cucumber (*Cucumis sativus*). Germplasm accessions of *C. sativus* var. *sativus* were mechanically infected with ToLCNDV and screened for resistance, by scoring symptom severity, tissue printing, and conventional PCR. The viral load of ToLCNDV was determined in a selected number of accessions using quantitative PCR. Severe symptoms and high load of viral DNA were found in plants following inoculation in a nuclear collection of 40 Spanish landraces and in 18 accessions of *C. sativus* from different geographical origins. Two Indian accession (CGN22297 and CGN22986) showed mild symptoms and intermediate viral load, whereas three Indian accessions (CGN23089, CGN23423 and CGN23633) were highly resistant to the mechanical inoculation, remaining symptomless and showing a reduced viral accumulation. The resistance was confirmed by mechanical inoculation with ToLCNDV in a second assay of seedlings obtained by selfing of one resistant plant of the accession CGN23423. To study the inheritance of the resistance to ToLCNDV, the resistant CGN23089 accession was crossed with the susceptible accession BGV011742. The progeny was mechanically inoculated with ToLCNDV and F_1 plants developed moderate symptomatology and showed high viral load, which suggests a recessive control of resistance. Finally, breeding for ToLCNDV cucumber resistant cultivars will be conducted through further genetic studies of the three newly promissing resistant *C. sativus* accessions.

INTRODUCTION

Cucurbits are cultivated in tropical, subtropical and temperate regions of the New and Old world and supply essential vitamins and minerals to current diets in countries around the world being a major source of food for man. Crops belonging to the three main genera in economic importance, *Cucumis* (melon and cucumber), *Citrullus* (watermelon), and *Cucurbita* (zucchini, pumpkin, squash and gourd), rank in the first positions in global vegetable and fruit production. Spain is one of the main world producers of cucurbits (FAO, 2018), and the first exporting country in Europe. However, the production of these crops has been severely affected by diseases, in particular those caused by viruses (Lecoq and Katis, 2014) that have a high economic impact. Aphid-borne transmitted potyviruses has been the most widespread and damaging threat in cucurbits until recent severe outbreaks of whitefly-transmitted viruses. Among them, tomato *leaf curl New Delhi virus* (ToLCNDV), a member of the genus *Begomovirus*, family *Geminiviridae*, has spread rapidly in southern Spain since the first detection in 2012 and represents a major risk in the production of zucchini, melon and cucumber. This virus is causing a great impact with important losses in this horticultural region, and is considered a serious threat to greenhouse and open-field cucurbit crops. ToLCNDV was first detected on tomato (*Solanum lycopersicum* L.) in India in 1995 (Srivastava et al. 1995), and later, it was found in other south and southeast Asian countries on several hosts, particularly species of the *Solanaceae* and *Cucurbitaceae* families (Moriones et al. 2017; Zaidi et al. 2017). ToLCNDV was limited to Asian countries until 2012, when it was reported affecting cucurbits (mainly zucchini [*Cucurbita pepo* L.], melon [*Cucumis melo* L.], and cucumber [*Cucumis sativus* L.]) in different Mediterranean countries, first in Spain and after in Tunisia, Italy, Morocco, Greece and Algeria (Juárez et al. 2014; Mnari-Hattab et al. 2015; Panno et al. 2016; Sifres et al. 2018; Orfanidou et al. 2019; Kheireddine et al. 2019). More recently, the virus has been identified in cucurbits plants in Portugal and Estonia (EPPO, 2019), and in species of the Solanaceae family in Italy (Luigi et al. 2019), so ToLCNDV is rapidly spreading through Europe.

ToLCNDV consists of two circular single-stranded DNA molecules of approximately 2.7 kb each (designated as DNA-A and DNA-B), both required for essential viral functions and encapsidated in geminate particles (Papidam et al. 1995; Jyothsna et al. 2013). The symptoms caused by ToLCNDV depend on the species and the time of infection, but usually induces curling, leaf mottling and mosaic of young leaves, short internodes and fruit skin roughness (Juárez et al. 2014) often resulting in a significant yield reduction. ToLCNDV is naturally transmitted by the whitefly *Bemisia tabaci* (Gennadius) byotipes MED and MEAM1 in a persistent manner (Chang et al. 2010; Islam et al. 2010; Khan et al. 2012; Jyothsna et al. 2013), although some isolates are also mechanically sap transmitted to different hosts (Usharani et al. 2004; Chang et al. 2010; Sohrab et al. 2013; López et al. 2015), even recently, it has been described a seed-transmissible strain of ToLCNDV infecting Chayote (*Sechium edule* [Jacq] Sw) in India (Sangeetha et al. 2018). Against this background, the European and Mediterranean Plant Protection Organization (EPPO) included this virus in the EPPO Alert List (EPPO, 2017).

ToLCNDV is currently managed based on cultural practices and chemical treatment against its vector. However, these control methods have limited effectiveness and can be expensive. Therefore, the development of resistant varieties through conventional breeding provide an effective and sustainable solution for reducing the impact of the disease caused by this virus. In cucurbits, monogenic resistance to ToLCNDV has been described in sponge gourd (*Luffa cylindrica* M. Roem.) (Islam et al. 2010; 2011), a popular cucurbit vegetable in India. In melon, resistance has been identified in five Indian melon genotypes belonging to the subsp. *agrestis* (Naudin) Pangalo (three accessions of the *momordica* horticultural group and two wild *agrestis*) (López et al. 2015). A major QTL in chromosome 11 was found controlling the resistance to ToLCNDV in one of the wild *agrestis* accession, with epistatic interactions of two additional regions in chromosomes 2 and 12 (Sáez et al. 2017). Finally, resistance has also been identified in pumpkin (*Cucurbita moschata*) accessions from diverse origins (Sáez et al. 2016). A major recessive gene located in chromosome 8, in a region syntenic to the candidate region in chromosome

11 of melon, was found controlling the resistance to ToLCNDV in this species (Sáez et al. 2020).

The first step for breeding cucumber resistant cultivars is the search of resistant sources. Cucumber germplasm has been screened for resistance to viral diseases such as several potyviruses (Provvidenti, 1985), *cucumber vein yellowing virus* (CVYV) (Picó et al. 2003), *cucurbit yellow stunting disorder virus* (CYSDV) (Aguilar et al. 2006), *cucumber mosaic virus* (CMV) (Munshi et al. 2008), *melon yellow spot virus* (MYSV) (Sugiyama et al. 2009), or *cucumber green mottle mosaic virus* (CGMMV) (Crespo et al. 2018), but to our knowledge no resistance has been described for ToLCNDV in cucumber. In this report, we evaluated the response to ToLCNDV of a cucumber germplasm collection by mechanical inoculation. The identification of three Indian *C. sativus* accessions highly resistant to the mechanical inoculation, which remained symptomless and showed a reduced viral accumulation, provides the first sources for breeding ToLCNDV-resistant cucumber cultivars.

MATERIALS AND METHODS

Plant material

A nuclear collection of 40 Spanish landraces of *C. sativus* var. *sativus* (Table 1), stored at the Genebank of the Institute for the Conservation and Breeding of Agricultural Biodiversity at the Polytechnic University of Valencia (COMAV-UPV), was first screened in a climatic chamber against ToLCNDV by mechanical inoculation. These accessions represent the variability of the full COMAV collection, consisting of 217 accessions collected from diverse Spanish origins and multiplied by COMAV (Valcárcel et al. 2018a; 2018b). This collection includes accessions belonging to the typical "short" (20) "long" (16) and "French" (4) cucumber types (Table 1), which are highly appreciated on national and international markets because of its quality. Also, 23 *C. sativus* var. *sativus* accessions from different geographical origins (Table 2) of the types "short" (12), "medium" (5), and "long" (5) cucumber types, and one of unknown type were also tested. Seeds of these

accessions were firstly provided by the Centre for Genetic Resources (CGN germplasm collection, Netherlands), and then multiplied at COMAV.

Virus source, mechanical inoculation and symptoms evaluation

As inoculum source, zucchini plants of MU-CU-16 accession were agroinoculated by injection into petioles with an infectious-clone of ToLCNDV (Sáez et al. 2016). ToLCNDV transmission to cucumber plants was performed by mechanical inoculation at the stage of one true leaf as described by López et al. (2015). Briefly, inoculum was prepared by grinding 1 g of symptomatic leaf tissue from agroinfiltrated plants in the presence of inoculation buffer in a 1:4 (w:v) proportion. The expanded true leaf and one cotyledon of each plant were dusted with carborundum (600 mesh) and then inoculated rubbing a cotton-bud sticks, gently soaked with the crude homogenated inoculums.

For the mechanical inoculation, seeds were disinfected in a 10% solution of sodium hypochlorite for 3 min and washed for 5 min in distilled water. Germination was performed in Petri plates with moistened cotton at 37ºC for 48 h. Seedlings were transplanted to pots in a growth chamber under a photoperiod of 16 h day at 25ºC and 8 h night at 18ºC and 70% relative humidity. Seedlings at the one true-leaf stage were mechanically inoculated, leaving two plants per genotype not inoculated as controls. Inoculated plants were individually evaluated at 15 and 30 days post inoculation (dpi) for the presence and severity of virus symptoms. Symptoms on upper leaves were recorded by visual evaluation using the following scale: 0, no symptoms; 1, mild symptoms; 2, moderate symptoms; 3, severe symptoms; 4, very severe symptoms or dead plant (Fig. 1). Additionally, every plant was assayed for the presence of virus using the tissue printing technique and conventional PCR with the protocols described below. Also, the viral load of ToLCNDV was determined by qPCR in a selected number of accessions with the best resistance response (CGN22297, CGN22986, CGN23089, CGN23423 and CGN23633). The number of plants tested of each accession varied between 3 and 6 due to seed availability and germination. The two most resistant accessions were selected for further analysis with additional plants.

ToLCNDV detection by *tissue-printing*

For detection of ToLCNDV in tissue prints, plant petioles of the upper leaves at 15 dpi were cut with a razor blade and cross-sections were blotted onto positively charged nylon membranes (Hybond-N, Amersham), immediately after cutting. Membranes were air dried, fixed by UV irradiation (700 × 100 mJ/cm^2) and hybridized with a digoxigenin-labelled RNA probe. The riboprobe was generated by transcription with T7 RNA polymerase from a recombinant pTZ57R plasmid (Fermentas) with an insert corresponding to the complete CP gene of the ToLCNDV in negative orientation, following the manufacturer's instructions (Roche Diagnostics). Prehybridization, hybridization and washing of the membranes were performed as previously reported (Sánchez-Navarro et al. 1999), except that the hybridization was conducted at 60ºC. Chemiluminiscent detection using CSPD reagent as substrate was performed as recommended by the manufacturer (Roche Diagnostics). Films were exposed to the membranes at room temperature for 30-60 min.

ToLCNDV detection by PCR and qPCR

To confirm the presence of the virus by PCR, total DNA from apical leaves of inoculated and control plants was extracted at 30 dpi using the CTAB method (Doyle and Doyle, 1990). DNA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific) and diluted with sterile-deionized water to a final concentration of 50 ng μL^{-1}. One microliter aliquots of total DNA (50 ng) were used as templates in PCR reactions with the ToLCNDV specific primer pairs: To-A1F and To-A1R from the DNA-A (Sáez et al. 2016). The resulting PCR products of 504 bp in length were analyzed by electrophoresis in 1.0% agarose gels in TAE buffer.

The relative ToLCNDV accumulation in individual plants of the most promising accessions was determined at 30 dpi by qPCR, and three susceptible plants were used as controls. DNA was diluted to a final concentration of 5 ng μL^{-1} and all plants were analyzed in two technical replicates using a *LightCycler*® 480 System (Roche). In each qPCR reaction, 15 ng of genomic DNA were used as templates, in

a final volume of 10 µL. We used 2.5 µL of MasterMix qPCR No-ROX PyroTaq EvaGreen 5x (Cmb bioline) and 0.35 µL (10 µM) of each primer and 3.8 µL of H_2O. Primers ToLCNDVF1 (5'-AATGCCGACTACACCAAGCAT-3', positions 1145–1169) and ToLCNDVR1 (5'-GGATCGAGCAGAGAGTGGCG-3', positions 1399–1418), derived from the Spanish isolate Murcia 11.1 (segment DNA-A, accession number KF749225), were used for the amplification of a 273 bp fragment of viral DNA-A. The *β-actine* of *C. sativus* gene was amplified in all samples as reference control using an adapted design of primers used in watermelon previous works (Kong et al. 2015) (ClACT-F (5'-CCATGTATGTTGCCATTCAG-3'), and ClACT-R (5'-GGATAGCATGGGGAAGAGCA -3'. Cycling conditions consisted of incubation at 95ºC for 5 min, 45 cycles of 95ºC for 5 s, 60ºC for 30 s and 72ºC for 30 s. Relative ToLCNDV levels were calculated using the $2^{-\Delta\Delta Ct}$ expression of the Livak method (Livak and Schmittgen, 2001), where ΔΔCt is the difference between the ΔCt of each sample and the ΔCt of the calibrator sample.

Validation of response to the virus infection and generation of one F_1 population

One plant of one of the accessions with the best resistant behavior, CGN23089-2, was transplanted and grown in a whitefly-proof greenhouse and crossed with plants of the accession BGV011742, highly susceptible to ToLCNDV. Also, one plant of a second highly resistant accession, CGN23423-2, was selfed. Seeds of the F_1 hybrid and of the selfing offspring were disinfected and seedlings were transplanted to pots and grown in climatic chamber under controlled conditions. All plants were mechanically inoculated with ToLCNDV and phenotyped according to symptomatology and viral accumulation determined by qPCR, at 15 and 30 dpi, following the procedure described above.

Statistical analysis

Means of $2^{(-\Delta\Delta Ct)}$ were analyzed by ANOVA and least significance difference (LSD) multiple range test using STATGRAPHICS Centurion XVI.I to evaluate statistically significant differences between them, with a level of confidence of 95%.

RESULTS

Response of the Spanish landraces of *C. sativus* to the mechanical transmission of ToLCNDV

A core collection of 40 accessions from different Spanish provinces held at the COMAV Genebank was assayed. Most of the 40 tested cucumber accessions were highly susceptible to the mechanical transmission of ToLCNDV, showing from moderate symptoms of mottling to severe symptoms characterized by mosaic and yellowing of young leaves (Fig. 1). Symptoms started to appear at different days after mechanical inoculation. On average, symptoms score in plants of the "short", "long" and "French" types of *C. sativus* increased from 15 to 30 dpi, with average scores from 1.78±0.19 to 2.1±0.2, 1.52±0.19 to 1.9±0.19 and 2.43±0.31 to 2.8±0.31 in each group respectively, on a range of 0 to 4 (Table 1). As it had already been observed in the cucumber growing areas of the southeast of Spain, plants of the "French" type were the most susceptible.

Fig. 1. Symptom scoring in cucumber plants showing ToLCNDV symptoms corresponding to the scale 0 abence of symptoms, 1 mild symptoms, 2 moderate symptoms, 3 severe symptoms and 4 very severe symptoms or dead plant.

To further characterize the response to ToLCNDV, the viral load of all plants at 15 dpi was evaluated by molecular hybridization by tissue printing. To carry out a more precise confirmation, viral accumulation was determined by semi-quantitative PCR at 30 dpi. Viral load ranged from intermediate to high (Table 1) in most of the genotypes, increasing or remaining constant from 15 to 30 dpi. Only accessions BGV000479, BGV002495, BGV002494, BGV004851 and BGV011586 developed mild symptoms at the end of the trial, although with moderate or high viral accumulation.

Table 1. Response of Spanish landraces of *C. sativus* to the mechanical inoculation with ToLCNDV. Mean score of viral load detected in each plant of the assayed accessions by tissue printing at 15 dpi and by PCR at 30 dpi are shown according to the scale of high (+++), intermediate (++), low (+) or absent (-) viral accumulation. Data not available is shown as n/a.

			Symptoms at 15 dpi		**Symptoms at 30 dpi**		**Viral load**	
Type	**Genebank code**	**Spanish province**	**Mean**	**Range**	**Mean**	**Range**	**Tissue *Printing***	***PCR***
Short	BGV000047	Zaragoza	1.2	(1-2)	1.8	(1-2)	+++	++
	BGV000408	Cádiz	1.6	(1-2)	1.6	(1-3)	+++	+++
	BGV000437	Jaén	1.2	(0-3)	1.8	(1-2)	++	++
	BGV000467	Jaén	1.0	(0-2)	1.4	(0-3)	+++	n/a
	BGV000479	Córdoba	1.0	(1)	1.0	(0-2)	+++	+++
	BGV000512	Huelva	1.0	(0-2)	1.4	(1-2)	+++	n/a
	BGV002495	Tenerife	n/a		0.6	(0-2)	++	++
	BGV003714	Cuenca	2.0	(1-3)	1.7	(1-2)	+++	+
	BGV004026	Cáceres	1.4	(1-2)	2.5	(1-3)	+++	+++
	BGV004304	Murcia	n/a		2.0	(1-3)	+++	+++
	BGV008299	Valencia	2.0	(1-3)	2.4	(2-3)	++	+++
	BGV010301	Guadalajara	1.4	(0-3)	1.6	(0-3)	+++	+++
	BGV010314	Guadalajara	1.7	(1-2)	1.2	(0-2)	+	++
	BGV010636	Soria	2.4	(1-4)	3.4	(2-4)	++	+++
	BGV011582	Teruel	0.8	(0-2)	1.8	(1-2)	++	+++
	BGV011734	Valladolid	2.0	(0-4)	3.2	(3-4)	++	+
	BGV011736	Ávila	2.6	(0-4)	2.8	(2-4)	++	+++
	BGV011742	Albacete	3.4	(3-4)	3.8	(3-4)	++	+++
	BGV014959	Huesca	3.8	(3-4)	3.6	(3-4)	++	++
	BGV015469	Cáceres	1.6	(0-2)	2.8	(2-4)	+	++
Long	BGV000372	Granada	0.6	(0-1)	1.2	(0-2)	++	+++
	BGV000381	Málaga	0.6	(0-1)	2.4	(2-3)	+++	n/a
	BGV000416	Cádiz	1.4	(0-2)	2.0	(1-3)	++	+++
	BGV001310	Asturias	1.0	(0-2)	1.6	(1-2)	+++	+++
	BGV002494	Tenerife	2.0	(2)	1.0	(0-2)	+++	++
	BGV004305	Murcia	1.6	(1-2)	1.2	(1-2)	+++	+++
	BGV004309	Murcia	1.4	(1-2)	3.0	(3)	+++	+++
	BGV004851	Castellón	0.0	(0)	1.0	(0-2)	++	++
	BGV004926	Valencia	2.0	(2)	2.0	(2)	+++	n/a
	BGV004936	Valencia	1.4	(1-2)	1.3	(1-2)	++	n/a
	BGV011586	Orense	0.6	(0-3)	0.6	(0-3)	+	+++
	BGV011724	Teruel	1.8	(0-4)	1.8	(0-4)	+	+++
	BGV014967	Guadalajara	2.2	(0-4)	2.4	(1-4)	++	+++
	BGV015229	Vizcaya	2.5	(1-3)	2.5	(2-3)	++	++
	BGV015696	Alicante	2.4	(0-4)	2.6	(0-4)	++	+++
	BGV015700	Girona	2.8	(1-4)	3.4	(2-4)	++	+++
French	BGV010290	Granada	2.8	(0-4)	3.8	(3-4)	+++	+++
	BGV011735	Zaragoza	2.3	(0-3)	2.3	(0-4)	++	++
	BGV014961	Castellón	3.0	(0-4)	2.6	(0-4)	+++	+++
	BGV014969	Cantabria	1.6	(0-3)	2.4	(0-4)	+++	++

Response of the *C. sativus* accessions from different origins to the mechanical transmission of ToLCNDV

The cucumber accessions from different countries showed variable responses to ToLCNDV infection. Susceptible accessions behaved similarly to the Spanish landraces, displaying moderate to severe yellowing and mottling that in most cases increased from 15 to 30 dpi. Accessions belonging to the "long" type, all original from China, had on average the higher symptoms scores (Table 2) at 15 and 30 dpi (means of 1.86±0.2 and 2.14±0.31, respectively). Similarly, the only accession of unknown type (CGN19655, original from U.S.A) was highly susceptible with symptoms score means rising from 1.4 at 15 dpi to 2.4 at 30 dpi (Table 2). Viral titers detected with both tissue printing and PCR were high or very high in these accessions (Table 2).

Interestingly, lower severity of the ToLCNDV infection was observed in some Indian genotypes of the "medium" and "short" types. Accession CGN22297 was symptomless or had very mild symptoms at 15 dpi (mean symptoms scores of 0.4, in a range between 0 and 1), although some plants developed moderate symptomatology at the end of the assay (plants with symptoms scores ranging from 0 to 2) (Table 2). All plants of accessions CGN22986, CGN23089, CGN23423 and CGN23633 remained symptomless, or had symptoms score lower than one, throughout all the screening assay. On these five accessions, ToLCNDV titers were low or not detected by probe hybridization at 15 dpi, although were low or moderate at 30 dpi after PCR analysis (Table 2).

Among the remaining "medium" and "short" type accessions from different countries assayed, some initially had a promising behavior (mean symptom score 1.14 and 1.36, respectively, ranging from 0.3 to 3.5), but typical ToLCNDV severe symptomatology and high or very high viral titers were identified in all of them at the different stages of the disease (Table 2).

Table 2. Response of *C. sativus* accessions from different origins to the mechanical inoculation with ToLCNDV. Mean score of viral load detected in each plant of the assayed accessions by tissue printing at 15 dpi and by PCR at 30 dpi are shown according to the scale of high (+++), intermediate (++), low (+) or absent (-) viral accumulation. Data not available is shown as n/a.

				Symptoms 15 dpi		**Symptoms 30 dpi**		**Viral Load**	
Type	**Genebank code**	**Country**	**Local name**	**Mean**	**Range**	**Mean**	**Range**	***Tissue Printing***	**PCR**
Short	CGN19748	India	Khira Cucumber	3.5	(3-4)	3.0	(3)	++	++
	CGN19817	India	Medium	1.4	(0-3)	3.0	(2-4)	+++	++
	CGN20512	Netherlands	752	2.5	(2-3)	2.8	(2-3)	++	++
	CGN20517	Sri Lanka	Yellow 1	1.0	(0-2)	1.3	(0-2)	+	++
	CGN21585	India	Saharanpur	0.3	(0-1)	2.3	(1-4)	n/a	++
	CGN21691	D.R Congo	N2/81	2.8	(0-4)	3.2	(1-4)	+++	+++
	CGN22280	India	Shuei Huang Kua	1.0	(0-3)	1.0	(1)	+++	+++
	CGN22986	India	Smallgreen Anthracnose	0.4	(0-1)	0.4	(0-1)	++	+
	CGN23089	India	197087 Khira	0.2	(0-1)	0.0	(0)	-	++
	CGN23411	India	Cheshuicchatyi	0.6	(0-2)	1.0	(0-3)	++	+++
	CGN23423	India	JL-2 Dhillon	0.0	(0)	0.3	(0-1)	-	++
	CGN23633	India	Jaipur Balam	0.0	(0)	0.7	(0-1)	-	++
Medium	CGN19819	India	Puneri Klura	0.8	(0-1)	1.3	(1-2)	+++	++
	CGN20853	Japan	Sagami Hanpaku Fushinari Kyuri	1.5	(0-2)	1.5	(1-2)	+++	+++
	CGN21616	Iran	Rasht	3.3	(2-4)	3.7	(3-4)	+++	+++
	CGN22281	India	Long Green	0.8	(0-2)	1.5	(0-2)	++	++
	CGN22297	India	K-75	0.4	(0-1)	0.8	(0-2)	+	++
Long	BGV015107	China	Hei Wu She	1.2	(0-2)	1.6	(1-3)	++	+++
	BGV015113	China	Shou Guang Qiu Gua	2.0	(1-3)	1.6	(1-2)	++	+++
	BGV015115	China	Long Quan Qing Huang Gua	1.6	(0-4)	1.6	(1-3)	++	+++
	BGV015116	China	De Hui Huang Gua	2.0	(0-3)	2.6	(1-4)	+++	+++
	BGV015118	China	San Ye Zao	2.5	(0-4)	3.3	(1-4)	+++	+++
-	CGN19655	U.S.A	SC 53-B (6)	1.4	(0-4)	2.4	(0-4)	+++	+

ToLCNDV quantification in resistant genotypes

Individual plants of the five Indian accessions with better response after infection with ToLCNDV were tested by qPCR to further determine the viral accumulation at 30 dpi. The susceptible plants BGV002494-5, BGV011742-1 and BGV014959-4 used as controls showed the highest level of relative viral titers, but the resistant genotypes presented variability between their relative ToLCNDV accumulations (Figure 2A).

Plants of CGN23089, CGN23423 and CGN23633 accessions had uniformly low viral loads, with $2^{(-\Delta\Delta Ct)}$ values 3.4 10^3 times lower than the levels accumulated by the susceptible plants, on average. Instead, in both CGN22297 and CGN22986

accessions some plants were identified with low $2^{(-\Delta\Delta Ct)}$ values and some with high viral load, similar to that detected in one of the susceptible genotypes (Figure 2A). After this further characterization, the accessions CGN23089 and CGN23423, that were those with the lowest symptoms scores at 15 and 30 dpi and with the lowest viral titers, estimated with different methods were selected for further characterization.

Response of self-pollinated and F1 progenies to the mechanical transmission of ToLCNDV

The selfing offspring of the plant CGN23423-2 and the F_1 hybrid derived from the cross CGN23089-2 x BGV011742 (one of the most susceptible Spanish landraces selected as susceptible parent for this cross) were mechanically inoculated with ToLCNDV in a second assay to confirm the resistance. As was expected, all plants of the self-pollinated offspring of CGN23423-2 had a similar behavior to that observed in the resistant plants of the first assay, displaying from mild to no symptoms at 15 and 30 dpi (Figure 3A). ToLCNDV titers determined in fourteen plants remained low in most of the cases, with a general declining of the viral load between 15 and 30 dpi (Figure 2B). However, two plants (3 and 13) reached intermediate $2^{(-\Delta\Delta Ct)}$ values, between those observed in resistant and susceptible plants of the first assay (Figure 2A). Even so, statistically significant differences were not identified by LSD multiple range test between the relative viral accumulation means of these plants (at 15 and 30 dpi) and means of the five asymptomatic Indian accessions evaluated in the first assay.

The F_1 (CGN23089-2 x BGV011742) plants developed moderate symptomatology (two in the symptoms scale) at 15 dpi and same behavior was observed up to the end of the assay (Figure 3B). The qPCR results showed high viral load, with not statistically significant differences between $2^{(-\Delta\Delta Ct)}$ means at 15 and 30 dpi. On average, viral titer in the F_1 hybrid overcome those of CGN23089, CGN23423 and CGN23633 accessions in one thousand times, but it was similar to the high viral accumulation detected in some plants of the CGN22297 and

CGN22986 accessions. Nevertheless, the average of viral load in the F_1 hybrid was more than three times lower than in the susceptible accessions and both means were grouped at different levels of significance (Figure 2B).

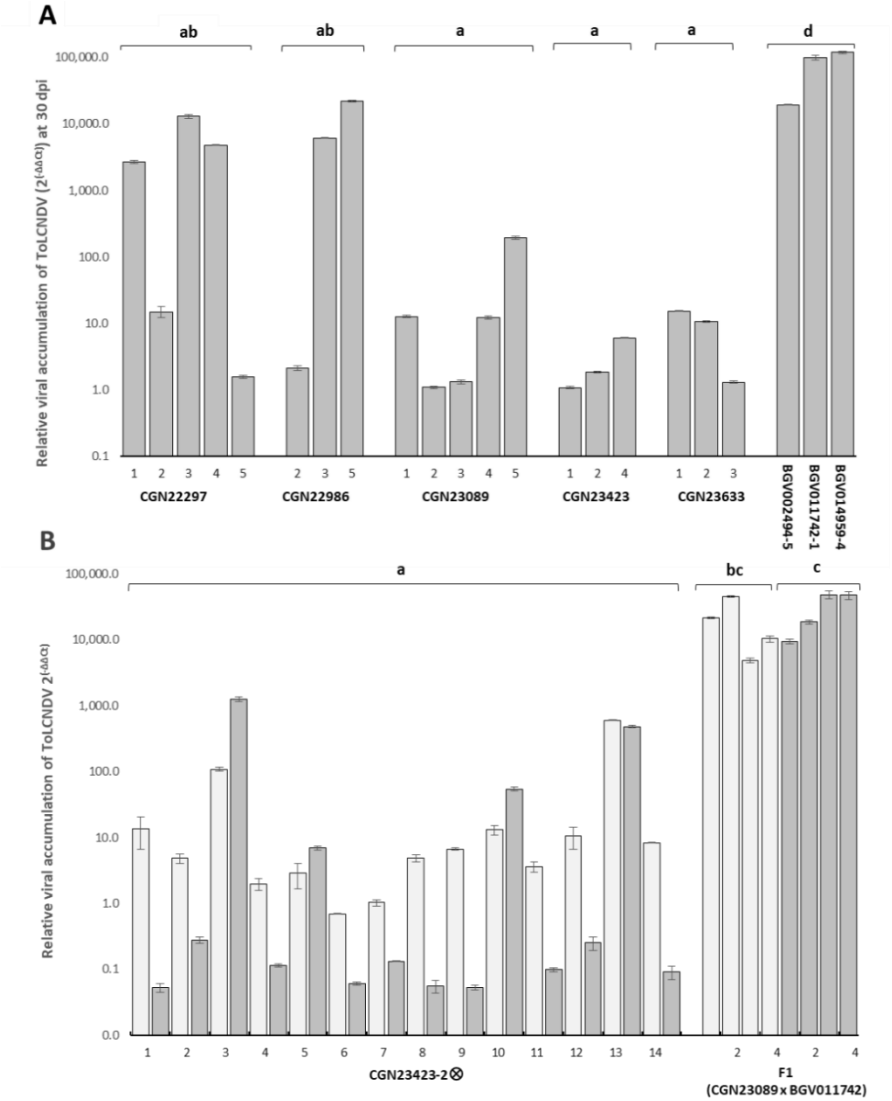

Figure 2.A: Relative ToLCNDV accumulation ($2^{(-\Delta\Delta Ct)}$) at 30 days after mechanical inoculation (dpi) with ToLCNDV in the five asymptomatic Indian accessions (CGN22297, CGN22986, CGN23089, CGN23423 and CGN23633) and in three susceptible controls (BGV002494-5, BGV011742-1 and BGV014959-4). **B:** Relative ToLCNDV accumulation ($2^{(-\Delta\Delta Ct)}$) at 15 and 30 dpi (light and dark bars, respectively) of plants obtained by selfing the CGN23423-2 genotype and of the four F1 (CGN23089-2 x BGV011742) hybrids. Values in the *y* axis are shown in a log-10 scale. In *x* axis accessions and number of plants of each accession are indicated. There are not statistically significant differences between means of $2^{(-\Delta\Delta Ct)}$ in those bars groups with the same lowercase letters over them.

DISCUSSION

Forty accessions of cucumber collected from different provinces of Spain were screened in order to find sources of resistance against ToLCNDV, but none of the accessions showed immunity or high resistance to the virus. Most accessions were highly susceptible to the virus after mechanically inoculation, and only five showed intermediate level symptoms and less load viral. The high susceptibility observed across this collection representative of the cucumber Spanish diversity, reveals that ToLCNDV represents a major threat to cucumber cultivation.

The cucumber accessions of other origins showed variable results. All accessions from China, and the single accessions from Japan, Sri Lanka, Iran, United States and D.R. Congo used in this study were susceptible to ToLCNDV. Interestingly, we have identified resistance in Indian accessions. CGN22297 and CGN22986 showed variable response in symptom development and in viral load, suggesting that the resistance was not fixed in these accessions. The accessions CGN23089, CGN23423 and CGN23633 were uniformly resistant, symptomless and with very low ToLCNDV accumulation compared to susceptible controls.

Finding virus resistance in *C. sativus* is not unexpected as this species has been often used as a source of resistance to different cucurbit viruses. For example, resistance genes to different potyviruses has been identified in mainly three cucumber accessions: 'Suriman', 'Taichung Mou Gua' (TMG-1) and 'Dina-1' (Weng and Wehner 2017). In the inbred cucumber line '02245' one locus controlling resistance to papaya ring spot virus (PRSV) and another controlling resistance to watermelon mosaic virus (WMV), both recessives, were found by Tian et al. (2015; 2016). In the same line, resistance to the cucumovirus CMV is quantitatively inherited (Shi et al. 2018) and in *C. sativus* var. *hardwickii* Munshi et al. (2008) identified CMV resistance controlled by a single recessive gene. Additionally, resistance has been reported to CVYV in the Spanish landrace C.sat-10 (Picó et al. 2003), to CYSDV controlled by more than one recessive gene (Aguilar et al. 2006) and in two Indian accessions of *C. sativus* to CGMMV (Crespo et al. 2018).

To date, most of the sources of resistance identified in cucurbits against ToLCNDV come from India. For instance, resistance to sponge gourd was identified in germplasm collected from different regions in India (Islam et al., 2010). A dominant allele was found controlling the resistance (Islam et al. 2011). In *Cucumis melo*, resistance to ToLCNDV was found in three accessions of the *momordica* horticultural group and two accessions of the wild *agrestis* group, all from India (López et al. 2015). A major *locus* in chromosome 11 and two additional regions in chromosomes 2 and 12 were found controlling the resistance of the wild *agrestis* accession WM-7 (Sáez et al. 2017). In a recent publication, in the same Indian accession WM-7 one recessive (*bgm-1*) and two dominant (*Bgm-2* and *Tolcndv*) genes were found controlling the resistance to ToLCNDV (Romay et al. 2019). Finally, in *Cucurbita moschata*, genetic resistance to ToLCNDV was identified in four accessions from different origins, one of them from India (Sáez et al. 2016). In this case, a major recessive gene located in chromosome 8 was found controlling the resistance in the Indian accession. Also, the candidate region of the chromosome 8 of this *C. moschata* accession is syntenic to the region responsible of ToLCNDV resistance in the chromosome 11 of melon (Sáez et al. 2020). The fact that most the ToLCNDV resistant cucurbit accessions come from India, can be related with the co-evolution of host and pathogen in this part of the world where ToLCNDV was detected infecting cucurbits many years ago (Dhillon et al. 2012).

The analysis of the F1 generation derived from the resistant accession CGN23089 suggests that the resistance to ToLCNDV found in cucumber is also recessive. It is interesting to note that recessive control of resistance is frequent in several virus resistance systems. Recessive resistance genes interfere the viral life cycle at different levels: single-cell, cell-to-cell movement, long-distance transport through the plant and/or preventing high levels of virus accumulation (Díaz-Pendón et al. 2003). In cucumber, the mechanism of resistance to ToLCNDV is characterized by a drastic and significant reduction of virus titer and infected plants are asymptomatic or exhibit mild disease symptoms. This type of resistance is similar to that observed in the rest of the resistances identified in the pathosystem ToLCNDV-

host. In cucurbits, the high level of ToLCNDV DNA accumulation in plant tissue results in the development of severe symptoms and leads to a major reduction in yield in case of susceptible cultivars, but this is not true for the cultivars showing resistance. The virus DNA remains low and approximately constant and has minimal effect on the yield and health of plants (Islam et al. 2010; 2011; López et al. 2015; Sáez et al. 2016; 2017; 2020). In tomato, ToLCNDV viral DNA also determined the level of resistance and yield loss in test varieties of tomato under same environmental conditions. Resistant cultivars showed a low level of viral DNA in their tissue when compared to other susceptible cultivars (Ali et al. 2019), and the same results had been reported previously by Wege (2007) for resistant plants in which they observed the positive correlation between symptoms severity and level of virus accumulation. This also happens for example in the case of CGMMV in cucumber (Crespo et al. 2018), CYSDV and WMV (Marco et al. 2003; Díaz-Pendón et al. 2005), and PRSV in squash and watermelon (Pacheco et al. 2003). Further studies will be needed to establish the mechanism that is limiting ToLCNDV accumulation in resistant plants.

In conclusion, the accessions identified in this study are good candidates for breeding programs to avoid damage caused by ToLCNDV in *C. sativus*. How this resistance is inherited remains to be determined, but our initial analyses suggest that the resistance to ToLCNDV in these accessions is controlled by one (o more) recessive gene. Given the importance of ToLCNDV and the scarcity of sources of resistance to ToLCNDV in cucumber, the virus resistance found in accessions CGN23089, CGN23423 and CGN23633 should be introgressed into commercial cultivars. We are now constructing segregant populations for inheritance studies to select molecular markers linked to the ToLCNDV resistance highly useful in marker-assisted breeding for ToLCNDV resistance in cucumber.

REFERENCES

Aguilar, J. M., Abad, J., & Aranda, M.A. 2006. Resistance to cucurbit yellow stunting disorder virus in cucumber. *Plant Disease,* 90, 583-586.

Ali, A., & Kobayashi, M. 2010. Seed transmission of cucumber mosaic virus in pepper. *Journal of Virological Methods*, 163, 234–237.

Chang, H. H., Ku, H. M., Tsai, W. S., Chien, R. C., & Jan, F. J. 2010. Identification and characterization of a mechanical transmissible begomovirus causing leaf curl on oriental melon. *European Journal of Plant. Pathology*, 127, 219-228.

Crespo, O., Janssen, D., Robles, C., & Ruiz, L. 2018. Resistance to cucumber green mottle mosaic virus in *Cucumis sativus*. *Euphytica*, 214, 201.

Dhillon, N. P. S., Monforte, A. J., Pitrat, M., Pandey, S., Singh, P. K., Reitsma, K. R., Garcia-Mas, J., Sharma, A., & McCreight, J. D. 2012. Melon landraces of India: contributions and importance, in Janick, J. (ed). Wiley, Hoboken. *Plant Breeding Reviews*, 35, 85–150.

Díaz-Pendón, J. A., Fernández-Muñoz, R., Gómez-Guillamón, M. L., & Moriones, E. 2005. Inheritance of resistance to watermelon mosaic virus in *Cucumis melo* that impairs virus accumulation, symptom expression, and aphid transmission. *Phytopathology*, 95, 840–846.

Doyle, J. J., & Doyle, J. L. 1990. Isolation of plant DNA from fresh tissue. *Focus*, 12, 13–15.

EPPO. 2017. European and Mediterranean Plant Protection Organization. https://www.eppo.int/QUARANTINE/Alert_List/viruses/ToLCNDV.htm (accessed 28 February 2017).

EPPO. 2019. European and Mediterranean Plant Protection Organization. Available at https://gd.eppo.int/taxon/TOLCND/distribution (accessed 20 November 2019).

FAO. 2018. Food and Agriculture Organization of the United Nations. Food and Agriculture data. http://www.fao.org/faostat/en/#data/QC (accessed 5 March 2020).

Islam, S., Munshi, A. D., Verma, M., Arya, L., Mandal, B., Behera, T. K., Kumar, R., & Lal S. K. 2010. Genetics of resistance in *Luffa cylindrica* Roem. against tomato leaf curl New Delhi virus. *Euphytica*, 174, 83-89.

Islam, S., Munshi, A. D., Verma, M., Arya, L., Mandal, B., Behera, T. K., Kumar, R., & Lal, S. K. 2011. Screening of *Luffa cylindrica* Roem. for resistance against tomato leaf curl New Delhi virus, inheritance of resistance, and identification of SRAP markers linked to the single dominant resistance gene. *Journal of Horticultural Science and Biotechnology*, 86, 661-667.

Juárez, M., Tovar, R., Fiallo-Olivé, E., Aranda, M. A., Gosálvez, B., Castillo, P., Moriones, E., & Navas-Castillo, J. 2014. First detection of tomato leaf curl New Delhi virus infecting zucchini in Spain. *Plant Disease*, 98, 857-858.

Jyothsna, P., Haq, Q. M. I., Singh, P., Sumiya, K. V., Praveen, S., Rawat, R., Briddon, R. W., & Malathi, V. G. 2013. Infection of tomato leaf curl New Delhi virus (ToLCNDV), a bipartite begomovirus with betasatellites, results in enhanced level of helper virus components and antagonistic interaction between DNA B and betasatellites. *Applied Microbiology* and *Biotechnology*, 97, 5457-5471.

Khan, M. S., Ji, S. H., & Chun, S. C. 2012. Begomoviruses and their emerging threats in South Korea: a review. *Plant Pathology*, 28, 123-136.

Kheireddine, A., Sifres, A., Sáez, C., Picó, B., & López, C. 2019. First report of tomato leaf curl New Delhi virus infecting cucurbit plants in Algeria. Plant Disease, 103, 3291.

Kong, Q., Yuan, J., Gao, L., Zhao, L., Cheng, F., Huang, Y., & Bie, Z. 2015. Evaluation of appropriate reference genes for gene expression normalization during watermelon fruit development. *PLoS One*, 10, e0130865.

Lecoq, H., & Katis, N. 2014. Control of cucurbit viruses, in Loebenstein, G., Nikolaos, K. (eds.), *Advances in Virus Research*, Chapter 5. Oxford, UK: Elsevier Inc., pp. 255-296.

Livak, K. J., & Schmittgen, T. D. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. *Methods*, 25, 402-408.

López, C., Ferriol, M., & Picó, M. B. 2015. Mechanical transmission of tomato leaf curl New Delhi virus to cucurbit germplasm: selection of tolerance sources in *Cucumis melo*. *Euphytica*, 204, 279-691.

Luigi, M., Bertin, S., Manglli, A., Troiano, E., Davino, S., Tomassoli, L., & Parrella, G. 2019. First report of tomato leaf curl New Delhi virus causing yellow leaf curl of pepper in Europe. *Plant Disease*, 103, 2970.

Marco, C. F., Aguilar, J. M., Abad, J., Gómez-Guillamón M. L., & Aranda, M. A. 2003. Melon resistance to cucurbit yellow stunting disorder virus in characterized by reduced virus accumulation. *Phytopathology*, 93, 844–852.

Mnari-Hattab, M., Zammouri, S., Belkadhi, M. S., Bellon Doña, D., ben Nahia, E., & Hajlaoui, M. R. 2015. First report of tomato leaf curl New Delhi virus infecting cucurbits in Tunisia. *New Disease Reports*, 31, 21.

Moriones, E., Praveen, S., & Chakraborty, S. 2017. Tomato leaf curl New Delhi virus: an emerging virus complex threatening vegetable and fiber crops. *Viruses*, 9, 264.

Munshi, A. D., Pandam, B., Mandal, B., Bisht, I. S., Rao, E. S., & Kumar, R. 2008. Genetics of resistance to cucumber mosaic virus in *Cucumis sativus* var. *hardwickii* R. Alef. *Euphytica*, 164, 501– 506.

Orfanidou, C. G., Malandraki, I., Beris, D., Keksidou, O., Vassilakos, N., Varveri, C., katis, N., & Maliogka, N. I. (2019). First report of tomato leaf curl New Delhi virus in zucchini crops in Greece. *Journal of Plant Pathology*, 101, 799.

Pacheco, D. A., Rezende, J. A. M., & Piedade, S. M. dS. 2003. Biomass, virus concentration, and symptomatology of cucurbits infected by mild and severe strains of papaya ringspot virus. *Scientia Agricola*, 60, 691–698.

Panno, S., Iacono, G., Davino, M., Marchione, S., Zappardo, V., Bella, P., Tomassoli, L., Accotto, G. P., & Davino, S. 2016. First report of tomato leaf curl New Delhi virus affecting zucchini squash in an important horticultural area of southern Italy. *New Disease Reports*, 33, 6.

Papidam, M., Beachy, R. N., & Fauquet, C. M. 1995. Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not essential for infectivity. *Journal of General Virology*, 76, 25-35.

Picó, B., Villar, C., & Nuez, F. 2003. Screening *Cucumis sativus* landraces for resistance to cucumber vein yellowing virus. *Plant Breeding*, 122, 426-430.

Provvidenti, R. 1985. Sources of resistance to viruses in two accessions of *Cucumis sativus*. *Cucurbit Genetics Cooperative Report*, 8, 9-12.

Romay, G., Pitrat, M., Lecoq, H., Millot, P., Girardot, G., & Desbiez, C. 2019. Resistance against melon chlorotic mosaic virus and tomato leaf curl New Delhi virus in melon. *Plant Disease*, 103, 2913-2919.

Sáez, C., Esteras, C., Martínez, C., Ferriol, M., Narinder, P. S. D., López, C. & Picó, B. 2017. Resistance to tomato leaf curl New Delhi virus in melon is controlled by a major QTL located in chromosome 11. *Plant Cell Reports*, 36, 1571–1584.

Sáez, C., Martínez, C., Ferriol, M., Manzano, S., Velasco, L., Jamilena, M., López, C. & Picó, B. 2016. Resistance to tomato leaf curl New Delhi virus in *Cucurbita* spp. *Annals of Applied Biology*, 169, 91–105.

Sáez, C., Martínez, C., Montero-Pau, J., Esteras, C., Blanca, J., Sifres, A., Ferriol, M., López, C. & Picó, B. 2020. A major QTL located in chromosome 8 of *Cucurbita moschata* is responsible for resistance to tomato leaf curl New Delhi virus (ToLCNDV). *Frontiers in Plant Science*, 11:207.

Sánchez-Navarro, J. A., Cañizares, M. C., Cano, E. A., & Pallás, V. 1999. Simultaneous detection of five carnation viruses by non-isotopic molecular hybridization. *Journal of Virological Methods*, 82, 167-175.

Sangeetha, B., Malathi, V. G., Alice, D., Suganthy, M., & Renukadevi. P. 2018. A distinct seed-transmissible strain of tomato leaf curl New Delhi virus infecting chayote in India. *Virus Research*, 251: 81-91.

Shi, L., Yang, Y., Xie, Q., Miao, H., Bo, K., Song, Z., Wang, Y., Xie, B., Zhang, S., & Gu, X. 2018. Inheritance and QTL mapping of cucumber mosaic virus resistance in cucumber (*Cucumis Sativus* L.). *PLoS ONE*, 13, e0200571.

Sifres, A., Sáez, C., Ferriol, M., Selmani, E. A., Riado, J., Picó, B., & López, C. 2018. First report of tomato leaf curl New Delhi virus infecting zucchini in Morocco. *Plant Disease*, 102, 1045.

Sohrab, S. S., Karim, S., Varma, A., Abuzenadah, A. M., Chaudhary, A. G., Damanhouri, G. A., & Mandal, B. 2013. Characterization of tomato leaf curl New Delhi virus infecting cucurbits: Evidence for sap transmission in a host specific manner. *African Journal of Biotechnology*, 12, 5000-5009.

Srivastava, K. M., Hallan, V., Raizada, R. K., Chandra, G., Singh, B. P., & Sane, P. V. 1995. Molecular cloning of Indian tomato leaf curl virus genome following a simple method of concentrating the supercoiled replicative form of viral DNA. *Journal of Virological Methods*, 51, 297-304.

Sugiyama, M., Okuda, M., & Sakata, Y. 2009. Evaluation of resistance to melon yellow spot virus in a cucumber germplasm collection. *Plant Breeding*, 128, 696–700.

Tian, G. L., Yang, Y. H., Zhang, S. P., Miao, H., Lu, H. W., Wang, Y., Xie, B. Y., & Gu, X. F. 2015. Genetic analysis and gene mapping of papaya ring spot virus resistance in cucumber. *Molecular Breeding*, 35, 110.

Tian, G. L., Miao, H., Yang, Y. H., Zhou, J., Lu, H. W., Wang, Y., Xie, B. Y., Zhang, S. P., & Gu, X. F. 2016. Genetic analysis and fine mapping of watermelon mosaic virus resistance gene in cucumber. *Molecular Breeding*, 36,131.

Usharani, K. S., Surendranath, B., Paul-Khurana, S. M., Garg, I. D., & Malathi, V. G. 2004. Potato leaf curl – a new disease of potato in northern India caused by a strain of tomato leaf curl New Delhi virus. *Plant Pathology*, 53, 235.

Valcárcel, J. V., Peiró, R. M., Pérez-de-Castro, A., & Díez, M. J. 2018a. Morphological characterization of the cucumber (*Cucumis sativus* L.) collection of the COMAV's Genebank. *Genetic Resources and Crop Evolution*, 65, 1293-1306.

Valcárcel, J. V., Pérez-de-Castro, A., Díez, M. J., & Peiró, R. M. 2018b. Molecular characterization of the cucumber (*Cucumis sativus* L.) accessions of the COMAV's genebank. *Spanish Journal and Agricultural Research*, 16, e0701.

Wege, C. 2007. Movement and localization of Tomato yellow leaf curl viruses in the infected plant. In *Tomato yellow leaf curl virus disease*, 185-206. Springer, Dordrecht.

Weng, Y., & Wehner, T.C. 2017. Cucumber gene catalog 2017. *Cucurbit Genetics Cooperative Report*, 39-40, 17-54.

Zaidi, S. S., Martin, D. P., Amin, I., Farooq, M., & Mansoor, S. 2017. Tomato leaf curl New Delhi virus: a widespread bipartite begomovirus in the territory of monopartite begomoviruses *Molecular Plant Pathology*, 18, 901-911.

Capítulo III

CAPÍTULO III

Resistance to *tomato leaf curl New Delhi virus* in melon is controlled by a major QTL located in chromosome 11

Cristina Sáez, Cristina Esteras, Cecilia Martínez, María Ferriol, Narinder P. S. Dhillon, Carmelo López & Belén Picó

Plant Cell Reports, 2017, 36, 1571–1584

ABSTRACT

Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite *begomovirus* that severely affects melon crop (*Cucumis melo*) in the main production areas of Spain since 2012. In this work, we evaluated the degree of resistance of four accessions (two belonging to the subsp. *agrestis* var. *momordica* and two to the wild *agrestis* group) and their corresponding hybrids with a susceptible commercial melon belonging to the subsp. *melo* (Piel de Sapo, PS). The analysis using quantitative PCR (qPCR) allowed us to select one wild *agrestis* genotype (WM-7) with a high level of resistance and use it to construct segregating populations (F_2 and backcrosses). These populations were phenotyped for symptom severity and virus content using qPCR, and genotyped with different sets of SNP markers. Phenotyping and genotyping results in the F_2 and BC1s populations derived from the WM-7 × PS cross were used for QTL analysis. Three genomic regions controlling resistance to ToLCNDV were found, one major locus in chromosome 11 and two additional regions in chromosomes 12 and 2. The highest level of resistance (no or mild symptoms and very low viral titer) was obtained with the homozygous WM-7WM-7 genotype at the major QTL in chromosome 11, even with PSPS genotypes at the other two *loci*. The resistance derived from WM-7 is useful to develop new melon cultivars and the linked SNPs selected in this paper will be highly useful in marker-assisted breeding for ToLCNDV resistance in melon.

INTRODUCTION

Tomato leaf curl New Delhi virus (ToLCNDV) causes a devastating viral disease that threatens the main cucurbit crops in Spain since 2012 (Juárez et al. 2014). It is a *Begomovirus* (family Geminiviridae) with two circular single-stranded DNA molecules of about 2.6–2.7 kb, referred to as DNA-A and DNA-B, both required for essential viral functions and encapsidated in geminate particles (Papidam et al. 1995; Jyothsna et al. 2013). The virus is transmitted by the whitefly *Bemisia tabaci* Genn. in a persistent manner (Chang et al. 2010; Rosen et al. 2015) and it can be artificially inoculated mechanically (López et al. 2015).

ToLCNDV was first detected on tomato (*Solanum lycopersicum* L.) in 1995 in north India (Srivastava et al. 1995) and soon after was found in other south and southeast Asian countries. The host range was extended to other plant species, mainly crops of the Cucurbitaceae and Solanaceae families (Chang et al. 2010; Pratap et al. 2011; Khan et al. 2012; Jyothsna et al. 2013; Bandaranayake et al. 2014). Before 2012, ToLCNDV was limited to Asian countries; thereafter, it was reported in different Mediterranean countries, affecting cucurbits [mainly zucchini (*Cucurbita pepo* L. subsp. *pepo*), melon (*Cucumis melo* L.), and cucumber (*Cucumis sativus* L.)]. It was initially detected in Spain and more recently in Tunisia and Italy (Juárez et al. 2014; Mnari-Hattab et al. 2015; Panno et al. 2016). In the background of its potential spread, European and Mediterranean Plant Protection Organization (EPPO) has added this virus to the EPPO Alert List (EPPO 2017).

The Spanish strain of ToLCNDV was first isolated from zucchini plants grown in Murcia and Almería in 2012–2013 (Juárez et al. 2014). Further analysis of Spanish isolates from different regions and hosts showed genetic uniformity, compatible with a recent introduction. This uniformity contrasts with the high heterogeneity found in isolates from other parts of the world (Fortes et al. 2016; Ruiz et al. 2016).

In southern Spain, severe epidemic outbreaks of the disease cause serious economic losses to greenhouses and open field zucchini squash and melon crops. In melon, the disease has spread to open fields in central Spain, the most traditional

Spanish area of extensive melon cultivation. Yield losses around 20% have been reported in that area (Hernández 2016). Melon plants affected by ToLCNDV show curled leaves with yellow mosaic, stunted growth, and longitudinal cracking of ovaries and fruits (Juárez et al. 2014; Font et al. 2015; EPPO 2017). Some control strategies are being applied to prevent infections, relying on whitefly control, structural measures in greenhouses, and elimination of infected plants, but resistant commercial cultivars have not been released yet (EPPO 2017).

The knowledge of the genetic basis of the resistance to ToLCNDV is necessary to perform breeding programs. Only in sponge gourd (*Luffa cylindrica* M. Roem.) and in some *Solanum* species, the resistance to ToLCNDV has been characterized. This resistance has been found to be monogenic in *Luffa cylindrica* and polygenic in *Solanum* spp. (Islam et al. 2010, 2011; Rai et al. 2013). In *Cucumis melo*, López et al. (2015) screened a large germplasm collection representing the diversity of the species as a previous step. They identified resistance to the Spanish isolate of ToLCNDV in five melon genotypes, all from India and belonging to the subsp. *agrestis* (Naudin) Pangalo (three accessions of the *momordica* horticultural group and two wild *agrestis*). In this work, we aimed to identify QTLs controlling the resistance to ToLCNDV in *C. melo* using segregating populations derived from one of these resistant sources and a susceptible Piel de Sapo melon (belonging to the subsp. *melo*).

MATERIALS AND METHODS

Plant material

Five Indian accessions of *Cucumis melo* subsp. a*grestis* were identified as resistant by López et al. (2015) after mechanical inoculation with ToLCNDV: three cultivated *momordica* and two wild *agrestis* types. In this work, to validate the resistant response to the virus infection, we assayed two *momordica* (PI-414723 and Kharbuja) and two wild types (WM-7 and WM-9). The cultivated accession Piñonet Piel de Sapo (PS) (*Cucumis melo* subsp. *melo* var. *inodorus*) was used as susceptible control.

Seeds of PI-414723 and Kharbuja were supplied by USDA-NPGS and PS by COMAV genebanks, and seeds of WM-7 and WM-9 came from the Indian collection described in Roy et al. (2012). All accessions were multiplied by selfing at COMAV. Seeds were disinfected in a 5% solution of sodium hypochlorite for 3 min and washed for 5 min in distilled water. Germination was performed in Petri plates with moistened cotton at 37 °C for 48 h. Seedlings were transplanted to pots in a growth chamber under a photoperiod of 16 h day at 25 °C and 8 h night at 18 °C and 70% RH. Eight plants per genotype were mechanically inoculated and assessed for resistance, using symptom scores and quantitative PCR (qPCR). Six additional plants per genotype were grown in a greenhouse with plants of the PS cultivar to generate the four F_1 hybrid progenies. Eight plants of each hybrid were phenotyped for resistance to ToLCNDV. Six plants of the PS cultivar were used as susceptible controls in each inoculation assay. The screening results suggested that wild *agrestis* had better resistance levels than *momordica* sources, so we selected one of the wild accessions (WM-7) to generate the segregating populations for genetic studies. We selected WM-7 after genotyping both wild accessions, because WM-9 was highly heterozygous, whereas WM-7 was homozygous for most of the analyzed *loci*. Plants of WM-7, PS, and their F_1 generation were cultivated in a greenhouse to generate F_2, $BC1_{PS,}$ and $BC1_{WM-7}$ (backcrosses to PS and to WM-7, respectively) segregating populations. Both F_2 and BC1 populations were screened against ToLCNDV (158 plants of the F_2 and 70 of each $BC1_{PS}$ and $BC1_{WM-7}$ populations, respectively).

Inoculation method

A ToLCNDV-infectious clone, with a 99% nucleotide identity with the sequence of the Spanish isolate KF749224 and KF749225 (Juárez et al. 2014), was agroinfiltrated by injection into petioles of MU-CU-16 zucchini plants, which showed ToLCNDV symptoms 15 days later (Sáez et al. 2016). ToLCNDV transmission to melon plants was performed by mechanical inoculation as described by López et al. (2015). Leaf tissue from the MU-CU-16 plants was mashed in the presence of inoculation buffer in a 1:4 (*w*:*v*) proportion. All plants were mechanically

inoculated at the stage of two true leaves, leaving one not inoculated as negative control. For each plant, one cotyledon and one fully expanded leaf, previously dusted with carborundum 600 mesh, were gently rubbed with a cotton-swab dipped in the homogenate inoculum. All plants were re-inoculated 10 days after the first mechanical inoculation to prevent them from escaping to the infection.

To evaluate the behavior of the F_1 WM-7 × PS under natural infection conditions, ten plants of the F_1 and PS were grown in a greenhouse located in El Ejido (Almería) along with 4000 commercial plans of *Cucumis melo* subsp. *melo* var. *cantalupensis*. ToLCNDV infection in this commercial greenhouse was due to the presence of viruliferous whiteflies. The F_1 plants and the PS controls were planted at the end of February (2016) and evaluated for symptomatology and virus titer at the beginning of May.

Disease assessment: symptoms and qPCR

All the mechanically inoculated plants and controls were grown in a climatic chamber. Symptoms of ToLCNDV were scored at 15 and 30 day post-inoculation (dpi), following the visual scale described in López et al. (2015), ranging from 0 (absence of symptoms) to 4 (highly severe symptoms). We used the Chi-squared (χ^2) test to assess whether the number of resistant/susceptible plants fitted the expected segregation.

Quantitative PCR was used to quantify the amount of virus. The five parents and the corresponding F_1s were analyzed at 15 and 30 dpi. Viral accumulation was also analyzed at 30 dpi in all plants of the segregating populations derived from the WM-7 × PS (F_2, $BC1_{PS,}$ and $BC1_{WM-7}$). Total DNA from apical leaves was extracted using the Cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990) and quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). DNA was diluted with sterile-deionized water to a final concentration of 5 ng μL^{-1}. Equal amounts of genomic DNA (15 ng) were used as templates in qPCR reactions of 15 µL, containing 7.5 µl of 2× iTaq™ universal SYBR® Green Supermix (Bio-Rad), 1.5 µl of each primer, and 1.5 µl of H_2O.

Primers ToLCNDVF1 (5′-AATGCCGACTACACCAAGCAT-3′, positions 1145–1169) and ToLCNDVR1 (5′-GGATCGAGCAGAGAGTGGCG-3′, positions 1399–1418) were used for the amplification of a 273 bp fragment of viral DNA-A. The two single copy *Cucumis melo* genes *CmPEROX* and *CmWIP1* (Mascarell-Creus et al. 2009) were amplified in all samples as internal DNA extraction controls using the primers CmPEROXF (5′-ACTCGATCAACTTCGAGCAAA-3′), CmPEROXR (5′-GCCTATCCAAAGACCTCGGCCTTCCC-3′), CmWIP1F (5′-TAGGGCTTCCAACTCCTTCCTCTT-3′), and CmWIP1R (5′CTTGCAATTGATGGGTGTGATCTTCTTG-3′). All samples were run in triplicate in a Roche Lightcycler 480. Cycling conditions consisted on an incubation step at 95 °C for 5 min, and 45 cycles at 95 ºC for 5 s and 60 ºC for 30 s to amplify *CmPEROX* and *CmWIP1*, and 40 cycles at the same conditions to amplify ToLCNDV DNA. The Ct (cycle threshold), which is the cycle number at which the fluorescence passes the threshold, was calculated for the viral target and the internal control genes in each sample. Relative accumulation of ToLCNDV was estimated by the comparative Ct method, using the formula $2^{-\Delta\Delta Ct}$, where ΔΔCt is the difference between the ΔCt of each sample (ΔCt_{sample}: $Ct_{reference\ genes} - Ct_{viral\ target}$) and the ΔCt of the calibrator sample ($\Delta Ct_{calibrator}$). The resistant parental WM-7 at 15 dpi was used as the calibrator sample in all assays.

SNP genotyping and QTL analysis

Plants of the WM-7 and PS parental accessions, and the derived F_1, F_2, $BC1_{PS}$, and $BC1_{WM-7}$ populations, were genotyped with a set of 124 SNPs evenly distributed throughout the melon genome. This SNP panel was designed in previous studies and validated to perform QTL analysis in subsp. *melo* × subsp. *agrestis* melon crosses and to perform association studies (Leida et al. 2015; Perpiñá et al. 2016). The genomic position in the melon genome version 3.5.1 (García-Mas et al. 2012) of the polymorphic markers and their genetic position, estimated with the genotype of the F_2 population employing MAPMAKER 3.0 (Van Ooijen and Voorrips 2001) with the Kosambi map function, are indicated in Additional file 1, along with their flanking sequences. Total DNA was extracted from young leaves tissue before

inoculation, using the protocol described in Esteras et al. (2012). DNA was quantified and adjusted to 15 ng μL^{-1}. Genotyping was done using the iPLEX® Gold MassARRAY® Sequenom system at the Epigenetic and Genotyping unit of the University of Valencia (Unitat Central d´Investigació en Medicina (UCIM), Spain).

To identify markers linked to the resistance to ToLCNDV derived from WM-7, a QTL analysis was performed using symptom score at 15 and 30 dpi and ToLCNDV relative accumulation at 30 dpi of the F 2 population. We used the Kolmogorov–Smirnov test to check the normality assumption of traits distribution. Since the traits were not normally distributed, Kruskal–Wallis non-parametric test was used for quantitative trait loci (QTL) detection using the MapQTL version 4.1 software (Van Ooijen 2009), considering as significant associations those with $p < 0.05$.

In addition, a composite interval mapping approach (CIM, Zeng 1994) was applied. For the relative viral accumulation, the transformed distribution of ΔΔCt (ln (ΔΔCt) +2) instead of $2^{-\Delta\Delta Ct}$ was used. It was implemented in Windows QTL Cartographer 2.5 (Wang et al. 2012), using a windows size of 15 cM and five cofactors. QTLs having LOD scores greater than a threshold value that had been determined by a permutation test were retained (1000 permutations were applied at the genome-wide level or each linkage group separately). *Loci* identified with both methods (Kruskal–Wallis and CIM) were considered true QTLs. For each QTL peak, we estimated the percentage of phenotypic variation explained (R^2), the additive and dominance effects, as well as the degree of dominance. A drop interval of 1.5 from the peak LOD was used to map the position of QTLs.

Additional mapping in chromosome 11

A new set of SNPs was designed to confirm QTL analysis and refine the position of the main locus associated with resistance to ToLCNDV located in chromosome 11. These new SNPs were obtained from a resequencing assay of the two parents, WM-7 and PS, which provided 12,927 polymorphisms between them. A set of 19 SNPs uniformly covering the chromosome 11 was implemented in a new

Sequenom assay and used to genotype parents, F_1 and F_2 populations. These genotyping results were used to construct a new genetic map of chromosome 11. MAPMAKER 3.0 (Van Ooijen and Voorrips 2001) software and the Kosambi map function was employed to generate the new map. The genetic distances of the new map were used in a second QTL analysis following the same procedure described above. The genetic and genomic positions of these markers and their flanking sequences are shown in Additional file 2. Increasing the marker density in the candidate region is useful to narrow the size of the QTL confidence interval. However, CIM results can be affected by an uneven distribution of markers. We adjusted the CIM model to these new set of markers. The best results were obtained using a windows size of 10 cM and three cofactors. Those markers closely linked (<1 cM) that cause collinearity, and those having more than a 10% of missing data were removed from the analysis. In addition, 50 plants of the $BC1_{PS}$ and 50 of $BC1_{WM\text{-}7}$ populations were genotyped to validate QTL analysis with both SNPs sets (the first background SNPs and the additional set of chromosome 11 SNPs).

Study of QTL interactions

Epistatic interactions between the main QTLs involved in ToLCNDV resistance were investigated by two-way ANOVA with the STATGRAPHIC Centurion XVI.I statistic software and using the genotypic data from the linked markers in the F_2 population. These markers were D16 for the QTL in chromosome 11, CMPSNP658 for the QTL in chromosome 2, and AI_35-A08 for the QTL in chromosome 12. Interactions were considered to be statistically significant when $p \leq 0.05$.

RESULTS

Resistance of the different melon sources and their F_1 progeny

All the assayed accessions showed resistance to ToLCNDV, with no symptoms or only mild-to-moderate symptoms (most scores from 0 to 2), compared to the severe symptoms found in the susceptible PS cultivar (scores from 3 to 4), and with viral

titers that were on average 15 times lower than those of the PS control (Fig. 1). However, differences in the response to ToLCNDV infection were observed between the wild *agrestis* and the *momordica* types. Plants of the Kharbuja accession developed mild symptoms at 30 dpi, and showed the highest ToLCNDV virus titer among the assayed resistant sources (Fig. 1). Most plants of PI-414723 remained symptomless, and although a few showed moderate symptoms at 15 dpi, all recovered and were symptomless at the end of the assay. The F_1 hybrids of these two *momordica* accessions with PS showed similar behavior. In general, symptoms in F_1 plants were slightly more severe than those of the corresponding resistant parents, but remained mild to moderate at the end of the assay. These *momordica* derived F_1 had viral titers significantly higher than those of the resistant parents, increasing from 15 to 30 dpi, but still around 4–6 times lower than PS.

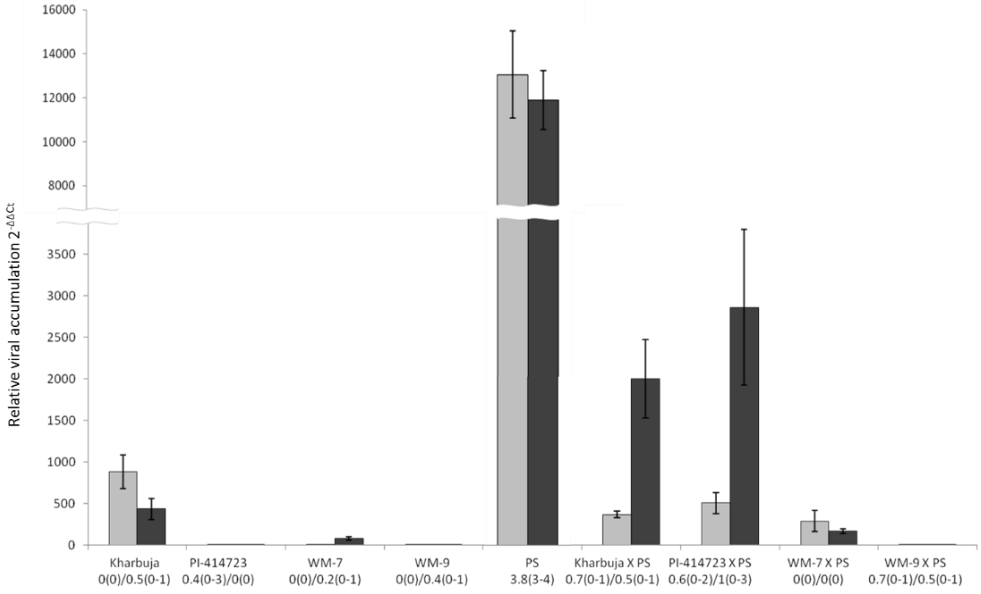

Figure 1. Relative ToLCNDV accumulation (calculated as $2^{-\Delta\Delta Ct}$) at 15 and 30 days after mechanical inoculation (dpi) with ToLCNDV (*light and dark bars*, respectively), in four *Cucumis melo* subsp. *agrestis* resistant accessions (two *momordica*, Kharbuja and PI414723, and two wild types WM-7 and WM-9), the susceptible control *C. melo* subsp. *melo* Piñonet Piel de sapo (PS) and their respective hybrids. Mean and range of symptom score at 15/30 dpi are indicate in the *x* axis legend.

The two wild *agrestis* WM-7 and WM-9 exhibited a higher level of resistance. Symptoms were milder (scores 0–1) than those of the *momordica* accessions, and viral titers were very low, both in the parents and F_1 progenies (Figure 1). The fact that resistance to ToLCNDV remained in F_1 progenies suggests a dominant genetic control of this trait. The genetic control of virus resistance was studied in the F_2 and BC1 populations obtained from WM-7 × PS.

In the greenhouse assay, F_1 plants derived from WM-7 remained symptomless and with a low viral titer in the greenhouse (symptom score of 0 and $2^{-\Delta\Delta Ct}$ value of 210 ± 35), whereas the PS controls and the commercial cantaloupes showed severe symptoms (including fruit cracking) and high viral accumulation (scores 1–3, and 3–4, and $2^{-\Delta\Delta Ct}$ values 11,250 ± 532 and 14,325 ± 450, respectively) after the natural infection with viruliferous whiteflies.

Response of segregating populations

The F_2 and $BC1_{PS}$ populations derived from WM-7 × PS segregated for both symptoms severity and ToLCNDV accumulation. All plants of the $BC1_{WM-7}$ generation remained symptomless at the end of the assay.

In the F_2 population, 108 plants remained symptomless or with mild symptoms at the end of the assay (scores 0 and 1), whereas 50 plants showed moderate to very severe symptoms at 30 dpi (scores 2–4), with leaf curling and yellow spotting typical of ToLCNDV infection. Plants with symptom scores of 0 and 1 (similar to those found in the resistant parent and in the corresponding F_1) were classified as resistant, and those with symptom scores between 2 and 4 as susceptible. The χ^2 test indicated that this segregation fitted to a 3:1 (resistant:susceptible) ratio expected for a single dominant gene for resistance ($p = 0.07$) (Table 1).

$BC1_{PS}$ also segregated for symptoms severity with 38 resistant (scores 0–1) and 32 susceptible (scores 2–4) plants at 30 dpi. This segregation also fitted to a 1:1 ratio expected for a single dominant gene ($p = 0.4$) (Table 1).

Table 1. Segregation of resistant/susceptible plants in F_2, $BC1_{PS}$, and $BC1_{WM-7}$ progenies (derived from the cross WM-7 × PS) 30 days after mechanical inoculation with ToLCVND.

Progeny	Symptoms segregation		Expected proportion	$\chi 2$ test*
	Resistant	Susceptible		
F_2	108	50	3:01	3.4 (p = 0.07)
BC_{1PS}	38	32	1:01	0.7 (p = 0.4)
BC_{1WM-7}	70	0	1:00	–

* Probability of the χ^2 value calculated for a dominant monogenic expected ratio

To further characterize the response to ToLCNDV, virus accumulation was estimated in segregating populations by qPCR (Figure 2). Viral titer positively correlated with symptoms severity (r^2 = 0.9301 and 0.978, p = 0.0002 and 0.0215, in F_2 and $BC1_{PS}$, respectively). In both populations F_2 and $BC1_{PS}$, the resistant plants had viral amounts significantly lower than susceptible plants (mean $2^{-\Delta\Delta Ct}$ values 263.7 versus 1037.8, and 1687.8 versus 5778.4 for F_2 and $BC1_{PS}$, respectively), and a high variability in the viral titer was found in susceptible plants.

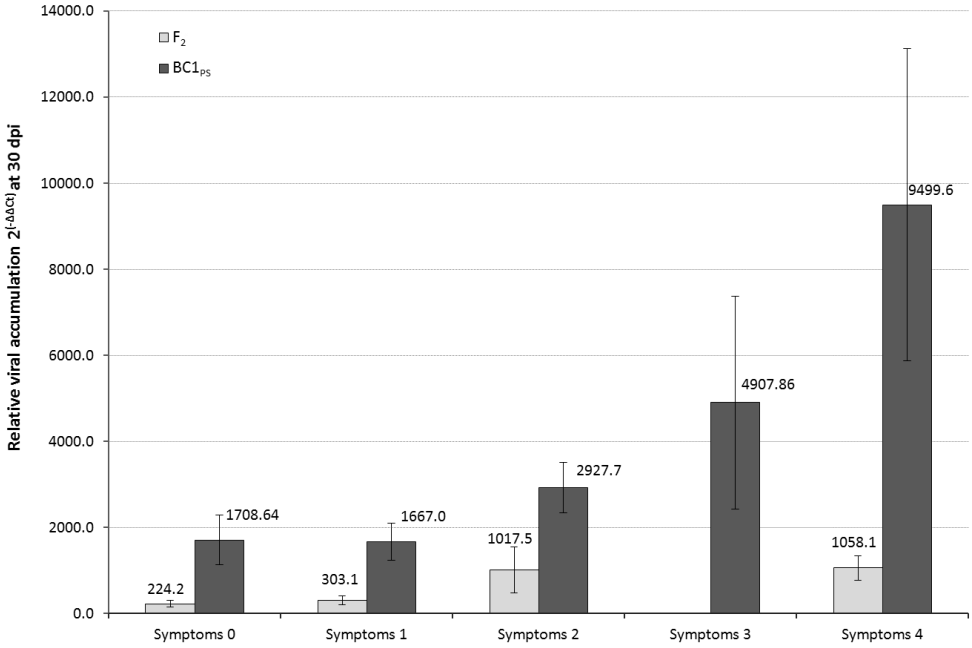

Figure 2. Mean relative ToLCNDV accumulation (calculated as $2^{-\Delta\Delta Ct}$) in plants within each symptomatic class in F_2 and $BC1_{PS}$ populations (derived from the cross WM-7 × PS) at 30 dpi.

Mean viral titer of the $BC1_{PS}$ population was significantly higher than that of the F_2. This effect was observed in the resistant and susceptible categories (Figure 2). Variability was also found in virus titer among plants of the $BC1_{WM-7}$ population, with a mean $2^{-\Delta\Delta Ct}$ value of 140.75, ranging from 0.002 to 4083.8. Taking together all these results, an effect of the genetic background (additional genes from the resistant parent) on the viral accumulation is suggested.

QTL analysis of F_2 population

To study the contribution of different genomic regions to ToLCNDV resistance, a QTL analysis was performed, using genotypic and phenotypic data of the F_2 population. Genotyping results of the F_2 population with the background set of SNPs and phenotypes for symptom score at 15 and 30 dpi and virus titer at 30 dpi were used for the analysis. Since the studied traits did not fit to a normal distribution, the non-parametric Kruskal–Wallis test (KW) was used to identify QTLs using untransformed data, and Composite Interval Mapping Method (CIM) was used with transformed data. Three genomic regions involved in the variation of these traits were detected with both methods (Table 2).

Three major overlapping QTLs were found in chromosome 11, one for each trait (*ToLCNDVSy15*_11, *ToLCNDVSy30*_11, and *ToLCNDVVT30*_11), located between CMPSNP30 and CMPSNP475 markers, with LOD peaks of 19.3, 50.7, and 12.9 (located at 60.5, 57.4, and 59.5 cM). These QTLs explained 67, 67, and 54% of the observed variation of symptoms at 15 dpi, 30 dpi, and viral accumulation, respectively (Table 2). We found additional overlapping QTLs for all traits in chromosome 12 (*ToLCNDVSy15*_12, *ToLCNDVSy30*_12, and *ToLCNDVVT30*_12) and for viral accumulation in chromosome 2 (*ToLCNDVVT30*_2). The QTLs in chromosome 12, with 5.3, 3.9, and 4.6 LOD scores were located close to the AI_35-A08 marker (LOD peaks at 14.9, 15.7, and 12.9 cM), and explained percentages of variation of 18, 10, and 10%, respectively, for each trait. The QTL in chromosome 2 (LOD peak 4.8 at 82.1 cM, closest marker CMPSNP658) was only significant for virus titer, and explained 13% of the observed variance for this trait.

Table 2. Quantitative trait loci (QTLs) identified in the F_2 segregating population genotyped with background SNPs, using the non-parametric Kruskal–Wallis test with untransformed data, and composite interval mapping method with transformed data.

Trait	Chr[a]	Interval[b] (cM)	Nearest marker[c]	QTL name	Kruskal–Wallis			Composite interval mapping				
					*K**[d]	Mean WM-7[e]	Mean PS[f]	LOD[g]	Add[h]	Dom[i]	*d*/*a*[j]	R^{2k}
Symptoms 15 dpi	11	52.2–65.3	CMPSNP475	*ToLCNDVSy15_11*	*****	0.035	1.29	19.3	1.14	−1.21	−1.06	0.67
	12	0–34.5	AI_35-A08	*ToLCNDVSy15_12*	****	0.087	1.25	5.3	0.63	−0.16	−0.25	0.18
Symptoms 30 dpi	11	51.0–63.8	CMPSNP475	*ToLCNDVSy30_11*	******	0	2	50.7	1.8	−2.00	−1.11	0.67
	12	0–32.1	AI_35-A08	*ToLCNDVSy30_12*	***	0.17	1.83	3.9	0.74	0.2	0.27	0.1
Mean of ΔΔCt	2	70.9–100.3	CMPSNP658	*ToLCNDVVT30_2*	**	−1.59	−4.76	4.8	−3.36	0.23	−0.07	0.13
	11	46.9–68.8	CMPSNP475	*ToLCNDVVT30_11*	*******	2.48	−5.21	12.9	−5.82	−0.53	0.09	0.54
	12	0–18.7	AI_35-A08	*ToLCNDVVT30_12*	**	−0.007	−4.75	4.6	−2.71	−0.76	0.28	0.1

[a]Chromosome

[b]Interval position of the putative QTL, identified in the WM-7 × PS *F* 2 by CIM, in cM on the genetic map according with a LOD drop of 1.5

[c]The closest marker to LOD peak

[d]*K**: significant level in the Kruskal–Wallis test **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001, ******: 0.0005, *******: 0.0001

[e]Mean of the genetic class WM-7 in each marker

[f]Mean of the genetic class PS in each marker

[g]*LOD* higher logarithm of the odds score

[h]*Add* additive effect of the PS allele

[i]*Dom* dominant effect of the PS allele

[j]*d/a* degree of dominance

[k]R^2 percentage of phenotypic variance explained by the QTL

Further analysis of the major QTL in chromosome 11

The resequencing data of the parental lines provided additional SNPs evenly distributed in the candidate region of chromosome 11 that were used to genotype the F_2 population. Genotyping results were employed to generate a new genetic map of this region covering 112.2 cM, with an average distance between consecutive markers of 4.7 cM (Figure 3).

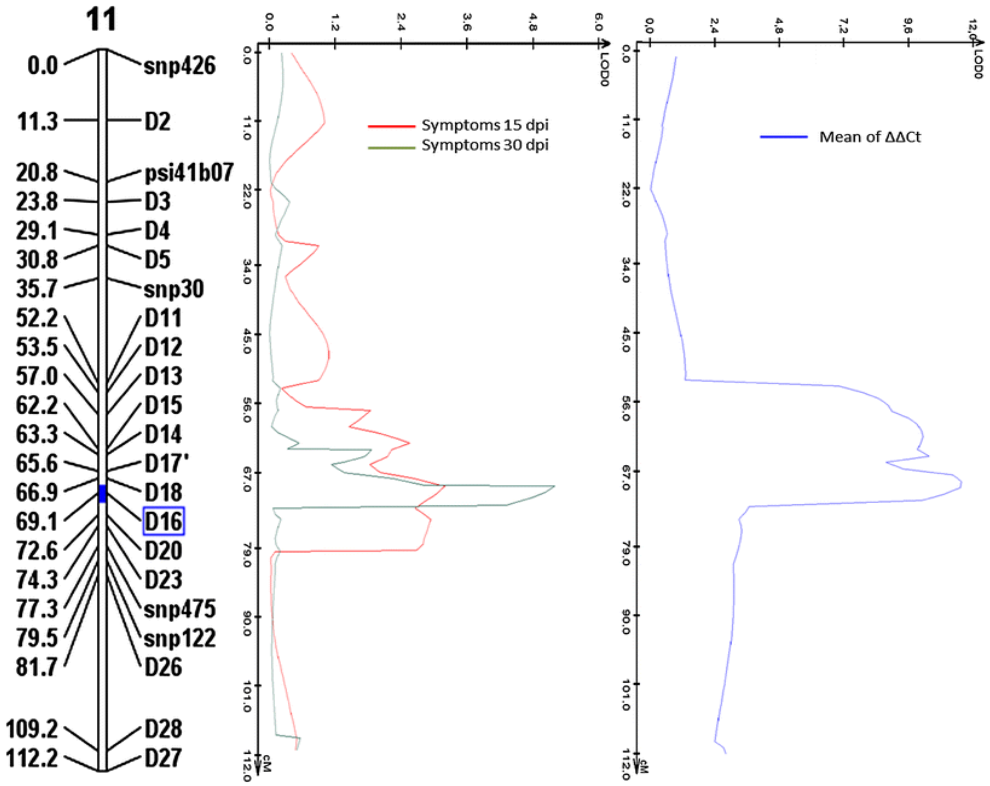

Figure 3. Genetic map of the chromosome 11 constructed with the genotyping data of the F_2 population (derived from the cross WM-7 × PS). The position of the major QTLs for the three traits (symptoms at 15 and 30 dpi and virus titer at 30 dpi) associated with ToLCNDV resistance are indicated. Map distances (cM) were obtained using Kosambi's function and QTL location was obtained by Composite Interval Mapping (CIM) method.

The new QTL analysis confirmed the occurrence of major QTLs in chromosome 11 (*ToLCNDVSy15*_11, *ToLCNDVSy30*_11, and *ToLCNDVVT30*_11), with LOD scores of 3.2, 5.2, and 11.6 (LOD peaks located at 68.0, 69.0, and 68.4 cM) and 11, 21, and 32 of explained variance, respectively (Table 3; Figure 3). Despite this second mapping experiment decreased the proportion of explained

Table 3. Confirmation of the major QTLs for ToLCNDV resistance *ToLCNDVSy15_11*, *ToLCNDVSy30_11*, and *ToLCNDVVT30_11* after genotyping the F_2 population with new markers evenly distributed in chromosome 11 using the non-parametric Kruskal–Wallis test with untransformed data and composite interval mapping method with transformed data.

Trait	Lg[a]	Interval[b] (cM)	Nearest marker[c]	QTL mane	Kruskal–Wallis			Composite Interval Mapping (CIM)				
					*K**[d]	Mean WM-7[e]	Mean PS[f]	LOD[g]	Add[h]	Dom[i]	*d/a* [j]	R^{2k}
Symptoms 15 dpi	11	61.0–79.0	D16	*ToLCNDVSy15_11*	*****	0.48	0.65	3.2	0.56	0.054	0.096	0.11
Symptoms 30 dpi	11	68.9–72.7	D16	*ToLCNDVSy30_11*	*******	0.03	1.98	5.2	0.95	0.24	0.25	0.21
Mean of ΔΔCt	11	66.4–71.0	D16	*ToLCNDVVT30_11*	*******	−2.71	−3.56	11.6	−0.34	−0.33	0.97	0.32

[a]Chromosome

[b]Interval position of the putative QTL, identified in the WM-7 × PS *F* 2 by CIM, in cM on the genetic map according with a LOD drop of 1.5

[c]The closest marker to LOD peak

[d]*K**: significant level in the Kruskal–Wallis test **: 0.05, ***: 0.01, ****: 0.005, *****: 0.001, ******: 0.0005, *******: 0.0001

[e]Mean of the genetic class WM-7 in each marker

[f]Mean of the genetic class PS in each marker

[g]*LOD* higher logarithm of the odds score

[h]*Add* additive effect of the PS allele

[i]*Dom* dominant effect of the PS allele

[j]*d/a* degree of dominance

[k]R^2 percentage of phenotypic variance explained by the QTL

variance, it allowed the delimitation of the position interval of the *ToLCNDVSy30*_11 and *ToLCNDVVT30*_11 QTLs between the new D14 and D23 markers (located in chromosome 11 physical positions 29,550,418 and 30,192,781, respectively), being the D16 (29,690,406) the closest marker to the LOD peaks.

Epistatic interactions between QTLs involved in ToLCNDV resistance

To detect the putative effects of the regions of chromosomes 2 and 12 on the major QTL in chromosome 11, the digenic interactions between them were studied. Those that were statistically significant ($p \leq 0.05$) are shown in Table 4.

Table 4. Significant epistatic interactions ($p \leq 0.05$) between SNPs linked to the three regions involved in ToLCNDV resistance, calculated with data from the F_2 segregating population.

Traits	Markers (Chr)	*p* value
15 dpi	D16 × AI_35-A08 (12)	0.005
30 dpi	D16 × AI_35-A08 (12)	0.0425
Relative viral titer $2^{(-\Delta\Delta Ct)}$	D16 × CSMSNP658 (2)	0.0168

QTL in chromosome 11 versus chromosome 12

Significant interactions were identified between *ToLCNDVSy15*_11 and *ToLCNDVSy15*_12 ($p = 0.005$) and between *ToLCNDVSy30*_11 and *ToLCNDVSy30*_12 ($p = 0.0425$) (Table 4; Figure 4), which explained 10.8 and 7.12% of the variance of each trait, respectively. The effect of interaction was similar for both traits (Figure 4). The occurrence of the WM-7 homozygous genotype at D16 resulted in a high level of resistance (no or mild symptoms), independently of the genotype at the chromosome 12 *locus*. However, the symptom severity of plants both heterozygous and PSPS at D16 was dependent on the genotype at *locus* AI_35-A08. The D16 heterozygous plants displayed no or mild symptoms when combined with WM-7WM-7 AI_35-A08 genotype, but mild to moderate when combined with PSPS or heterozygous genotype at chromosome 12. Only the D16 PSPS/AI_35-A08PSPS plants were highly susceptible (severe symptoms) as the presence of the WM-7 alleles at the AI_35-A08 *locus* (both in heterozygosity or homozygosity) in D16 PSPS plants resulted in intermediate symptoms.

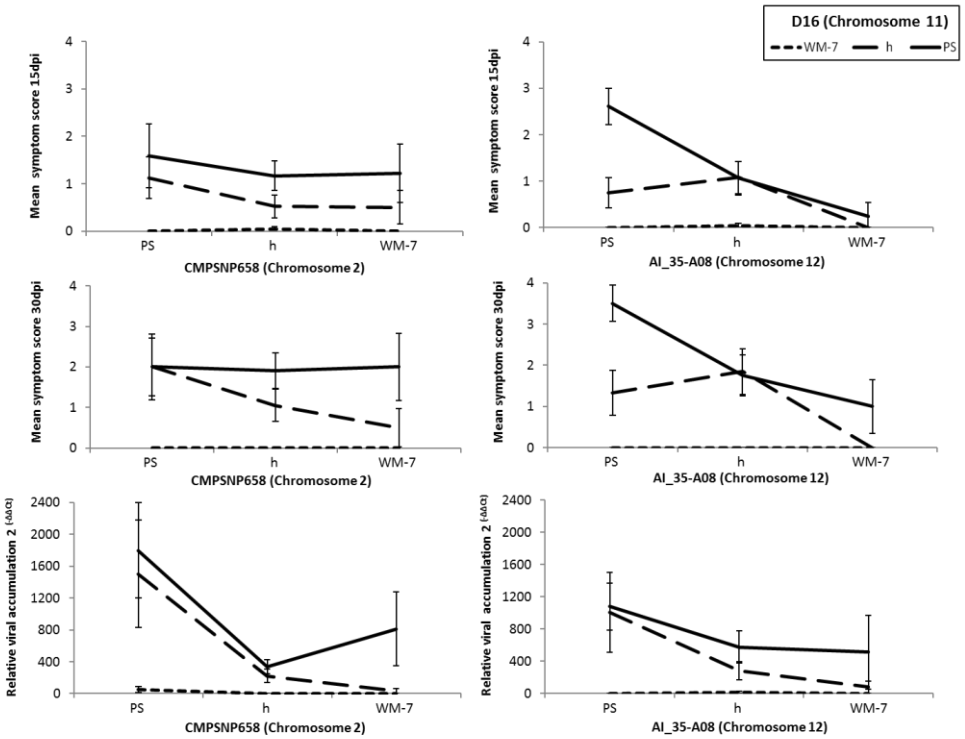

Figure 4. Epistatic interactions identified in F_2 population between D16 marker (chromosome 11) and CMPSNP658 and AI_35-A08, of chromosome 2 and 12, respectively, for symptoms at 15 and 30 dpi and viral titer at 30 dpi (genotypic classes of D16 marker: *PS* plants homozygous for the PS allele, *h* heterozygous plants, *WM-7* plants homozygous for the WM-7 allele).

QTL in chromosome 11 versus chromosome 2

A significant interaction was also identified between *ToLCNDVVT30*_11 and *ToLCNDVVT30*_2 (p = 0.0168) (Table 4), which explained 2.8% of the genetic variance of this trait. The homozygous plants for the WM-7 allele at marker D16 were all resistant with a very low viral titer, independently of the genotype at marker CMPSNP658 (Figure 4). However, the response of the heterozygous genotype at D16 was dependent on the genotype at CMPSNP658. Viral titer was low, although higher than that of the resistant WM-7WM-7 D16 genotype when the genotype at the CMPSNP658 *locus* was homozygous for the WM-7 allele or heterozygous, but high viral titers were found when the CMPSNP658 genotype was homozygous for the PS allele.

Validation of the resistance in the BC_{1PS} and BC_{1WM-7} populations

To confirm the effect of the chromosome 11 major QTL in $BC1_{PS}$ and $BC1_{WM-7}$ populations, the means of symptom score at 15 and 30 dpi and viral accumulation ($2^{-\Delta\Delta Ct}$) were calculated for each genotypic class of the D16 marker (Figure 5), and compared with those of the F_2 generation. In addition, interactions with QTLs of chromosomes 2 and 12 were studied in backcrosses (Figure 6).

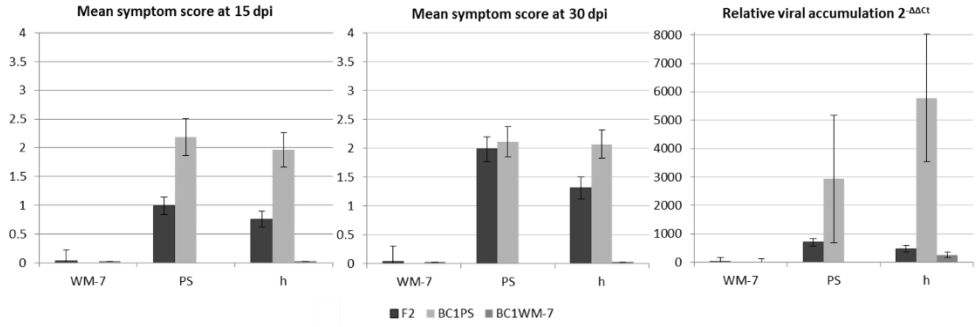

Figure 5. Mean of symptom score at 15 and 30 dpi and viral titers in F_2, $BC1_{PS,}$ and $BC1_{WM-7}$ populations, according to each genotypic class of D16 marker (chromosome 11). Genotypic classes of D16 marker are as in Figure 4

The high level of resistance of the WM-7 homozygous genotype at D16 was confirmed at the $BC1_{WM-7}$ population. All the WM-7WM-7 $BC1_{WM-7}$ plants had no symptoms and viral titers as low as the WM-7WM-7 F_2 plants (Figure 5). Intermediate levels of resistance were also found, as expected, in D16 heterozygous $BC1_{WM-7}$ plants. The heterozygous $BC1_{WM-7}$ plants showed less symptoms than F_2 plants but similar viral titers. A reduction of viral titer, similar to that found in the F_2, was observed in the $BC1_{WM-7}$ population due to the presence of the WM-7WM-7 genotype in the two additional *loci*, more pronounced with *locus* CMPSNP658 (Figure 6a, b).

The D16 heterozygous plants of the $BC1_{PS}$ generation showed symptoms and viral titers significantly higher than that of heterozygous F_2 plants (Figure 5). The effect of the other two *loci* was also confirmed in $BC1_{PS,}$ where a significant drop of virus titer caused by heterozygous genotypes in chromosomes 2 and 12 was observed (Figure 6a, b).

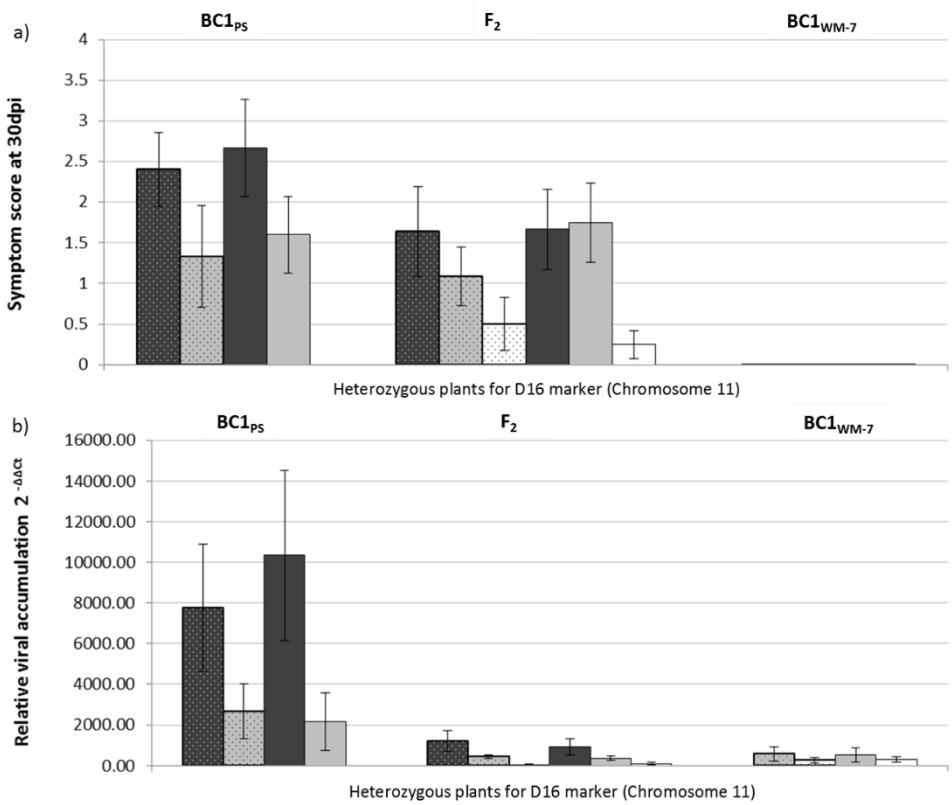

Figure 6. Mean of symptom score and viral titers at 30 dpi in plants, of the $BC1_{PS}$, F_2, and $BC1_{WM-7}$ populations, heterozygous for the D16 marker (chromosome 11) and classified according their genotype in markers CMPSNP658 (chromosome 2, *dotted bars*) and AI_35-A08 (chromosome 12, *solid bars*). For these markers, *dark bars* represent PSPS genotype, *grey bars* heterozygous genotype, and *white bars* WM-7WM-7 genotype.

DISCUSSION

In the present work, we studied the genetic control of resistance to ToLCNDV from the wild *agrestis* Indian melon accession WM-7 using mechanical inoculation. WM-7 was first selected as resistant after screening a wide collection of *C. melo* (López et al. 2015). In agreement with this previous work, the two *agrestis* accessions used here were found to be more resistant that the two *momordica* accessions. In the current paper, the resistant response of the F_1 derived from WM-7 to natural whitefly inoculation is also demonstrated. Indian germplasm has been previously used as source of resistances to viral and fungal pathogens for breeding melons (Dhillon et al. 2012; McCreight et al. 2017).

However, this germplasm has not yet been exploited for breeding melons against ToLCNDV.

Mendelian analysis of symptom segregation in F_2 and BC1s populations derived from WM-7, as well as QTL results, suggested the presence of a major dominant gene in chromosome 11 controlling symptoms development and virus titer and an effect of additional genomic regions (in chromosomes 12 and 2) on disease progress. Those results are consistent with the existence of a major dominant gene reported to control the resistance to whitefly transmission of ToLCNDV in sponge gourd (*Luffa cylindrica*), another cucurbit crop popular in India (Islam et al. 2010), for which two linked SRAP markers have been reported (Islam et al. 2011). In our study, the use of mechanical inoculation could have increased the genetic complexity of the trait. Any of the minor genes could, for example, be implicated in resistance to mechanical transmission (i.e., in the restriction of the virus movement from cell to cell in the leaf parenchyma), which would be not important for the disease when inoculated in the phloem directly by the whitefly. A similar oligogenic control, three dominant genes, has been reported in *Solanum habrochaites* S. Knapp & D. M. Spooner, a wild species related to tomato, after ToLCNDV agroinoculation (Rai et al. 2013).

Our study also indicates the existence of epistatic interactions between these three regions, both for symptoms development and for viral accumulation. Similarly, Palomares-Rius et al. (2016) described two genomic regions involved in the resistance to Cucurbit yellow stunting disorder virus (CSYDV), one responsible for virus multiplication and the other controlling symptoms development, also derived from a subsp. *agrestis* melon type. The knowledge of these epistatic interactions between the three *loci* involved in the resistance to ToLCNDV will be necessary to develop new melon cultivars derived from WM-7. In this context, the linked SNPs selected in this paper will be highly useful in managing these *loci* in breeding programs.

Available background SNPs derived from previous resequencing experiments (Blanca et al. 2011, 2012; Esteras et al. 2013; Leida et al. 2015), and the additional SNPs generated in new resequencing assays, allowed an accurate analysis of the region of chromosome 11. Among the genes annotated in the candidate region of chromosome 11 (Additional file 3), we have not found nucleotide binding site-leucine-rich repeat gene sequences, present in the majority of plant R genes. These sequences were strongly associated with resistance to ToLCNDV in *Luffa cylindrica* Roem (Saha et al. 2013), and were also found to be induced in *Capsicum annuum* following ToLCNDV infection (Kushwaha et al. 2015). In addition, the leucine-rich repeat receptor-like kinase NIK confers high resistance to begomoviruses by trigging translational suppression on begomovirus infection (Brustolini et al. 2015; Zorzatto et al. 2015). In tomato, defense-related host gene expression in response to ToLCNDV includes one gene differentially expressed in a resistant cultivar that encodes a 26S proteasomal subunit RPT4a (SlRPT4) that interferes with the ToLCNDV genome transcription and activates hypersensitive response (Sahu et al. 2016). We did not find either sequences related to factors involved in recessive resistance to viruses, such as eukaryotic translation initiation factors (Yeam 2016), or messenger RNA surveillance factors, like Pelota (located in locus *Ty-5*), which confers resistance to geminiviruses in tomato (Lapidot et al. 2015). Further analysis of this region is necessary to check if it contains genes similar to those mentioned above that have not been annotated yet.

However, interestingly, the sequence analysis of this region revealed a predicted transcription factor similar to the TIFY4B from *Arabidopsis thaliana* (MELO3C022348, located at chromosome 11 position 30,052,212–30,054,984) (Chung and Sunter, 2014) that has shown a potential role in host defense against geminiviruses. TIFY4B is a plant-specific DNA binding protein that interacts with begomoviruses for their transcription activity (Lacatus and Sunter 2009). The overexpression of this factor may delay symptom development by inhibiting viral replication, determining as a consequence the degree of symptom severity to geminiviruses depending on the host. For example, the expression of TIFY4B

in *Nicotiana benthamiana* and tomato increased in response to geminivirus infection, reducing viral replication (Chung and Sunter 2014). In addition, a protein similar to the serine/threonine-protein kinase PBS1 of *A. thaliana* (MELO3C022340, located at chromosome 11 position 29,959,231–29,964,198) is annotated in this region. PBS1 is required for plant defense mechanism mediated by R proteins, and appeared to be highly upregulated in tomato cultivars that were resistant to ToLCNDV (Sahu et al. 2010). A similar protein *OsPBL1* (PBS1-like) is involved in antiviral defense signaling pathways in rice (Lee and Kim 2015).

In the candidate region of chromosome 2, there is an RNA-dependent RNA polymerase 2 gene (MELO3C017106, located in chromosome 2 in position 25,048,095–25,052,883). This function has been described to contribute to basal virus resistance in many species (Hunter et al. 2016). For example, the widely used *Solanum chilense*-derived *Ty-1* and *Ty-3* alleles, which confer resistance to the geminivirus tomato yellow leaf curl virus (TYLCV) and Tomato mottle virus (ToMoV) (Zamir et al. 1994; Ji et al. 2007), also encode for an RNA-dependent RNA polymerase involved in the RNAi response to virus infections (Verlaan et al. 2013). RNAi has a key role in the defense of plants against begomoviruses (Zaidi et al. 2016). In tomato, both virus-specific siRNAs and miRNAs seem to be involved in the resistance against ToLCNDV (Naqvi et al. 2010; Sahu et al. 2010; Pradhan et al. 2015), and transgenic plants expressing RNAs for silencing were highly resistant (Mubin et al. 2007; Vu et al. 2013; Sharma et al. 2015). Most of these resistance sources are known to support virus replication. However, the level of virus accumulation is lower than the levels in susceptible cultivars. The candidate region of chromosome 12 contains a CCR4-associated factor 1 (CAF1) (MELO3C025580, located at chromosome 12 in position 13,943,018–13,944,220). This factor has been reported to be associated with plant defense responses (Sarowar et al. 2007; Chou et al. 2014).

Preliminary approximation to candidate genes involved in virus resistance and disease defense provides interesting information to identify the gene/s involved in resistance to ToLCNDV. Future work will focus on fine mapping the three QTLs responsible for the resistance and in expression studies and co-segregation assays of the candidate genes to facilitate the transfer of these QTLs into elite breeding lines.

REFERENCES

Bandaranayake WMEK, Wickramarachchi WART, WickramasingheHAM, Rajapakshe RGAS, Dissanayake DMKK (2014) Moleculardetection and characterization of begomoviruses associatedwith cucurbitaceae vegetables in Sri Lanka. J Natl Sci Found SriLanka 42:265–271. doi:10.4038/jnsfsr.v42i3.7400

Blanca J, Cañizares J, Ziarsolo P, Esteras C, Mir G, Nuez F, García-Mas J, Picó B (2011) Melon transcriptome characterization:simple sequence repeats and single nucleotide polymorphismsdiscovery for high throughput genotyping across the species.Plant Genome 4:118–131. doi:10.1186/1471-2164-12-104

Blanca J, Esteras C, Ziarsolo P, Pérez D, Fernández V, Collado C,Rodríguez R, Ballester A, Roig C, Cañiizares J, Picó B (2012)Transcriptome sequencing for SNP discovery across Cucumismelo. BMC Genom 13:280. doi:10.1186/1471-2164-13-280

Brustolini OJB, Machado JPB, Condori-Apfata JA, Coco D, DeguchiM, Loriato VAP, Pereira WA, Alfenas-Zerbini P, Zerbini FM,Inoue-Nagata AK, Santos AA, Chory J, Silva FF, Fontes EPB(2015) Sustained NIK-mediated antiviral signalling confersbroad-spectrum tolerance to begomoviruses in cultivated plants.Plant Biotechnol J 13:1300–1311. doi:10.1111/pbi12349

Chang HH, Ku HM, Tsai WS, Chien RC, Jan FJ (2010) Identificationand characterization of a mechanical transmissible begomoviruscausing leaf curl on oriental melon. Eur J Plant Pathol127:219–228. doi:10.1007/s10658-010-9586-0

Chou WL, Huang LF, Fang JC, Yeh CH, Hong CY, Wu SJ, Lu CA (2014) Divergence of the expression and subcellular localizationof CCR4-associated factor 1 (CAF1) deadenylase proteins inOryza sativa. Plant Mol Biol 85:443–458. doi:10.1007/s11103-014-0196-7

Chung HY, Sunter G (2014) Interaction between the transcriptionfactor AtTIFY4B and begomovirus AL2 protein impactspathogenicity. Plant Mol Biol 86:185–200. doi:10.1007/s11103-014-0222-9

Dhillon NPS, Monforte AJ, Pitrat M, Pandey S, Singh PK, ReitsmaKR, Garcia-Mas J, Sharma A, McCreight JD (2012) Melonlandraces of India: contributions and importance. In: Janick J(ed) Plant breeding reviews, vol 35. Wiley, New Jersey, pp 85–150. doi:10.1002/9781118100509

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13-15

EPPO (2017) European and Mediterranean Plant Protection Organization.https://www.eppo.int/QUARANTINE/Alert_List/viruses/ToLCNDV.htm. Accessed 28 Feb 2017

Esteras C, Gómez P, Monforte AJ, Blanca J, Vicente-Dólera N, Roig C, Nuez F, Picó B (2012) High-throughput SNP genotyping inCucurbita pepo for map construction and quantitative trait loci mapping. BMC Genom 22(13):80. doi:10.1186/1471-2164-13-80

Esteras C, Formisano G, Roig C, Díaz A, Blanca J, García-Mas J,Gómez-Guillamón ML, López-Sesé AI, Lázaro A, Monforte AJ,Picó B (2013) SNP genotyping in melons: genetic variation,population structure, and linkage disequilibrium. Theor ApplGenet 126:285–303. doi:10.1007/s00122-013-2053-5

Font San Ambrosio MI, Alfaro Fernández AO (2015) El virus deNueva Delhi (Tomato leaf curl New Delhi virus, ToLCNDV) amplía su gama de hospedantes en los cultivos españoles. Phytoma 272:25–30

Fortes IM, Sánchez-Campos S, Fiallo-Olivé E, Díaz-Pendón JA, Navas-Castillo J, Moriones E (2016) A novel strain of Tomato leaf curl New Delhi virus has spread to the Mediterranean basin. Viruses 8:307. doi:10.3390/v8110307

García-Mas J, Benjak A, Sanseverino W, Bourgeois M, Mir G,González VM et al (2012) The genome of melon (Cucumis meloL). Proc Natl Acad Sci 109:11872–11877. doi:10.1073/pnas.1205415109

Hernández V (2016) El melón deja espacio a la sandía en Castilla La Mancha. MERCADOS. http://www.revistamercadoscom/noticia/melon-deja-espacio-la-sandia-castilla-la-mancha/. Accessed 24Feb 2017

Hunter LJR, Brockington SF, Murphy AM, Pate AE, MacFarlane SA,Palukaitis P, Carr JP (2016) RNA-dependent RNA polymerase 1in potato (Solanum tuberosum) and its relationship to other plantRNA-dependent RNA polymerases. Sci Rep 6:23082. doi:10.1038/srep23082

Islam S, Munshi AD, Mandal B, Behera TK, Kumar R (2010)Genetics of resistance in Luffa cylindrical Roemagainst tomatoleaf curl New Delhi virus. Euphytica 174:83–89. doi:10.1007/s10681-010-0138-7

Islam S, Munshi AD, Verma M, Arya L, Mandal B, Behera TK,Kumar R, Lal SK (2011) Screening of Luffa cylindrical Roemfor resistance against Tomato leaf curl New Delhi virus,inheritance of resistance, and identification of SRAP markerslinked to the single dominant resistance gene. J Hortic SciBiotechnol 86:661–667Ji Y, Schuster DJ, Scott JW (2007) Ty-3, a begomovirus resistancelocus near the Tomato yellow leaf curl virus resistance locus Ty-1 on chromosome 6 of tomato. Mol Breed 20:271–284. doi:10.1007/s11032-007-9089-7J

Juárez M, Tovar R, Fiallo-Olivé E, Aranda MA, Gosálvez B, Castillo P, Moriones E, Navas-Castillo J (2014) First detection of tomatoleaf curl New Delhi virus infecting Zucchini in Spain. Plant Dis 98:857–858. doi:10.1094/pdis-10-13-1050-PDN

Jyothsna P, Haq QMI, Singh P, Sumiya KV, Praveen S, Rawat R,Briddon RW, Malathi VG (2013) Infection of tomato leaf curlNew Delhi virus (ToLCNDV), a bipartite

begomovirus withbetasatellites, results in enhanced level of helper virus componentsand antagonistic interaction between DNA B andbetasatellites. Appl Microbiol Biotechnol 97:5457–5471.doi:10.1007/s00253-012-4685-9

Khan MS, Ji SH, Chun SC (2012) Begomoviruses and their emergingthreats in South Korea: a review. Plant Pathol 28:123–136.doi:10.5423/PPJ.2012.28.2.123

Kushwaha N, Singh AK, Basu S, Chakraborty S (2015) Differentialresponse of diverse solanaceous hosts to tomato leaf curl NewDelhi virus infection indicates coordinated action of NBS-LRRand RNAi-mediated host defense. Arch Virol 160:1499–1509.doi:10.1007/s00705-015-2399-x

Lacatus G, Sunter G (2009) The Arabidopsis PEAPOD2 transcriptionfactor interacts with geminivirus AL2 protein and the coatprotein promoter. Virology 392:196–202. doi:10.1016/j.virol.2009.07.004

Lapidot M, Karniel U, Gelbart D, Fogel D, Evenor D, Kutsher Y,Makhbash Z, Nahon S, Shlomo H, Chen L, Reuveni M, Levin I(2015) A novel route controlling begomovirus resistance by themessenger RNA surveillance factor pelota. PLoS Genet11:e1005538. doi:10.1371/journal

Lee KJ, Kim K (2015) The rice serine/threonine protein kinaseOsPBL1 (ORYZA SATIVA ARABIDOPSIS PBS1-LIKE 1) ispotentially involved in resistance to rice stripe disease. PlantGrowth Regul 77(1):67–75. doi:10.1007/s10725-015-0036-z

Leida C, Moser C, Esteras C, Sulpice R, Lunn JE, De Langen F et al(2015) Variability of candidate genes, genetic structure andassociation with sugar accumulation and climacteric behavior ina broad germplasm collection of melon (Cucumis melo L). BMCGenet 16:28. doi:10.1186/s12863-015-0183-2

López C, Ferriol M, Picó MB (2015) Mechanical transmission ofTomato leaf curl New Delhi virus to cucurbit germplasm:selection of tolerance sources in Cucumis melo. Euphytica204:279–691. doi:10.1007/s10681-015-1371-x

Mascarell-Creus A, Cañiizares J, Vilarrasa-Blasi J, Mora-Garcia S, Blanca J, Gonzalez-Ibeas D, Saladie M, Roig C, Picó B, Lopez-Vigas N, Aranda MA, Garcia-Mas J, Nuez F, Puigdomenech P,Caño-Delgado AI (2009) An oligo-based microarray offers noveltranscriptomic approaches for the analysis of pathogen resistanceand fruit quality traits in melon (Cucumis melo L.). BMC Genom10:467. doi:10.1186/1471-2164-10-467

McCreight JD, Wiutemantel WM, Natwick ET, Sinclair JW, CrosbyKM, Go´mez-Guillamo´n ML (2017) Recessive resistance toCYSDV in melon TGR 1551. Acta Hortic 1151:101–108. doi:10.17660/ActaHortic.2017.1151.17

Mnari-Hattab M, Zammouri S, Belkadhi MS, Bellon Doña D, benNahia MR, Hajlaoui E (2015) First report of Tomato leaf curlNew Delhi virus infecting cucurbits in Tunisia. New Dis Rep31:21. doi:10.5197/j2044-05882015031021

Mubin M, Mansoor S, Hussain M, Zafar Y (2007) Silencing of AV2gene by antisense RNA protects transgenic plants against abipartite begomovirus. Virol J 4:10. doi:10.1186/1743-422X-4-10

Naqvi AR, Haq QMR, Mukherjee SK (2010) MicroRNA profiling of Tomato leaf curl New Delhi virus (ToLCNDV) infected tomatoleaves indicates that deregulation of mir159/319 and mir172might be linked with leaf curl disease. Virol J 7:281. doi:10.1186/1743-422X-7-281

Palomares-Rius FJ, Garcés-Claver A, Gómez-Guillamón ML (2016) Detection of two QTLs associated with resistance to cucurbit yellow stunting disorder virus in Melon Line TGR 1551. In: Kozik EU, Paris HS (eds) XIth EUCARPIA Meeting on Geneticsand Breeding of Cucurbitaceae, pp 334–337Panno S, Iacono G, Davino M, Marchione S, Zappardo V, Bella P,Tomassoli L, Accotto GP, Davino S (2016) First report ofTomato leaf curl New Delhi virus affecting zucchini squash in animportant horticultural area of southern Italy. New Dis Rep 33:6.doi:10.5197/j2044-05882016033006

Papidam M, Beachy RN, Fauquet CM (1995) Tomato leaf curlgeminivirus from India has a bipartite genome and coat protein isnot essential for infectivity. J Gen Virol 76:25–35. doi:10.1099/0022-1317-76-1-25

Perpiñá G, Esteras C, Gibon Y, Monforte AJ, Picó B (2016) A newgenomic library of melon introgression lines in a cantaloupegenetic background for dissecting desirable agronomical traits.BMC Plant Biol 16:154. doi:10.1186/s12870-016-0842-0

Pradhan B, Naqvi AR, Saraf S, Mukherjee SK, Dey N (2015) Prediction and characterization of Tomato leaf curl New Delhivirus (ToLCNDV) responsive novel microRNAs in Solanumlycopersicum. Virus Res 195:183–195. doi:10.1016/j.virusres.2014.09.001

Pratap D, Kashikar AR, Mukherjee SK (2011) Molecular characterizationand infectivity of a Tomato leaf curl New Delhi virusvariant associated with newly emerging yellow mosaic diseaseof eggplant in India. Virol J 8:305. doi:10.1186/1743-422X-8-305

Rai NK, Sahu PP, Gupta S, Reddy MK, Ravishankar KV, Singh M,Sadashiva AT, Prasad M (2013) Identification and validation ofan ISSR marker linked to Tomato leaf curl New Delhi virusresistant gene in a core set of tomato accessions. Veg Sci 40:1–6Rosen R, Kanakala S, Kliot A, Pakkianathan BC, Farich BA, Santana-Magal N, Elimelech M, Kontsedalov S, Lebedev G, Cilia M,Ghanim M (2015) Persistent, circulative transmission of begomovirusesby whitefly vectors. Curr Opin Virol 15:1–8. doi:10.1016/j.coviro.2015.06.008

Roy A, Bal SS, Fergany M, Kaur S, Singh H, Malik AA, Singh J,Monforte AJ, Dhillon NPS (2012) Wild melon diversity in India (Punjab State). Genet Resour Crop Evol 59:755–767. doi:10.1007/s10722-011-9716-3

Ruiz L, Simon A, Velasco L, Janssen D (2016) Biological characterizationof Tomato leaf curl New Delhi virus from Spain. PlantPathol. doi:10.1111/ppa12587

Sáez C, Martínez C, Ferriol M, Manzano S, Velasco L, Jamilena M, López C, Picó B (2016) Resistance to Tomato leaf curl NewDelhi virus in Cucurbita spp. Ann Appl Biol 169:91–105. doi:10.1111/aab12283

Saha D, Rana RS, Sureja AK, Verma M, Arya L, Munshi AD (2013)Cloning and characterization of NBS-LRR encoding resistancegene candidates from Tomato Leaf Curl New Delhi Virusresistant genotype of Luffa cylindrica Roem. Physiol Mol PlantPathol 81:107–117. doi:10.1016/j.pmpp.2012.11.007

Sahu PP, Rai NK, Chakraborty S, Singh M, Chandrappa PH, RameshB, Chattopadhyay D, Prasad M (2010) Tomato cultivar tolerantto Tomato leaf curl New Delhi virus infection induces virusspecificshort interfering RNA accumulation and defenceassociatedhost gene expression. Mol Plant Pathol 11:531–544.doi:10.1111/j.1364-3703.2010.00630.x

Sahu PP, Sharma N, Puranik S, Chakraborty S, Prasad M (2016) Tomato 26S Proteasome subunit RPT4a regulates ToLCNDVtranscription and activates hypersensitive response in tomato. SciRep 6:27078. doi:10.1038/srep27078

Sarowar S, Oh HW, Cho HS, Baek KH, Seong ES et al (2007) Capsicum annuum CCR4-associated factor CaCAF1 is necessaryfor plant development and defence response. Plant J 51:792–802.doi:10.1111/j.1365-313X.2007.03174.x

Sharma V, Basu S, Chakraborty S (2015) RNAi mediated broad spectrum transgenic resistance in Nicotiana benthamiana tochilli-infecting begomoviruses. Plant Cell Rep 34:1389–1399.doi:10.1007/s00299-015-1795-8

Srivastava KM, Hallan V, Raizada RK, Chandra G, Singh BP, SanePV (1995) Molecular cloning of Indian tomato leaf curl virusgenome following a simple method of concentrating thesupercoiled replicative form of viral DNA. J Virol Methods51:297–304

Van Ooijen JW (2009) MapQTL_ 6 Software for the mapping ofquantitative trait loci in experimental population of diploidspecies Kayzma BV. Wageningen, The Netherlands

Van Ooijen JW, Voorrips RE (2001) JoinMap Version 30, Softwarefor the calculation of genetic linkage maps Release 30 PlantResearch International Wageningen, The Netherlands

Verlaan MG, Hutton SF, Ibrahem RM, Kormelink R, Visser RGF, Scott JW, Edwards JD, Bai Y (2013) The tomato yellow leaf curlvirus resistance genes Ty-1 and Ty-3 are allelic and code forDFDGD-class RNA–dependent RNA polymerases. PLOSGenetics 9:e1003399. doi:10.1371/journal.pgen.1003399

Vu TV, Roy Choudhury N, Mukherjee SK (2013) Transgenic tomatoplantsexpressing artificial microRNAs for silencing the pre-coatand coat proteins of abegomovirus, Tomato leaf curl New Delhivirus, show tolerance to virus infection. Virus Res 172:35–45.doi:10.1016/j.virusres.2012.12.008

Wang S, Basten CJ, Zeng Z-B (2012) Windows QTL cartographer 25department of statistics, North Carolina State University, Raleigh, NC. http://statgen.ncsu.edu/qtlcart/WQTLCart.htm.Accessed 20 Feb 2017

Yeam I (2016) Current advances and prospectus of viral resistance inhorticultural crops. Hortic Environ Biotechnol 57:113–122.doi:10.1007/s13580-016-0105-x

Zaidi SSEA, Martin DP, Amin I, Farooq M, Mansoor S (2016)Tomato leaf curl New Delhi virus: a widespread bipartitebegomovirus in the territory of monopartite begomoviruses. MolPlant Pathol. doi:10.1111/mpp12481

Zamir D, Ekstein-Michelson I, Zakay Y et al (1994) Mapping andintrogression of a tomato yellow leaf curl virus tolerance gene,TY-1. Theor Appl Genet 88:141–146. doi:10.1007/BF00225889

Zeng ZB (1994) Precision mapping of quantitative trait loci. Genetics136:1457–1468Zorzatto C, Machado JP, Lopes KV, Nascimento KJ, Pereira WA,Brustolini OJ, Reis PA, Calil IP, Deguchi M, Sachetto-MartinsG, Gouveia BC, Loriato VA, Silva MA, Silva FF, Santos AA,Chory J, Fontes EP (2015) NIK1-mediated translation suppressionfunctions as a plant antiviral immunity mechanism. Nature520:679–682. doi:10.1038/nature14171

ELECTRONIC SUPPLEMENTARY MATERIAL

Below is the link to the electronic supplementary material.

Supplementary material 1

https://static-content.springer.com/esm/art%3A10.1007%2Fs00299-017-2175-3/MediaObjects/299_2017_2175_MOESM1_ESM.xlsx

Supplementary material 2

https://static-content.springer.com/esm/art%3A10.1007%2Fs00299-017-2175-3/MediaObjects/299_2017_2175_MOESM2_ESM.xlsx

Supplementary material 3

https://static-content.springer.com/esm/art%3A10.1007%2Fs00299-017-2175-3/MediaObjects/299_2017_2175_MOESM3_ESM.xlsx

Capítulo IV

CAPÍTULO IV

A major QTL located in chromosome 8 of Cucurbita moschata is responsible of for resistance to tomato leaf curl New Delhi virus (ToLCNDV)

Cristina Sáez, Cecilia Martínez, Javier Montero-Pau, Cristina Esteras, José Blanca, María Ferriol, Carmelo López and Belén Picó

Frontiers in Plant Science 11:207

ABSTRACT

Tomato leaf curl New Delhi virus (ToLCNDV) is a bipartite whitefly-transmitted begomovirus, responsible since 2013 of severe damages in cucurbit crops in Southeastern Spain. Zucchini (*Cucurbita pepo*) is the most affected species, but melon (*Cucumis melo*) and cucumber (*Cucumis sativus*) are also highly damaged by the infection. The virus has spread across Mediterranean basin and European countries and integrated control measures are not being enough to reduce economic losses. The identification of resistance genes is required to develop resistant cultivars. In this assay, we studied the inheritance of the resistance to ToLCNDV previously identified in two *C. moschata* accessions. We generated segregating populations crossing both resistant pumpkins, an American improved cultivar Large Cheese (PI 604506) and an Indian landrace (PI 381814), with a susceptible *C. moschata* genotype (PI 419083). The analysis of symptoms and viral titers of all populations established the same monogenic recessive genetic control in both resistant accessions, and the allelism tests suggest the occurrence of alleles of the same *locus*. By genotyping with a SNPs collection evenly distributed along the *C. moschata* genome, a major QTL was identified in chromosome 8 controlling resistance to ToLCNDV. This major QTL was also confirmed in the interspecific *C. moschata* x *C. pepo* segregating populations, although *C. pepo* genetic background affected the resistance level. Molecular markers here identified, linked to the ToLCNDV resistance *locus*, are highly valuable for zucchini breeding programs, allowing the selection of improved commercial materials. The duplication of the candidate region within the *C. moschata* genome was studied, and genes with paralogs or single copy genes were identified. Its synteny with the region of chromosome 17 of the susceptible *C. pepo* revealed an INDEL including interesting candidate genes. The chromosome 8 candidate region of *C. moschata* was also syntenic to the region in chromosome 11 of melon, previously described as responsible of ToLCNDV resistance. Common genes in the candidate regions of both cucurbits, with high or moderate impact polymorphic SNPs between resistant and susceptible *C. moschata* accessions, are interesting to study the mechanisms involved in this recessive resistance.

INTRODUCTION

Tomato leaf curl New Delhi virus (ToLCNDV) is an economically important begomovirus (family *Geminiviridae*) with two circular single-stranded DNA genome components of about 2.7 kb, designated as DNA-A and DNA-B (Padidam et al., 1995; Jyothsna et al., 2013). ToLCNDV is transmitted in nature by the whitefly *Bemisia tabaci* biotypes MEAM1 and MED (Chang et al., 2010; Rosen et al., 2015; Janssen et al., 2017), but some isolates of this virus can also be transmitted by mechanical inoculation (Usharani et al., 2004, Chang et al., 2010; Sohrab et al., 2013; López et al., 2015).

ToLCNDV has a wide host range. It affects crops of the *Solanaceae* family, such as tomato (*Solanum lycopersicum* L.), potato (*Solanum tuberosum* L.), chili pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.) (Padidam et al., 1995; Usharani et al., 2004; Hussain et al., 2004; Pratap et al., 2011). It is also highly damaging to crops of the *Cucurbitaceae* family, including luffa (*Luffa cylindrica* M. Roem.), ash gourd (*Benincasa hispida* (Thunb.) Cogn.), cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* L), melon (*Cucumis melo* L.) and different types of squashes (*Cucurbita* spp.) (Sohrab et al., 2003; Ito et al., 2008; Singh et al., 2009; Chang et al., 2010; Roy et al., 2013). Recently, it has been reported affecting species of other plant families, such as opium poppy (*Papaver somniferum* L., Papaveraceae) (Srivastava et al., 2016), cotton (*Gossypium hirsutum* L., Malvaceae) (Zaidi et al., 2016), soybean (*Glycine max*, Fabaceae L. Merr.) (Jamil et al., 2017) and firecracker flower (*Crossandra infundibuliformis* L. Nees, *Acanthaceae*) (Sundararaj et al., 2019). Furthermore, some weeds as black nightshade (*Solanum nigrum* L.), thorn apple (*Datura stramonium* L.), squirting cucumber (*Ecballium elaterium* (L.) A. Rich), smooth sowthistle (*Sonchus oleraceus* L.), false daisy (*Eclipta prostrata* (L.) L.) and apple of Sodom (*Calotropis procera* (Aiton) Dryand.) (Haider et al., 2006; Moriones et al., 2017; Zaidi et al., 2017; Juárez et al., 2019) have been found to be hosts of the virus, acting as reservoirs during the whole year.

ToLCNDV was first detected in North India in 1995 (Srivastava et al., 1995), from where it spread to South and Southeast Asian countries. It was limited to Asia until 2012, when it was reported affecting cucurbits in different Mediterranean countries, first in Spain (Juárez et al., 2014) and later in Tunisia (Mnari-Hattab et al., 2015), Italy (Panno et al., 2016), Morocco (Sifres et al., 2018) Greece (Orfanidou et al., 2019) and Algeria (Kheireddine et al., 2019). More recently, the virus has been identified in cucurbits plants in Portugal and Estonia (EPPO, 2019), which is indicative of the rapid spread of ToLCNDV through Europe. The most affected crop in European countries is Zucchini squash (*Cucurbita pepo* L. subsp. p*epo*). In this crop, the virus causes severe stunting of plants, which exhibit upward and downward curling of the leaves, severe mosaic and fruit skin roughness (Juárez et al., 2014). Infected plants often present partial or complete yield loss and fruits with lower market value. Zucchini is one of the most widely grown crops and appreciated vegetable in the Mediterranean basin. This region produced nearly 300,000 tm of this vegetable and other species of the *Cucurbita* genus (pumpkins, squash and gourds) in 2017 (FAOSTAT), representing almost 24% of world production, excluding China and India. Before the arrival of ToLCNDV, the aphid-borne potyvirus *Zucchini yellow mosaic virus* (ZYMV) was the major viral pathogen of this crop (Capuozzo et al., 2017). Since 2013, ToLCNDV is the most prevalent virus in the area, where it is an important constraint to zucchini production. In the background of the severe epidemic outbreaks of ToLCNDV in cucurbits, both in greenhouses and in open fields, European and Mediterranean Plant Protection Organization (EPPO) has added this virus to the EPPO Alert List (EPPO, 2017).

Cultural practices, such as the control of the whitefly vector, the elimination of infected plants, and the avoidance of the most susceptible cultivars are not very effective in preventing ToLCNDV outbreaks (EPPO, 2017). In fact, breeding resistant varieties is considered the most economical and effective method to control virus diseases. Genetic resistance to ToLCNDV has been identified in some accessions of the *Cucurbita* genus (Sáez et al., 2016). In that work, authors screened for ToLCNDV resistance a large collection of *Cucurbita* spp. accessions including

landraces and commercial varieties of the cultivated species (*C. pepo* L., *C. moschata* Duchesne and *C. maxima* Duchesne) and wild *Cucurbita* species. All the *C. pepo* and *C. maxima* accessions behaved as highly susceptible, but four *C. moschata* accessions were highly resistant, two of them after both mechanical and whitefly inoculation, remaining symptomless with a reduced viral accumulation (Sáez et al., 2016).

Genetic resistance to ToLCNDV has also been characterized in some other species belonging to different families. In *Solanum habrochaites* S. Knapp & D.M. Spooner, a wild species related to tomato, three dominant genes are responsible for the resistance (Rai et al., 2013). In *Luffa cylindrica*, a popular cucurbit vegetable in India, a dominant monogenic resistance was reported (Islam et al., 2010; 2011). More recently, in melon, Sáez et al., (2017) found one major *locus* in chromosome 11 and two additional regions in chromosomes 12 and 2 that control resistance to ToLCNDV. In this context, the purpose of this study was to map the quantitative trait loci (QTL) associated with the resistance to ToLCNDV in *C. moschata* using segregating populations derived from these resistant sources and a susceptible accession of this species, and to confirm this resistance in interspecific *C. moschata* x *C. pepo* populations as the first step to transfer the resistance to zucchini.

MATERIAL AND METHODS

Plant material

In this work we selected two *Cucurbita moschata* accessions (PI 604506 and PI 381814), previously reported (Sáez et al., 2016) as symptomless or with slight symptoms after whitefly and sap inoculation with ToLCNDV, to study the genetic control of the resistance. PI 604506 is the improved pumpkin cultivar Large Cheese from the USA and PI 381814 an Indian landrace. The Chinese *C. moschata* accession PI 419083 was used as susceptible control. Seeds of the three accessions were firstly provided by USDA-NPGS genebank, then fixed by selfing and multiplied by the cucurbits breeding group at the Institute for the Conservation and Breeding of Agricultural Biodiversity (COMAV), and stored at the COMAV germplasm bank.

Virus source and mechanical inoculation

To generate the viral inoculum source, susceptible zucchini plants were agroinfiltrated with an infectious clone based on the Spanish isolate of ToLCNDV (99% nucleotide identity with the sequences of the A and B viral genomic particles: KF749224 and KF749225 (Juárez et al., 2014), following the procedure described in Sáez et al. (2016).

Tissue of symptomatic leaves from 15 days post ToLCNDV agroinoculation plants was crushed in a mortar together with inoculation buffer (50 mM potassium phosphate [pH 8.0], 1 % polyvinylpyrrolidone 10, 1 % polyethylene glycol 6000, 10 mM 2-mercaptoethanol and 1 % activated charcoal) in a 1:4 (w:v) proportion (López et al., 2015). The homogenate was used to mechanically inoculate all plants at the stage of one true-leaf, dusting on the true-leaf and on one cotyledon with Carborundum 600 mesh and scratching with a cotton-swab dipped in the blend. Inoculated plants were grown in a climatic chamber and disease progression was monitored. Symptomless plants 15 days after mechanical inoculation (dpi) were re-inoculated to avoid escaping to the infection.

Generation of F_1 and segregating populations

Ten seeds of each *C. moschata* accession were disinfected and germinated as described by Sáez et al. (2016). Seedlings were transplanted to pots and grown in climatic chamber under controlled conditions (photoperiod of 16 h day at 25 ºC and 8 h night at 18 ºC and 70% of relative humidity). Subsequently, plants were moved to a greenhouse and crossed to obtain three F_1 progenies: F_1 PI 419083 x PI 604506, F_1 PI 419083 x PI 381814 and F_1 PI 604506 x PI 381814. Eight plants of each parent and the corresponding hybrids were mechanically inoculated with ToLCNDV as described above and phenotyped according to symptomatology and viral accumulation as described below.

Eight additional plants of the *C. moschata* parents were cultivated in a greenhouse along with eight plants of the F_1 progenies. To generate segregating populations, F_1 plants were selfed to obtain F_2 progenies and backcrossed to plants of

PI 604506, PI 381814 and PI 419083 to generate the $BC1_{PI\ 604506}$, $BC1_{PI\ 381814}$ and $BC1_{PI\ 419083}$ populations, respectively. All these segregating populations were screened against ToLCNDV with the same inoculation and phenotyping methodology, using three plants of each *C. moschata* accession as controls. F_2 and BC1 derived from F_1 PI 419083 x PI 381814 were obtained later because of the influence of the local climate conditions in PI 381814 vegetative growth, causing late-flowering and slow development of fruits. Hence, we studied firstly the genetic control of the resistance to ToLCNDV in the segregating populations derived from PI 604506 and results were validated in F_2 and BC1 coming from F_1 PI 419083 x PI 381814.

Symptoms evaluation and quantification of the viral accumulation

Symptomatology was evaluated in all plants at 15 and 30 dpi using the visual scale described by López et al. (2015). Symptoms score ranged from zero (absence of symptoms) to four (highly severe symptoms), classifying as resistant those plants with symptoms scored zero or one and as susceptible those with symptoms scored from two to four. The goodness-of-fit between the expected and observed segregation ratios resistant:susceptible plants was analyzed by chi-squared (χ^2) test ($p < 0.05$) in the F_2 and BC1 segregating populations.

The relative ToLCNDV accumulation in each plant was determinated at 30 dpi by quantitative PCR (qPCR). Total DNA from apical leaves was extracted using the Cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990) and quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). DNA was diluted with sterile-deionized water to a final concentration of 5 ng·μL^{-1}. Three biological replicates were done for each parental genotype, and all plants of the assay were analyzed in three technical replicates using a *LightCycler*® 480 System (Roche). In each qPCR reaction, 15 ng of genomic DNA were used as templates, in a final volume of 15 µL. We used 7.5 µl of 2X iTaq™ universal SYBR® Green Supermix (BIO-RAD) and 1.5 µl (100 nM) of each primer and 1.5 µl of H_2O. Primers ToLCNDVF1 (5'-AATGCCGACTACACCAAGCAT-

3', positions 1145–1169) and ToLCNDVR1 (5'-GGATCGAGCAGAGAGTGGCG-3', positions 1399–1418) were used for the amplification of a 273 bp fragment of viral DNA-A. The single copy gene *CpACS2* was amplified in all samples as internal control using the primers CpACS2F (5'-ACTCGATCAACTTCGAGCAAA-3'), CpACS2R (5'-GCCTATCCAAAGACCTCGGCCTTCCC-3'). Both ToLCNDVF1/R1 and *CpACS2* primers were used in previous works by Sáez et al. (2016). Cycling conditions consisted of incubation at 95 ºC for 5min, 45 cycles of 95 ºC for 5s and 60 ºC for 30s. Relative ToLCNDV levels were calculated using the $2^{-\Delta Ct}$ expression, a variation of the Livak method (Bio-Rad Laboratories, 2006; Livak and Schmittgen, 2001), where Ratio (reference/target) = $2^{-\Delta Ct} = 2^{-(Ct\ (viral\ target) - Ct\ (reference\ gen))}$.

QTL analysis in *C. moschata* F_2 population derived from PI 419083 x PI 604506

PI 604506 and PI 419083 accessions were included in an RNAseq analysis, performed in the frame of a *de-novo* assembly of the zucchini genome project (Montero-Pau et al., 2018), and their transcriptome sequences were used to generate the SNP panel here used. SNPs were selected by aligning each sequence to the version 1 of the *C. moschata* cv. *Rifu* genome (Sun et al., 2017), available at the Cucurbit Genomics Database (http://cucurbitgenomics.org). We used Bowtie2 tool with the-very-sensitive-local argument. Variant calling was performed using Freebayes version 1.0.2 (Garrison and Marth, 2012), excluding alignments from analysis if they had a mapping quality less than 40, alleles with quality under 20 and filtering SNPs with minimum count 10. A set of 137 SNPs evenly distributed throughout the *C. moschata* genome (additional file 1) were selected and used to genotype PI 604506, PI 419083, their derived F_1 and 134 plants of the corresponding F_2 population.

All plants were genotyped using the Agena Bioscience iPLEX® Gold MassARRAY (Agena Biosciences) system at the Epigenetic and Genotyping unit of the University of Valencia (Unitat Central d´Investigació en Medicina (UCIM), Faculty of Medicine, Spain). Total DNA was extracted from young leaves tissue,

using the protocol described above, and quantified and adjusted to 15 ng·μL^{-1}. F_2 genotyping results were run in MAPMAKER 3.0 (Lander et al., 1987; Lincoln et al., 1992) with the Kosambi map function, obtaining the genetic position of each marker.

To identify markers linked to the resistance to ToLCNDV derived from the PI 604506 accession, a quantitative trait locus (QTL) analysis was performed using symptoms at 15 and 30 dpi and ToLCNDV relative accumulation at 30 dpi as quantitative traits, and a qualitative score of resistance (0 susceptible phenotype and 1 resistant phenotype) assigned to each plant according to symptoms and viral accumulation. We used the Kolmogorov-Smirnov test to check the normality assumption of traits distribution. Since the traits were not normally distributed, Kruskal-Wallis non-parametric test was used for QTLs detection using MapQTL version 4.1 software (Van Ooijen, 2009), considering as significant associations those with $p < 0.05$. Since $2^{(-\Delta Ct)}$ values have a skewed distribution, we used the original ΔCt data for QTL analysis. The binary qualitative trait of resistance was also analyzed by logistic regression model, with a significance level of $\alpha=0.05$.

Additionally, a composite interval mapping approach (CIM, Zeng, 1994) was applied in Qgene 4.0 (Joehanes and Nelson, 2008), using the genetic map previously generated with this F_2. The LOD threshold was calculated using a 1000 permutations test per trait, for a $p < 0.05$. The percentage of phenotypic variance explained (R^2), the additive and dominance effects, degree of dominance and the interval position of the QTL according with a 2 units LOD drop was estimated for the highest significant peak LOD. *Loci* identified with both methods (Kruskal-Wallis and CIM) were considered true QTLs of putative interest.

Validation of the QTL of chromosome 8 in additional *C. moschata* segregating populations derived from PI 419083, PI 604506 and PI 381814

The previous analysis allowed detecting a major QTL responsible for the resistance in chromosome 8. In order to confirm this QTL in additional *C. moschata* segregating populations and to introgress the candidate region in chromosome 8 of *C. moschata* in the zucchini (*C. pepo*) background (the cucurbit crop more severely

affected by ToLCNDV), a new set of 19 SNPs of the chromosome 8 candidate region was implemented in a new Agena Bioscience platform. These new SNPs were selected to be useful for both purposes. The transcriptomic sequences of PI 604506, PI 381814 and PI 419083 (obtained in the RNAseq analysis by Montero-Pau et al., 2018), were aligned to the *C. pepo* genome (Zucchini accession MU-CU-16), available at the Cucurbit Genomics Database (http://cucurbitgenomics.org), using Bowtie2. Integrative Genomics Viewer (IGV) (Thorvaldsdóttir et al., 2013) was used to detect variations between sequences, and those polymorphic SNPs between resistant (PI 604506 and PI 381814) and susceptible (PI 419083 and MU-CU-16) genotypes were selected. This Agena platform was employed to genotype a subset of 131 plants of the previously genotyped F_2 (PI 419083 x PI 604506), 121 of F_2 (PI 419083 x PI 381814), 31 $BC1_{PI\ 604506}$ and 73 of $BC1_{PI\ 381814}$.

For further saturation of the candidate region, five additional SNPs, not integrated in the new Agena Bioscience set, were designed with the same requirements and used to genotype the F_2 (PI 419083 x PI 604506) population by High Resolution Melting (HRM) (Vossen et al., 2009). PRIMER3 software (Untergasser et al., 2012) was employed to design the oligonucleotides for the HRM analysis. The genomic positions of all these new SNPs (Agena Bioscience platform and HRM markers) and their flanking sequences are shown in additional file 2.

A new map of chromosome 8 was constructed with 24 SNPs markers (3 and 16 SNPs from the first and second Agena platforms, respectively, and 5 HRM), using genotyping results of F_2 (PI 419083 x PI 604506). MAPMAKER 3.0 (Lander et al., 1987; Lincoln et al., 1992) software and the Kosambi map function were employed to generate the new map. The genetic distances of the new map were used in a second QTL analysis, with the F_2 (PI 419083 x PI 604506) population, following the same procedure described above. Means of symptom scores at 30 dpi of plants from F_2 (PI 419083 x PI 381814), $BC1_{PI\ 604506}$ and $BC1_{PI\ 381814}$ populations classified according to the marker classes (a, b and h for F_2 and h and a for BC1) were analyzed by ANOVA and Bonferroni multiple range tests using STATGRAPHIC Centurion

XVI.I statistic software, to evaluate differences between means, considering statistically significant differences when $p \leq 0.01$

Validation of the QTL in the interspecific cross *C. pepo* x *C. moschata*

An interspecific cross between the ToLCNDV susceptible *C. pepo* accession MU-CU-16 (Sáez et al., 2016) and the resistant *C. moschata* accession PI 604506, provided five F_1 seeds that were germinated as described above. Four seedlings were moved to a greenhouse and selfed to obtain F_2 (MU-CU-16 x PI 604506) generation. The remaining F_1 seedling and 176 plants of F_2 (MU-CU-16 x PI 604506) were screened by mechanical inoculation of ToLCNDV. Symptoms and viral titers were determined by the same procedure described above.

This *Cucurbita* interspecific F_2 population was genotyped with the new Agena Bioscience platform and the 5 HRM SNPs markers of chromosome 8. The genotyping results were used to construct a new genetic map of chromosome 8 and to perform an additional QTL analysis as described above.

Genomic variation, structural variants and synteny

In order to obtain a more detailed view of the underlying genomic variation in the candidate region, both *C. moschata* resistant and susceptible parents (PI 604506 and PI 419083) were fully sequenced. Raw reads are deposited in NCBI under BioProject PRJNA604046. Genomic DNA was obtained from fresh tissue using CTAB extraction, and a pair end library (2 x 150 bp) was built for each accession. Libraries were sequenced as part of an Illumina HiSeq 2000 lane by Polar Genomics (Ithaca, USA). Reads were clean using *ngs_crumbs* software (https://github.com/JoseBlanca/) to eliminate adapters, low-quality bases (Phred quality <25 in a 5 bp window), reads shorter than 50 bp and duplicated sequences. Clean reads were mapped against the reference *C. moschata* genome using *bwa-mem* (Li, 2013) and variant calling was performed using Freebayes version 1.1.0 (Garrison and Marth, 2012) after filtering reads with a MAPQ lower than 57. To study the potential effect of the genetic changes, SNPs were annotated based on its predicted effect on the gene using SNPEff v4.3 (Cingolani et al., 2012). Differences

in sequencing genome coverage between both accessions were studied to explore possible genomic deletions. Read coverage along the candidate region was calculated using samtools v.1.9 (Li et al. 2009), and we checked if coverage deviated from the 99% confidence interval of the observed coverage for each accession assuming a log-normal distribution. Confidence interval for the log-normal distribution was calculated using function *elnorm* of R package 'EnvStats' (Millard, 2013). In addition to that, the structural variant caller Manta v.1.6 (Chen et al., 2016) was used to check for differential large insertion/deletions. Identification of putative paralogs of the genes in the candidate region was done with OrthoMCL (Li et al., 2003).

Identification of syntenic regions between *C. moschata* and *C. pepo* and *Cucumis melo* was done by nucleotide BLAST of each gene within the candidate region of *C. moschata* against the other two genomes. BLAST hits were filtered using an E-value cutoff of 10^{-20} and a minimum overlap between sequences of 70%. For *C. pepo,* to inspect for possible insertion/deletions, a dot plot comparing chromosome 17 region of *C. pepo* and chromosome 8 of *C. moschata* was built based on the alignment of both sequences using LAST (Kielbasa et al., 2011). For *C. melo,* module of Tripal 'SyntenyViewer', available in cucurbitgenomics.com, was used to visualize the sinteny.

New *C. moschata* and *C. pepo* genome assemblies have become recently available (https://www.dnazoo.org/assemblies/Cucurbita_moschata, https://www.dnazoo.org/assemblies/Cucurbita_pepo, online availability since November 2019), but after finishing the analysis we showed here. Our results were checked through alignments with the new assemblies to avoid misinformation.

Additionally to the analysis of the genomic sequences, SNPs discovered using the available RNAseq data (Montero-Pau et al., 2018) from the three *C. moschata* accessions used as parentals in the previous crosses and six additional *C. moschata* from different origins that exhibited susceptibility to ToLCNDV in previous works (López et al., 2015; Sáez et al., 2016), were also annotated using SNPEff.

RESULTS

Response to ToLCNDV of F_1 progenies

The inoculation assay showed that the two *C. moschata* accessions resistant to ToLCNDV, PI 604506 and PI 381814, remained totally symptomless or with only slight symptoms (score from 0 to 1) at 30 dpi, contrasting with the severe mosaic developed in the susceptible control (score 4), PI 419083 (Figure 1). F_1 plants of the two susceptible x resistant crosses were highly susceptible, displaying a similar symptomatology as PI 419083 at 30 dpi. Conversely, F_1 progeny derived from the cross between the two *C. moschata* resistant accessions remained symptomless throughout the essay (Figure 1).

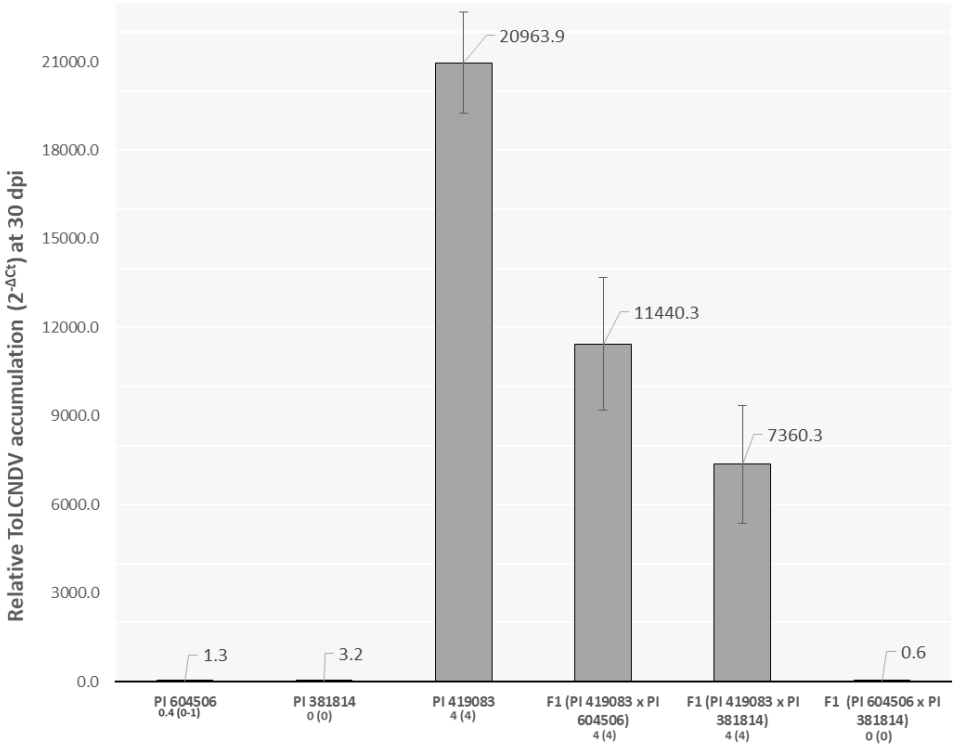

Figure 1. Relative ToLCNDV accumulation (calculated as $2^{-\Delta Ct}$) at 30 days after mechanical inoculation (dpi) with ToLCNDV in the resistant *Cucurbita moschata* accessions PI 604506 and PI 381814, the susceptible control PI 419083 and their respective hybrids. Mean and range of symptoms scores at 30 dpi are indicated in the x axis legend.

Strong correlation between symptoms severity and viral titers was observed (r^2= 0.73, p= 0.030) after measuring relative ToLCNDV accumulation by qPCR. According with their resistant behavior, PI 604506, PI 381814 and the F_1 (PI 604506 x PI 381814) had viral titers, on average, 7.8 x 10^3 times lower than those of the susceptible control PI 419083 and the two F_1 derived from it (Figure 1).

The fact that F_1 progenies derived from the two susceptible x resistant crosses were susceptible, while the F_1 derived from the resistant x resistant cross was resistant, suggests a recessive genetic control of the resistance in both accessions, controlled by common genes. A further analysis of the genetic control of the resistance was performed in F_2 and BC1 populations.

Response to ToLCNDV of segregating populations derived from the cross between resistant and susceptible *C. moschata* accessions

F_2 and $BC1_{PI\ 604506}$ populations, derived from the F_1 PI 419083 x PI 604506, segregated for symptoms severity. Table 1 shows resistant:susceptible plants segregation, according to symptomatology at 30 dpi. At the end of the assay, 38 plants of F_2 remained symptomless (score 0) and 96 showed severe symptomatology (scores 2 to 4). The X^2 test indicated that this segregation fitted to a 1:3 (resistant: susceptible) ratio expected for a single recessive gene for resistance (p= 0.43) (Table 1). To further characterize the response to ToLCNDV, virus accumulation was estimated in the segregating population F_2 (PI 419083 x PI 604506) by qPCR (Figure 2). On average, viral titer strongly correlated to symptoms severity following an exponential model (r^2= 0.82, p= 0.035).

All plants developing mosaic, deformation or short internodes had high viral titers, whereas in the symptomless plants ToLCNDV accumulation was detected at very low concentrations. On average, the viral accumulation ($2^{-\Delta Ct}$) in susceptible plants was 2.2 x10^3 times higher than in resistant plants. Since viral titer is in concordance with symptoms development, symptom scores were used to phenotype the response to ToLCNDV in plants of the remaining F_2 and BC1 populations. In $BC1_{PI\ 604506}$, 33 plants were resistant (score 0) and 26 susceptible (scores 2 to 4). This

segregation also fitted to a 1:1 ratio expected for a single recessive gene (p= 0.44) (Table 1). In accordance with the occurrence of a single recessive gene controlling the resistance, all plants of the $BC1_{PI\ 419083}$ generation had severe symptoms at the end of the assay.

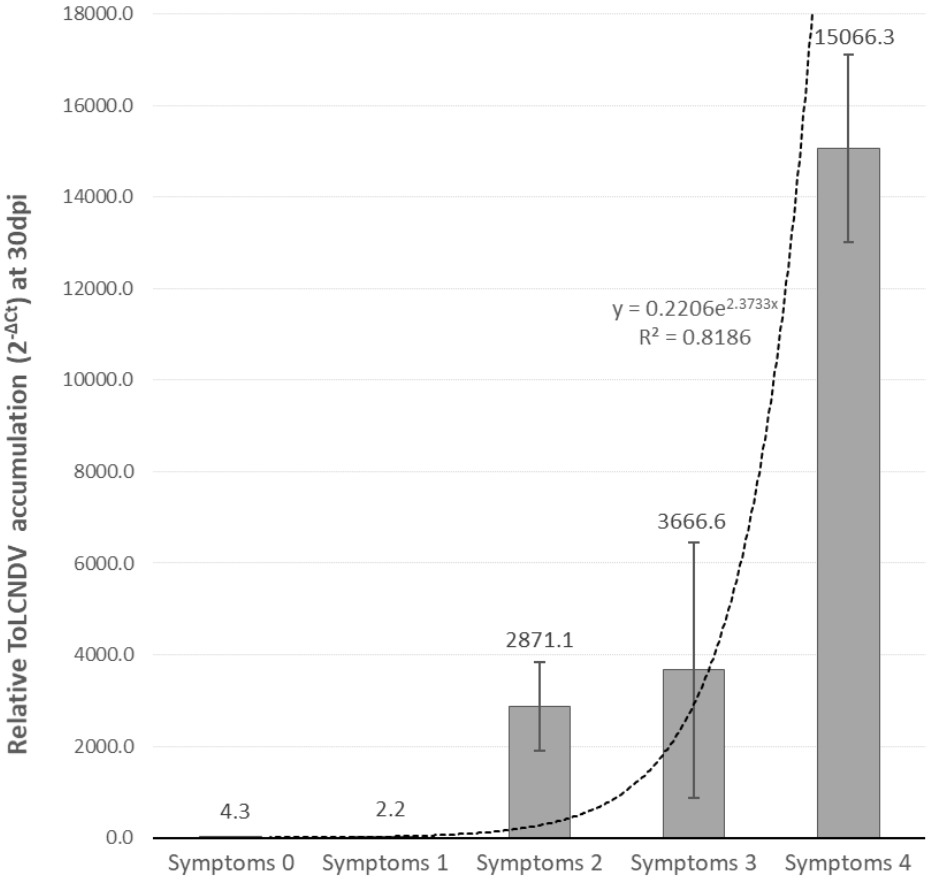

Figure 2. Mean of relative ToLCNDV accumulation (calculated as $2^{-\Delta Ct}$) in plants within each symptomatic class in F_2 (PI 419083 x PI 604506) at 30 days after mechanical inoculation. Dot line represents the exponential relationship between both variables, which was statistically significant for a confident level of α= 0.05.

Symptom segregation ratios observed in the F_2 (PI 419083 x PI 381814) and $BC1_{PI\ 381814}$ populations also fitted to one recessive gene for resistance null hypothesis in X^2 test (Table 1). Forty and 43 plants of F_2 and $BC1_{PI\ 381814}$, respectively, remained symptomless (score 0), and 81 and 30 plants showed severe symptoms (scores 2 to 4), with p= 0.047 and p= 0.16, in both respective populations

(Table 1). Accordingly with the F_1 results, the 160 plants of the F_2 derived from the resistant x resistant cross PI 604506 x PI 381814 remained totally symptomless along all the assay.

Table 1. Segregation of resistant/susceptible plants in F2 and BC progenies at 30 days after mechanical inoculation with ToLCNDV.

Female parent	Male parent	Progeny	Resistant: susceptible segregation	Ratio	X^2 test[a]
PI 419083 (S)[b]	PI 604506 (R)[b]	F_2	38:96	1:3	0.6368 (p= 0.43)
PI 419083 (S)	PI 604506 (R)	$BC_{PI\ 419083}$	0:90	0:1	-
PI 419083 (S)	PI 604506 (R)	$BC_{PI\ 604506}$	33:26	1:1	0.6102 (p=0.44)
PI 419083 (S)	PI 381814 (R)	F_2	40:81	1:3	3.7713 (p=0.047)
PI 419083 (S)	PI 381814 (R)	$BC_{PI\ 381814}$	43:30	1:1	1.9726 (p=0.16)
PI 604506 (R)	PI 381814 (R)	F_2	160:0	1:0	-

[a] Probability of the $\chi 2$ value calculated for a recessive monogenic expected ratio.
[b] (S) = susceptible genotype, (R) = resistant genotype.

QTL analysis in F_2 (PI 419083 x PI 604506) population

The F_2 (PI 419083 x PI 604506) population was genotyped with the 137 SNPs markers evenly distributed throughout *C. moschata* genome, and used to construct a linkage map that included 20 linkage groups and spanned a total of 2681.5 cM of genetic distance, with an average of 22.92 cM between markers (Additional file 1). The linkage map was used to identify QTLs involved in ToLCNDV resistance in *C. moschata*, based on genotyping and phenotyping results (symptoms scores at 15 and 30 dpi, virus titer at 30 dpi and the qualitative resistance score) of F_2 (PI 419083 x PI 604506) population. QTL analysis, performed using non-parametric Kruskal–Wallis test (KW) followed by Composite Interval Mapping (CIM), resulted in the detection of a major QTL in chromosome 8 (Table 2), validated by logistic regression of the qualitative trait of resistance (data not shown). Four QTLs, all located in almost the same genetic position, showed significant association with all the traits evaluated, explaining a proportion between 29.0 and 45.0% of the observed phenotypic variance. All QTLs (ToLCNDVCm_Sy15-8, ToLCNDVCm_Sy30-8,

ToLCNDVCm_VT30-8, ToLCNDVCm_Re-8) were located close to D133 (physical position 1,366,729 bp), with LOD peaks between 10.06 and 17.31.

Narrowing the candidate region in chromosome 8

To validate the major QTL identified in the previous analysis and to increase marker density in the candidate region, F_2 (PI 419083 x PI 604506) population was genotyped with the new Agena Bioscience-HRM SNPs set of Chromosome 8. Twenty-one out of the 24 new markers (Supplementary file 2) were polymorphic in this population, despite all of them were selected *in silico* as SNPs variants between both parents using IGV software. Genotyping results were employed to generate a new linkage map in this region (Supplementary Table 2), covering 72.5 cM, with an average distance between consecutive markers of 3.15 cM, and two clusters of linked markers at 0 and 11.7 cM genetic positions. The QTL analysis was performed using the new map and the new genotyping results (using one selected marker of each of the two clusters of completely linked SNPs) (Table 3). ToLCNDVCm_Sy15-8 QTL, associated to the variation of symptoms at 15 dpi, was identified again with both non-parametric Kruskal-Wallis and CIM analysis, near D133 (located at 18.8 cM in this new map) and with similar explained variance, LOD peak, additive and dominant effects. However, ToLCNDVCm_Sy30-8, ToLCNDVCm_VT30-8 and ToLCNDVCm_Re-8 QTLs, corresponding to traits measured at the end of the assay (30 dpi), when differences are clearer between resistant and susceptible plants, were closely linked to a new marker with both analysis methods. The closest markers (those linked at 11,7 cM DPM37, DMP39, DMP11, DMP10, DMP42, DMP43, DMP44, DMP41 and snp_8202510 markers) are included in the interval position of the same QTLs identified with Kruskal-Wallis and CIM (between 8 and 14 cM) (Figure 3.A), and validated with logistic regression of the qualitative trait of resistance, according to their physical and genetic position.

Table 2. Quantitative trait loci (QTLs) identified in the F_2 (PI 419083 x PI 604506) segregating population genotyped with the set of 137 SNPs evenly distributed through the *C. moschata* genome, using the non-parametric Kruskal–Wallis test and composite interval mapping method.

			Kruskal-Wallis					
Trait	**Chr[a]**	**QTL name**	**cM (peak position)**	**Nearest marker[b]**	**K[c]**	**Mean a[d]**	**Mean h[e]**	**Mean b[f]**
Symptoms at 15 dpi	8	ToLCNDVCm_Sy15-8	0	D133	35.51	2.78	2.20	0.46
Symptoms at 30 dpi	8	ToLCNDVCm_Sy30-8	0	D133	55.88	4.00	3.44	1.10
Viral titer at 30 dpi (ΔCt)	8	ToLCNDVCm_VT30-8	0	D133	33.11	-12.76	-10.67	-4.24
Resistance (qualitative trait)	8	ToLCNDVCm_Re-8	0	D133	59.14	0.00	0.13	0.73

			CIM (Qgene)							
Trait	**Chr[a]**	**QTL name**	**cM (peak position)**	**Interval (cM)[g]**	**Nearest marker[b]**	**LOD[h]**	**Add[i]**	**Dom[j]**	**[d/a][k]**	***R*²[l]**
Symptoms at 15 dpi	8	ToLCNDVCm_Sy15-8	4.00	0-21.27	D133	10.06	1.52	0.84	0.55	0.29
Symptoms at 30 dpi	8	ToLCNDVCm_Sy30-8	0.00	0-5.43	D133	16.52	1.45	0.90	0.62	0.44
Viral titer at 30 dpi (ΔCt)	8	ToLCNDVCm_VT30-8	0.00	0-6.82	D133	13.97	-4.26	-2.20	0.52	0.39
Resistance (qualitative trait)	8	ToLCNDVCm_Re-8	0.00	0-5.17	D133	17.31	-0.37	-0.23	0.64	0.45

[a]Chromosome
[b]The closest marker to LOD peak
[c] *K**: the Kruskal-Wallis test statistic, with a significant level of 0.0001
[d]Mean of the PI 419083 genetic class in each marker
[e]Mean of the PI 419083/PI 604506 genetic class in each marker
[f]Mean of the PI 604506 genetic class in each marker
[g]Interval position of the putative QTL, identified in the F_2 (PI 419083 x PI 604506) in cM on the genetic map according with a LOD drop of 2
[h] *LOD* higher logarithm of the odds score
[i] *Add* additive effect of the PI 419083 allele
[j] *Dom* dominant effect of the PI 419083 allele
[k] *d/a* degree of dominance
[l] R^2 percentage of phenotypic variance explained by the QTL

Table 3. Quantitative trait loci (QTLs) identified in the F_2 (PI 419083 x PI604506) segregating population genotyped with markers of chromosome 8 of *C. moschata*, using the non-parametric Kruskal–Wallis test and composite interval mapping (CIM). Genetic positions are according with the new *C. moschata* x *C. moschata* linkage map of chromosone 8.

			Kruskal-Wallis					
Trait	**Chr[a]**	**QTL name**	**cM (peak position)**	**Nearest marker[b]**	**K[c]**	**Mean a[d]**	**Mean h[e]**	**Mean b[f]**
Symptoms 15 dpi	8	ToLCNDVCm_Sy15-8	18.8	D133	35.58	2.84	2.22	0.46
Symptoms 30dpi	8	ToLCNDVCm_Sy30-8	11.7	DMP39 [m]	82.96	4.00	3.60	0.46
Viral titer at 30 dpi (ΔCt)	8	ToLCNDVCm_VT30-8	11.7	DMP39 [m]	50.38	-12.63	-11.14	-2.42
Resistance (qualitative trait)	8	ToLCNDVCm_Re-8	11.7	DMP39 [m]	86.71	0.00	0.10	0.89

m: markers with same genetic position and significance in the analysis: DPM37, DMP39, DMP11, DMP10, DMP42, DMP43, DMP44, DMP41

			CIM (Qgene)							
Trait	**Chr[a]**	**QTL name**	**cM (peak position)**	**Interval (cM)[g]**	**Nearest marker[b]**	**LOD[h]**	**Add[i]**	**Dom[j]**	**[d/a][k]**	***R²*[l]**
Symptoms 15 dpi	8	ToLCNDVCm_Sy15-8	18.00	4.53-24.00	D133	9.96	1.25	0.60	0.48	0.30
Symptoms 30dpi	8	ToLCNDVCm_Sy30-8	12.00	8.01-13.46	DMP39	29.76	1.79	1.38	0.77	0.65
Viral titer at 30 dpi (ΔCt)	8	ToLCNDVCm_VT30-8	12.00	10.85-14.15	DMP39	24.37	-5.18	-3.64	0.70	0.59
Resistance (qualitative trait)	8	ToLCNDVCm_Re-8	12.00	8.13-13.83	DMP39	30.92	-0.45	-0.35	0.76	0.66

[a]Chromosome
[b]The closest marker to LOD peak
[c] *K**: the Kruskal-Wallis test statistic, with a significant level of 0.0001
[d]Mean of the PI 419083 genetic class in each marker
[e]Mean of the PI 419083/PI 604506 genetic class in each marker
[f]Mean of the PI 604506 genetic class in each marker
[g]Interval position of the putative QTL, identified in the F_2 (PI 419083 x PI 604506) in cM on the genetic map according with a LOD drop of 2
[h] *LOD* higher logarithm of the odds score
[i] *Add* additive effect of the PI 419083 allele
[j] *Dom* dominant effect of the PI 419083 allele
[k] *d/a* degree of dominance
[l] R^2 percentage of phenotypic variance explained by the QTL

Validation of the major QTL in chromosome 8 in $BC_{1PI\ 604506}$, F_2 (PI 419083 x PI 381814) and $BC_{1PI\ 381814}$ segregating populations

The Agena Bioscience SNPs panel of chromosome 8 was used to genotype the $BC1_{PI\ 604506}$ derived from the PI 419083 x PI 604506 cross, and the F_2 and $BC1_{PI\ 381814}$ populations derived from the PI 419083 x PI 381814 cross. Mean of symptoms scores at 30 dpi were calculated for each genotypic class of selected SNPs located within the defined QTL interval (DMP35 and DMP39), and compared in the figures 3.B, C and D.

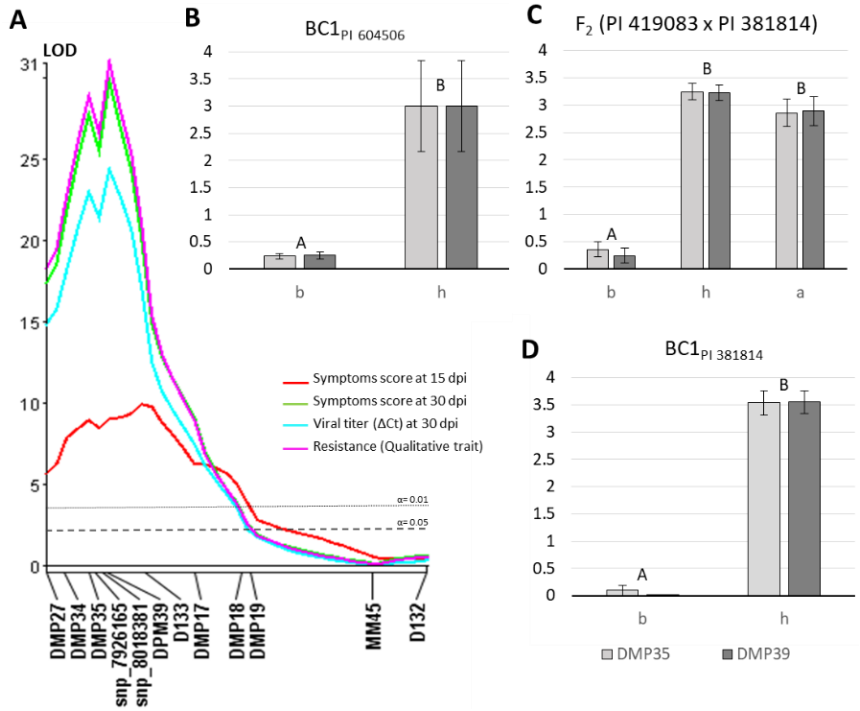

Figure 3. A: Molecular markers linked to QTLs for the four traits (symptoms at 15 and 30 days after mechanical inoculation (dpi), virus titer at 30 dpi and the qualitative resistance trait) associated with ToLCNDV resistance. QTL location was obtained by Composite Interval Mapping (CIM) method using F_2 (PI 419083 x PI 604506). **B, C and D:** Mean of symptom score at 30 dpi in $BC_{PI\ 604506}$, F_2 (PI 419083 x PI 381814) and $BC_{PI\ 381814}$ populations, respectively, according to each genotypic class DMP35 (light gray bars) and DMP39 (dark gray bars) markers (chromosome 8). PI 604506 or PI 381814 homozygous genotype is represented as "b", heterozygous as "h" and PI 419083 homozygous plants as "a". Bars with same capitals letters are not significantly different at $p \leq 0.01$.

The lowest level of symptoms was observed when plants in the three populations had the PI 604506 or PI 381814 homozygous genotype (b), in DPM35

or DPM39 indistinctly. Plants heterozygous (h) or PI 419083 homozygous (a) in both markers displayed significantly more severe symptomatology.

QTL analysis and validation of the candidate region in *C. pepo*

Consistently with the results obtained in F_1 from susceptible x resistant *C. moschata* crosses, severe symptoms were developed by F_1 *C. pepo* MU-CU-16 x *C. moschata* PI 604506 plants at 15 and 30 dpi (Figure 4). This result supports that resistance in PI 604506 has a recessive genetic control. F_2 (MU-CU-16 x PI 604506) plants segregated for symptomatology and viral accumulation. Symptoms including upward and downward curling and severe mosaic of young leaves, short internodes and bad distorted development, were observed in 124 and 151 F_2 (MU-CU-16 x PI 604506) plants at 15 and 30 dpi, respectively. The number of resistant plants decreased from 52 to 25 between 15 and 30 dpi. Nine plants had bad development or died in the course of the infection. On average, virus titers determined by qPCR at 30 dpi were in concordance with symptoms development, with mean of relative viral accumulation expressed as $2^{(-\Delta Ct)}$ of 1.04 ± 0.31 and 49571.67 ± 9670.31 in resistant and susceptible plants, respectively. The observed segregation proportion was adjusted to the expected ratio resistant:susceptible plants, in case of one recessive gene responsible on the genetic control of resistance to ToLCNDV at 15 dpi (X^2 =2.1894, p= 0.14), but not at 30 dpi (X^2 =10.312, p= 0.0014).

The genetic map of chromosome 8 generated with the genotyping results of the Agena Bioscience-HRM SNPs in the F_2 (MU-CU-16 x PI 604506) gave a total genetic length of 21.4 cM, with an average genetic distance between successive markers of 0.98 cM (Supplementary Table 2).

The QTL analysis performed in this population show that the QTLs identified in the *C. moschata* populations were stable in the cross with the *C. pepo* accession MU-CU-16. ToLCNDVCm_Sy15-8, ToLCNDVCm_Sy30-8, ToLCNDVCm_VT30-8, ToLCNDVCm_Re-8 were located in the same region that in *C. moschata* (Table 4), physically mapped in chromosome 17.

Figure 4. Plants of the interspecific F_1 resulting from the cross *C. pepo* MU-CU-16 x *C. moschata* PI 604506. **A)** Typical **s**ymptoms of *tomato leaf curl New Delhi virus* including curling, severe mosaic and short internodes. **B)** Healthy plant used to obtain F_2 progeny by selfing in a greenhouse. **C)** Detail of F_1 (MU-CU-16 x PI 604506) fruit obtained by selfing.

The highest R^2 value (65%) was explained by the ToLCNDVCm_Sy15-8, associated to DMP39 as nearest marker to the peak LOD. R^2 values were lower in QTLs related to advanced stages of the ToLCNDV infection, mainly in the viral titer at 30 dpi (ΔCt) trait. In these cases, nearest markers to the LOD peaks were DMP39 and snp_7926165 in ToLCNDVCm_Sy30-8 (Kruskal-Wallys and CIM tests, respectively), snp_7926165 in ToLCNDVCm_VT30-8 and DMP35 and snp_7926165 in ToLCNDVCm_Re-8 (Kruskal-Wallys and CIM tests, respectively). Logistic regression validate the occurrence of ToLCNDVCm_Re-8 QTL. According to the 2-LOD drop confidence intervals, the position interval where the four QTLs are co-mapping in chromosome 17 of *C. pepo* genome (v.4.1) is delimited between DMP34 (7,658,175 bp) and DMP41 (8,165,929).

After both QTLs analysis of chromosome 8, a consensus candidate region considered as responsible of ToLCNDV resistance in *C. moschata*, was established between DMP34 (561,788) and snp_8202510 (1,116,660).

Table 4. Quantitative trait loci (QTLs) identified in the F_2 (MU-CU-16 x PI 604506) segregating population genotyped with markers evenly distributed in chromosome 8 of *C. moschata*, using the genetic map obtained with this population, using the non-parametric Kruskal–Wallis test and composite interval mapping (CIM).

			Kruskal-Wallis					
Trait	**Chr[a]**	**QTL name**	**cM (peak position)**	**Nearest marker[b]**	**K[c]**	**Mean a[d]**	**Mean h[e]**	**Mean b[f]**
Symptoms 15 dpi	17	ToLCNDVCm_Sy15-8	10.5	DMP39	109.65	3.78	3.49	0.25
Symptoms 30 dpi	17	ToLCNDVCm_Sy30-8	10.5	DMP39	75.99	3.87	3.84	1.73
Viral titer at 30 dpi (ΔCt)	17	ToLCNDVCm_VT30-8	8.3	snp_7926165	28.89	-14.51	-13.83	-6.02
Resistance (qualitative)	17	ToLCNDVCm_Re-8	7.2	DMP35	63.18	0.02	0.02	0.53

			CIM (Qgene)							
Trait	**Chr[a]**	**QTL name**	**cM (peak position)**	**Interval (cM)[g]**	**Nearest marker[b]**	**LOD[h]**	**Add[i]**	**Dom[j]**	**[d/a][k]**	***R*²[l]**
Symptoms 15 dpi	17	ToLCNDVCm_Sy15-8	10	8.22-10.70	DMP39	40.57	1.76	1.49	0.84	0.65
Symptoms 30 dpi	17	ToLCNDVCm_Sy30-8	8	6.98-11.32	snp_7926165	20.70	1.08	1.05	0.97	0.42
Viral titer at 30 dpi (ΔCt)	17	ToLCNDVCm_VT30-8	8	6.57-11.62	snp_7926165	15.32	-4.25	-3.56	0.84	0.33
Resistance (qualitative)	17	ToLCNDVCm_Re-8	8	6.92-11.33	snp_7926165	17.28	-0.25	-0.25	1.00	0.37

[a]Chromosome

[b]The closest marker to LOD peak

[c] *K**: the Kruskal-Wallis test statistic, with a significant level of 0.0001

[d]Mean of the MU-CU-16 genetic class in each marker

[e]Mean of the MU-CU-16/PI 604506 genetic class in each marker

[f]Mean of the PI 604506 genetic class in each marker

[g]Interval position of the putative QTL, identified in the F_2 (MU-CU-16 x PI 604506) in cM on the genetic map according with a LOD drop of 2

[h] *LOD* higher logarithm of the odds score

[i] *Add* additive effect of the MU-CU-16 allele

[j] *Dom* dominant effect of the MU-CU-16 allele

[k] *d/a* degree of dominance

[l] R^2 percentage of phenotypic variance explained by the QTL

Genomic variation, structural variants and synteny

The alignment between the reference assemblies of *C. moschata* and *C. pepo* used for mapping purposes in the current paper (http://cucurbitgenomics.org) and the new assemblies available in november 2019 (https://www.dnazoo.org/assemblies/) showed no significant effect on the QTL region here studied (Supplementary Figure 1). Consequently, we keep working with the previous reference versions of both genomes.

A total of 53.2 and 31.5 million genomic clean reads were obtained from PI 604506 and PI 419083, respectively, and approximately more than 97% of them mapped against the *C. moschata* v.1 reference genome. No large structural variants were found between both accessions, and the read genome coverage was similar among them (Figure 5), which indicates that there is no deletions causing the observed phenotype. Some genomic positions show significant deviations for the expected coverage in both accessions (Figure 5), which could indicate some assembly errors on the reference genome.

After filtering for mapping quality, 28.2 and 18.6 million reads were kept. A total of 1,220,940 SNPs were found to be variable between both parental accessions and 2,748 were located in the candidate region in chromosome 8. Out of them, nine SNPs had a predicted high impact (either a frameshift or missense variant, a stop codon gain/loss or a splice site variant) and located within six genes (Supplementary Table 3). Two of these markers are located in the same genes where SNPs used in mapping (snp_7926165 and DMP44) were detected to be linked to ToLCNDV resistance (CmoCh08G001470 encoding a_BZIP transcription factor bZIP80 (835,327 to 841,749 bp) and CmoCh08G001770 encoding an unknown protein (1,047,526 to 1,051,835 bp)). The remaining seven SNPs with predicted high impact were located in three additional genes of this interval (CmoCh08G001130 encoding a Ribosome inactivating protein (583200 to 588238 bp), CmoCh08G001780 encoding a putative transmembrane protein (1,051,479 to 1,053,847 bp) and CmoCh08G001880 coding a IQ-DOMAIN 14-like protein (1,097,864 to 1,102,974)).

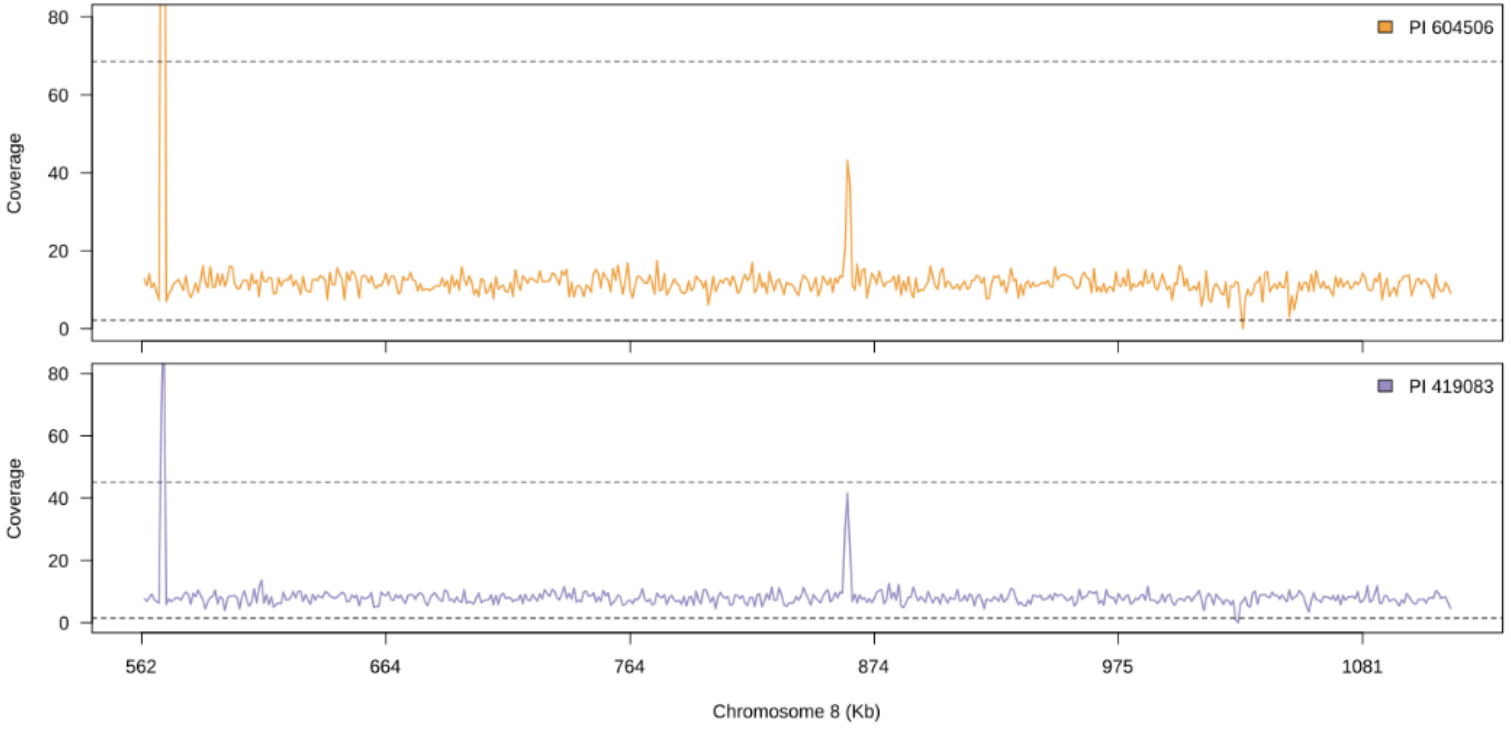

Figure 5. Genomic coverage along candidate region of chromosome 8 of the two accession used as parents for QTL mapping. Solid line shows the average coverage for 1 Kb windows. Dashed line shows the upper and lower 99% confidence interval for the observed coverage for the whole genome.

Also, some other SNPs with low, moderate or unknown modifying effect are placed in genes related to plant virus resistance (Supplementary Table 3).

Additionally to the genomic SNPs, the transcriptomic sequences of the 3 parentals and the six additional susceptible *C. moschata* accessions provided 731 SNPs in the candidate region, 94 of them were fixed for different alleles in the PI 604506 resistant accession and in the seven susceptible accessions (Supplementary Table 3). PI 381814 transcriptomic sequence had a low coverage in the candidate region and it was not possible to identify common polymorphisms between the two resistant accessions, PI 604506 and PI 381814. Three SNPs were detected with high predicted effect, all of them were common to those found in the genomic sequences analyzed and were located in three genes (Supplementary Table 3) (CmoCh08G001130 encoding a Ribosome inactivating protein, CmoCh08G001470 encoding a_BZIP transcription factor bZIP80 and CmoCh08G001770 encoding an unknown protein).

The structure of the candidate region was studied in more detail. A whole genome duplication likely occurred in the species that originated the *Cucurbita* genus (Montero-Pau et al., 2018). In fact, the search of putative paralogs of the genes in the chromosome 8 region indicated that 68 out of 86 genes in the chromosome 8 candidate region could be assigned to an orthogroup, and 58 of them presented at least one paralog gene. These paralog genes are widespread along the genome (Figure 6), although it seems that there is a conserved duplicated region of chromosome 8 on chromosome 17. Interestingly, some genes of the candidate region have been identified as single copy in chromosome 8 (Supplementary Table 4), without paralog genes in other chromosomes, which is consistent with a major QTL responsible of ToLCNDV resistance.

We also studied the synteny of this region with the susceptible *C. pepo*, which is phylogenetically closely related to *C. moschata.* BLAST alignment showed

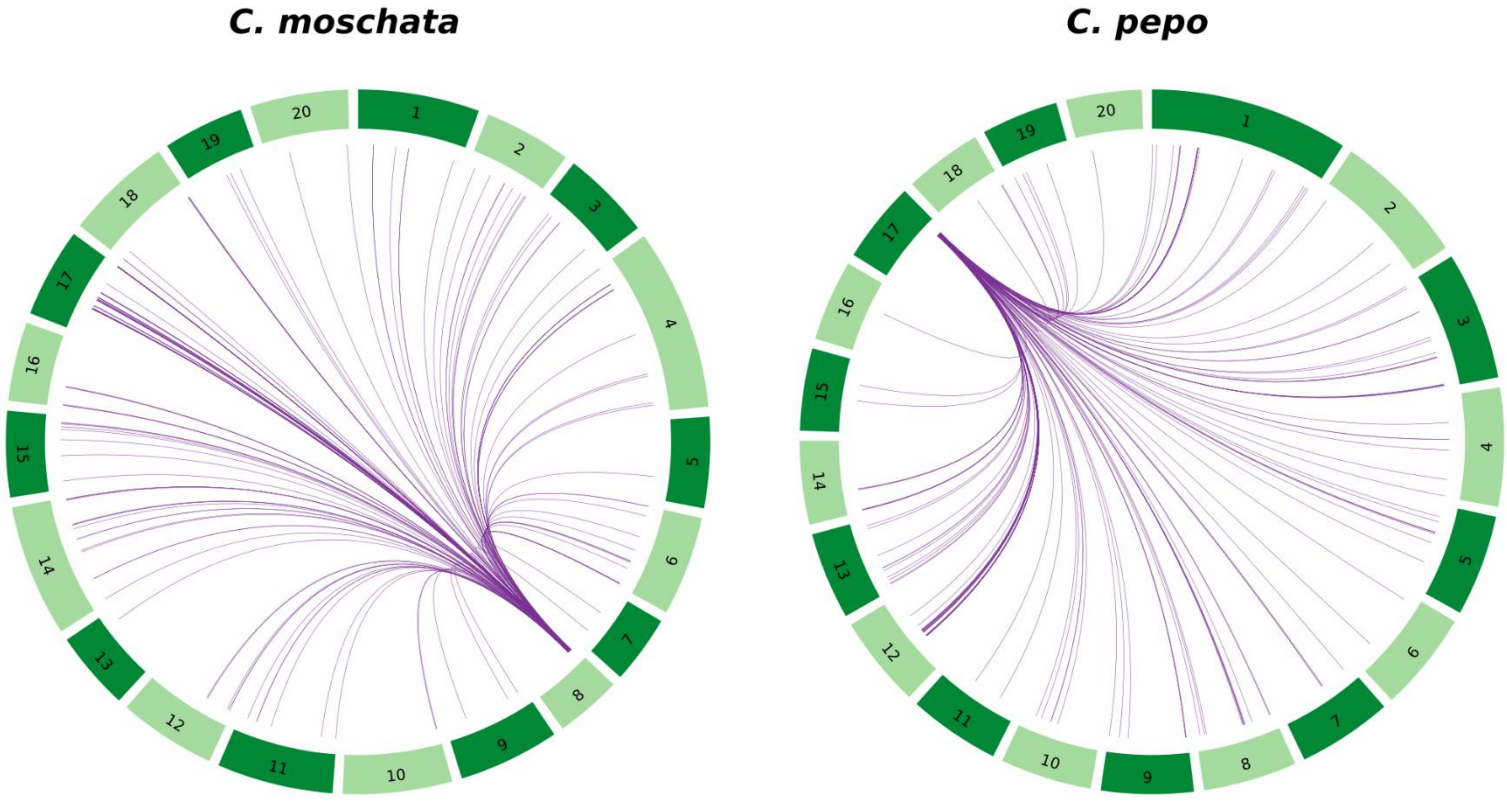

Figure 6. Circos plot showing the location of the duplicated genes located in *C. moschata*'s candidate region of chromosome 8 and in *C. pepo* syntenic region to *C. moschata* located in chromosome 17.

synteny between chromosome 8 region and chromosome 17 from 7,658,023 to 8,205,474 bp of *C. pepo* (see Figure 7 and Supplementary Table 4).

Gene order and orientation is preserved for most genes, but there is one region showing INDELs. Interestingly, the region with a major insertion in *C. pepo*, from 8,108,962 to 8,113,419 bp, is the region in which the MAD-box transcription factor CmoCh08G001760 maps. This region correspond to position 1,024,011 bp of *C. moschata*, located between the 5' UTR and the first exonic region of this gene. Specific analysis of this *C. pepo* insertion sequence allowed to detect a Long Terminal Repeats (LTR) retrotransposon of Ty1-copia Retrofit/Ale kind, of 3,692 bp of length located from 8,109,186 to 8,113,548 bp. This transposable sequence was previously annotated using the annotation procedure for repetitive sequences described in Montero-Pau et al.(2018). Supplementary Table 6 shows the annotation results and the fasta sequence of the region. Although this insertion is absent in both resistant (PI 604506) and susceptible (PI 419083) *C. moschata* accessions (Figure 5), many polymorphic SNPs between them are located in this gene, including 5´UTR and 3´UTR variants (1,023,872 and 1,047,775 bp), and a missense variant with moderate effect (1,043,369).

BLAST search of the *C. moschata* QTL region against *C. melo* found several syntenic regions. In the case of *C. melo* highly significant alignments where obtained against chromosome 11 where a major QTL associated with resistance to ToLCNDV is located (Sáez et al., 2017). Results show inversions in SNPs positions between both species, with at least two points of inversion events and loss of information regions (Supplementary table 2). This syntenic relationship was confirmed with the information displayed by the SyntenyViewer of cucurbitgenomics.org tool. Using chromosome 8 of *C. moschata* as query genome and location, circular representation showed regions of synteny with eight chromosomes of melon DHL92 (v3.6.1), including the candidate region of chromosome 11 (Figure 8.A). Figures 8.B and 8.C show syntenic blocks where are located ToLCNDV resistance-linked QTLs (coded as cmomedB906 and cmomedB910 in the database), the genomic position covered

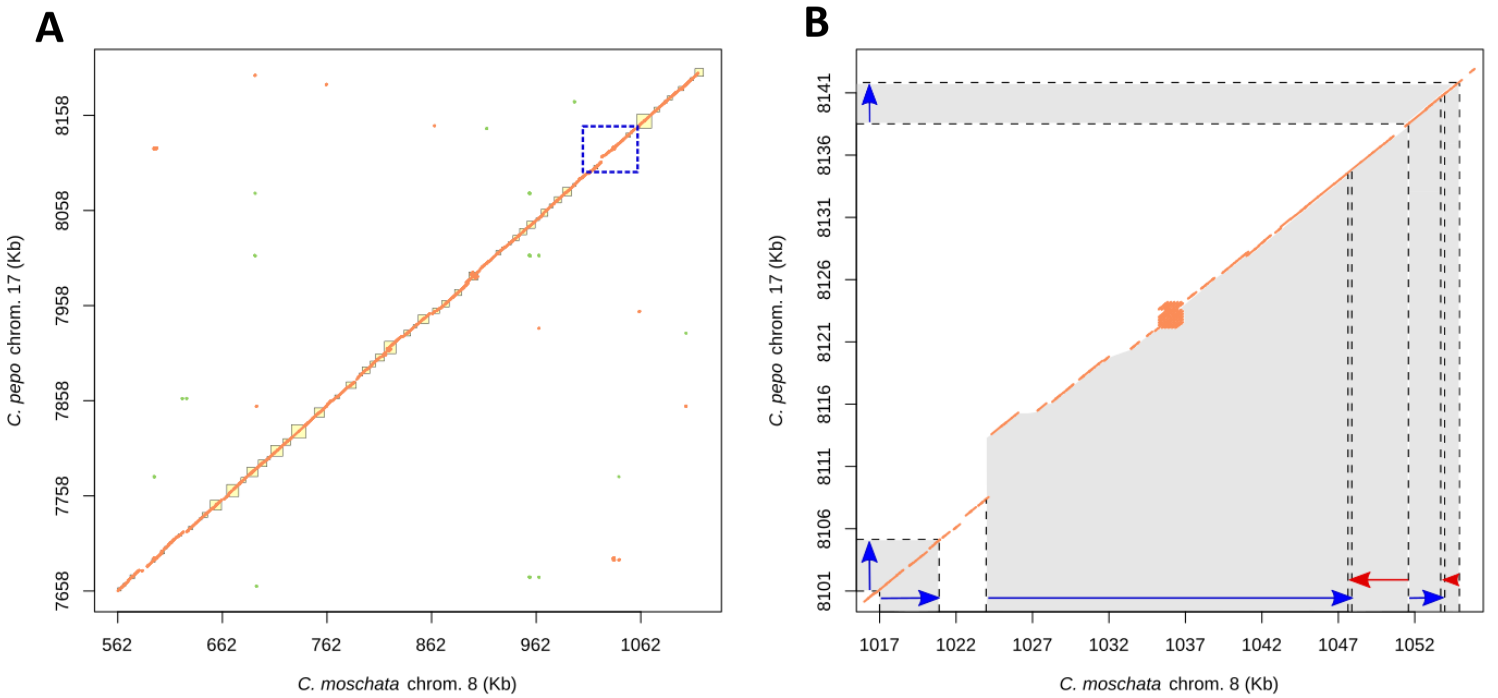

Figure 7. A: Dot plot showing the alignment between the Chromosome 8 of *C. moschata* assembly v.1 and chromosome 17 of *C. pepo* v.4.1. **B:** Expanded syntenic region where large INDELs have been detected. Blue and red arrows points genes sense.

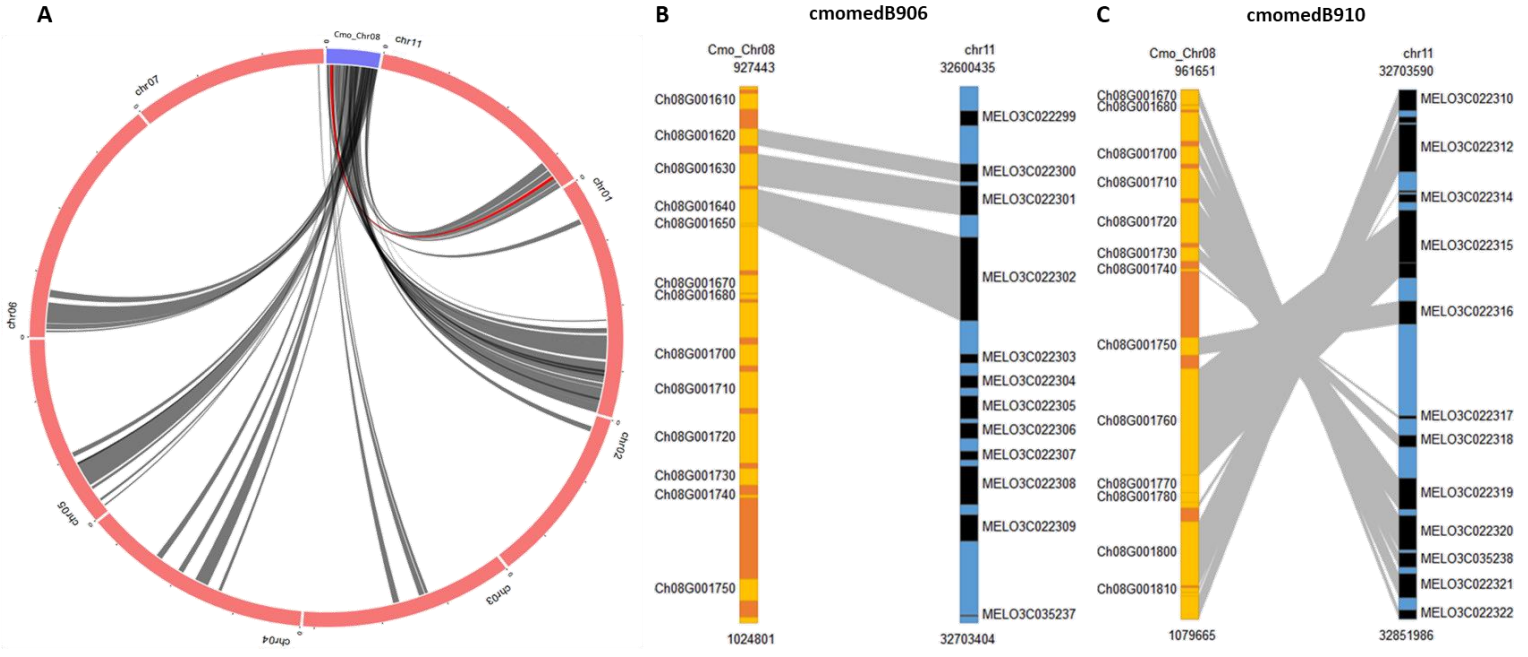

Figure 8. Synteny between the interval regions of QTLs detected in chromosome 8 of *C. moschata* and the major QTL in chromosome 11 of *C. melo.* **A:** Circular representation showing synteny with genome of melon DHL92 (v3.6.1). **B and C:** synteny blocks where QTLs linked to ToLCNDV resistance are located (coded as cmomedB906 and cmomedB910 in the database).

and graphic synteny relationship in both blocks. Furthermore, statistical significance of synteny between homologous genes in candidate region of *C. moschata* and *C. melo* is presented in Supplementary Table 5. Seventeen genes are shared by both candidate regions, including the MAD-box transcription factor CmoCh08G001760 and the Transmembrane protein CmoCh08G001780 where INDELs or high effect SNPs have been identified.

DISCUSSION

In this work, we evaluated the resistance to ToLCNDV previously described in the two *C. moschata* accessions PI 604506 and PI 381814 using mechanical inoculation (Sáez et al., 2016). Our results confirmed that both genotypes remain symptomless after inoculation assays. The Large Cheese improved cultivar PI 604506 originated in the USA (Burpee Company). Even though the primary center of *C. moschata* diversity is located in Northern South America and Central America, it spread soon to Mexico and later to the Caribbean area and USA, where it diversified (Decker-Walters and Walters, 2000). The landrace PI 381814 was collected in India, a secondary center of *C. moschata* variation, where resistance to ToLCNDV was found in melon accessions (López et al., 2015). This fact can be related with the co-evolution of host and pathogen in this area, in which ToLCNDV was detected for the first time infecting cucurbits many years ago. Indian cucurbits germplasm has been previously used as source of resistances to viral and fungal pathogens (Dhillon et al., 2012; McCreight et al., 2017). Mendelian analysis of symptom segregation in F_2 and BC_1s populations derived from PI 604506 and PI 381814, as well as QTL results, suggested the presence of a major recessive gene in chromosome 8 of *C. moschata* controlling symptoms development and virus titer. Allelism test results, that show resistance in all plants of F_2 (PI 604506 x PI 381814), suggests that alleles of the same *locus* control ToLCDV resistance in both accessions.

The occurrence of a major gene controlling ToLCNDV resistance derived from *C. moschata* sources is consistent with the existence of a major QTL reported to

control the resistance to ToLCNDV in melon, derived from the wild Indian accession of *Cucumis melo* subsp. *agrestis* WM-7 (Sáez et al., 2017). Resistance to whitefly transmission of ToLCNDV in sponge gourd (*Luffa cylindrica*), a cucurbit crop widely cultivated in India (Islam et al., 2010), has also been described to be regulated by a main dominant gene, for which two linked SRAP markers were reported (Islam et al., 2011).

Even though the major QTL linked to the resistance in *C. moschata* was stable in the *C. pepo* x *C. moschata* interspecific progeny, the mendelian segregation of symptoms only fitted to one recessive gene at 15 dpi. The effect of additional minor genes contributing to ToLCNDV resistance that are segregating in this interspecific population could account for these differences. In fact, in melon, besides the major QTL of chromosome 11, two additional minor regions in chromosomes 12 and 2 modifying the resistant response were identified (Sáez et al., 2017). In a recent publication (Romay et al., 2019), one recessive (*bgm-1*) and two dominant (*Bgm-2* and *Tolcndv*) genes were also found controlling resistance to ToLCNDV in the same Indian accession WM-7. A similar oligogenic control, three dominant genes, has been reported in *Solanum habrochaites* S. Knapp & D. M. Spooner, a wild species related to tomato, after ToLCNDV agroinoculation (Rai et al., 2013).

The role of the genetic background in resistance to plant viruses is considered determinant in breeding programs when transferring QTLs from one species to another. Gallois et al. (2018) have studied and reviewed the effect of epistatic relationship with QTL analysis on virus resistance, suggesting that a major-effect QTL (proportion of phenotypic variance explained by the QTL $R^2 > 0.60$) could be more susceptible to genetic background influence than minor-effect QTLs. This statement supports the incomplete penetrance obtained when we tried to transfer the QTL conferring resistance to ToLCNDV from chromosome 8 of *C. moschata* into *C. pepo* background. In this work, R^2 percentages of QTLs detected in the F_2 (PI 419083 x PI 604506) at 30 dpi ranged between 53-64% (Table 3), while in F_2 (MU-CU-16 x PI 604506) the R^2 percentages of QTLs linked to the same candidate region

decreased from 15 dpi (R^2 = 65%) to 30 dpi (R^2 = 33%-42%). These results suggest the requirement of other *loci* fixed in the *C. moschata* genetic background, needed in the mechanism of resistance to ToLCNDV, but segregating in *C. pepo*. With this information, it is recommended selecting for resistance at 30 dpi in populations coming from interspecific crosses, as it is the final stage of infection that better reflect the final response of the plants to the virus.

Resistance to ZYMV was found in different *C. moschata* accessions (Munger and Provvidenti, 1987; Paris et al., 1988; Wessel-Beaver, 2005). The resistance in the Portuguese *C. moschata* accession, Menina, is conferred by one dominant gene, Zym-1, in the cross with the susceptible *C. moschata* Waltham Butternut (Paris et al., 1988). However, when the resistance from Menina was introgressed into the *C. pepo*, segregation did not adjust to a single-gene ratio, and other additional dominant genes, Zym-2 and Zym-3, seemed to be involved in the resistance (Paris and Cohen, 2000). According with Pachner et al. (2011), even Zym-1, Zym-2, and Zym-3 together in *C. pepo* do not confer the same level of resistance seen in 'Menina'. Studies of inheritance of ZYMV resistance showed that the presence of Zym-1 is essential, but must be combined with other six genes to obtain different levels of expression and durability of resistance in *C. pepo* (Pachner et al., 2015; Capuzzo et al., 2017). According with these works, future QTLs analysis of F_2 (MU-CU-16 x PI 604506), including genotyping with SNPs covering the whole *Cucurbita* genome are crucial to reveal epistatic effects of other *loci* affecting ToLCNDV resistance.

The major *locus* for resistance to ToLCNDV in chromosome 8 of both *C. moschata* sources, PI 604506 and PI 381814 is recessively inherited. Recessive resistance genes, or susceptibility genes, because their presence conditions virus susceptibility (Garcia-Ruiz., 2018), are a common defense strategy against plant viruses (Diaz-Pendón et al., 2004; Kang et al., 2005). In cucurbits, recessive resistance genes have been reported in several viruses. Translation initiation factors eIF(iso)4E and eIF4G confer recessive resistance against a subset of viruses in several crop species (Hashimoto et al., 2016). The *nsv* recessive gene, encoding an

eIF4E factor, confers resistance to *melon necrotic spot virus* (Nieto et al., 2006), preventing the accumulation of viral RNA at the single-cell level (Díaz et al., 2002). In potyvirus-infected *Nicotiana benthamiana* leaf tissues, DEAD-box RNA helicase RH8, that share sequence homology with eIF4A, a component of the eIF4F multiprotein complex, is involved in viral genome translation and replication (Huang et al., 2010). We searched for putative eIF4E and eIF4F at the candidate region for ToLCNDV resistance of *C. moschata* annotation reference genome, and found that two genes (CmoCh08G001290 and CmoCh08G001490) encoding an ATP-dependent RNA helicase and Chromodomain-helicase-DNA-binding protein 1-like protein, respectively, mapped on the candidate region of chromosome 8. Concretely, CmoCh08G001490 is a single copy gene in *C. moschata*, with a 3' UTR SNP variant in PI 604506 sequence and is syntenic with a BZIP domain class transcription factor gene (MELO3C022278) of the chromosome 11 of *C. melo*.

Additionally, other strategies have been reported for recessive resistance against viruses. The recessive *cmv1* gene that confers resistance to *cucumber mosaic virus* in melon, encodes a vacuolar protein sorting 41 (VPS41) (Giner et al., 2017) involved in membrane trafficking to the vacuole. Membrane components are key factors required for plant infection success, and viral replication is associated with host intracellular membranes (Nicaise, 2014). In the case of *tom1* and *tom2A Arabidopsis* mutants, *tobacco mosaic virus* (TMV) accumulation is suppressed in single cells. Both genes encode transmembrane proteins localized in the tonoplast that are required for tobamovirus replication (Ishibashi et al., 2012). Among the annotated genes within the *C. moschata* candidate region here identified, several genes are related with membrane components. CmoCh08G001420 encodes a Vesicle transport protein and CmoCh08G001500 an Autophagy-related protein 3. Interestingly, two of the genes where high impact SNPs have been detected are annotated as putative Transmembrane protein (CmoCh08G001780 and CmoCh08G001790), included in the syntenic region between both candidate regions with resistance to ToLCNDV in *C. moschata* and *C. melo*.

Comparative physical mapping revealed a high level of synteny between the candidate regions with the major QTLs controlling ToLCNDV resistance of chromosomes 8 and 11 of *C. moschata* and *C. melo*, respectively (Sáez et al., 2017). The interval of approximately 118 kb encompasses genes from CmoCh08G001670 to CmoCh08G001830 of *C. moschata*. Comparing the orientations of this syntenic block, physical positions of genes in both genomes is reversed. Inversions are believed to play an important role in speciation and local adaptation by reducing recombination and protecting genomic regions from introgression (Yang et al., 2014a). The cluster of genes within this syntenic region contains transcription factors that have been described to confer resistance to viruses in different crops.

Genes of the same family of the WRKY transcription factor-like protein of *C. moschata* (CmoCh08G001670) appears to be involved in and defense responses upon TMV infection in *Capsicum annuum* (Huh et al., 2012). In PI 604506, six 3' UTR variants are affecting this gene. Moreover, a basic leucine zipper (BZIP) transcription factor gene (CmoCh08G001710) is placed close to SNP_8061105. Although CmoCh08G001470 is not placed in the syntenic region with *C. melo*, it also encodes a BZIP transcription factor gene. Particularly, a stop codon lost has been detected in this gene of PI 604506, which could alter the primary structure of the protein.

Two genes encoding MADS-box transcription factors are in this same region (CmoCh08G001750 and CmoCh08G001760). This gene family has been associated to different virus-resistance mechanisms. A MADS-box transcription factor was described as the *Ty-2* candidate, involved in the tomato resistance to *tomato yellow leaf curl virus* (TYLCV) (Yang et al,. 2014b), and recently, a MADS-box gene has been reported to be up-regulated in the *Sw-7* resistance to *tomato spotted wilt tospovirus* (TSWV) (Padmanabhan et al., 2019). No SNPs with high impact predicted effect were identified in CmoCh08G001760 between *C. moschata* accessions, but changes in 5´ and 3´UTRs and a missense mutation with predicted moderate effect were polymorphic between resistant and susceptible accessions.

This gene has no ortholog in *C. pepo* chromosome 17, likely due to the insertion affecting this region of the genome. The possible involvement of this gene in ToLCNDV resistance, would explain the total susceptibility to ToLCNDV found within *C. pepo* species (Sáez et al., 2016) and the difficulties to introgress the resistance *locus* from *C. moschata* to *C. pepo.*

The CmoCh08G001760 gene has paralogues in different chromosomes of *C. moschata*. OrthoMCL detected 8 putative paralogs in different chromosomes of *C. moschata* (Chr1, Chr8, Chr12, Chr14, Chr17 and Chr18). The alignment of the aa sequences of the *C. moschata* paralogs and all MADs-box genes of *Arabidopsis thaliana* shows that CmoCH08G001760.1 is most similar to the *C. moschata* paralog located in Chr17, CmoCh17G013780.1. Both genes clustered together and apart from *A. thaliana* genes (Supplementary Figure 2). The detailed comparison of the aa sequences of both genes showed significant length differences (169 aa *versus* 71 aa, for CmoCH08G001760 and CmoCh17G013780.1 respectively). Both proteins have a common MADs motif at the N-terminus of the protein, but differ in the rest of the sequence. These results are consistent with a different function of both genes. The sequence comparison of the CmoCh17G013780.1 gene of both parentals, PI 604506 and PI 419083 (done using the genomic sequences available at NCBI under BioProject PRJNA604046) does not provide SNP variants between them, also supporting the absence of a role of this paralog in ToLNDV resistance.

Molecular markers located close to the QTLs detected here can be used in marker-assisted selection in breeding ToLCNDV-resistant pumpkins and squash. Further genetic and transcriptomic studies of the candidate genes for resistance to ToLCNDV in the different cucurbit sources of resistance analyzed to date, are needed to develop strategies to control virus useful in different species of this crop family.

REFERENCES

Bio-Rad Laboratories (2006). Real-Time PCR Applications Guide. Bio-Rad Laboratories, Inc. 5279, 42-43.

Capuozzo, C., Formisano, G., Iovieno, P., Andolfo, G., Tomassoli, L., Barbella, M.M., et al. (2017). Inheritance analysis and identification of SNP markers associated with ZYMV resistance in *Cucurbita pepo*. Mol. Breeding 37:99. doi:10.1007/s11032-017-0698-5.

Chang, H.H., Ku H.M., Tsai W.S., Chien R.C., Jan F.J. (2010). Identification and characterization of a mechanical transmissible begomovirus causing leaf curl on oriental melon. Eur. J. Plant Pathol. 127, 219-228. doi:10.1007/s10658-010-9586-0.

Chen, X., Schulz-Trieglaff, O., Shaw, R., Barnes, B., Schlesinger, F., Källberg, M., et al. (2016). Manta: rapid detection of structural variants and indels for germline and cancer sequencing applications. Bioinformatics 32, 1220-1222. doi:10.1093/bioinformatics/btv710.

Cingolani, P., Platts, A., Wang, L.L., Coon, M., Nguyen, T., Wang, L., et al. (2012). A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. Fly 6, 80-92. doi:10.4161/fly.19695.

Decker-Walters, D. and Walters, T. (2000). "Squash", in The Cambridge World History of Food, eds. K. Kiple and K. Ornelas (Cambridge: Cambridge University Press), 335-351. doi:10.1017/CHOL9780521402149.034.

Dhillon, N.P.S., Monforte, A.J., Pitrat, M., Pandey, S., Singh, P.K., Reitsma, K.R., et al. (2012). Melon landraces of India: contributions and importance. Plant breed.35, 85-150. doi:10.1002/9781118100509.ch3.

Diaz-Pendon, J.A., Trunger, V., Nieto, C., García-Mas, J., Bendahmane, A. Aranda, M.A. (2004). Advances in understanding recessive resistance to plant viruses. Mol. Plant Pathol. 5, 223-233. doi:10.1111/j.1364-3703.2004.00223.x.

Díaz, J.A., Nieto, C., Moriones, E., Aranda, M. A. (2002). Spanish *Melon necrotic spot virus* isolate overcomes the resistance conferred by the recessive *nsv* gene of melon. Plant Dis. 86: 694.

Doyle, J.J., Doyle, J.L. (1990). Isolation of plant DNA from fresh tissue. Focus 12, 13-15.

EPPO (2017). European and Mediterranean Plant Protection Organization https://www.eppo.int/QUARANTINE/Alert_List/viruses/ToLCNDV.htm (Accessed on 28 August 2017).

EPPO (2019). European and Mediterranean Plant Protection Organization https://gd.eppo.int/taxon/TOLCND/distribution (Accessed on 20 November 2019).

FAO. 2017. FAOSTAT http://www.fao.org/faostat/en/#data/QC (Accessed 5 Feb 2020)

Gallois, J.L., Moury, B., German-Retana, S. (2018). Role of the Genetic Background in Resistance to Plant Viruses. Int. J. Mol. Sci. 19, 2856. doi: 10.3390/ijms19102856.

Garcia-Ruiz, H. (2018). Susceptibility Genes to Plant Viruses. Viruses, 10: 484. doi:10.3390/v10090484.

Garrison, E., Marth, G. (2012). Haplotype-based variant detection from short-read sequencing.arXiv:1207.3907.

Giner, A., Pascual, L., Bourgeois, M., Gyetvai, G., Rios, P., et al. (2017). A mutation in the melon vacuolar protein sorting 41 prevents systemic infection of *Cucumber mosaic virus*. Sci. Rep. 7:10471. doi:10.1038/s41598-017-10783-3.

Haider, M.S., Tahir, M., Latif, S., Briddon, R.W. (2006). First report of tomato leaf curl New Delhi virus infecting *Eclipta prostrata* in Pakistan. Plant Pathol. 55:285 doi:10.1111/j.1365-3059.2005.01278.x.

Hashimoto, M., Neriya, Y., Yamaji, Y., Namba, S. (2016). Recessive Resistance to Plant Viruses: Potential Resistance Genes Beyond Translation Initiation Factors. Front. microbiol. 7:1695. doi:10.3389/fmicb.2016.01695.

Huang, T.S., Wei, T., Laliberté, J.F., Wang, A. (2010). A Host RNA Helicase-Like Protein, AtRH8, Interacts with the Potyviral Genome-Linked Protein, VPg, Associates with the Virus Accumulation Complex, and Is Essential for Infection. Plant Physiol. 152, 255-266. doi:10.1104/pp.109.147983.

Huh, S.U., Choi, L.M., Lee, G.J., Kim, Y.J. (2012). *Capsicum annuum* WRKY transcription factor d (CaWRKYd) regulates hypersensitive response and defense response upon tobacco mosaic virus infection. Plant Sci. 197, 50-58. doi:10.1016/j.plantsci.2012.08.013.

Hussain, M., Mansoor, S., Iram, S., Zafar, Y., Briddon, R.W. (2004). First report of *tomato leaf curl New Delhi virus* affecting chilli pepper in Pakistan. Plant Pathol. 53: 794. doi:10.1111/j.1365-3059.2004.01073.x.

Ishibashi, K., Miyashita, S., Katoh, E., Ishikawa, M. (2012). Host membrane proteins involved in the replication of tobamovirus RNA. Curr. Opin. Virol. 2, 693–698. doi:10.1016/j.coviro.2012.09.011.

Islam, S., Munshi, A.D., Mandal, B., Behera, T.K., Kumar, R. (2010). Genetics of resistance in *Luffa cylindrical* Roemagainst *tomato leaf curl New Delhi virus.* Euphytica 174, 83-89. doi: 10.1007/s10681-010-0138-7.

Islam, S., Munshi, A.D., Verma, M., Arya, L., Mandal, B., Behera, T.K., et al. (2011). Screening of *Luffa cylindrical* Roem for resistance against tomato leaf curl New Delhi virus, inheritance of resistance, and identification of SRAP markers linked to the single dominant resistance gene. J. Hortic. Sci. Biotechnol. 86, 661–667. doi:10.1080/14620316.2011.11512819.

Ito, T., Sharma, P., Kittipakorn, K., Ikegami, M. (2008). Complete nucleotide sequence of a new isolate of *Tomato leaf curl New Delhi virus* infecting cucumber, bottle gourd and muskmelon in Thailand. Arch. 153, 611-613. doi:10.1007/s00705-007-0029-y.

Jamil, N., Rehman, A., Hamza, M., Hafeez, A., Ismail, H., Zubair, M., et al.,(2017) First report of *Tomato leaf curl New Delhi virus*, a bipartite begomovirus, infecting soybean (*Glycine max*). Plant dis. 101:845. doi:10.1094/PDIS-09-16-1267-PDN.

Janssen, D., Simon, A., Crespo, O., Ruiz, L. (2017). Genetic population structure of *Bemisia tabaci* in Spain associated with Tomato leaf curl New Delhi virus. Plant Protect. Sci. 53: 25-31. doi:10.17221/62/2016-PPS.

Joehanes, R., Nelson, J.C., (2008). QGene 4.0, an extensible Java QTL-analysis platform. Bioinformatics 24, 2788-2789. doi:10.1093/bioinformatics/btn523.

Juárez, M., Tovar, R., Fiallo-Olivé, E., Aranda, M.A., Gosálvez, B., Castillo, P., et al. (2014). First detection of *tomato leaf curl New Delhi virus* infecting Zucchini in Spain. Plant Dis. 98:857–858. doi:10.1094/pdis-10-13-1050-PDN.

Juárez, M., Rabadán, M.P., Díaz Martínez, L., Tayahi, M., Grande-Pérez, A. and Gómez, P. (2019). Natural Hosts and Genetic Diversity of the Emerging Tomato Leaf Curl New Delhi Virus in Spain. Front Microbiol. 2019; 10: 140. doi:10.3389/fmicb.2019.00140.

Jyothsna, P., Haq, Q.M.I., Singh, P., Sumiya, K.V., Praveen, S., Rawat, R., Briddon, R.W., Malathi, V.G. (2013). Infection of *tomato leaf curl New Delhi virus* (ToLCNDV), a bipartite begomovirus with betasatellites, results in enhanced level of helper virus components and antagonistic interaction between DNA B and betasatellites. Appl. Microbiol. Biotechnol. 97, 5457-5471. doi:10.1007/s00253-012-4685-9.

Kang, B.C., Yeam, I., Jahn, M.M. (2005). Genetics of plant virus resistance. Annu. Rev. Phytopathol. 43, 581-621. doi:10.1146/annurev.phyto.43.011205.141140.

Kielbasa, S.M., Wan, R., Sato, K., Horton, P., Frith, M.C. (2011). Adaptive seeds tame genomic sequence comparison. Genome Res. 21, 487-93. doi:10.1101/gr.113985.110.

Kheireddine, A., Sifres, A., Sáez, C., Picó, B., López C. (2019). First Report of Tomato Leaf Curl New Delhi Virus Infecting Cucurbit Plants in Algeria. APS. 103, 3291. doi.org/10.1094/PDIS-05-19-1118-PDN.

Lander, E.S., Green, .P, Abrahamson, J., Barlow, A., Daly, M.J. (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1, 174–181. doi:10.1016/0888-7543(87)90010-3.

Li, H. (2013). Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv:1303.3997.

Li, H., Handsaker, B., Wysoker, A., Fennell T., Ruan, J., Homer, N., et al., (2009) The Sequence alignment/map (SAM) format and SAMtools. Bioinformatics 25, 2078-9. doi:10.1093/bioinformatics/btp352.

Li, L., Stoecker,t C.J.Jr., and Roos, D.S. (2003). OrthoMCL: Identification of Ortholog Groups for Eukaryotic Genomes, Genome Res. 13, 2178-2189. doi:10.1101/gr.1224503.

Lincoln, S., Daly, M., & Lander, E. (1992). Constructing genetic maps with MAPMAKER/EXP 3.0. Whitehead Institute Technical Report 3rd edn. Whitehead Institute, Cambridge, Massachusetts.

Livak, K.J., Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2–DDCt method. Methods 25, 402–408. doi: 10.1006/meth.2001.1262

López, C., Ferriol, M., Picó, M.B. (2015). Mechanical transmission of *Tomato leaf curl New Delhi virus* to cucurbit germplasm: selection of tolerance sources in *Cucumis melo.* Euphytica 204,279–691. doi:10.1007/s10681-015-1371-x.

McCreight, J.D., Wiutemantel, W.M., Natwick, E.T., Sinclair, J.W., Crosby, K.M., Gómez-Guillamón, M.L. (2017). Recessive resistance to CYSDV in melon TGR 1551. Acta Hortic. 1151, 101–108. doi: 10.17660/ActaHortic.2017.1151.17.

Millard, S.P. (2013). EnvStats: An R Package for Environmental Statistics. Springer, New York. ISBN 978-1-4614-8455-4.

Mnari-Hattab, M., Zammouri, S., Belkadhi, M.S., Bellon, Doña, D., Ben Nahia, E., et al. (2015). First report of *Tomato leaf curl New Delhi virus* infecting cucurbits in Tunisia. New Dis. Rep. 31:21. doi:10.5197/j2044-05882015031021.

Montero-Pau, J., Blanca, J., Bombarely, A., Ziarsolo, P., Esteras, C., Martí-Gómez, C., et al. (2018). De-novo assembly of zucchini genome reveals a whole genome duplication associated with the origin of the *Cucurbita* genus. Plant Biotechnol. J. 16, 1161-1171. doi: 10.1111/pbi.12860.

Moriones, E., Praveen, S., Chakraborty, S. (2017). Tomato Leaf Curl New Delhi Virus: An Emerging Virus Complex Threatening Vegetable and Fiber Crops. Viruses 9:264. doi:10.3390/v9100264.

Munger, H.M., Provvidenti, R. (1987) Inheritance of resistance to zucchini yellow mosaic virus in *Cucurbita moschata*. Cucurbit Genet. Coop. Rep 10, 8–81.

Nguyen, L.T., Schmidt, H.A., Von Haeseler, A., Minh B.Q. (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Mol. Biol. Evol. 32, 268-274. https://doi.org/10.1093/molbev/msu300.

Nicaise, V. (2014). Crop immunity against viruses: outcomes and future challenges. Front. Plant Sci. 5:660. doi:10.3389/fpls.2014.00660.

Nieto, C., Morales, M., Orjeda, G., Clepet, C., Monfort, A., Sturbois, et al. (2006). An *eIF4E* allele confers resistance to an uncapped and non-polyadenylated RNA virus in melon. Plant J. 48, 452-462. doi:10.1111/j.1365-313X.2006.02885.x.

Orfanidou, C.G., Malandraki, I., Beris, D., Kektsidou, O., Vassilakos, N., Varveri, C., et al, (2019). First report of tomato leaf curl New Delhi virus in zucchini crops in Greece. J. Plant Pathol. 101:799. (in press). doi:10.1007/s42161-019-00265-y.

Pachner, M., Paris, H.S., Lelley, T. (2011). Genes for resistance to zucchini yellow mosaic in tropical pumpkin. J. Hered. 102,330-335. doi:10.1093/jhered/esr006.

Pachner, M., Paris, H.S., Winkler, J., Lelley, T. (2015). Phenotypic and marker-assisted pyramiding of genes for resistance to zucchini yellow mosaic virus in oilseed pumpkin (*Cucurbita pepo*). Plant Breed. 134:121-128. doi:10.1111/pbr.12219.

Padidam, M., Beachy, R.N., Fauquet, C.M. (1995). Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not essential for infectivity. J Gen. Virol. 76, 25–35. doi:10.1099/0022-1317-76-1-25.

Padmanabhan, C., Ma, Q., Shekasteband, R., Stewart, K. S., Hutton, S. F., Scott, J. W., et al. (2019). Comprehensive transcriptome analysis and functional characterization of PR-5 for its involvement in tomato Sw-7 resistance to tomato spotted wilt tospovirus. Sci. rep. 9:7673. doi:10.1038/s41598-019-44100-x.

Panno, S., Iacono, G., Davino, M., Marchione, S., Zappardo, V., Bella, P., et al. (2016). First report of *Tomato leaf curl New Delhi virus* affecting zucchini squash in an important horticultural area of southern Italy. New Dis. Rep. 33:6. doi:10.5197/j2044-05882016033006.

Paris, H.S., Cohen, S., Burger, Y., Joseph, R. (1988). Single-gene resistance to zucchini yellow mosaic virus in *Cucurbita moschata*. Euphytica 37:27-29. doi:10.1007/BF00037219.

Paris, H.S., Cohen, S. (2000). Oligogenic inheritance for resistance to zucchini yellow mosaic virus in *Cucurbita pepo*. Ann. Appl. Biol. 136,209–214. doi:10.1111/j.1744-7348.2000.tb00027.x.

Pratap, D., Kashikar, A.R., Mukherjee, S.K. (2011). Molecular characterization and infectivity of a *Tomato leaf curl New Delhi virus* variant associated with newly emerging yellow mosaic disease of eggplant in India. Virol. J. 8:305. doi: 10.1186/1743-422X-8-305.

Rai, N.K., Sahu, P.P., Gupta, S., Reddy, M.K., Ravishankar, K.V., Singh, M., Sadashiva, A.T., et al. (2013). Identification and validation of an ISSR marker linked to Tomato leaf curl New Delhi virus resistant gene in a core set of tomato accessions. Vegetable Sci. 40, 1-6.

Romay, G., Pitrat, M., Lecoq, H., Wipf-Scheibel, C., Millot, P., Girardot G., et al. (2019). Resistance Against Melon Chlorotic Mosaic Virus and Tomato Leaf Curl New Delhi Virus in Melon. Plant Dis. 103, 2913-2919. doi: 10.1094/PDIS-02-19-0298-RE.

Rosen, R., Kanakala, S., Kliot, A., Pakkianathan, B.C., Farich, B.A., Santana-Magal, N., et al. (2015). Persistent, circulative transmission of begomoviruses by whitefly vectors. Curr. Opin. Virol. 15, 1-8. doi: 10.1016/j.coviro.2015.06.008.

Roy, A., Spoorthi, P., Panwar, G., Bag, M.K., Prasad, T.V., Kumar, G., et al. (2013). Molecular evidence for occurrence of *Tomato leaf curl New Delhi virus* in ash gourd (*Benincasa hispida*) germplasm showing a severe yellow stunt disease in India. Indian J. Virol. 24, 74-77. doi: 10.1007/s13337-012-0115-y.

Sáez, C., Martínez, C., Ferriol, M., Manzano, S., Velasco, L., Jamilena, M., et al. (2016). Resistance to *Tomato leaf curl New Delhi virus* in *Cucurbita* spp. Ann. Appl. Biol. 169:91-105. doi: 101111/aab12283.

Sáez, C., Esteras, C., Martínez, C., Ferriol, M., Narinder, P.S.D, López, C., et al. (2017). Resistance to Tomato Leaf Curl New Delhi Virus in melon is controlled by a major QTL located in chromosome 11. Plant Cell Rep. 36, 1571-1584. doi: 10.1007/s00299-017-2175-3.

Sifres, A., Sáez, C., Ferriol, M., Selmani, E., Riado, J., Picó, B., et al. (2018). First Report of Tomato leaf curl New Delhi virus Infecting Zucchini in Morocco. Plant Dis. 102:1045. doi:10.1094/PDIS-10-17-1600-PDN.

Singh, A.K., Mishra, K.K., Chattopadhyay, B., Chakraborty, S. (2009). Biological and molecular characterization of a begomovirus associated with yellow mosaic vein mosaic disease of pumpkin from Northern India. Virus Genes 39, 359–370. doi:10.1007/s11262-009-0396-4.

Sohrab, S.S., Mandal, B., Pant, R.P., Varma, A. (2003). First report of association of *Tomato leaf curl virus-New Delhi* with yellow mosaic disease of *Luffa cylindrica* in India. Plant Dis. 87:1148. doi:10.1094/PDIS.2003.87.9.1148A.

Sohrab, S.S., Karim, S., Varma, A., Abuzenadah, A.M., Chaudhary, A.G., Damanhouri, G.A., et al. (2013). Characterization of *Tomato Leaf Curl New Delhi Virus* infecting cucurbits: Evidence for sap transmission in a host specific manner. Afr. J. Biotechnol. 12,5000-5009. doi: 10.5897/AJB2013.12012.

Srivastava, K.M., Hallan, V., Raizada, R.K., Chandra, G., Singh, B.P., Sane, P.V. (1995). Molecular cloning of Indian tomato leaf curl virus genome following a simple method of concentrating the supercoiled replicative form of viral DNA. J. Virol. Methods 51,297-304.

Srivastava, A., Kumar, S., Jaidi, M., Raj, S.K., Shukla, S.K. (2016). First report of *Tomato leaf curl New Delhi virus* on opium poppy (*Papaver somniferum*) in India. Plant Dis. 100:232. doi:10.1094/PDIS-08-15-0883-PDN.

Sun, H., Wu, S., Zhang, G., Jiao, C., Guo, S., Ren, Y., et al. (2017). Karyotype Stability and Unbiased Fractionation in the Paleo-Allotetraploid Cucurbita Genomes. Mol. Plant. 10, 1293-1306. doi: 10.1016/j.molp.2017.09.003.

Sundararaj, D., Jesse, M., Gunasekaran, D., Riyaz, M., Thangavelu, R., et al. (2019). First report of tomato leaf curl New Delhi virus infecting Crossandra infundibuliformis (L.) in India. Plant Dis., in press. doi: 10.1094/PDIS-08-19-1764-PDN.

Thorvaldsdóttir, H., Robinson, J.T., Mesirov, J.P. (2013). Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Brief. Bioinform. 14, 178-192. doi: 10.1093/bib/bbs017.

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., et al, (2012). Primer3--new capabilities and interfaces. Nucleic Acids Res. 40:e115.

Usharani, K.S., Surendranath, B., Paul-Khurana, S.M., Garg, I.D., Malathi, V.G. (2004). Potato leaf curl – a new disease of potato in northern India caused by a strain of *Tomato leaf curl New Delhi virus.* Plant Pathol. 53:235. doi: 10.1111/j.0032-0862.2004.00959.x.

Van Ooijen, J.W. (2009). MapQTL® 6 Software for the mapping of quantitative trait loci in experimental population of diploid species Kayzma BV. Wageningen, The Netherlands.

Vossen, R.H., Aten, E., Roos, A., den Dunnen, J.T. (2009). High-resolution melting analysis (HRMA) - More than just sequence variant screening. Hum Mutat. 30, 860–6. doi:10.1002/humu.21019.

Wessel-Beaver, L. (2005). Cultivar and germplasm release. Release of 'Soler' tropical pumpkin. J. Agric. Univ. P.R. 89, 263–266.

Yang, L., Koo, D., Li, D., Zhang, T., Jiang, J., Luan, F., et al. (2014a). Next-generation sequencing, FISH mapping and synteny-based modeling reveal mechanisms of decreasing dysploidy in *Cucumis*. Plant J. 77, 16-30. doi:10.1111/tpj.12355.

Yang, X., Yang, X., Caro, M., Hutton, S., Scott, J., Guo , Y., et al. (2014b). Fine mapping of the tomato yellow leaf curl virus resistance gene Ty-2 on chromosome 11 of tomato. Mol. Breed. 34, 749-760. Doi:10.1007/s11032-014-0072-9.

Zaidi, S.S., Shafiq, M., Amin, I., Scheffler, B.E., Scheffler, J.A., Briddon, R.W., et al. (2016). Frequent occurrence of *Tomato Leaf Curl New Delhi Virus* in cotton leaf curl disease affected cotton in Pakistan. PLoS ONE 1:e0155520. doi:10.1371/journal.pone.0155520.

Zaidi, S.S., Shakir, S., Malik, H.J., Farooq, M., Amin, I., Mansoor ,S. (2017). First Report of *Tomato leaf curl New Delhi virus* on *Calotropis procera*, a Weed as Potential Reservoir Begomovirus Host in Pakistan. Plant dis. 101, 1071-1072. doi: 10.1094/PDIS-10-16-1539-PDN.

Zeng, Z.B. (1994). Precision mapping of quantitative trait loci. Genetics 136, 1457–1468.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2020.00207/full#supplementary-material

Capítulo V

CAPÍTULO V

RNA-seq transcriptome analysis provides candidate genes for resistance to *tomato leaf cur New Delhi virus* in melon

Cristina Sáez, Alejandro Flores-León, Alicia Sifres, Narinder P S Dhillon, Carmelo López & Belén Picó

In preparation to Frontiers in Plant Sciences - Emerging and Reemerging Plant Viruses in a Context of Global Change

ABSTRACT

Tomato leaf curl New Delhi virus (ToLCNDV) emerged in the Mediterranean Basin in 2012 as the first DNA bipartite begomovirus (*Geminiviridae* family) causing severe yield and economic losses in cucurbits crops. A major locus for resistance was identified in the wild melon accession WM-7 (*Cucumis melo* kachri group), but mechanism involved in the resistant response remained unknown. In this work, we used RNA-sequencing technology to obtain a deep insight into candidate genes of this region and resistance machinery activated in the course of ToLCNDV infection. Transcriptomes of the resistant WM-7 genotype and the susceptible cultivar Piñonet Piel de Sapo (PS) (*C. melo* ibericus group) in ToLCNDV and mock inoculated plants were compared, at four stages of infection (0, 3, 6, ad 12 days post inoculation (dpi)). Different patterns of genes expression were observed between each genotype, with their respective controls and at the different points of the disease. Differentially expressed genes (DEGs) in ToLCNDV infected plants were classified into GO terms, and those associated with DNA transcription, replication and helicase activity, were down-regulated in WM-7 but up-regulated in PS, indicating that restriction of ToLCNDV replication and intercellular spread might be responsible of the observed resistant behavior. DEGs involved in hormonal signaling of jasmonic acid pathway, photosynthesis, RNA silencing route, transmembrane and sugar transporters entail adverse consequences for systemic infection in the resistance genotype, but promoting it in the susceptible PS. Expression level was validated by qRT-PCR in selected genes related with virus resistance or located in candidate regions for resistance. The results here obtained suppose a great contribution to understand the altered genes in response to begomoviruses, spotlighting a complex genetic regulation of ToLCNDV resistance in melon. This information will be relevant to cucurbit breeders in the assisted transference of ToLCNDV resistance to commercial cultivars.

INTRODUCTION

Melon (*Cucumis melo* L.) is one of the major cucurbits crops, worldwide cultivated and with large fruit and botanical diversification. Highly appreciated by its nutritional profile and aromatic sweet flavor, its production generates high imputs to farmers in industrialized and developing countries.

Besides years of selection and breeding, melon sustainable production around the globe is compromised by several pathogens and diseases. The occurrence of new viruses emerging in melon growing regions is one of the main farmers and seed companies concerns, because of the ability to limit productions and yields of these biological agents (Annu et al., 2019).

Tomato Leaf curl New Delhi virus (ToLCNDV) is a species of bipartite begomovirus (family *Geminiviridae*) naturally transmitted by the whitefly *Bemisia tabaci* (Gennadius) in a persistent manner. This virus has supposed a constricting threat of melon crops in many countries of the Indian subcontinent since the earlies 2000s, but recently a new recombinant strain has appeared in the Mediterranean basin generating a devasting disease to mainly melon and zucchini squash (*Cucurbita pepo*) crops (Juárez et al., 2014). The new strain was first identified in Spain, but all the isolates detected across the Mediterranean countries share a conserved genomic sequence (Juárez et al., 2014, 2019; Fortes et al., 2016; Panno et al., 2019). Consequently, the Mediterranean ToLCNDV strain has been recently designated as ToLCNDV-ES (Moriones et al., 2017).

As the Asiatic strains, ToLCNDV-ES infection in melon results in severe symptomatology, including leaf curling and distortion, green and yellow spotting conforming mosaic, changes in leaves shape and stunting. Infection at young stages of the plant avoid correct vegetative and flowering development, with decreased or completely lost of fruit quality due to skin roughness, longitudinal cracking and small size, making them unmarketable (Panno et al., 2016). Melon production losses may arrive to 80% in greenhouse and open-field exploitations if integrated control measures are not adopted (Messelink et al., 2020). To ToLCNDV-ES management,

genetic resistance is the most efficient approach for farmers and environment, avoiding pest and insecticides treatments.

Melon accessions belonging to momordica, kachri, acidulus and agrestis groups from India have been reported as resistant to begomoviruses (McCreight et al., 2008; Yousif et al., 2007; López et al., 2015; Pitrat, 2016; Romay et al., 2019), and several QTL and molecular markers has been identified on this germplasm for resistance to ToLCNDV-ES (Sáez et al., 2017; Romay et al., 2019).

In previous works, we mapped a major gene with incomplete dominance conferring resistance to ToLCNDV-ES on chromosome 11 of the Indian accession WM-7 (kachri group) (Sáez et al., 2017). However, two minor modifiers on chromosomes 2 and 12 modulate the response to viral infection. Functional characterization of the genes included on these regions are required to enhance the understanding of the molecular resistance mechanisms in WM-7.

Whole transcriptome sequencing by RNA-seq technology has increased popularity to explore genes expression changes as reaction of cucurbits plants during viruses infections (Sun et al., 2017; Li et al., 2017a; Sun et al., 2019; Lou et al., 2020). This tool offers a global view of basal defense response and help elucidating complex mechanisms of resistance in plants.

Transcriptome analysis and genes expression studies have been performed to evaluate interactions between ToLCNDV and solanaceous crops. Sahu et al., (2010) compared transcripts level between tolerant and susceptible to ToLCNDV tomato genotypes, finding induced genes in the tolerant genotype related with cell cycle, transcription factors, DNA/RNA processing and molecular signal and transport. The over-expression of two of the main candidates for resistant, a DEAD-box RNA helicase gene (*SlDEAD35*) and the 26S proteasome subunit RPT4a (*SlRPT4*), have been recently proved as inhibitor of ToLCNDV symptoms and infection and involved in hypersensitivity response and cell death, respectively (Sahu et al., 2016; Pandey et al., 2019). In pepper (*Capsicum annuum*) and potato (*Solanum tuberosum*)

resistant genotypes to ToLCNDV induced transcripts level of R-genes (NBS-LRR) (Kushwaha et al., 2015; Jeevalatha et al., 2017).

In melon, Román et al. (2019) evaluated differences of expression of twelve candidate genes between a resistant and a susceptible accession to ToLCNDV. Two genes, a NAC transcription factor and an actine related protein, resulted strongly induced in the susceptible genotype and were associated with disease development. Besides those studies, global transcriptional and molecular characterization of ToLCNDV resistance in cucurbits has not been conducted.

In this study, we perform an RNAseq assay to compare transcripts level between healthy and infected plants of the resistant WM-7 genotype and a susceptible to ToLCNDV-ES melon accession. To further study the changing expression patter along the course infection, we evaluated deregulated genes at different stages.

MATERIALS AND METHODS

Plant material and mechanical inoculation

The ToLCNDV-resistant wild *C. melo* accession WM-7 of the kachri group and the susceptible Spanish traditional cultivar Piñonet Piel de Sapo (PS), belonging to ibericus group, were used as plant materials in this study. WM-7 is an Indian accession of the collections described by Roy et al. (2012), and selfed in successive growing cycles to increase the homozygosity level. Seeds of both accessions were disinfected in a 10% solution of sodium hypochlorite for 1 min and twice washed with distilled water for 5 min. To guarantee homogenous germination all seeds were opened by forceps, placed over moistened cotton in Petri plates and incubated in darkness at 37 °C for 48 h.

At the stage of two expanded true-leaves, 226 seedlings of both resistant and susceptible accessions were mechanical inoculated following the procedure described in López et al., (2015). Symptomatic leaf tissue from zucchini plants agroinfiltrated with an infective clone of ToLCNDV-ES (Sáez et al., 2016) was used as inoculum source. The tissue was mashed in an iced mortar together with

inoculation buffer in a 1:4 (*w*:*v*) proportion (López et al., 2015), and the mix was scrubbed with a cotton swab over one cotyledon and one true leaf, previously dusted with Carborundum 600 mesh. Same number of plants were mock-inoculated following the same protocol but rubbing with only inoculation buffer and Carborundum. Seedlings were growth in a climatic chamber for 20 days after mechanical inoculation (dpi) at 25 °C and 60% of relative humidity, under a photoperiod of 16 h day/8 h night.

Sampling design

Whole plant leaf tissue of six healthy seedlings was sampled just before mechanical inoculation and used as control treatment (0 dpi stage). Same collection was repeated with six mock and virus-inoculated seedlings, respectively, at 3, 6, 9, 12, 15 and 18 dpi. Six different plants were sampled at every stage. Samples were frozen immediately in liquid nitrogen before storage at -80 ºC. Once sampled, all plants were maintained to 20 dpi to test the correct performance of ToLCNDV and mock inoculation, and then, the tissue of the six plants in each time point was pooled in a single sample. All temporal treatments were repeated in three biological replicates and processed independently.

In the RNA-seq assay two biological replicates of 0, 3, 6 and 12 dpi WM-7 and PS mock and inoculated treatments were used, corresponding to 28 samples (Table 1). Results obtained were validated in the third biological replicate by qRT-PCR.

Evaluation of response to ToLCNDV

Symptoms of ToLCNDV were assessed in all plants at each sampling stage and at 20 dpi, according with the visual scale described in López et al. (2015), where score 0 means absence of symptoms and score 4 highly severe symptoms displayed. To verify presence or absence of ToLCNDV infection in all plants at 20 dpi, *tissue printing* hybridation by dygoxigenin-labelled ToLCNDV-RNA probe was performed following the procedure described by Sánchez-Navarro et al. (1999).

Quantitative PCR (qPCR) was used to determine the viral titers evolution at 3, 6 and 12 dpi. Total DNA was extracted from each pooled sample of inoculated WM-7 and PS accessions in the three biological replicates using the Cetyltrimethyl ammonium bromide (CTAB) method (Doyle & Doyle 1990). ToLCNDV amount was analyzed by qPCR in two technical replicates, following the same procedure described in Sáez et al. (2017).

RNA extraction

The 28 samples of processed leaves tissue were sent to the Biomarker Technologies Co. Ltd (Beijing) for cDNA library construction.

Total RNA intended to sequencing was extracted using a phenol–chloroform method and DNAse treatment was performed on 6 µg of total RNA following the manufacturer's protocol (TURBO DNA-free™ kit, Ambion, Life technologies). RNA was purified with RNeasy Kit columns (Qiagen) and quality was tested for a minimum RNA integrity number (RIN) score of 7 using a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). 500 ng total RNA were used to prepare RNA-seq libraries using the TruSeq Stranded mRNA Library Prep Kit (Illumina) according to the manufacturer's protocol.

To qRT-PCR validation, total RNA was isolated using 700 µL of Extrazol® EM30–200 (Blirt DNA, Gdansk, Poland), according to manufacturer's specifications. Integrity was checked by 1 % agarose gel electrophoresis and purity and quantity was determined spectrophotometrically at 260 and 280 nm wavelengths using a NanoDrop 1000 (Thermo Scientific, Waltham, MA, USA). Total RNA (1 µg) treated with PerfeCTa® DNase I (RNase-free) (Quanta Biosciences, Gaithersburg, MD, USA) was used as template with the RevertAid™ First Strand cDNA Synthesis Kit (ThermoFisher Scientific) with Oligo dT primers.

RNAseq data analysis and evaluation of expression differences

After three HiSeq 2500 Illumina (Illumina, CA, USA) sequencing rounds (single-end 50 pb), data files were filtered by Trimmomatic (Bolger et al., 2014) to

remove adapters and low quality reads. Quality of clean reads was checked by FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) (Andrews, 2010).

Trimmed reads were aligned to the last version of melon reference genome (v4.0), recently updated and publicly available at melonomics.net website (Castanera et al., 2020), using STAR (Spliced Transcripts Alignment to a Reference) (Version 2.02.01) (Dobin et al., 2012). This tool is specific for RNA-seq assays and employ genomic annotation (v4.0) to selective map reads against exonic regions (Schaarschmidt et al., 2020). Subsequently, RSEM (version 1.3.1) (RNA-Seq by Expectation Maximization) was used to quantify the abundance of transcript reads assigned to each one out the 28.299 genes included in the complete genome annotation, released on the genome assembly v4.0 (Li et al., 2011). Detection of differentially expressed genes (DEGs) was assessed by DESeq2 package (version 1.26.0) (Love et al., 2014), comparing raw counts between samples at 3, 6 or 12 dpi against 0 dpi. DEGs with adjusted *p-values* under 0.05 and |log2 fold changes ≥ 1| were considered as significant. Additionally, transcriptional changes comparing inoculated and mock treatments at the same post inoculation date were considered when the difference between their respective |log2 fold change| values were over 1.5.

DEGs with log2 fold changes ≥ 1 at 3, 6 and 12 dpi were considered as "up-regulated", while those with log2 fold changes ≤ -1 were "down-regulated".

Common DEGs at the different stages of the disease and those shared between genotypes were represented by Venn Diagrams, displayed with jvenn tool (Bardou e al., 2014), freely available at http://bioinfo.genotoul.fr/jvenn.

The Cucurbits Genomics Database (CuGenDB, http://cucurbitgenomics.org/) was employed to determine biological functions and pathways of the identified DEGs. GO term enrichment analysis was performed unploading DEGs lists, considering significantly enriched those with padj < 0.05.

Validation of DEGs by qPCR

To validate the RNA-seq results, quantitative real-time PCR (qRT-PCR) was conducted on five candidate DEGs suspected to be involved in the resistance to ToLCNDV.

PCR reactions were performed on a Roche LightCycler1480 RT-PCR System (Roche Diagnostics, Rotkreuz, Switzerland) using the The FastStart Essential DNA Green Master (Roche Molecular Systems, Rotkreuz, Switzerland). Each qPCR reaction contained 1.5 µL of cDNA, 7.5 µL of FastStart Essential DNA Green Master (Roche Diagnostics, Rotkreuz, Switzerland) and 1.5 µL (10 µM) of each primer and of H_2O to a final volume of 15 µL. Primers design was made using Primer 3Pus software (Untergasser et al., 2007) and listed in Supplementary file 1. Gene expression analysis was conducted on all WM-7 and PS samples obtained at 0, 3, 6 and 12 dpi. Three technical replicates were set up. Expression levels were analyzed using relative quantitative accumulation by ΔΔCt method (González-Ibeas et al., 2007). As endogenous controls the melon Peptidyl-prolyl cis-trans isomerase gene (*Cyclophilin* CYP7, MELO3C025848.2) (González-Ibeas et al., 2007), was used to reference the level expression of each gene in every condition.

A specific fragment was amplified in each reaction using the following conditions: 95 °C for 5 min, followed by 40 cycles of 95°C for 15 sec, 60 °C for 30 sec, and 72 °C for 15 sec. A melting curve (60 °C-95 °C) was obtained after the amplification cycles to check specificity of the reaction.

RESULTS

Assessment of WM-7 and PS response to ToLCNDV infection

All the assayed WM-7 plants inoculated with ToLCNDV remained resistant to the end of the assay with symptoms scores between 0 and 1 (Figure 1A). Conversely, early mild symptoms (score 2) were identified in PS at 6 dpi in a small number of plants. This score increased at 9 dpi. At 12, 15, and 20 dpi all PS plants showed

severe symptoms (scores from 2 to 4) including mosaic, curling and yellowing (Figure 1A).

Qualitative viral titers at 20 dpi were determined by tissue-print hybridization (data not shown). No signal was detected in control and mock inoculated WM-7 and PS plants, whereas in all PS infected plants there was a high level of ToLCNDV. Only, some WM-7 plants shown low viral titers.

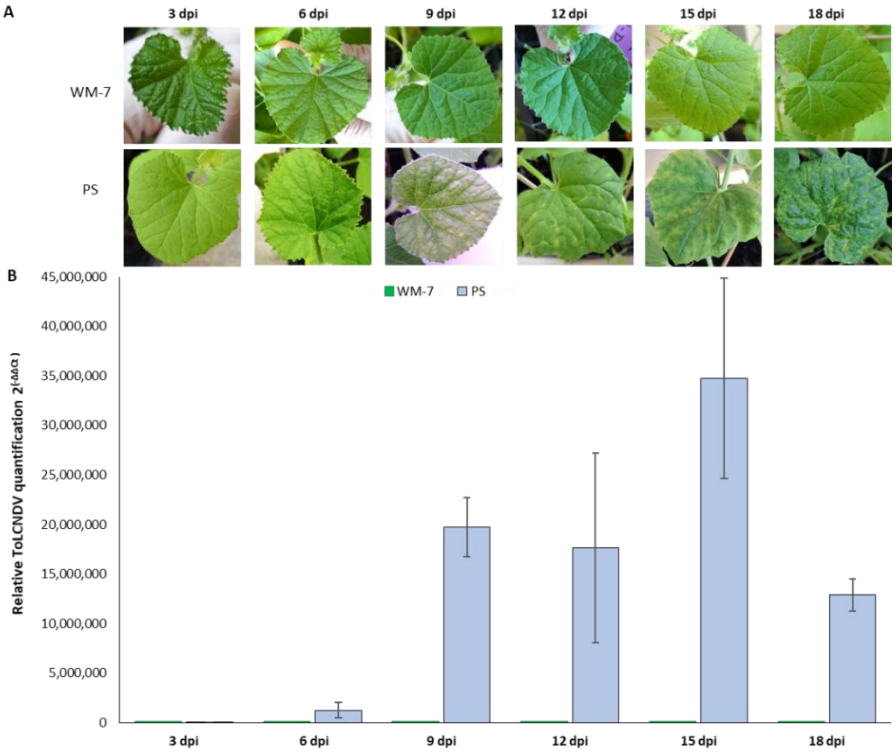

Figure 1. Assessment of WM-7 and PS response to ToLCNDV-ES infection. Temporal evolution of symptomatology **(A),** and mean of viral titers (**B**) in WM-7 and PS.

To quantify the relative viral accumulation of ToLCNDV in both WM-7 and PS infected treatments (mean of three biological replicates), a qPCR assay was performed, using healthy plants (0 dpi) as controls. ToLCDNV was identified at all stages in both genotypes, unless at 0 dpi. Viral accumulation was similarly low in WM-7 and PS at the early stage of 3 dpi, but significant differences were observed in the course of disease (Figure 1B). Although WM-7 viral accumulation levels

remained low to the end of the assay, viral load in PS continued increasing at 6 dpi to reach high levels at 9 dpi, achieving the highest accumulation at 15 dpi (Figure 1B).

According to these results, ToLCNDV was capable of replicating in both resistant and susceptible genotypes at early stages. Between 3 and 6 dpi, virus transcription and/or movement were suppressed in WM-7, while in PS the infection become systemic after 6 dpi.

RNA sequencing and reads alignment and differentially expressed genes

Twenty-eight libraries were sequenced, generating almost 2,600 million high quality trimmed reads. On average, 95% of the clean reads were mapped to the melon reference genome (v4.0) in only one *locus* (Table 1). The sample (WM7_B3dpi_Rep2) presented a 58.95 % of reads uniquely mapped, consequence of ribosomal RNA excess in this sample. It was corrected by STAR outfilter multimap nmax function, selecting only those reads with single mapping (Table 1).

In general, deregulated gene expression in PS was higher than in WM-7 at the same periods, with 2,137 and 1,204 DEGs, respectively, during ToLCNDV infection. Number of final genes considered as DEGs at 3, 6 and 12 dpi to PS and WM-7 is shown in Table 1, and complete lists are located at Supplementary files 2 and 3, respectively.

At each time point, DEGs were visualized by volcano plots (Figure 2), representing up and down regulated genes to each genotype. Lowest number of DEG was observed at 3 dpi to both WM-7 and PS, in a similar level. At this stage, all plants of the resistant and susceptible genotypes were asymptomatic and with slight viral titers. The number of up-regulated genes in PS plants was increased as infection progressed (159, 312 and 800 up-regulated genes at 3, 6 and 12 dpi, respectively), following the same trend than symptoms development and viral amounts. At 12 dpi, when most of the PS plants were symptomatic, a very high number of genes were overexpressed. Instead, the highest number of down-regulated genes was detected at

6 dpi in this accession (245, 440 and 181 down-regulated genes at 3, 6 and 12 dpi, respectively).

Similarly, the amount of genes with altered expression pattern in WM-7 was increased over time, but with humbler and more balanced levels (146, 222 and 343 DEGs were up–regulated, and 146, 132 and 215 DEGs were down-regulated, at 3, 6 and 12 dpi, respectively). Therefore, ToLCNDV infection generate higher transcriptional deregulation in the susceptible than in the resistant genotype.

Table 1. Summary of the RNA-Seq experimental design, reads alignment and DEGs. Assigned name to each sample, their temporal and inoculation treatment and replicates performed are shown. Proportion of uniquely clean reads mapped against the reference melon genome (v4.0), and differentially expressed genes comparing firstly with 0 dpi control (stage column), and then filtering after compare mock and inoculated treatments (Inoculation column).

Sample name	Inoculation treatment	Time point	Replicate	Cleaned reads	Mapped reads	% Mapped reads	DEGs	
							Stage[a]	Inoculation[b]
WM-7_0dpi_1	Not inoculated	0 dpi	1	96,571,093	92,933,140	96.23	-	-
WM-7_0dpi_2		0 dpi	2	89,738,162	86,520,235	96.41		
WM-7_I3dpi_1	ToLCNDV inoculated	3 dpi	1	96,017,670	92,139,780	95.96	6009	292
WM-7_I3dpi_2		3 dpi	2	90,412,987	87,094,110	96.33		
WM-7_B3dpi_1	Mock inoculated	3 dpi	1	97,337,221	93,893,779	96.46	6002	
WM-7_B3dpi_2		3 dpi	2	90,083,950	53,101,818	58.95		
WM-7_I6dpi_1	ToLCNDV inoculated	6 dpi	1	95,502,062	88,222,091	92.38	4816	354
WM-7_I6dpi_2		6 dpi	2	89,625,183	74,603,092	83.24		
WM-7_B6dpi_1	Mock inoculated	6 dpi	1	96,044,324	92,625,513	96.44	5207	
WM-7_B6dpi_2		6 dpi	2	90,375,930	86,463,397	95.67		
WM-7_I12dpi_1	ToLCNDV inoculated	12 dpi	1	96,637,024	92,925,369	96.16	4976	558
WM-7_I12dpi_2		12 dpi	2	90,324,017	86,939,559	96.25		
WM-7_B12dpi_1	Mock inoculated	12 dpi	1	97,444,807	94,078,242	96.55	5296	
WM-7_B12dpi_2		12 dpi	2	91,063,342	87,570,888	96.16		
PS_0dpi_1	Not inoculated	0 dpi	1	102,027,470	95,339,552	93.44	-	-
PS_0dpi_2		0 dpi	2	90,578,076	87,236,559	96.31		
PS_I3dpi_1	ToLCNDV inoculated	3 dpi	1	95,503,001	91,778,653	96.1	6746	404
PS_I3dpi_2		3 dpi	2	60,411,004	57,587,132	95.33		
PS_B3dpi_1	Mock inoculated	3 dpi	1	97,063,008	93,062,108	95.88	6674	
PS_B3dpi_2		3 dpi	2	92,073,644	88,433,510	96.05		
PS_I6dpi_1	ToLCNDV inoculated	6 dpi	1	96,678,397	92,088,929	95.25	6229	752
PS_I6dpi_2		6 dpi	2	93,655,611	89,233,728	95.28		
PS_B6dpi_1	Mock inoculated	6 dpi	1	96,653,614	92,903,742	96.12	5401	
PS_B6dpi_2		6 dpi	2	91,746,137	88,232,317	96.17		
PS_I12dpi_1	ToLCNDV inoculated	12 dpi	1	95,935,797	92,131,302	96.03	6789	981
PS_I12dpi_2		12 dpi	2	91,460,724	87,827,401	96.03		
PS_B12dpi_1	Mock inoculated	12 dpi	1	96,368,610	92,693,418	96.19	5992	
PS_B12dpi_2		12 dpi	2	89,485,860	85,736,511	95.81		

[a]*Stage*: DEGs comparing treatments 3, 6 or 12 dpi to 0 dpi, with not filtering criteria applied.
[b]*Inoculation*: DEGs applying all filtering criteria and comparing mock and ToLCNDV inoculated treatments.

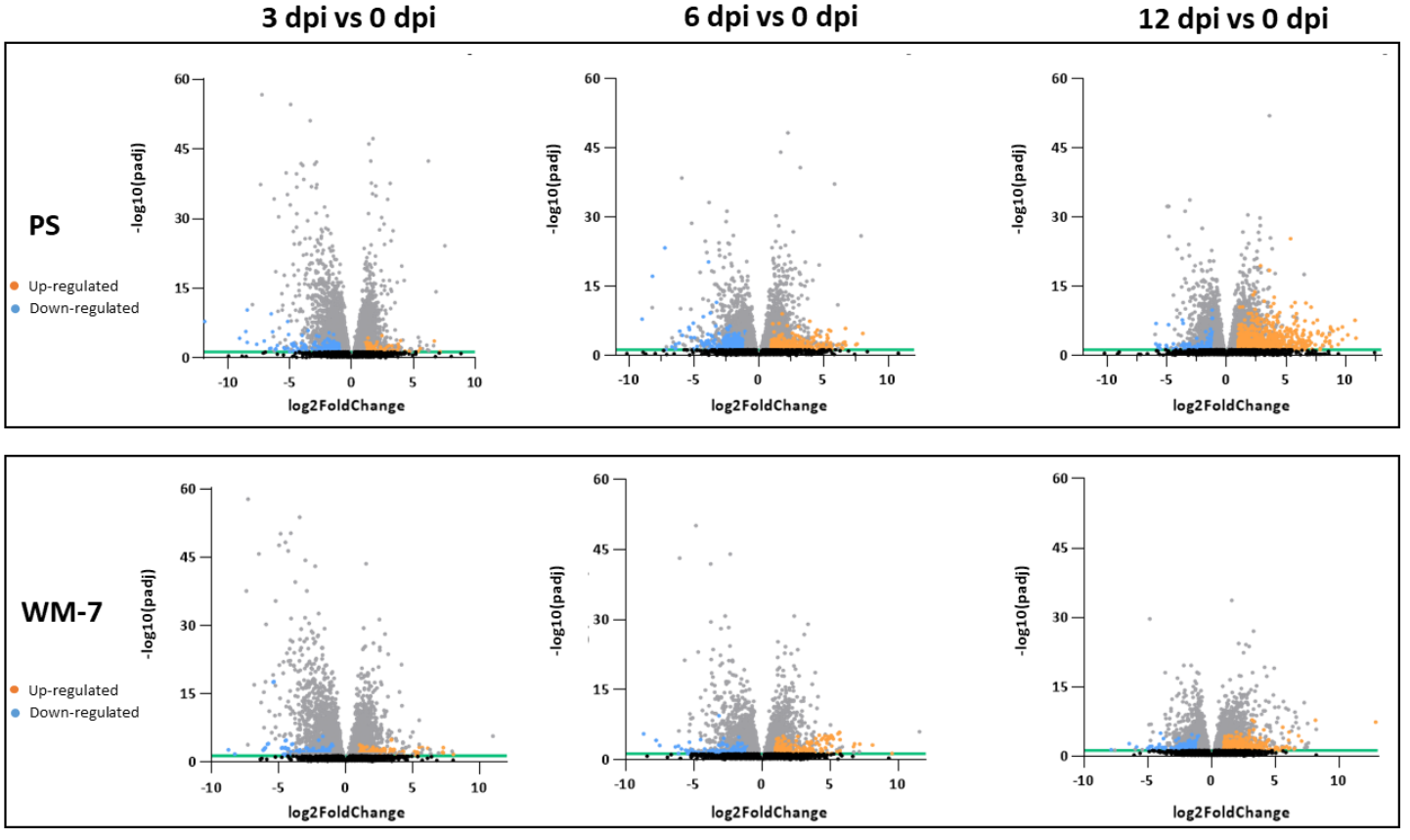

Figure 2. Volcano plots display DEGs distribution for each time point at 3, 6 and 12 dpi, in both PS and WM-7. Orange dots represent the up-regulated genes and blue dots down-regulated genes. X-axis correspond to log2 (fold change) and y-axis to -log10 (*p-adjusted*). Gray dots represent those DEGs not considered significant with *p-adjusted* ≥ 0.05 (green line shows the cut-off) or differing from mock inoculated treatments in 1.5 < log2 (fold change) >1.5.

Common DEGs between genotypes and time stages

PS and WM-7 shared 328 DEGs during ToLCNDV infection (Figure 3A). Interestingly, 56 of these DEGs had an opposite expression pattern, being 26 overexpressed in the susceptible genotype and repressed in the resistance one, and 30 up-regulated in WM-7 but down-regulated in PS (Figure 3A, Supplementary file 4). These common DEGs include genes related with resistance to pathogens, suggesting that there is a group of genes directly implicated in the different phenotypic responses to ToLCNDV. Resistance or susceptibility could be determined by the transcription activation or suppression of these genes. Another set of genes were deregulated in only one of both genotypes (721 in WM-7 and 1,531 in PS).

There were nine DEGs in WM-7 deregulated at the three stages evaluated (Figure 3B, Supplementary file 5), seven up-regulated and two down. A multiprotein-bridging factor 1c (MELO3C004553.2) in chromosome 5 was the gene with highest differences at 3, 6 and 12 dpi (log2 (Fold Change) of 3.525, 3.419 and 4.071, respectively), with no variation in those mock inoculated plants. Similar response was observed in a gene codifying a Cysteine proteinase inhibitor (MELO3C002921.2) in chromosome 9, whose family has been described conferring resistance to viruses in plants (Gutiérrez-Campos et al., 1999; Gholizadeh et al., 2005). Interestingly, two Cysteine-rich receptor-kinase-like protein genes (CRKs) (MELO3C018796.2 in chromosome 1, and MELO3C002492.2 in chromosome 12) were strongly deregulated at all stages in infected WM-7 plants. CRKs involvement in pathogen resistance and cell death in plants has been well described (Lu et al., 2017; Yadeta et al., 2017; Quezada et al., 2019). Nine genes related with heat response to stress were altered at some of the three stages, and a great number of photosynthetic genes had enhanced expression at 6 and 12 dpi (Supplementary file 2). DNA-directed RNA polymerases have been associated with defense responses (Nenchinov et al., 2016), and a gene encoding this kind of protein (MELO3C027682.2, chromosome 7) was remarkably overexpressed at 6 and 12 dpi (Supplementary file 5).

Likewise, ten genes in PS plants were common DEGs at the three evaluated stages of ToLCNDV disease development (Figure 3B, Supplementary file 6). Expression profile of two of them (MELO3C004399.2 and MELO3C018799.2, in chromosomes 5 and 1, respectively) resulted especially engaging, as they were highly down-regulated at 3 dpi but changed to be very overexpressed at 6 and 12 dpi, when this genotype develops symptoms and systemic infection of the virus. Interestingly, these genes are also annotated codifying ACCELERATED CELL DEATH 6-like and Cysteine-rich receptor-like kinase proteins, same functions described above in WM-7.

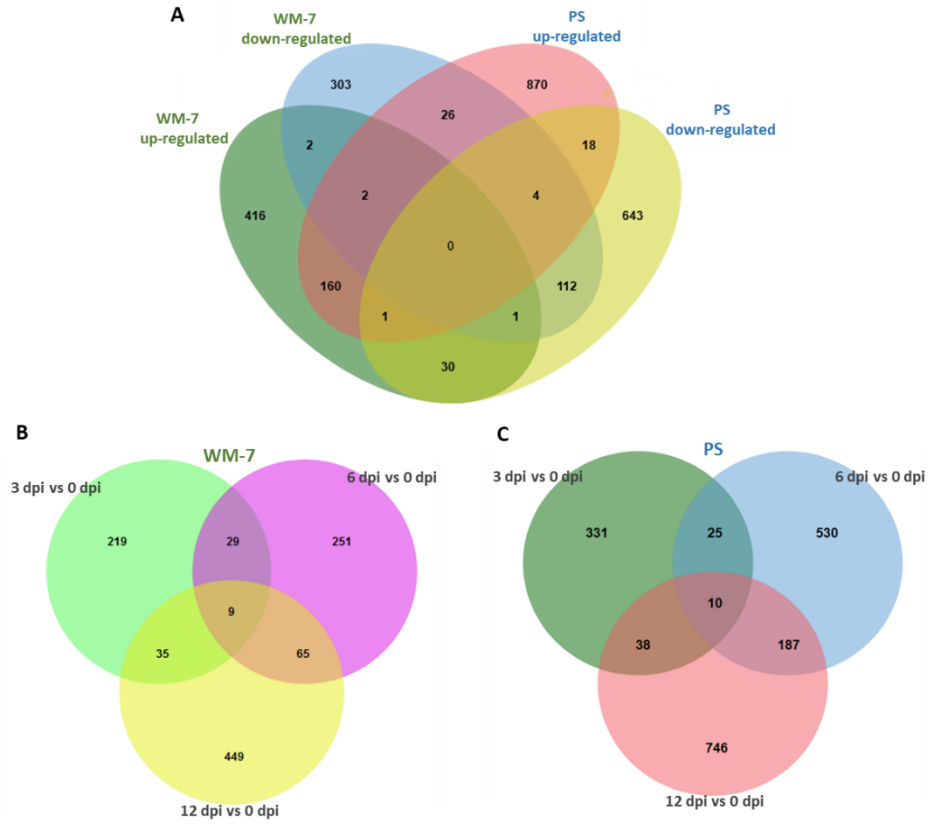

Figure 3. Venn diagrams representing common and specific up and down DEGs identified in WM-7 and PS (A); common and specific DEGs at 3, 6, and 12 dpi in WM-7 (B) and PS (C).

Functional classification of DEGs by GO enrichment

Gene ontology enrichment was used to analyze and functionally group the up and down-regulated genes identified in WM-7 and PS. Classification was made in three ontological categories: biological processes, cellular components, and molecular function.

We identified GO terms shared between the resistant and susceptible genotypes (Figure 4). Among the biological process group, those terms involved in chromosome organization and replication, metabolism and conformation of DNA, contained DEGs that were induced at 3 dpi in PS but were repressed in WM-7 at 12 dpi. In cellular component category, among the up-regulated genes, those coding membrane components were the most enriched in PS at 6 and 12 dpi. Although, there was WM-7 membrane genes over expressed too, the number in PS was much greater than in the resistant genotype. A high number of up- regulated genes in WM-7 were represented by intracellular organelles, that were repressed in PS at 6 dpi. Genes codifying proteins integrated in the nucleus or chromosomes were induced in PS at 3 dpi but down-expressed at 6 dpi. By contrast, transcription of these genes in WM-7 was suppressed at 12 dpi. Although less in number represented, genes of the minichromosome maintenance protein complex (MCM) were repressed in WM-7 at 12 dpi and up-regulated in PS at 3 dpi. MCM proteins play an important role in replication of DNA and plasmids (Maine et al., 1984; Sinha et al., 1986; Tye, 1999) and have been described interacting with Rep protein of geminiviruses to facilitate their replication (Rizvi et al., 2015).

Examining the GO terms concerning molecular components, those related with binding were the most enriched in PS, showing genes with high-altered expression pattern. Few genes related with DNA helicase activity were early induced in PS (3 dpi) but repressed at 12 dpi in WM-7.

In any category was possible to identify common GO term evolving WM-7 DEGs at 3 dpi and DEGs in PS at any stage, suggesting that at early stages in WM-7 act a different genes configuration that is absent in PS.

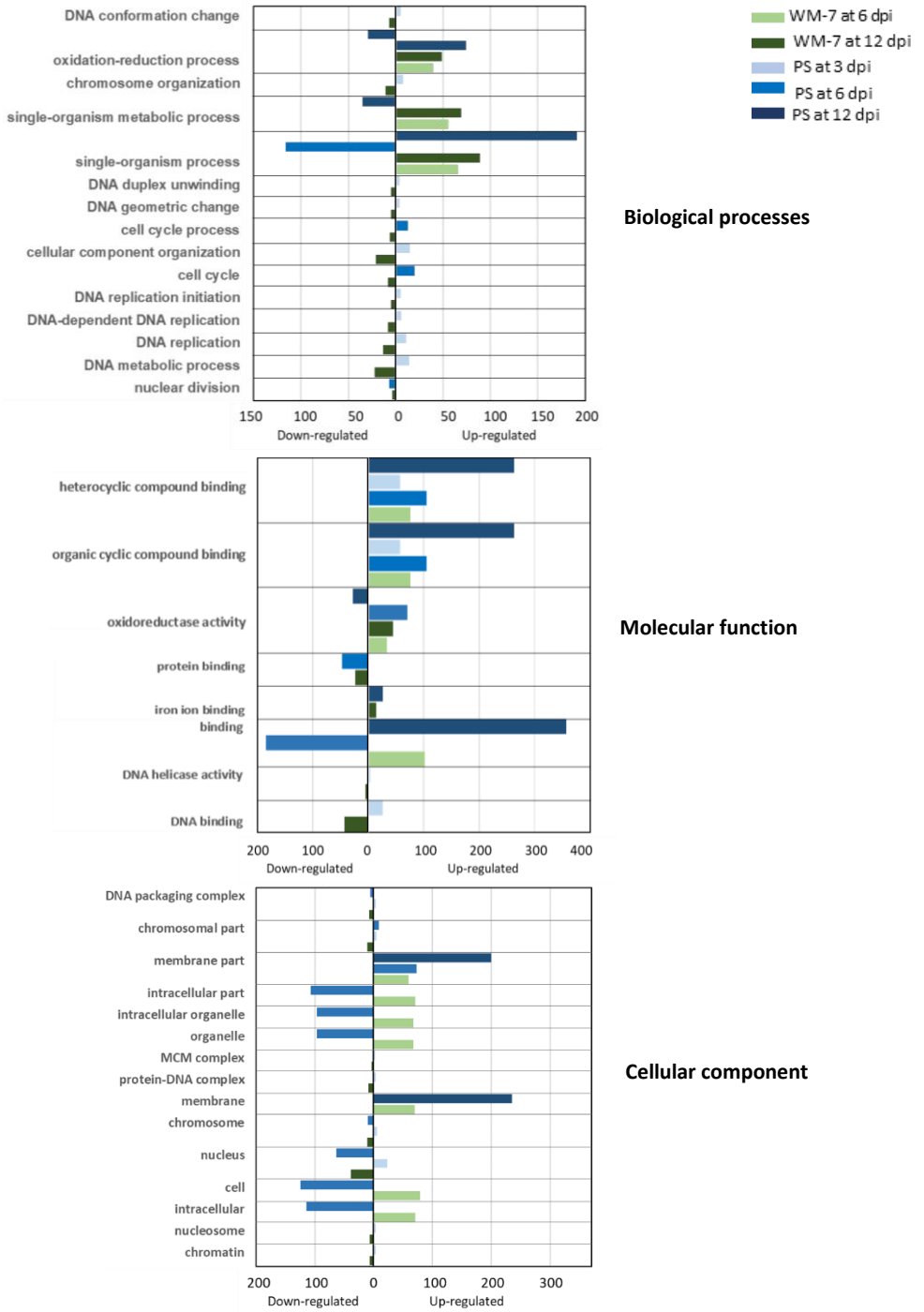

Figure 4. Gene Ontology (GO) classification. The number of up or down-regulated genes assigned to each class were calculated at 3, 6, and 12 dpi to WM-7 and PS and represented in colours according with the legend.

REVIGO (Supek et al., 2011) was used to group similar categories of GO term identified exclusively in WM-7 or PS, but not in common. Scatterplots were constructed to present these results (Figure 5).

Genes related to defense response with altered expression by ToLCNDV infection

To further investigate and identify candidate genes involve in ToLCNDV resistance, we analyzed the expression profile of 70 R-genes of melon genome characterized in Islam et al. (2020), and other categories of genes including gene silencing, pathogen resistant proteins and hormonal response.

R-genes

Among the 70 R-genes set, 19 were DEGs in the susceptible genotype, distributed in seven chromosomes, and three genes were deregulated in chromosomes 4, 5 and 11 of the resistant genotype (Table 2). In PS all DEGs followed the same trend, with repressed expression at 3 dpi but up regulated at 6 and 12 dpi, independently of the chromosome. In WM-7 one R-gene with LRR domain on chromosome 11 was heavily repressed at 6 dpi, near the candidate region of resistance to ToLCNDV-ES described in Sáez et al., (2017).

Expression pattern of additional selected genes with resistance to pathogens annotated function was studied (Table 3). Most of the PS genes were deregulated like R-genes described above, while DEGs in WM-7 follow different deregulation trends. In both genotypes, pathogenesis-related protein 1 (PR-1) family genes were altered. These proteins are the most abundant produced when pathogen infect plants and have been considered as hallmarks of hypersensitive response and broad-spectrum systemic acquired resistance, in association with salicilic acid (SA) (Balint-Kurti et al., 2019; Guerrero et al., 2020).

Some defense related proteins were up-regulated at 6 and 12 dpi in PS, including seven *tobacco mosaic virus* (TMV) resistance genes (chromosomes 5 and 9), several transcription factors as NAC, MYB, bHLH, genes of hormonal response to ethylene and a NEGATIVE REGULATOR OF RESISTANCE protein (Supplementary file 6).

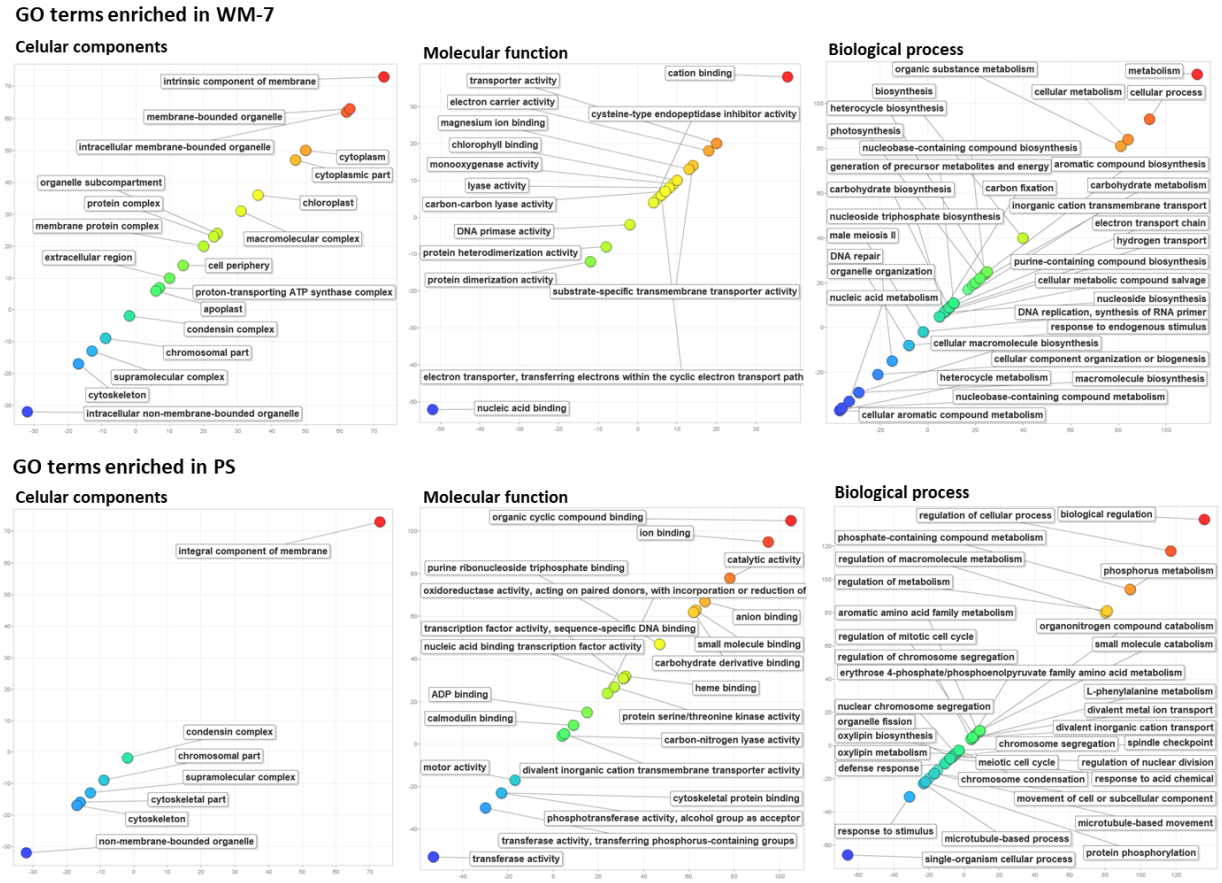

Figure 5. Scatterplot showing grouped GO terms according to the most representative categories. Figures were generated using REVIGO program loading the list of enriched GO terms of WM-7 and PS by separate. Positive and negatives numbers in both axis represent up and down-regulated genes, respectively.

Table 2. Expression profile of the deregulated R-genes characterized in Islam et al. (2020).

	log2 (Fold Change)						Gene				Functional disease resistance-related domains (Nou et al., 2020)	
	3 dpi		6 dpi		12 dpi							
	ToLCNDV inoculated	Mock inoculated	ToLCNDV inoculated	Mock inoculated	ToLCNDV inoculated	Mock inoculated	Chr	Start	End	Function	Domain	Function
PS												
MELO3C023579.2			1.305	-	1.492	-	1	32,573,956	32,576,619	Disease resistance protein RGA2-like isoform X1	LRR	Recognition of pathogen and Plant Defense
MELO3C023578.2	-1.063	-					1	32,588,913	32,593,637	Disease resistance protein	NB-ARC	Molecular switch in activating defenses
MELO3C023441.2	-1.232	-					1	33,623,112	33,627,816	Receptor-kinase, putative	LRR	Recognition of pathogen and Plant
MELO3C010826.2					3.548	-	3	28,214,790	28,218,285	Receptor-kinase, putative	LRR	Recognition of pathogen and Plant Defense
MELO3C010825.2					6.754	4.610	3	28,218,855	28,226,256	Receptor-kinase, putative	LRR	Recognition of pathogen and Plant
MELO3C009693.2			1.112	-	1.407	-	4	28,945,912	28,948,344	Disease resistance protein	NB-ARC	Molecular switch in activating defenses
MELO3C009179.2			1.429	-			4	32,531,221	32,534,345	Receptor-kinase, putative	LRR	Recognition of pathogen and Plant
MELO3C004289.2	-1.145	-			1.062	-	5	25,863,643	25,869,731	TMV resistance protein N-like	TIR-NBS-LRR	Pathogen specificity and defense
MELO3C004303.2			1.697	-	1.664	-	5	26,036,017	26,040,632	TMV resistance protein N-like	LRR	Recognition of pathogen and Plant
MELO3C004311.2			1.560	-	2.700	-	5	26,076,967	26,095,083	TMV resistance protein N-like	TIR-NBS-LRR	Pathogen specificity and defense
MELO3C004313.2			2.177	-	1.771	-	5	26,106,563	26,109,785	TMV resistance protein N-like	TIR-NBS-LRR	Pathogen specificity and defense
MELO3C017703.2	-1.062	-					7	24,126,814	24,129,634	Disease resistance protein RGA2-like	NB-ARC	Molecular switch in activatingdefenses
MELO3C022146.2					2.410	-	9	744,523	750,028	TMV resistance protein N-like	TIR-NBS-LRR	Pathogen specificity anddefense
MELO3C022144.2			1.048	-	1.406	-	9	767,040	775,410	TMV resistance protein N-like	TIR-NBS-LRR	Pathogen specificity anddefense
MELO3C005450.2			2.717	-	3.153	-	9	20,427,143	20,430,013	LRR receptor-like kinase family protein	LRR	Recognition of pathogen and Plant Defense
MELO3C005451.2			5.330	-	7.293	3.030	9	20,434,381	20,437,380	LRR receptor-like kinase	LRR	Recognition of pathogen and Plant Defense
MELO3C002506.2			1.722	-	3.179	-	12	22,229,817	22,238,994	Receptor-like protein kinase	RLK	Signaling and plantdefense
MELO3C002504.2	-1.634	-			2.234	-	12	22,242,891	22,252,225	cysteine-rich receptor-like protein kinase 28	RLK	Signaling and plantdefense
MELO3C002501.2					2.578	-	12	22,262,412	22,265,400	cysteine-rich receptor-like protein kinase 26 isoform X1	RLK	Signaling and plantdefense
WM7												
MELO3C009694.2					1.795	-	4	28,938,791	28,941,261	Disease resistance protein	NB-ARC	Molecular switch in activatingdefenses
MELO3C004309.2					2.092	-	5	26,060,336	26,066,739	TMV resistance protein N-like	TIR-NBS-LRR	Pathogen specificity anddefense
MELO3C022449.2			-4.838	-			11	31,293,359	31,296,020	Receptor-like protein	LRR	Recognition of pathogen and Plant Defense

TIR-NBS-LRR: Toll/interleukin-1 receptor homology nucleotide-binding site leucine-rich repeat
LRR: Leucine-rich repeat
NB-ARC: Nucleotide-binding adaptor shared by APAF-1, R proteins and CED-4
RLK: Protein kinase

Table 3. Expression pattern of additional selected genes with resistance to pathogens**.**

	log2 (Fold Change)						Gene			
	3 dpi		6 dpi		12 dpi					
	ToLCNDV inoculated	Mock inoculated	ToLCNDV inoculated	Mock inoculated	ToLCNDV inoculated	Mock inoculated	Chr	Start	End	Function
PS										
MELO3C018539.2					6.232	-	1	1,023,454	1,024,330	Pathogenesis-related protein 1-like
MELO3C018540.2					4.757	-	1	1,030,810	1,031,348	pathogenesis-related protein 1-like
MELO3C018544.2			1.148	-			1	1,042,545	1,043,212	Pathogenesis-related protein 1
MELO3C018547.2					2.416	-	1	1,051,651	1,052,250	Pathogenesis-related protein 1
MELO3C018878.2					3.820	-	1	3,559,447	3,560,191	Pathogen-induced protein CuPi1
MELO3C023694.2					3.590	-	1	6,394,591	6,396,233	Pathogen-related protein
MELO3C023578.2	-1.063	-					1	32,588,913	32,593,637	Disease resistance protein
MELO3C017322.2	-1.803	-					2	24,275,353	24,280,275	LEAF RUST 10 DISEASE-RESISTANCE LOCUS RECEPTOR-LIKE PROTEIN KINASE-like 1.2 isoform X2
MELO3C008149.2			1.310	-	2.130	-	3	1,163,259	1,172,229	LEAF RUST 10 DISEASE-RESISTANCE LOCUS RECEPTOR-LIKE PROTEIN KINASE-like 1.4 isoform X1
MELO3C031443.2					1.049	-	5	18,385,406	18,392,554	TMV resistance protein N
MELO3C004262.2			4.967	-	7.110	-	5	25,614,803	25,621,533	TMV resistance protein N-like
MELO3C004265.2					-3.999	-	5	25,650,358	25,651,288	TMV resistance protein N-like isoform X2
MELO3C004291.2			3.994	-	5.272	-	5	25,906,332	25,908,625	TMV resistance protein N-like
MELO3C031332.2	6.714	4.701					5	26,375,103	26,376,034	Disease resistance protein
MELO3C004354.2			1.032	-	1.137	-	5	26,592,987	26,596,766	CC-NBS-LRR resistance protein
MELO3C004385.2					5.488	-	5	26,848,627	26,849,611	Pathogenesis-related protein PR-4-like
MELO3C019482.2			-1.096	-			6	10,571,172	10,573,959	Pathogenesis-related thaumatin-like protein
MELO3C016941.2					1.139	-	7	947,005	948,144	Protein ENHANCED DISEASE RESISTANCE 2
MELO3C017692.2					4.161	-	7	24,081,397	24,084,555	Disease resistance protein RGA2-like

Table 3. Continued.

MELO3C017703.2	-1.062	-					7	24,126,814	24,129,634	Disease resistance protein RGA2-like
MELO3C033615.2			1.350	-	1.808	-	9	6,658,398	6,659,268	Disease resistance protein RGA2-like
MELO3C029858.2	-1.042	-					9	20,748,481	20,749,427	NBS resistance-like protein
MELO3C020880.2			-1.323	-			11	3,294,068	3,294,968	Pathogenesis-related protein 1
MELO3C024731.2					3.364	-	11	7,170,632	7,173,642	Disease resistance protein RGA2-like
MELO3C002084.2			2.412	-	2.657	-	12	24,991,237	24,991,695	Protein NEGATIVE REGULATOR OF RESISTANCE
WM-7										
MELO3C018796.2	-2.636	-			-6.437	-	1	2,864,020	2,864,927	Cysteine-rich receptor-kinase-like protein
MELO3C018540.2	5.297	-			5.506	-	1	1,030,810	1,031,348	Pathogenesis-related protein 1-like
MELO3C018539.2					5.704	-	1	1,023,454	1,024,330	Pathogenesis-related protein 1-like
MELO3C012701.2			1.438	-			1	21,798,030	21,798,830	Cysteine proteinase inhibitor
MELO3C012702.2			1.869	-			1	21,826,195	21,827,118	Cysteine proteinase inhibitor
MELO3C017497.2					5.171	-	2	22,319,516	22,320,537	Pathogenesis-related protein PR-1
MELO3C008149.2					1.128	-	3	1,163,259	1,172,229	LEAF RUST 10 DISEASE-RESISTANCE LOCUS RECEPTOR-LIKE PROTEIN KINASE-like 1.4 isoform X1
MELO3C031111.2	1.548	-	1.533	-			5	7,065,995	7,068,688	Negative regulator of systemic acquired resistance SNI1
MELO3C004261.2					2.559	-	5	25,594,168	25,594,730	TMV resistance protein N-like
MELO3C004309.2			2.056	-	2.092	-	5	26,060,336	26,066,739	LOW QUALITY PROTEIN: TMV resistance protein N-like
MELO3C016128.2			-1.373	-			7	19,211,072	19,212,632	Pathogen-related protein
MELO3C017071.2			1.514	-			7	123,638	125,187	Cysteine/Histidine-rich C1 domain family protein
MELO3C022150.2					2.260	-	9	703,234	708,384	TMV resistance protein N-like
MELO3C033944.2	-3.577	-7.025			-4.180	-	9	16,122,393	16,123,197	NBS-LRR type resistance protein
MELO3C002921.2	1.250	-	1.610	-	1.477	-	9	7,641,728	7,642,167	Cysteine proteinase inhibitor
MELO3C002492.2	4.266	-	3.409	-			12	22,299,334	22,300,624	cysteine-rich receptor-like protein kinase 25

Transcription factors

In geminivirus infections, transcription factors (TFs) involved in plant development are differentially regulated (Kumar, 2019). We have search for DEGs coding TFs and 17 MYB were altered at all stages in both genotypes (Supplementary file 2 and 3). In PS, 23 DEGs were of WRKY type and 16 were NACs TFs, all deregulated at mainly 6 and 12 dpi. Out 16 NAC altered, 12 were up-regulated (Supplementary file 2). Conversely, only four WRKY were deregulated in WM-7, one repressed at 3 dpi (Supplementary file 3). In this genotype there were also three altered NAC TFs, out them 2 were down-regulated (Supplementary file 3). Only one MYC-2 transcription factor (MELO3C022250.2) was suppressed at 6 dpi in chromosome 11 of PS (Supplementary file 2). MYC-2, belongs to the basic helix-loop-helix (bHLH) TFs family, and interacts with the BV1 protein of bipartite begomoviruses (Li et al., 2014). This protein directly regulates terpenes transcription genes, and mediated whitefly resistance achieving vector-virus mutualism. A member of terpene cyclase/mutase genes family (MELO3C022374.2) was strongly suppressed in PS at 6 dpi but highly induced in WM-7 at 12 dpi (Supplementary file 4). Similarly, 11 DEGs were bHLH in PS at 6 or 12 dpi (7 down regulated). In WM-7 plants six bHLH genes were identified, all up-regulated when compare with healthy controls. Therefore, ToLCNDV-ES generates a stronger readjustment of TFs expression in PS than in WM-7.

Hormones

Lipoxygenases (LOX) are essential enzymes required in the jasmonic acid (JA) hormone synthesis (Wastenack & Song, 2017), and associated to systemic defense, hypersensitive response and cell death when pathogens infect plants (Hwang & Hwang, 2010, Vicente et al., 2012). LOX genes induction or deregulation has been reported after geminivirus infection (Góngora-Castillo et al., 2012; Allie et al., 2013). In the susceptible and resistant genotypes, 13 and 6 LOXs were deregulated at all stages, respectively. Interestingly, all the genes coding these proteins were located into two clusters in chromosome 5 (Supplementary files 2 and 3). Among them, four were common DEGs to PS and WM-7 (MELO3C004244.2,

MELO3C014630.2, MELO3C027325.2 and MELO3C031318.2) (Supplementary file 4), and were early induced in the resistant genotype but suppressed in the susceptible melon.

SA and JA pathways promote resistance to begomoviruses but also to heat stress, and both responses are mediated by common genes families (Tsai et al., 2019). Geminivirus coat protein interacts with Heat Shok Proteins (HSPs) and recruits them to their own viral regulation, interfering with host antiviral response (Gorovits et al., 2013; Jeevalatha et al., 2017, Gorovits et al., 2019; Kumar, 2019). HSPs downregulation in resistant plants restricts begomovirus movement in plasmodesmata (Naqvi et al., 2017). However, out 19 and 32 HSPs deregulated in PS and WM-7, 17 and 30 had a strong induction, respectively (Supplementary files 2 and 3).

Some defense related proteins were up-regulated at 6 and 12 dpi in PS, including genes of hormonal response to ethylene and a NEGATIVE REGULATOR OF RESISTANCE protein (Supplementary file 6).

Ubiquitination and UPS complex

In plants, ubiquitination contributes to resistance geminivirus interactions (Czosnek et al., 2013). RING-type E3 ubiquitin ligase and F-box genes are components of proteasomal ubiquitination complex (UP) and have been described interacting during begomovirus infection (Correa et al., 2013). Nine genes RING-type E3 ubiquitin ligase were altered in PS, five of them down-regulated and four induced, while only one RING-type E3 ubiquitin transferase (MELO3C003458.2) was repressed in WM-7 (Supplementary files 2 and 3). F-box genes were altered in both susceptible and resistant genotypes. In PS, eight F-box genes were induced and five under-expressed, and similarly, three F-box genes had repressed expression in WM-7 and four were induced (Supplementary files 2 and 3). F-box proteins regulate diverse cellular processes, including cell cycle transition, transcriptional regulation and signal transduction, which mediates ubiquitination of proteins targeted for degradation by the proteasome.

RNA Silencing

As RNA silencing constitute one of the major strategies to develop resistance response against geminiviruses, we search for DEGs related to RNA silencing.

Calmodulin proteins regulate RNA silencing machinery, and their induction increase geminivirus accumulation in plants (Chung et al., 2014). Calmodulin proteins can reduce the expression of RNA-Dependent-RNA-Polymerases (RDRs) proteins and interact with Suppressor of Gene Silencing 3 (SGS3) and degrade it by autophagy. We detected four calmodulin DEGs down-regulated at 3 dpi in PS and 4 up-regulated at 6 and 12 dpi. In WM-7 just one calmodulin gene was induced at 12 dpi (Supplementary files 2 and 3). One autophagy protein (MELO3C031521.2) was also up-regulated in WM-7 at 12 dpi.

Expression of two cytosine-specific methyltransferases (CMT3), implicated in transcriptional gene silencing (TGS), was deregulated in PS and WM-7 (Supplementary files 2 and 3). In the susceptible genotype, the gene MELO3C015649.2 of chromosome 2 encodes this kind of enzyme and was repressed at 6 dpi. Conversely, a gene with similar function (MELO3C026448.2, chromosome 10) was early induced at 3 dpi in WM-7. CMT3 also interact with autophagy and ubiquitin pathways (You et al., 2019). Two cytosine-specific methyltransferases are also deregulated, one induced in WM-7 at 3 dpi (MELO3C026448.2) and one repressed in PS at 6 dpi (MELO3C015649.2) (Supplementary files 2 and 3). These enzymes have homology with MET1, also a methyltransferase involved in TGS.

Chromatin and histone methylation are mechanisms used by plants to regulates gene expression of invasive viral DNAs (Castillo-González et al., 2015). A histone-lysine N-methyltransferases (MELO3C025676.2) was induced in PS at 3 dpi, whereas same gene was repressed in WM-7 at 12 dpi (Supplementary file 4). In the resistant WM-7 genotype (Supplementary file 3), two additional Histone-lysine N-methyltransferases genes were one induced (MELO3C011304.2) and the second repressed (MELO3C012115.2) at 12 dpi. A histone acetyltransferase coding gene (MELO3C011266.2) was also repressed at 3 dpi in this genotype. Additionally, in

the course of assay, seven genes codifying for histones appeared down-regulated and two induced in this resistance accession. In PS (Supplementary file 2), MELO3C022387.2 another histone-lysine N-methyltransferase is induced at 3 dpi in chromosome 11. A histone acetyltransferase and a histone demethylase (MELO3C018028.2 and MELO3C017723.2, respectively) were also repressed in this genotype at 6 dpi and 10 genes coding histones were deregulated, five induced and five under-expressed. Reduction in transcripts levels of genes related to histone have been related with suppression of chromatin organization and DNA methylation (Choi et al., 2015).

AGO4 reduce geminivirus infection by viral DNA methylation (Mallory & Vaucheret, 2010), but this protein may be also recruited by geminiviruses to enhance its transcription (Vinutha et al., 2018). ToLCNDV AC4 protein suppresses RNA silencing by interaction with host argonaute 4 protein (AGO4) of tomato (Vinutha et al., 2018). Its orthologue protein in melon (MELO3C014440.2) was up regulated at 3 dpi in both, susceptible and resistant genotypes.

RNA-Dependent-RNA-polymerases (RDRs) play a key role impairing resistance to geminiviruses amplifying RNA antiviral silencing (Prakash et al., 2020). In melon genome, there are eight genes functionally annotated as RDRs and distributed by chromosomes 2, 9 and 10. Two of them were higly up regulated at 6 and 12 dpi in chromosome 9 of PS (MELO3C005284.2 and MELO3C005257.2). An additional one (MELO3C015406.2, chromosome 2) was also induced in this genotype at only 12 dpi. Changes of transcripts level in these genes were not observed in the resistant to ToLCNDV genotype WM-7.

Numerous genes coding polymerases enzymes were deregulated in both resistant and susceptible genotypes (Supplementaty files 2 and 3), but a DNA-directed RNA polymerase gene (MELO3C027682.2) was induced at 6 and 12 dpi in WM-7 genotype.

Analysis of DEGs in the candidate regions for ToLCNDV-ES resistance

We focused our expression analyses on those genes included in the three candidate regions of the *C. melo* genome conferring resistance to ToLCNDV-ES (Sáez et al., 2017).

Transcription changes in chromosome 11

The main locus involved in the resistance identified in WM-7 was located in chromosome 11, between 30,112,560 bp and 30,737,924 bp. On this interval, seven genes were deregulated in the PS and three in WM-7 (Table 4).

Table 4. DEGs in the candidate region for ToLCNDV resistance of *C. melo* chromosome 11.

		log2 (Fold Change)					
Gen	**Stage**	**ToLNDV**	**Mock**	**Chr**	**Start**	**End**	**Function**
PS							
MELO3C022339.2	6 dpi	-3.080	-	11	30,494,799	30,495,239	Glutaredoxin
MELO3C022300.2	12 dpi	2.179	-	11	30,156,612	30,160,071	probably inactive receptor-like protein kinase At2g46850
MELO3C022324.2	12 dpi	1.888	-	11	30,394,274	30,397,789	proton pump-interactor 2-like
MELO3C022341.2	12 dpi	10.806	-	11	30,509,871	30,512,205	Bidirectional sugar transporter SWEET
MELO3C022358.2	12 dpi	1.451	-	11	30,691,737	30,692,270	ethylene-responsive transcription factor ERF113-like
MELO3C022365.2	12 dpi	1.743	-	11	30,715,606	30,716,293	UPF0481 protein
MELO3C022367.2	12 dpi	1.168	-	11	30,725,006	30,743,460	UPF0481 protein At3g47200
WM-7							
MELO3C022327.2	3 dpi	-2.809	-	11	30,409,446	30,410,934	Transmembrane protein, putative
MELO3C022337.2	3 dpi	-1.075	-	11	30,481,662	30,482,590	auxin-responsive protein SAUR36
MELO3C022327.2	6 dpi	-3.596	-	11	30,409,446	30,410,934	Transmembrane protein, putative
MELO3C022319.2	12 dpi	-1.075	-	11	30,347,186	30,355,934	DNA primase large subunit

Expression of MELO3C022339.2 gene is suppressed in PS at 6 dpi. This gene code for a glutaredoxin protein, which has been described interacting with JA-SA pathway (Li et al., 2019). At 12 dpi, in this candidate region of the susceptible accession one gene coding a bidirectional sugar transporter SWEET (MELO3C022341.2) was more than ten times induced and a proton pump-interactor 2-like isoform X2 gene (MELO3C022324.2), described as plasma membranes regulator (Bonza et al., 2009), was also over-expressed. Ethylene-responsive transcription factor ERF113-like (MELO3C022358.2) is also up-regulated in the candidate region of PS. Common transcriptional induction was observed in infected

plants with TYLCV in previous works (Wu et al., 2019). The gene MELO3C022300.2, although has an altered level of transcripts, codify a probable inactivated receptor-like protein kinase, not described in defense response. Two uncharacterized proteins where also deregulated in PS in this region (MELO3C022365.2 and MELO3C022367.2).

In WM-7, all DEGs identified in the candidate region for resistance of chromosome 11 were down-regulated (Table 2). One of them (MELO3C022327.2) was repressed at 3 and 6 dpi, and encode a transmembrane protein, putative. Auxin-responsive protein SAUR36 (MELO3C022337.2) was repressed at the beginning of ToLCNDV-ES infection. Auxines and JA pathways are interconnected, and both regulate defense response to begomoviruses (Ramesh et al., 2017). The gene coding a DNA primase large subunit (MELO3C022319.2) was down-regulated in WM-7 at 12 dpi, likely involved in geminivirus replication.

Transcription changes in chromosomes 2 and 12

Candidate regions for ToLCNDV-ES resistance in chromosomes 2 and 12 were less narrowed than region of the major QTL (Sáez e al., 2017), hence, a larger list of DEGs was obtained to both regions (Supplementary file 7).

In chromosome 2, transcription factors and disease proteins were down-regulated in PS at 3 dpi, but up-regulated at 6 and 12 dpi. MELO3C017185.2 is a NAC transcription factor highly induced at 12 dpi in PS, transcription factor bHLH35 (ELO3C017424.2) also induced in PS at 6 and 12 dpi.

In WM-7 photosynthetic proteins were highly induced at 6 and 12 dpi. Modulation of resistance by this locus could be influenced by an enhanced photosynthetic capability in the resistance genotype WM-7. MELO3C017283.2 encode a transmembrane protein in chromosome 2, highly induced at 12 dpi in PS, as well as the up-regulated MELO3C017356.2 at 3 dpi, encoding a phosphoethanolamine n-methyltransferase, implicated in methylation of DNA.

A second QTL linked to ToLCNDV-ES resistance was mapped at the beginning of the chromosome 12 (Sáez et al., 2017) (Supplementary file 7). In this region, a cytochrome P450 gene (MELO3C004742.2) was strongly repressed at 3 dpi in PS, while the gene Photosystem II protein D1 (MELO3C027714.2) located in the same region was over-expressed in WM-7 at 12 dpi. MELO3C021758.2 encodes a DNA-directed RNA polymerase II and IV subunit 5A, and was down regulated in WM-7 at 6 dpi.

Mitogen-activated protein kinases are proteins implicated in the signal pathway of JA biosynthesis and interact with MYC2. Overexpression of these proteins induce SA and JA genes expression and promote TYLCV resistance (Li et al., 2017b). MELO3C026848.2 encode this kind of protein and resulted down-regulated in the susceptible genotype PS at the beginning of infection (3 dpi).

Additionally, candidate region in chromosome 12 include a NAC transcription factor (MELO3C004694.2), highly induced in PS at 12 dpi.

DEGs validation by qPCR

To validate the RNA-Seq data, qRT-PCR was performed for five candidate genes (MELO3C022315.2 coding a MADS-box transcription factor 8-like; MELO3C022322.2 coding a protein LAZY1; MELO3C022327.2 conding a Transmembrane protein and MELO3C022341.2 coding a Bidirectional sugar transporter SWEET; and MELO3C022348.2 coding a Protein TIFY 9;) for resistance to ToLCNDV in chromosome 11 of *C. melo*. Expression pattern in the assayed genes was consistent with those obtained by RNA sequencing (Figure 6).

Bidirectional sugar transporter SWEET (MELO3C022341.2) induced expression in PS was confirmed. Interestingly, MELO3C022348.2, was repressed at 12 dpi in PS. A similar TIFY4B gene acts as a geminiviral resistance factor and was down-regulated in the compatible interaction with begomoviruses (Kumar, 2019). These results must be confirmed in additional replicates and stages.

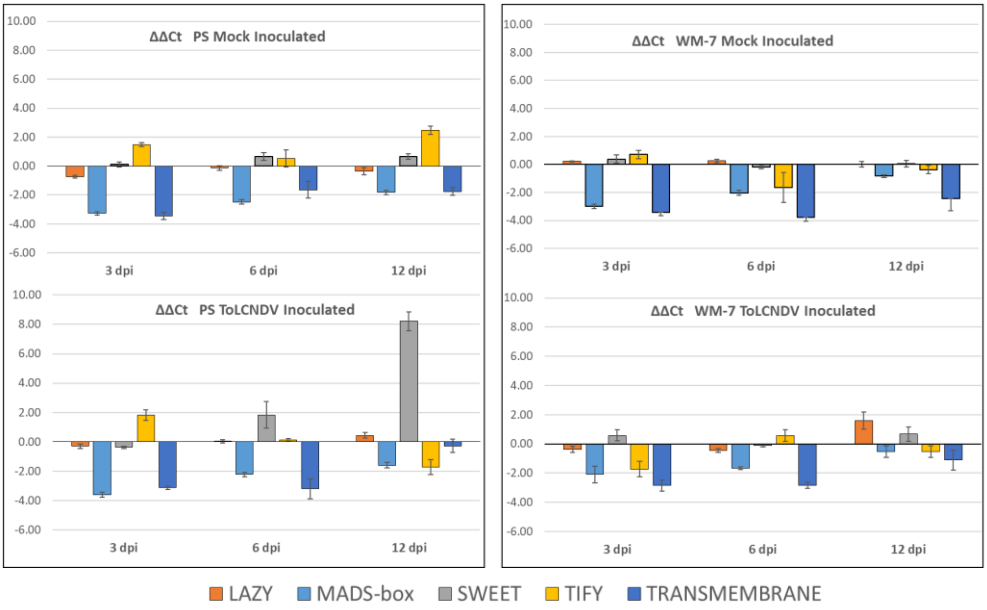

Figure 6. Relative expression (ΔΔCt) of five candidate genes for ToLCNDV resistance.

DISCUSSION

ToLCNDV strains generate devastating damaged in solanaceae and cucurbits crops, mainly in the Indian subcontinent and Mediterranean basin countries. Genetic resistance has been previously identified in different species of both horticultural families, but characterization of the molecular mechanism regulating resistance has been preferentially studied in *Solanaceae* family accessions (Sahu et al., 2010).

In this study, we investigate the process of ToLCNDV infection in a resistant and susceptible genotype of *C. melo*, trying to correlate resistance or susceptibility with changes in transcripts expression pattern, comparing them at different temporal stages. The accession WM-7 was identified as resistant to ToLCNDV, remaining symptomless till 30 days after mechanical inoculation (López et al., 2015; Sáez et al., 2017). In this study we have observed that virus replication is allowed at the beginning of the infection, but virus propagation is impaired by host genetic response. Conversely, systemic infection and symptoms development was increased in PS, where ToLCNDV accumulation was enhanced in the course of disease. The response observed in WM-7 reflect a fast and persistent basal immunity.

After bioinformatics analysis of RNAseq data, a large amount of DEGs were obtained in both resistance and susceptible ToLCNDV inoculated genotypes. The amount of DEGs was increasing with the disease course, and at all stages it was higher in PS than in WM-7, consistently with similar studies comparing response to begomoviruses in resistant and susceptible accessions (Allie et al., 2014; Zaidi et al., 2020). This behavior implied that ToLCNDV infection cause a lower impact and transcriptomic reorganization in WM-7 genotype than in PS.

GO enrichment analyses showed common functional and biological processes altered after ToLCNDV inoculation in WM-7 and PS. Categories related to DNA conformation, replication, unwinding and cell cycle were the most remarkable, as genes included were induced in PS but repressed in WM-7. Geminiviruses require the host factors to their own profit and spread, consequently recruitment and re-localization of host genes involved in DNA replication is a frequent viral strategy to enhance their replication machinery (Preiss & Jeske, 2003; Kushwaha et al., 2017; Maio et al., 2020). Interference in virus replication has been proposed as plant mechanism to confer resistance to begomoviruses (Ullah, 2014). Resistance to *mungbean yellow mosaic India virus* (MYMIV) was molecular and biochemical characterized in *Vigna mungo* (L.) R.Wilczek, revealing that viral DNA replication restriction accounts for resistance (Chakraborty & Newton, 2018). *Tomato yellow leaf curl virus* (TYLCV) induced transcriptional reprogramming in susceptible *Nicotiana benthamiana* plants, promoting DNA transcription genes expression (Wu et al., 2019). In our work, DNA helicase activity and MCM complex are GO categories included in molecular function and cellular component ontologies, respectively, also repressed in WM-7 but induced in PS at 3 dpi.

DNA helicases are required to strand separation during DNA replication. The geminiviral Rep protein is essential for replication initiation and has also helicase activity (Clérot & Bernardi, 2006). Their homologues, RNA helicases, have been described as proteins hijacked by plant viruses to assist their replication (Ranji & Boris-Lawrie, 2010, Sharma & Boris-Lawrie, 2012). During ToLCNDV infection, a

DEAD-box RNA helicase was up-regulated in a tolerant tomato cultivar (Sahu et al., 2010), and its silencing confers susceptibility (Pandey et al., 2019). Although scarce information about host DNA helicases and geminivirus has been published, interaction between geminiviral AC3 protein (Ren) and DNA helicases has been described assisting DNA replication (Praham et al., 2017). Host DNA helicase proteins could interact with the viral proteins to further strengthen the helicase activity of Rep for generate unwinding and initiate the stem-loop formation on the viral DNA (Brister & Muzyczka, 1999, Kazlaukas et al., 2016).

MCM complex consist in 2-7 subunits of proteins with minichromosome maintenance role, with direct implication in the initiation of DNA synthesis at replication origins and putative helicase function in eukaryotes (Forsburg, 2004; Bochman & Schwacha, 2008). Despite Rep helicase activity, there are reports pointing the MCM2 protein role in geminivirus replication efficiency (Rizvi et al., 2015), but molecular mechanisms remain unknown (Cho et al., 2008; Suyal et al., 2013). The 26S proteasomal subunit RPT4a (*SlRPT4*) is a component of ubiquitin/26S proteasome (UPS) pathway and acts into the intergenic regions (DNA-A and DNA-B) of ToLCNDV and inhibits bi-directional transcription of genome (Sahu et al., 2016). In this regard, interference in viral replication and transcription has been widely described conferring resistance to begomoviruses. Most of the functionally described resistance genes to ToLCNDV are involved in DNA replication, in accordance with altered transcriptional pathways identified in this work.

Among GO enrichment in WM-7, those up-regulated genes concerning photosynthesis conformed the most represented categories at 6 and 12 dpi. In plant-virus interactions, the chloroplast is strategically manipulated and damaged by viruses, affecting large proportions of genes that could suppose a valuable source of resistant for plant breeding (Bhattacharyya & Chakraborty, 2018; Rossitto de Marchi et al., 2020; Zhai et al., 2020). Chlorosis and mosaics in *cucumber mosaic virus* (CMV) infected tobacco leaves were associated with down-regulation of

photosynthetic and chloroplastic genes (Mochizuki et al., 2014) and similar genes had a reduced transcription in chlorotic tissues of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) systemically infected with *cucumber green mottle mosaic virus* (CGMMV) (Sun et al., 2019). Among begomoviruses, transcriptional reprogramming caused by TYLCV infection in *N. benthamiana* generated an expression reduction of photosynthesis genetic pathways (Wu et al., 2019). Plant defense against virus infection usually suppose expensive energetic costs, as antiviral RNA silencing. Consequently, multiple and complementary resistance mechanisms are activated to minimize this consumption (Souza et al., 2019). The high induction of chloroplastic and photosynthetic genes detected in WM-7, takes place at the stages when yellowing and mosaics, typical symptoms of begomovirus infection, are developed in PS. These genes could provide additional energy to the resistant plant cells to address the viral attack and avoiding chloroplast damage and leaves symptoms development. Promoting induction of these genes could increase resistance to ToLCNDV and yield traits in melon breeding programs.

Defense genes implicated in plant–pathogen interactions have been studied in this work. Although distributed along the whole melon genome, most of DEGs with described implication in viruses resistance were located in clusters at chromosomes 1, 5 and 9. The two largest NBS-LRR gene clusters in melon are located at chromosomes 5 and 9 where resistance to other cucurbits viruses have been mapped (Morata et al., 2017; Pérez-de-Castro et al., 2020). Chromosome 5 not only included deregulated pathogenesis and R genes, but also lipoxygenases hormones induced in WM-7 and down-regulated in PS. These hormones regulate cell death, jasmonic acid (JA) biosynthetic routes and conduct lipid peroxidation as response to pathogen infection (Hwang & Hwang, 2010). Plant-viruses interaction studies evidence how geminiviruses regulate JA signaling, hijacking implicated genes and increasing susceptibility (Guerrero et al., 2020; Yan & Chie, 2015; Zhang et al., 2017). In this work, we have identified transcriptional changes in JA-genes required for resistance to viruses. MYC-2 transcription factor (MELO3C022250.2), mitogen-activated protein kinases (MELO3C026848.2) and terpene cyclase/mutase genes family

(MELO3C022374.2) are down-regulated in the susceptible genotype PS at the beginning of infection (3 and 6 dpi). Conversely, some of these genes are induced in WM-7 at same stages.

In tomato (*Solanum lycopersicum*), induction of a mitogen-activated protein kinase 3 (*SlMPK3*) enhanced TYLCV resistance by increase of SA/JA-genes expression, as PR-1 or leucine aminopeptidases (Li et al., 2017b). Leucine aminopeptidases control defense and damages machinery in tomato, downstream of JA (Fowler et al., 2009). In this study, we have identified one gene (MELO3C004135.2) codifying this kind of protein in chromosome 5 of *C. melo*, highly induced in WM-7 at 3 dpi but down regulated in PS at 6 dpi (Supplementary file 4). Additionally, we have observed deregulation at early stages of other genes as E3 ligases, LAF 1 and transcription factors participating in ubiquitination and photomorphogenesis, all interconnected with jasmonic signaling (Seo et al., 2003; Kazan & Manners, 2011; Lozano-Durán et al., 2011; Correa et al., 2013). Viral replication, cell-to-cell movement and long-distance propagation are inhibited by SA and JA (Shang et al., 2011, Tsai et al., 2019). Thus, implication of all described genes in JA pathway suggest an interference and recruitment of this route by ToLCNDV in PS, as strategy to disrupt resistance response at early stages, whereas in WM-7 this hormonal pathway is promoted.

In chromosome 9, where is located the second higher R-genes cluster, two RDRs genes were over-expressed in PS (MELO3C005284.2 and MELO3C005257.2) at 6 and 12 dpi. Those genes are orthologues to RDR1 (RDRα) of *Arabidopsis thaliana*, involved in amplification of the gene silencing signal at post-transcriptional level in virus infection (Qui et al., 2009; Willmann et al., 2011; Islam et al., 2018). RDRs have been well described in the RNA silencing and their over-expression enhance resistance to begomoviruses, particularly to those of the tomato leaf cur viruses complex (Pakrash et al., 2020). This function of RDR1 in geminivirus resistance is variable and depends on the species of virus (Chen et al., 2010; Aregger et al., 2012). In *Nicotiana tabacum*, RDR1 was associated with symptoms recovery in plants infected with *tomato leaf curl Gujarat virus*

(ToLCGV), generating hypermethylation of the viral genome. However, in those *N. tabacum* plants infected with ToLCNDV, symptoms recovery was inhibited by AV2 viral protein, conducting to susceptible plants with high symptomatology and viral titers (Basu et al., 2018). In *N. benthamiana* a natural mutation of 72 bp insertion in RDR1 gene promote susceptibility to members of *Tobamovirus* genus (Yang et al., 2004), and might be responsible for high susceptibility to numerous viruses observed in this species, including geminiviruses (Akhtar et al., 2011). In cucumber (*Cucumis sativus* L.) RDR1 family is conformed by four subclasses (RDR1a, RDR1b, RDR1c1, RDR1c2). RDR1c1 and RDR1c2 are highly induced by the geminivirus *squash leaf curl virus* (SLCV) in susceptible plants, but in healthy plants remained at low levels (Leibman et al., 2018). Also, in *C. sativus*, RDR1 was notably induced by a viroid infection, suggesting the involvement of RDR1 in the anti-viroid defense (Xia et al., 2017). Our results concerning RDR1 expression in resistant and susceptible melon genotypes followed a similar expression trend, with very high expression in PS but unaltered in WM-7.

A third RDR gene (MELO3C015406.2) was up-regulated in PS in chromosome 2 at only 12 dpi. It is a RDR5 kind, with homologous sequence to Ty1/Ty3 gene conferring resistance to TYLCV by methylation of cytosines in the viral genome (Verlaan et al., 2013; Butterbach et al., 2014; Jackel et al., 2016; Gallego-Bartolomé et al., 2019). Despite their homology, MELO3C015406.2 was over-expressed in the susceptible genotype to ToLCNDV, conversely to was expected, as Ty1/Ty3 induction enhance resistance genotype. Tomato cultivars carrying Ty1/Ty3 genes have been described displaying resistant behavior after ToLCNDV inoculation (Fortes et al., 2016; Hussain et al., 2019). However, genotype background effect might modulate de effectiveness resistance to ToLCNDV of Ty1/Ty3 in tomato (Rai et al., 2013; Akhtar et al., 2019). Results obtained here suggested that infection of ToLCNDV induces and triggers silencing machinery in PS, but it is not efficient controlling virus spread. Another approach to explain this behavior is that RDRs are targeted and induced by ToLCNDV, interfering with silencing mechanism for defense and deregulating host factors, which result in symptomatology display.

Further studies must be conducted to characterize this potential begomovirus-cucurbits interaction promoting infection.

In transcriptional gene silencing (TGS), DNA methylation play crucial role inhibiting DNA virus transcription and replication. Plant host with this pathway suppressed result strongly susceptible to geminiviruses (Raja et al., 2014). Beside RDRs, other genes involved in RNA silencing-based resistance to geminiviruses have been here identified with altered expression, including histones, calmodulin proteins, AGO4 and methyltransferases. MET1 and CMT3 DNA methyltransferases are induced at early stages in WM-7 and repressed in PS after ToLCNDV inoculation. In geminiviruses infection, Rep protein interacts with CMT3 and MET1 in *N. benthamiana* and *A. thaliana* suppressing their expression and the maintenance of DNA methylation (Rodríguez-Negrete et al., 2013).

A phosphoethanolamine n-methyltransferase (MELO3C017356.2.1) was up-regulated in PS at early stages in the candidate region of chromosome 2. This protein has homology with the S-adenosyl-L-methionine-dependent methyltransferases of *A. thaliana.* Methylation of DNA is performed by cellular methyltransferases, using S-adenosyl methionine (SAM). *Arabidopsis* plants with mutated genes of SAM enzymes production are hypersensitive to geminiviruses (Mäkinen & De, 2019). *Beet severe curly top virus* (BSCTV) C2 protein interacts and inhibit SAM by descarboxilation reducing methylation of the viral DNA and promoting infection (Zhang et al., 2011). C4 protein of *cotton leaf curl multan virus* (CLCuMuV) interacts with SAMS of *N. benthamiana,* reducing its activity, DNA methylation and promoting accumulation of CLCuMuV (Ismayil et al., 2018). Alteration in PS of this kind of enzyme suggest its interaction with ToLCNDV, even in susceptible response.

DNA-directed RNA polymerases are components of the RNA directed DNA methylation (RdDM) epigenetic process, mediating geminiviruses silencing and conferring resistance (Jackel et al., 2017). Although several genes of this family are altered in both PS and WM-7 (Supplementary files 2 and 3), three DNA-directed RNA polymerases coding genes (MELO3C027682.2, MELO3C000330.2 and

MELO3C028015.2, and clustering together in chromosome 7) where very high induced only in WM-7 at 6 or 12 dpi.

These results highlight that viral silencing mediated by RNA strategy is likely contributing in resistant or susceptible response to ToLCNDV in *C. melo*. Even so, in candidate regions of chromosome 2, 11 and 12, where QTLs linked to ToLCNDV resistance have been identified (Sáez et al., 2017) we only could identify scarce genes directly involved on this pathway.

Instead, in candidate regions most transcriptomic alteration concerned to genes coding transcription factors, transmembrane transporters and photo-regulators. In the main locus conferring resistance to ToLCNDV described by Sáez et al., (2017), expression of MELO3C022339.2 gene is inhibited in PS at 6 dpi. This gene code for a glutaredoxin protein, which has been described interacting with JA-SA pathway (Li et al., 2017c). Moreover, MELO3C022339.2 is orthologue to the Thioredoxin superfamily proteins of *Arabidopsis thaliana*. Thioredoxin proteins have been described interacting with begomoviruses and also impairing resistance to potyviruses (Luna-Rivero et al., 2016; Liu et al., 2017; Mathioudakis et al., 2018). In response to TYLCV, thioredoxin and TCP proteins in tomato interacts with transcription factors that regulate defense mechanisms (Huang et al., 2016). In the same candidate region of melon for resistance to ToLCNDV, a transcription factor TCP20-like (MELO3C022331.2) is located, but we have not identified expression changes during the viral infection course. TCP transcription factors have been described interacting with ubiquitination, SUMO and MYC2 pathways, and with lipooxygenases encoded at chloroplast and implicated in JA biosynthesis (Schommer et al., 2008). A TCP gene was also proposed as responsible candidate of leaf curling during ToLCNDV infection (Naqvi et al., 2010).

In this region, Bidirectional sugar transporter SWEET (MELO3C022341.2) was the gene with higher induction in the susceptible response of PS at 12 dpi. SWEET transporters facilitate sugars transference into the phloem and promote their transport (Chen, 2014) and some studies have reported changes in the transcription

pattern of various SWEET genes in plants after pathogens attack (Antony et al., 2010; Chen, 2014; Chandran, 2015; Naqvi et al., 2017; Breia et al., 2020). SWEETs deregulation has been previously reported in plant-begomovirus interactions, and their suppression enhance the resistant phenotype (Naqvi et al., 2017). In an additional study, melon plants infected CMV accumulated more sugars in the phloem of leaves as result of sucrose transporter effect (Gil et al., 2011). In Cotton (*Gossypium* spp.), a SWEET transporter gene was downregulated in resistant plants after *cotton leaf curl disease* (CLCuD) infection (Naqvi et al., 2017), and involvement of sugar-signaling mechanisms has been observed in resistance plants to TYLCV (Sade et al., 2020). In *C. melo* genome there are 24 genes coding for SWEET transporters, among which MELO3C005869.2 and MELO3C002381.2 were also up regulated in PS at 6 dpi and 12 dpi in chromosome 9 and 12, respectively. Conversely, MELO3C001650.2 was repressed at 12 dpi in chromosome 12. Interestingly, MELO3C005869.2 is located at same region were a R-gene cluster has been described, and where another SWEET transporter gene (MELO3C005758.2) of WM-7 is deregulated at 12 dpi.

MELO3C022327.2 code a transmembrane protein and is located in candidate region for ToLCNDV resistance in chromosome 11. In WM-7 this gene is highly down-regulated at 3 ant 6 dpi. Out of 24 transmembrane genes deregulated in PS after ToLCNDV inoculation, 16 were induced, including the gene MELO3C017283.2 located at candidate region for resistance in chromosome 2. In candidate region for resistance of chromosome 12, MELO3C026071.2 was induced in WM-7 at 12 dpi. These results suggest that transport processes are implicated in immunity response against ToLCNDV and might enhance virus propagation across host plant.

Geminiviruses employ the host mechanism for triggering their own replication. Plant DNA primases have been described as catalyst enzymes used in the first step of geminivirus replication (Saunders et al., 1992; Alberter et al., 2005). Consistently, in the candidate region of chromosome 11, the gene coding a DNA primase large

subunit (MELO3C022319.2) is repressed in the resistant genotype WM-7. This gene is also synthenic with a DNA primase gene in chromosome 8 of *Cucurbita moschata*, were a mayor QTL controlling resistance to ToLCNDV has been identify (Sáez et al., 2020).

Additionally, candidate region in chromosome 12 include a NAC transcription factor (MELO3C004694.2), highly induced in PS at 12 dpi. Same induction has been reported in NAC transcriptions factors after begomoviruese infection. Some examples are a NAC1 gene associated with *ty-5* gene conferring resistance to TYLCV (Anbinder et al., 2009) and over-expression of two NAC TFs membrane linked after ToLCNDV infection (Bhattacharjee et al., 2017). Román et al. (2019) validated by qPCR a NAC transcription factor highly induced in the same susceptible accession of *C. melo* here evaluated (PS), evidencing the implication of this gene family in the increasement of viral accumulation in plant, and correlating its expression with begomoviral symptoms severity.

Resistance identify in WM-7 accession come conferred by a complicated and interconnected net of transcriptional genetic rearrangements. Our results highlight the key role of jasmonic, photosynthetic and RNAi pathways at the beginning of ToLCNDV infection, but further studies are required to identify essential key factors to trigger the resistance response. Fine mapping and functional characterization by CRISPR/Cas edition assays might provide useful information to breeding resistance to ToLCNDV in melon. Both methodologies could allow the validation of candidate genes here proposed and narrowing the current candidate regions of resistance.

REFERENCES

Akhtar, S., Briddon, R. W., & Mansoor, S. (2011). Reactions of *Nicotiana* species to inoculation with monopartite and bipartite begomoviruses. *Virology journal*, *8*(1), 1-9. https://doi.org/10.1186/1743-422X-8-475

Akhtar, K. P., Akram, A., Ullah, N., Saleem, M. Y., & Saeed, M. (2019). Evaluation of Solanum species for resistance to Tomato leaf curl New Delhi virus using chip grafting assay. *Scientia Horticulturae*, *256*, 108646. https://doi.org/10.1016/j.scienta.2019.108646

Alberter, B., Rezaian, M. A., & Jeske, H. (2005). Replicative intermediates of *Tomato leaf curl virus* and its satellite DNAs. *Virology*, *331*(2), 441-448. https://doi.org/10.1016/j.virol.2004.10.043

Allie, F., & Rey, M. E. C. (2013). Transcriptional alterations in model host, *Nicotiana benthamiana*, in response to infection by *South African cassava mosaic virus*. *European Journal of Plant Pathology*, 137, 765–785. https://doi.org/10.1007/s10658-013-0286-4

Allie, F., Pierce, E. J., Okoniewski, M. J., & Rey, M. E. C. (2014). Transcriptional analysis of *South African cassava mosaic virus*-infected susceptible and tolerant landraces of cassava highlights differences in resistance, basal defense and cell wall associated genes during infection. *BMC Genomics*, *15*, 1006. https://doi.org/10.1186/1471-2164-15-1006

Anbinder, I., Reuveni, M., Azari, R., Paran, I., Nahon, S., Shlomo, H., Chen, L., Lapidot, M., & Levin, I. (2009). Molecular dissection of *Tomato leaf curl virus* resistance in tomato line TY172 derived from *Solanum peruvianum*. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik*, *119*(3), 519–530. https://doi.org/10.1007/s00122-009-1060-z

Andrews S. (2010). FastQC: a quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Annu, P. K., Rani, R., & Rathi, A. S. (2019). Gemini Viruses-emerging threat to crops. *Journal of Pharmacognosy and Phytochemistry*, *8*(1), 2006-2012.

Antony, G., Zhou, J., Huang, S., Li, T., Liu, B., White, F., & Yang, B. (2010). Rice xa13 recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene Os-11N3. *The Plant cell*, *22*(11), 3864–3876. https://doi.org/10.1105/tpc.110.078964

Aregger, M., Borah, B. K., Seguin, J., Rajeswaran, R., Gubaeva, E. G., Zvereva, A. S., Windels, D., Vazquez, F., Blevins, T., Farinelli, L., & Pooggin, M. M. (2012). Primary and secondary siRNAs in geminivirus-induced gene silencing. *PLoS pathogens*, *8*(9), e1002941. https://doi.org/10.1371/journal.ppat.1002941

Balint-Kurti P. (2019). The plant hypersensitive response: concepts, control and consequences. *Molecular Plant Pathology*, *20*(8), 1163–1178. https://doi.org/10.1111/mpp.12821

Bardou, P., Mariette, J., Escudié, F., Djemiel, C., & Klopp, C. (2014). jvenn: an interactive Venn diagram viewer. *BMC Bioinformatics*, *15*(1), 1-7. https://doi.org/10.1186/1471-2105-15-293

Basu, S., Kumar Kushwaha, N., Kumar Singh, A., Pankaj Sahu, P., Vinoth Kumar, R., & Chakraborty, S. (2018). Dynamics of a geminivirus-encoded pre-coat protein and host RNA-dependent RNA polymerase 1 in regulating symptom recovery in tobacco. *Journal of experimental botany*, *69*(8), 2085–2102. https://doi.org/10.1093/jxb/ery043

Bhattacharjee, P., Das, R., Mandal, A., & Kundu, P. (2017). Functional characterization of tomato membrane-bound NAC transcription factors. *Plant molecular biology*, *93*(4-5), 511–532. https://doi.org/10.1007/s11103-016-0579-z

Bhattacharyya, D., & Chakraborty, S. (2018). Chloroplast: the Trojan horse in plant-virus interaction. *Molecular plant pathology*, *19*(2), 504–518. https://doi.org/10.1111/mpp.12533

Bochman, M. L., & Schwacha, A. (2008). The Mcm2-7 complex has in vitro helicase activity. *Molecular cell*, *31*(2), 287–293. https://doi.org/10.1016/j.molcel.2008.05.020

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bonza, M. C., Fusca, T., Homann, U., Thiel, G., De Michelis, MI. (2009). Intracellular localisation of PPI1 (proton pump interactor, isoform 1), a regulatory protein of the plasma membrane H(+)-ATPase of *Arabidopsis thaliana*. *Plant Biology*, 11(6):869–877. https://doi.org/10.1111/j.1438-8677.2008.00181.x.

Breia, R., Conde, A., Pimentel, D., Conde, C., Fortes, A. M., Granell, A., & Gerós, H. (2020). VvSWEET7 Is a Mono- and Disaccharide Transporter Up-Regulated in Response to *Botrytis cinerea* Infection in Grape Berries. *Frontiers in Plant Science*, 10:1753. https://doi.org/ 10.3389/fpls.2019.01753

Brister, J. R., & Muzyczka, N. (1999). Rep-mediated nicking of the adeno-associated virus origin requires two biochemical activities, DNA helicase activity and transesterification. *Journal of virology*, *73*(11), 9325–9336. https://doi.org/10.1128/JVI.73.11.9325-9336.1999

Butterbach, P., Verlaan, M. G., Dullemans, A., Lohuis, D., Visser, R. G., Bai, Y., & Kormelink, R. (2014). *Tomato yellow leaf curl virus* resistance by *Ty-1* involves increased cytosine methylation of viral genomes and is compromised by cucumber mosaic virus infection. *Proceedings of the National Academy of Sciences*, *111*(35), 12942-12947. https://doi.org/10.1073/pnas.1400894111

Castanera, R., Ruggieri, V., Pujol, M., García-Mas, J., & Casacuberta, J. M. (2019). An improved melon reference genome with single-molecule sequencing uncovers a recent burst of transposable elements with potential impact on genes. *Frontiers in Plant Science*, *10*. https://doi.org/10.3389/fpls.2019.01815

Castillo-González, C., Liu, X., Huang, C., Zhao, C., Ma, Z., Hu, T., Sun, F., Zhou, Y., Zhou, X., Wang, X. J., & Zhang, X. (2015). Geminivirus-encoded TrAP suppressor inhibits the histone methyltransferase SUVH4/KYP to counter host defense. *eLife*, *4*, e06671. https://doi.org/10.7554/eLife.06671

Chakraborty, N., & Basak, J. (2018). Molecular and biochemical characterization of *mungbean yellow mosaic India virus* resistance in leguminous host *Vigna mungo*. *Journal of plant biochemistry and biotechnology*, *27*(3), 318-330. https://doi.org/10.1007/s13562-018-0441-2

Chandran D. (2015). Co-option of developmentally regulated plant SWEET transporters for pathogen nutrition and abiotic stress tolerance. *IUBMB life*, *67*(7), 461–471. https://doi.org/10.1002/iub.1394

Chen, H., Tamai, A., Mori, M., Ugaki, M., Tanaka, Y., Samadder, P. P., Hirohiko Hirochika, A. M., Yamaoka, N, & Nishiguchi, M. (2010). Analysis of rice RNA-dependent RNA polymerase 1 (OsRDR1) in virus-mediated RNA silencing after particle bombardment. *Journal of general plant pathology*, *76*(2), 152-160. https://doi.org/10.1007/s10327-010-0226-5

Chen, L. Q. (2014). SWEET sugar transporters for phloem transport and pathogen nutrition. *New Phytologist*, *201*(4), 1150-1155. https://doi.org/10.1111/nph.12445

Cho, J. H., Kim, H. B., Kim, H. S., & Choi, S. B. (2008). Identification and characterization of a rice MCM2 homologue required for DNA replication. *BMB reports*, *41*(8), 581–586. https://doi.org/10.5483/bmbrep.2008.41.8.581

Choi, H., Jo, Y., Lian, S., Jo, K. M., Chu, H., Yoon, J. Y., Choi, S. K., Kim, K. H., & Cho, W. K. (2015). Comparative analysis of chrysanthemum transcriptome in response to three RNA viruses: Cucumber mosaic virus, Tomato spotted wilt virus and Potato virus X. *Plant molecular biology*, *88*(3), 233–248. https://doi.org/10.1007/s11103-015-0317-y

Chung, H. Y., Lacatus, G., & Sunter, G. (2014). Geminivirus AL2 protein induces expression of, and interacts with, a calmodulin-like gene, an endogenous regulator of gene silencing. *Virology*, 460–461. 108–118. 10.1016/j.virol.2014.04.034.

Clérot, D., & Bernardi, F. (2006). DNA helicase activity is associated with the replication initiator protein rep of tomato yellow leaf curl geminivirus. *Journal of virology*, *80*(22), 11322-11330. https://doi.org/10.1128/JVI.00924-06

Correa, R. L., Bruckner, F. P., de Souza Cascardo, R., & Alfenas-Zerbini, P. (2013). The Role of F-Box Proteins during Viral Infection. *International Journal of Molecular Sciences*, *14*(2), 4030–4049. https://doi.org/10.3390/ijms14024030

Czosnek, H., Eybishtz, A., Sade, D., Gorovits, R., Sobol, I., Bejarano, E., & Lozano-Durán, R. (2013). Discovering host genes involved in the infection by the *Tomato Yellow Leaf Curl Virus* complex and in the establishment of resistance to the virus using *Tobacco Rattle Virus*-based post transcriptional gene silencing. *Viruses*, *5*(3), 998-1022. https://doi.org/10.3390/v5030998

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., & Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, *29*(1), 15–21. https://doi.org/10.1093/bioinformatics/bts635

Doyle, J. J., & Doyle. J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus 12*(1), 13–15.

Forsburg S. L. (2004). Eukaryotic MCM proteins: beyond replication initiation. *Microbiology and molecular biology reviews: MMBR*, *68*(1), 109–131. https://doi.org/10.1128/mmbr.68.1.109-131.2004

Fortes, I. M., Sánchez-Campos, S., Fiallo-Olivé, E., Díaz-Pendón, J. A., Navas-Castillo, J., & Moriones, E. (2016). A novel strain of tomato leaf curl New Delhi virus has spread to the Mediterranean basin. *Viruses*, *8*(11), 307. https://doi.org/10.3390/v8110307

Fowler, J. H., Narváez-Vásquez, J., Aromdee, D. N., Pautot, V., Holzer, F. M., & Walling, L. L. (2009). Leucine aminopeptidase regulates defense and wound signaling in tomato downstream of jasmonic acid. *The Plant cell*, *21*(4), 1239–1251. https://doi.org/10.1105/tpc.108.065029

Gallego-Bartolomé, J., Liu, W., Kuo, P. H., Feng, S., Ghoshal, B., Gardiner, J., Zhao, J. M., Park, S. Y., Chory, J., & Jacobsen, S. E. (2019). Co-targeting RNA Polymerases IV and V Promotes Efficient De Novo DNA Methylation in Arabidopsis. *Cell*, *176*(5), 1068–1082.e19. https://doi.org/10.1016/j.cell.2019.01.029

Gholizadeh, A., Santha, I. M., Kohnehrouz, B. B., Lodha, M. L., & Kapoor, H. C. (2005). Cystatins may confer viral resistance in plants by inhibition of a virus-induced cell death phenomenon in which cysteine proteinases are active: cloning and molecular characterization of a cDNA encoding cysteine-proteinase inhibitor (celostatin) from Celosia cristata (crested cock's comb). *Biotechnology and Applied Biochemique*, 42(Pt 3):197-204. doi:10.1042/BA20050029

Gil, L., Yaron, I., Shalitin, D., Sauer, N., Turgeon, R., & Wolf, S. (2011). Sucrose transporter plays a role in phloem loading in CMV-infected melon plants that are defined as symplastic loaders. *Plant Journal*, *66*, 366–374. https://doi.org/10.1111/j.1365-313X.2011.04498.x

Góngora-Castillo, E., Ibarra-Laclette, E., Trejo-Saavedra, D. L., & Rivera-Bustamante, R. F. (2012). Transcriptome analysis of symptomatic and recovered leaves of geminivirus-infected pepper (*Capsicum annuum*). *Virology journal*, *9*, 295. https://doi.org/10.1186/1743-422X-9-295

Gonzalez-Ibeas, D., Blanca, J., Roig, C., González-To, M., Picó, B., Truniger, V., Gómez, P., Deleu, W., Caño-Delgado, A., Arús, P., Nuez, F., Garcia-Mas, J., Puigdomènech, P., & Aranda, M. A. (2007). MELOGEN: an EST database for melon functional genomics. *BMC genomics*, *8*, 306. https://doi.org/10.1186/1471-2164-8-306

Gorovits, R., Moshe, A., Ghanim, M., & Czosnek, H. (2013). Recruitment of the host plant heat shock protein 70 by Tomato yellow leaf curl virus coat protein is required for virus infection. *PloS one*, *8*(7), e70280. https://doi.org/10.1371/journal.pone.0070280

Gorovits, R., & Czosnek, H. (2017). The involvement of heat shock proteins in the establishment of Tomato yellow leaf curl virus infection. *Frontiers in plant science*, *8*, 355. https://doi.org/10.3389/fpls.2017.00355

Guerrero, J., Regedanz, E., Lu, L., Ruan, J., Bisaro, D. M., & Sunter, G. (2020). Manipulation of the Plant Host by the Geminivirus AC2/C2 Protein, a Central Player in the Infection Cycle. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.00591

Gutiérrez-Campo, R., Torres-Acosta, J. A., Saucedo-Arias, L. J., & Gomez-Lim, M. A. (1999). The use of cysteine proteinase inhibitors to engineer resistance against

potyviruses in transgenic tobacco plants. *Nature Biotechnology*, 17(12):1223-1226. doi:10.1038/70781

Huang, Y., Zhang, B. L., Sun, S., Xing, G. M., Wang, F., Li, M. Y., & Xiong, A. S. (2016). AP2/ERF transcription factors involved in response to tomato yellow leaf curly virus in tomato. *The Plant Genome*, *9*(2), 1-15. https://doi.org/10.3835/plantgenome2015.09.0082

Hussain, Z., Lata, S., Kumar, P., Kumar, S., & Tomar, B. S. (2019) Recessive behavior of Ty-3 gene for ToLCNDV disease resistance in Tomato (*Solanum lycopersicum* L.). Congress communication. Accessed: https://www.researchgate.net/profile/Kumar_Sumit3/publication/340476078_Recessive_behavior_of_Ty-3_gene_for_ToLCNDV_disease_resistance/links/5e8bf9c2a6fdcca789fbec50/Recessive-behavior-of-Ty-3-gene-for-ToLCNDV-disease-resistance.pdf

Hwang, I. S., & Hwang, B. K. (2010). The pepper 9-lipoxygenase gene CaLOX1 functions in defense and cell death responses to microbial pathogens. *Plant Physiology*, *152*(2), 948-967. https://doi.org/10.1104/pp.109.147827

Islam, W., Noman, A., Qasim, M., & Wang, L. (2018). Plant responses to pathogen attack: small RNAs in focus. *International journal of molecular sciences*, *19*(2), 515. https://doi.org/10.3390/ijms19020515

Islam, M. R., Hossain, M. R., Jesse, D., Jung, H. J., Kim, H. T., Park, J. I., & Nou, I. S. (2020). Characterization, identification and expression profiling of genome-wide R-genes in melon and their putative roles in bacterial fruit blotch resistance. *BMC genetics*, *21*(1), 80. https://doi.org/10.1186/s12863-020-00885-9

Ismayil, A., Haxim, Y., Wang, Y., Li, H., Qian, L., Han, T., Chen, T., Jia, Q.,Liu, A.Y., Zhu, S., Deng, H., Gorovits, R, Hong, Y., Hanley-Bowdoin, L. & Liu, Y. (2018). *Cotton Leaf Curl Multan virus* C4 protein suppresses both transcriptional and post-transcriptional gene silencing by interacting with SAM synthetase. *PLoS pathogens*, *14*(8), e1007282. https://doi.org/10.1371/journal.ppat.1007282

Jackel, J. N., Storer, J. M., Coursey, T., & Bisaro, D. M. (2016). Arabidopsis RNA polymerases IV and V are required to establish H3K9 methylation, but not cytosine methylation, on geminivirus chromatin. *Journal of virology*, *90*(16), 7529-7540. https://doi.org/10.1128/JVI.00656-16

Jeevalatha, A., Siddappa, S., Kumar, A., Kaundal, P., Guleria, A., Sharma, S., & Singh, B. P. (2017). An insight into differentially regulated genes in resistant and susceptible genotypes of potato in response to *tomato leaf curl New Delhi virus*-[potato] infection. *Virus Research*, *232*, 22-33. https://doi.org/10.1016/j.virusres.2017.01.015.

Juárez, M., Tovar, R., Fiallo-Olivé, E., Aranda, M. A., Gosálvez, B., Castillo, P., & Navas-Castillo, J. (2014). First detection of *Tomato leaf curl New Delhi virus* infecting zucchini in Spain. *Plant Disease*, *98*(6), 857-857. https://doi.org/10.1094/PDIS-10-13-1050-PDN

Juárez, M., Rabadán, M. P., Martínez, L. D., Tayahi, M., Grande-Pérez, A., & Gómez López, P. (2019). Natural hosts and genetic diversity of the emerging *tomato leaf curl New*

Delhi virus in Spain. *Frontiers in Microbiology*, *10*, 140. https://doi.org/10.3389/fmicb.2019.00140

Kazan, K., & Manners, J. M. (2011). The interplay between light and jasmonate signalling during defence and development. *Journal of experimental botany*, *62*(12), 4087–4100. https://doi.org/10.1093/jxb/err142

Kazlauskas, D., Krupovic, M., & Venclovas, Č. (2016). The logic of DNA replication in double-stranded DNA viruses: insights from global analysis of viral genomes. *Nucleic acids research*, *44*(10), 4551–4564. https://doi.org/10.1093/nar/gkw322

Kumar, R. V. (2019) Plant Antiviral Immunity Against Geminiviruses and Viral Counter-Defense for Survival. *Frontiers in Microbiology*, 10:1460. https://doi.org/10.3389/fmicb.2019.01460

Kushwaha, N., Singh, A. K., Basu, S., & Chakraborty, S. (2015). Differential response of diverse solanaceous hosts to *tomato leaf curl New Delhi virus* infection indicates coordinated action of NBS-LRR and RNAi-mediated host defense. *Archives of Virology*, *160*(6), 1499-1509. https://doi.org/10.1007/s00705-015-2399-x

Kushwaha, N. K., Mansi, & Chakraborty, S. (2017). The replication initiator protein of a geminivirus interacts with host monoubiquitination machinery and stimulates transcription of the viral genome. *PLoS pathogens*, *13*(8), e1006587. https://doi.org/10.1371/journal.ppat.1006587

Leibman, D., Kravchik, M., Wolf, D., Haviv, S., Weissberg, M., Ophir, R., Paris, H. S., Palukaitis, P., Ding, S. W., Gaba, V., & Gal-On, A. (2018). Differential expression of cucumber RNA-dependent RNA polymerase 1 genes during antiviral defence and resistance. *Molecular plant pathology*, *19*(2), 300–312. https://doi.org/10.1111/mpp.12518

Li, B., & Dewey, C. N. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC bioinformatics*, *12*(1), 323. https://doi.org/10.1186/1471-2105-12-323

Li, R., Weldegergis, B. T., Li, J., Jung, C., Qu, J., Sun, Y., & Chua, N. H. (2014). Virulence factors of geminivirus interact with MYC2 to subvert plant resistance and promote vector performance. *The Plant Cell*, *26*(12), 4991-5008. https://doi.org/10.1105/tpc.114.133181

Li, X., An, M., Xia, Z., Bai, X., & Wu, Y. (2017a). Transcriptome analysis of watermelon (*Citrullus lanatus*) fruits in response to *Cucumber green mottle mosaic virus* (CGMMV) infection. *Scientific Reports*, *7*(1), 16747. https://doi.org/10.1038/s41598-017-17140-4

Li, Y., Qin, L., Zhao, J., Muhammad, T., Cao, H., Li, H., Zhang, Y., & Liang, Y. (2017b). SlMAPK3 enhances tolerance to tomato yellow leaf curl virus (TYLCV) by regulating salicylic acid and jasmonic acid signaling in tomato (Solanum lycopersicum). *PloS one*, *12*(2), e0172466. https://doi.org/10.1371/journal.pone.0172466

Li, Z., Feng, Z, Maeli, Melotto, J. Y., & Sheng Y. H. (2017c). Jasmonate signaling and manipulation by pathogens and insects, *Journal of Experimental Botany*, 68(6), 1371–1385, https://doi.org/10.1093/jxb/erw478

Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome biology*, *15*(12), 550. https://doi.org/10.1186/s13059-014-0550-8

Liu, Q., Liu, H., Gong, Y., Tao, Y., Jiang, L., Zuo, W., & Lübberstedt, T. (2017). An atypical thioredoxin imparts early resistance to Sugarcane mosaic virus in maize. *Molecular Plant*, *10*(3), 483-497. https://doi.org/10.1016/j.molp.2017.02.002

López, C., Ferriol, M., & Picó, M. B. (2015). Mechanical transmission of Tomato leaf curl New Delhi virus to cucurbit germplasm: selection of tolerance sources in *Cucumis melo*. *Euphytica 204*, 279–691. https://doi.org/10.1007/s10681-015-1371-x

Lou, L., Su, X., Liu, X., & Liu, Z. (2020). Transcriptome analysis of *Luffa cylindrica* (L.) Roem response to infection with *Cucumber mosaic virus* (CMV). *Gene*, *737*, 144451. https://doi.org/10.1016/j.gene.2020.144451

Lozano-Durán, R., Rosas-Díaz, T., Gusmaroli, G., Luna, A. P., Taconnat, L., Deng, X. W., & Bejarano, E. R. (2011). Geminiviruses subvert ubiquitination by altering CSN-mediated derubylation of SCF E3 ligase complexes and inhibit jasmonate signaling in Arabidopsis thaliana. *The Plant Cell*, *23*(3), 1014-1032.

Lu, S., Faris, J. D., & Edwards, M. C. (2017). Molecular cloning and characterization of two novel genes from hexaploid wheat that encode double PR-1 domains coupled with a receptor-like protein kinase. *Molecular Genetics and Genomics* 292, 435–452 https://doi.org/10.1007/s00438-017-1287-3

Luna-Rivero, M. S., Hernández-Zepeda, C., Villanueva-Alonzo, H., Minero-García, Y., Castell-González, S. E., & Moreno-Valenzuela, O. A. (2016). Expression of genes involved in the salicylic acid pathway in type h1 thioredoxin transiently silenced pepper plants during a begomovirus compatible interaction. *Molecular Genetics and Genomics*, *291*(2), 819-830. https://doi.org/10.1007/s00438-015-1148-x

Mäkinen, K., & De, S. (2019). The significance of methionine cycle enzymes in plant virus infections. *Current opinion in plant biology*, *50*, 67–75. https://doi.org/10.1016/j.pbi.2019.03.002

Maine, G. T., Sinha, P., & Tye, B. K. (1984). Mutants of S. cerevisiae defective in the maintenance of minichromosomes. *Genetics*, *106*(3), 365–385.

Maio, F., Helderman, T. A., Arroyo-Mateos, M., van der Wolf, M., Boeren, S., Prins, M., & van den Burg, H. A. (2020). Identification of tomato proteins that interact with Replication initiator protein (Rep) of the geminivirus TYLCV. *Frontiers in Plant Science*, *11*, 1069. https://doi.org/10.3389/fpls.2020.01069

Mallory, A., & Vaucheret, H. (2010). Form, function, and regulation of ARGONAUTE proteins. *The Plant cell*, *22*(12), 3879–3889. https://doi.org/10.1105/tpc.110.080671

Mathioudakis, M. M., Khechmar, S., Owen, C. A., Medina, V., Ben Mansour, K., Tomaszewska, W., Spanos, T., Sarris, P. F., & Livieratos, I. C. (2018). A Thioredoxin Domain-Containing Protein Interacts with *Pepino mosaic virus* Triple Gene Block Protein 1. *International Journal of Molecular Sciences*, *19*(12), 3747. https://doi.org/10.3390/ijms19123747

McCreight, J. D., Liu, H.-Y., & Turini, T. A. (2008). Genetic resistance to *Cucurbit Leaf Crumple Virus* in melon. *HortScience* 43, 122-126. https://doi.org/10.21273/HORTSCI.43.1.122

Messelink, G. J., Calvo, F. J., Marín, F., & Janssen, D. (2020). Cucurbits. In: Gullino, M. L., Albajes, R. & Nicot, P. C., (Eds.), *Integrated Pest and Disease Management in Greenhouse Crops* (pp. 537-566). Springer International Publishing. https://doi.org/10.1007/978-3-030-22304-5_19

Miozzi, L., Napoli, C., Sardo, L., & Accotto, G. P. (2014). Transcriptomics of the interaction between the monopartite phloem-limited geminivirus tomato yellow leaf curl Sardinia virus and Solanum lycopersicum highlights a role for plant hormones, autophagy and plant immune system fine tuning during infection. *PLoS One*, *9*(2), e89951.

Mochizuki, T., Ogata, Y., Hirata, Y., & Ohki, S. T. (2014). Quantitative transcriptional changes associated with chlorosis severity in mosaic leaves of tobacco plants infected with C ucumber mosaic virus. *Molecular plant pathology*, *15*(3), 242-254.

Morata, J., & Puigdomènech, P. (2017). Variability among Cucurbitaceae species (melon, cucumber and watermelon) in a genomic region containing a cluster of NBS-LRR genes. *BMC genomics*, *18*(1), 138. https://doi.org/10.1186/s12864-017-3529-5

Moriones, E., Praveen, S., & Chakraborty, S. (2017). Tomato Leaf Curl New Delhi Virus: An Emerging Virus Complex Threatening Vegetable and Fiber Crops. *Viruses*, *9*(10), 264. https://doi.org/10.3390/v9100264

Naqvi, A. R., Haq, Q. M., & Mukherjee, S. K. (2010). MicroRNA profiling of tomato leaf curl New Delhi virus (tolcndv) infected tomato leaves indicates that deregulation of mir159/319 and mir172 might be linked with leaf curl disease. *Virology journal*, *7*, 281. https://doi.org/10.1186/1743-422X-7-281

Naqvi, R. Z., Zaidi, S. S. E. A., Akhtar, K. P., Strickler, S., Woldemariam, M., Mishra, B., & Mueller, L. A. (2017). Transcriptomics reveals multiple resistance mechanisms against cotton leaf curl disease in a naturally immune cotton species, *Gossypium arboreum*. *Scientific Reports*, *7*(1), 1-15. https://doi.org/10.1038/s41598-017-15963-9

Nemchinov, L., Boutanaev, A. & Postnikova, O. (2016). Virus-induced gene silencing of the RPC5-like subunit of RNA polymerase III caused pleiotropic effects in *Nicotiana benthamiana*. *Scientific Rep*orts, 6, 27785. https://doi.org/10.1038/srep27785

Pradhan, B., Van Tien, V., Dey, N., & Mukherjee, S. K. (2017). Molecular Biology of Geminivirus DNA Replication. *Avid Science*, https://avidscience.com/book/viral-replication/

Prakash, V., Singh, A., Singh, A. K., Dalmay, T., & Chakraborty, S. (2020). Tobacco RNA-dependent RNA polymerase 1 affects the expression of defence-related genes in *Nicotiana benthamiana* upon *Tomato leaf curl Gujarat virus* infection. *Planta*, *252*(1), 11. https://doi.org/10.1007/s00425-020-03417-y

Pandey, S., Muthamilarasan, M., Sharma, N., Chaudhry, V., Dulani, P., Shweta, S., & Prasad, M. (2019). Characterization of DEAD-box family of RNA helicases in tomato provides insights into their roles in biotic and abiotic stresses. *Environmental and Experimental Botany*, *158*, 107-116. https://doi.org/10.1016/j.envexpbot.2018.11.018

Panno, S., Iacono, G., Davino, M., Marchione, S., Zappardo, V., Bella, P., Tomassoli, L., Accotto, G.P. & Davino, S. (2016). First report of *Tomato leaf curl New Delhi virus* affecting zucchini squash in an important horticultural area of southern Italy. *New Disease Reports*, *33*(6), 2044-0588. http://dx.doi.org/10.5197/j.2044-0588.2016.033.006

Panno, S., Caruso, A. G., Troiano, E., Luigi, M., Manglli, A., Vatrano, T., Iacono, G., Marchione, S., Bertin, S., Tomassoli, L., Parrella, G., & Davino, S. (2019), Emergence *of tomato leaf curl New Delhi virus* in Italy: estimation of incidence and genetic diversity. *Plant Pathology*, *68*, 601-608. https://doi.org/10.1111/ppa.12978

Pérez-de-Castro, A., López-Martín, M., Esteras, C., Garcés-Claver, A., Palomares-Ríus, F. J., Picó, M. B., & Gómez-Guillamón, M. L. (2020). Melon Genome Regions Associated with TGR-1551-Derived Resistance to *Cucurbit yellow stunting disorder virus*. *International journal of molecular sciences*, *21*(17), E5970. https://doi.org/10.3390/ijms21175970

Pitrat, M. (2016). Melon genetic resources: phenotypic diversity and horticultural taxonomy. In: Grumet R., Katzir, N., & Garcia-Mas, J., (Eds.), *Genetics and genomics of Cucurbitaceae* (Vol. 20, pp. 25-59). Cham, Switzerland: Springer Nature. https://doi.org/10.1007/7397_2016_10

Preiss, W., & Jeske, H. (2003). Multitasking in replication is common among geminiviruses. *Journal of virology*, *77*(5), 2972–2980. https://doi.org/10.1128/jvi.77.5.2972-2980.2003

Qi, X., Bao, F. S., & Xie, Z. (2009). Small RNA deep sequencing reveals role for Arabidopsis thaliana RNA-dependent RNA polymerases in viral siRNA biogenesis. *PloS one*, *4*(3), e4971. https://doi.org/10.1371/journal.pone.0004971

Quezada, E. H., García, G. X., Arthikala, M. K., Melappa, G., Lara, M., & Nanjareddy, K. (2019). Cysteine-Rich Receptor-Like Kinase Gene Family Identification in the Phaseolus Genome and Comparative Analysis of Their Expression Profiles Specific to Mycorrhizal and Rhizobial Symbiosis. *Genes*, *10*(1), 59. https://doi.org/10.3390/genes10010059

Rai, N., Sahu, P., Gupta, S., Reddy, M.K., Ravishankar, K., Singh, M., Sadashiva, A.T., & Prasad, M. (2013). Identification and validation of an ISSR marker linked to Tomato leaf curl New Delhi virus resistant gene in a core set of tomato accessions. *Vegetable Science, 40*, 1-6.

Raja, P., Jackel, J. N., Li, S., Heard, I. M., & Bisaro, D. M. (2014). Arabidopsis double-stranded RNA binding protein DRB3 participates in methylation-mediated defense against geminiviruses. *Journal of Virology*, *88*(5), 2611–2622. https://doi.org/10.1128/JVI.02305-13

Ramesh, S. V., Sahu, P. P., Prasad, M., Praveen, S., & Pappu, H. R. (2017). Geminiviruses and Plant Hosts: A Closer Examination of the Molecular Arms Race. *Viruses*, *9*(9), 256. https://doi.org/10.3390/v9090256

Ranji, A., & Boris-Lawrie, K. (2010). RNA helicases: emerging roles in viral replication and the host innate response. *RNA biology*, *7*(6), 775–787. https://doi.org/10.4161/rna.7.6.14249

Rizvi, I., Choudhury, N. R., & Tuteja, N. (2015). Insights into the functional characteristics of geminivirus rolling-circle replication initiator protein and its interaction with host factors affecting viral DNA replication. *Archives of Virology* 160, 375–387. https://doi.org/10.1007/s00705-014-2297-7

Rodríguez-Negrete, E., Lozano-Durán, R., Piedra-Aguilera, A., Cruzado, L., Bejarano, E. R., & Castillo, A. G. (2013). Geminivirus Rep protein interferes with the plant DNA methylation machinery and suppresses transcriptional gene silencing. *The New phytologist*, *199*(2), 464–475. https://doi.org/10.1111/nph.12286

Román, B., Gómez, P., Picó, B., López, C., & Janssen, D. (2019). Candidate gene analysis of *Tomato leaf curl New Delhi virus* resistance in *Cucumis melo*. *Scientia Horticulturae*, *243*, 12-20. https://doi.org/10.1016/j.scienta.2018.07.005

Romay, G., Pitrat, M., Lecoq, H., Wipf-Scheibel, C., Millot, P., Girardot, G., & Desbiez, C. (2019). Resistance against melon chlorotic mosaic virus and tomato leaf curl New Delhi virus in melon. *Plant Disease*, *103*(11), 2913-2919. https://doi.org/10.1094/PDIS-02-19-0298-RE

Rossitto De Marchi, B., Kinene, T., Krause-Sakate, R., Boykin, L. M., Ndunguru, J., Kehoe, M., Ateka, E., Tairo, F., Amisse, J., & Sseruwagi, P. (2020). Genetic diversity and SNP's from the chloroplast coding regions of virus-infected cassava. *PeerJ*, *8*, e8632. https://doi.org/10.7717/peerj.8632

Roy, S., Banerjee, A., Tarafdar, J., Senapati, B. K., & Dasgupta, I. (2012). Transfer of transgenes for resistance to rice tungro disease into high-yielding rice cultivars through gene-based marker-assisted selection. *The Journal of Agricultural Science*, *150*(5), 610-618. https://doi.org/10.1017/S0021859611000827

Sade, D., Sade, N., Brotman, Y., & Czosnek, H. (2020). Tomato yellow leaf curl virus (TYLCV)-resistant tomatoes share molecular mechanisms sustaining resistance with their wild progenitor Solanum habrochaites but not with TYLCV-susceptible tomatoes. *Plant science : an international journal of experimental plant biology*, *295*, 110439. https://doi.org/10.1016/j.plantsci.2020.110439

Sáez, C., Martínez, C., Ferriol, M., Manzano, S., Velasco, L., Jamilena, M., López, C., & Picó, B. (2016). Resistance to *Tomato leaf curl New Delhi virus* in *Cucurbita* spp. *Annals of Applied Biology*. *169*, 91-105. doi: 101111/aab12283. https://doi.org/10.1111/aab.12283

Sáez, C., Esteras, C., Martínez, C., Ferriol, M., Dhillon, N. P., López, C., & Picó, B. (2017). Resistance to tomato leaf curl New Delhi virus in melon is controlled by a major QTL located in chromosome 11. *Plant Cell Reports*, *36*(10), 1571-1584. https://doi.org/10.1007/s00299-017-2175-3

Sáez, C., Martínez, C., Montero-Pau, J., Esteras, C., Sifres, A., Blanca, J., Ferriol, M., López, C., & Picó, B. (2020). A Major QTL Located in Chromosome 8 of *Cucurbita moschata* Is Responsible for Resistance to *Tomato Leaf Curl New Delhi Virus*. *Frontiers in plant science*, *11*, 207. https://doi.org/10.3389/fpls.2020.00207

Sahu, P. P., Rai, N. K., Chakraborty, S., Singh, M., Chandrappa, P. H., Ramesh, B., Chattopadhyay, D., & Prasad, M. (2010). Tomato cultivar tolerant to *Tomato leaf curl New Delhi virus* infection induces virus-specific short interfering RNA accumulation and defence-associated host gene expression. *Molecular plant pathology*, *11*(4), 531–544. https://doi.org/10.1111/j.1364-3703.2010.00630.x

Sahu, P. P., Sharma, N., Puranik, S., Chakraborty, S., & Prasad, M. (2016). Tomato 26S Proteasome subunit RPT4a regulates ToLCNDV transcription and activates hypersensitive response in tomato. *Scientific reports*, *6*(1), 1-12. https://doi.org/10.1038/srep27078

Sánchez-Navarro, J. A., Cañizares, M. C., Cano, E. A., & Pallás, V. (1999). Simultaneous detection of five carnation viruses by non-isotopic molecular hybridization. *Journal of Virological Methods*, *82*(2), 167-175. https://doi.org/10.1016/s0166-0934(99)00097-x

Saunders, K., Lucy, A., & Stanley, J. (1992). RNA-primed complementary-sense DNA synthesis of the geminivirus African cassava mosaic virus. *Nucleic acids research*, *20*(23), 6311–6315. https://doi.org/10.1093/nar/20.23.6311

Schaarschmidt, S., Fischer, A., Zuther, E., & Hincha, D. K. (2020). Evaluation of Seven Different RNA-Seq Alignment Tools Based on Experimental Data from the Model Plant Arabidopsis thaliana. *International Journal of Molecular Sciences*, *21*(5), 1720. https://doi.org/10.3390/ijms21051720

Schommer, C., Palatnik, J. F., Aggarwal, P., Chételat, A., Cubas, P., Farmer, E. E., & Weigel, D. (2008). Control of jasmonate biosynthesis and senescence by miR319 targets. *PLoS Biology*, *6*(9), e230. https://doi.org/10.1371/journal.pbio.0060230

Shang, J., Xi, D. H., Xu, F., Wang, S. D., Cao, S., Xu, M. Y., Zhao, P. P., Wang, J. H., Jia, S. D., Zhang, Z. W., Yuan, S., & Lin, H. H. (2011). A broad-spectrum, efficient and nontransgenic approach to control plant viruses by application of salicylic acid and jasmonic acid. *Planta*, *233*(2), 299–308. https://doi.org/10.1007/s00425-010-1308-5

Sharma, A., & Boris-Lawrie, K. (2012). Determination of host RNA helicases activity in viral replication. *Methods in enzymology*, *511*, 405–435. https://doi.org/10.1016/B978-0-12-396546-2.00019-X

Sinha, P., Chang, V., & Tye, B. K. (1986). A mutant that affects the function of autonomously replicating sequences in yeast. *Journal of molecular biology*, *192*(4), 805–814. https://doi.org/10.1016/0022-2836(86)90030-6

Souza, P., Garcia-Ruiz, H., & Carvalho, F. (2019). What proteomics can reveal about plant-virus interactions? Photosynthesis-related proteins on the spotlight. *Theoretical and experimental plant physiology*, *31*(1), 227–248. https://doi.org/10.1007/s40626-019-00142-0

Sun, X., Wang, Z., Gu, Q., Li, H., Han, W., & Shi, Y. (2017). Transcriptome analysis of *Cucumis sativus* infected by *Cucurbit chlorotic yellows virus*. *Virology journal*, *14*(1), 18. https://doi.org/10.1186/s12985-017-0690-z

Sun, Y., Fan, M., & He, Y. (2019). Transcriptome Analysis of Watermelon Leaves Reveals Candidate Genes Responsive to *Cucumber green mottle mosaic virus* Infection. *International Journal of Molecular Sciences*, *20*(3), 610. https://doi.org/10.3390/ijms20030610

Supek, F., Bošnjak, M., Škunca, N., & Šmuc, T. (2011). REVIGO summarizes and visualizes long lists of gene ontology terms. *PloS one*, *6*(7), e21800. https://doi.org/10.1371/journal.pone.0021800

Suyal, G., Mukherjee, S. K., Srivastava, P. S., & Choudhury, N. R. (2013). Arabidopsis thaliana MCM2 plays role(s) in mungbean yellow mosaic India virus (MYMIV) DNA replication. *Archives of virology*, *158*(5), 981–992. https://doi.org/10.1007/s00705-012-1563-9

Tsai, W. A., Weng S. H., Chen, M. C., Lin, J. S., & Tsai, W. S. (2019). Priming of Plant Resistance to Heat Stress and *Tomato Yellow Leaf Curl Thailand Virus* With Plant-Derived Materials. *Frontiers in Plant Science* 10:906. https://doi.org/10.3389/fpls.2019.00906.

Tye B. K. (1999). MCM proteins in DNA replication. *Annual review of biochemistry*, *68*, 649–686. https://doi.org/10.1146/annurev.biochem.68.1.649

Ullah, R., Akhtar, K. P., Moffett, P., Mansoor, S., Briddon, R. W., & Saeed, M. (2014). An analysis of the resistance of Gossypium arboreum to cotton leaf curl disease by grafting. *European journal of plant pathology*, *139*(4), 837-847. https://doi.org/10.1007/s10658-014-0437-2

Untergasser, A., Nijveen, H., Rao, X., Bisseling, T., Geurts, R., & Jack A.M. (2007). Leunissen: Primer3Plus, an enhanced web interface to Primer3. *Nucleic Acids Research*, 35: W71-W74; doi:10.1093/nar/gkm306. https://doi.org/10.1093/nar/gkm306

Verlaan, M. G., Hutton, S. F., Ibrahem, R. M., Kormelink, R., Visser, R. G., Scott, J. W., Edwards, J. D., & Bai, Y. (2013). The *tomato yellow leaf curl virus* resistance genes *Ty-1* and *Ty-3* are allelic and code for DFDGD-class RNA–dependent RNA polymerases. *PLoS genetics*, *9*(3). https://doi.org/10.1371/journal.pgen.1003399

Vicente, J., Cascon, T., Vicedo, B., Garcia-Agustin, P., Hamberg, M., & Castresana, C. (2012). Role of 9-lipoxygenase and alpha-dioxygenase oxylipin pathways as modulators of local and systemic defense. *Molecular Plant* 5, 914-928. https://doi.org/10.1093/mp/ssr105

Vinutha, T., Kumar, G., Garg, V., Canto, T., Palukaitis, P., Ramesh, S. V., & Praveen, S. (2018). Tomato geminivirus encoded RNAi suppressor protein, AC4 interacts with

host AGO4 and precludes viral DNA methylation. *Gene*, *678*, 184-195. https://doi.org/10.1016/j.gene.2018.08.009

Wasternack, C., & Song, S. (2017). Jasmonates: biosynthesis, metabolism, and signaling by proteins activating and repressing transcription. *Journal of Experimental Botany*, *68*(6), 1303-1321. https://doi.org/10.1093/jxb/erw443

Willmann, M. R., Endres, M. W., Cook, R. T., & Gregory, B. D. (2011). The Functions of RNA-Dependent RNA Polymerases in Arabidopsis. *The arabidopsis book*, *9*, e0146. https://doi.org/10.1199/tab.0146

Wu, M., Ding, X., Fu, X., & Lozano-Durán, R. (2019). Transcriptional reprogramming caused by the geminivirus Tomato yellow leaf curl virus in local or systemic infections in Nicotiana benthamiana. *BMC Genomics*, *20*(1), 542.

Xia, C., Li, S., Hou, W., Fan, Z., Xiao, H., Lu, M., Sano, T., & Zhang, Z. (2017). Global Transcriptomic Changes Induced by Infection of Cucumber (*Cucumis sativus* L.) with Mild and Severe Variants of Hop Stunt Viroid. *Frontiers in microbiology*, *8*, 2427. https://doi.org/10.3389/fmicb.2017.02427

Yadeta, K. A., Elmore, J. M., Creer, A. Y., Feng, B., Franco, J. Y., Rufian, J. S., He, P., Phinney, B., & Coaker, G. (2017). A Cysteine-Rich Protein Kinase Associates with a Membrane Immune Complex and the Cysteine Residues Are Required for Cell Death. *Plant Physiology*, *173*(1), 771–787. https://doi.org/10.1104/pp.16.01404

Yan, C., & Xie, D. (2015). Jasmonate in plant defence: sentinel or double agent? *Plant biotechnology journal*, *13*(9), 1233–1240. https://doi.org/10.1111/pbi.12417

Yang, S. J., Carter, S. A., Cole, A. B., Cheng, N. H., & Nelson, R. S. (2004). A natural variant of a host RNA-dependent RNA polymerase is associated with increased susceptibility to viruses by Nicotiana benthamiana. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(16), 6297–6302. https://doi.org/10.1073/pnas.0304346101

You, W. J., Feng, Y. R., Shen, Y. H., Chen, Y. R., Chen, T. Y., & Fu, S. F. (2019). Silencing of NbCMT3s has Pleiotropic Effects on Development by Interfering with Autophagy-Related Genes in Nicotiana benthamiana. *Plant & cell physiology*, *60*(5), 1120–1135. https://doi.org/10.1093/pcp/pcz034

Yousif, M. T., Kheyr-Pour, A., Gronenborn, B., Pitrat, M., & Dogimont, C. (2007). Sources of 536 resistance to Watermelon Chlorotic Stunt Virus in melon. *Plant Breeding*, 126, 422-427. https://doi.org/10.1111/j.1439-0523.2007.01366.x

Zaidi, S. S., Naqvi, R. Z., Asif, M., Strickler, S., Shakir, S., Shafiq, M., Khan, A. M., Amin, I., Mishra, B., Mukhtar, M. S., Scheffler, B. E., Scheffler, J. A., Mueller, L. A., & Mansoor, S. (2020). Molecular insight into cotton leaf curl geminivirus disease resistance in cultivated cotton (Gossypium hirsutum). *Plant biotechnology journal*, *18*(3), 691–706. https://doi.org/10.1111/pbi.13236

Zhai, Y., Peng, H., Neff, M. M., & Pappu, H. R. (2020). Emerging Molecular Links Between Plant Photomorphogenesis and Virus Resistance. *Frontiers in plant science*, *11*, 920. https://doi.org/10.3389/fpls.2020.00920

Zhang, Z., Chen, H., Huang, X., Xia, R., Zhao, Q., Lai, J., Teng, K., Li, Y., Liang, L., Du, Q., Zhou, X., Guo, H., & Xie, Q. (2011). BSCTV C2 attenuates the degradation of SAMDC1 to suppress DNA methylation-mediated gene silencing in Arabidopsis. *The Plant cell*, *23*(1), 273–288. https://doi.org/10.1105/tpc.110.081695

Zhang, L., Zhang, F., Melotto, M., Yao, J., & He, S. Y. (2017). Jasmonate signaling and manipulation by pathogens and insects. *Journal of experimental botany*, *68*(6), 1371–1385. https://doi.org/10.1093/jxb/erw478

SUPPLEMENTARY MATERIAL

The Supplementary Material for this chapter can be found online at:

https://upvedues-my.sharepoint.com/:u:/g/personal/crisaesa_upv_edu_es/EWmMiDOfvAdEvE1GEgPB_hMB46NZ8bQCxt-aa6sUgsU4rQ?e=iC0PpT

Discusión general

El virus de la hoja rizada del tomate de Nueva Delhi (ToLCNDV) constituye una de las principales limitaciones a las que se enfrenta el cultivo de las cucurbitáceas en diferentes países del subcontinente indio desde hace más de dos décadas (Varma et al., 2013). En 2012 el virus se detectó en España y desde entonces se ha convertido en una seria enfermedad emergente que amenaza la producción de cucurbitáceas en los principales países productores de la cuenca del Mediterráneo (López et al., 2015; Zaidi et al., 2017b; Panno et al., 2019).

Los graves daños generados por la cepa ToLCNDV-ES en los cultivos de calabacín y melón han ocasionado serias pérdidas agroeconómicas en el área mediterránea, que indirectamente afectan al resto de Europa, al ser ésta su principal abastecedora (Velasco et al., 2020). La uniformidad genética de las variedades comerciales empleadas hoy en día en la horticultura intensiva (Dempewolf et al., 2014) y la eficiencia en la transmisión del virus por su vector *Bemisia tabaci* (uno o pocos individuos pueden generar una epidemia) (EFSA PLH Panel, 2013), han favorecido la rápida propagación del virus. La implantación de medidas eficientes de control integrado y la mejora en el manejo del cultivo han permitido reducir la incidencia de la infección, y aunque se han desarrollado algunas variedades con resistencia parcial, hasta el momento no se cuenta con cultivares comerciales con resistencia completa a la enfermedad.

La identificación de genotipos resistentes al ToLCNDV dentro de las diferentes especies de la familia de las cucurbitáceas, reproductivamente compatibles con las variedades comerciales, fue el primer objetivo de esta tesis doctoral. El ToLCNDV-ES afecta principalmente a calabacín y melón, y en menor medida a pepino. En contra de los pronósticos iniciales, la sandía puede ser hospedante pero no se ve afectada por esta virosis. Acorde a este escenario, el objetivo global de esta tesis doctoral ha sido el aprovechamiento de la variabilidad natural en los géneros *Cucurbita* y *Cucumis* para la identificación de resistencias al ToLCNDV y el análisis de las bases genéticas y moleculares que las regulan.

1. Recursos fitogenéticos en cucurbitáceas para la mejora de la resistencia al ToLCNDV

Hasta la detección del ToLCNDV en plantas de la familia de las cucurbitáceas en España y el resto de los países mediterráneos, la principal amenaza vírica para esta familia hortícola la constituían los virus pertenecientes a los géneros *Cucumovirus*, *Potyvirus* y *Tobamovirus* (Pitrat, 2008; Lecoq & Desbiez, 2012), para los que se han identificado fuentes de genes de resistencia en diferentes especies de cucurbitáceas.

Al abordar un programa de mejora de resistencia a virus, resulta fundamental conocer en qué materiales se ha descrito previamente resistencia, incluyendo ancestros silvestres, tipos exóticos, variedades locales tradicionales y modernas, con el fin de seleccionar la estructura de las poblaciones de germoplasma a cribar y accesiones portadoras de genes candidatos de resistencia. En melón, la mayoría de las resistencias descritas se localizan en tipos exóticos de la India o Asia oriental (Singh et al., 2020), mientras que en el género *Cucurbita* las especies *C. moschata* y *C. ecuadorensis* han resultado ser un reservorio de genes de resistencia transferibles a *C. pepo* (Paris, 2016b).

Las accesiones procedentes de los centros geográficos de origen o diversificación de cada especie suelen ser reservorio de genes de resistencia, debido a que no han sufrido un proceso de domesticación en el que es común la selección de caracteres involucrados en el rendimiento de la planta o la calidad de fruto, en detrimento de otros caracteres de interés como la resistencia a enfermedades (Gómez et al., 2009).

Puesto que el calabacín (*C. pepo*) es el cultivo más afectado por el ToLCNDV-ES (Juárez et al., 2014), y uno de los más rentables económicamente en nuestro país, este trabajo se inició con el cribado para la identificación de resistencia de una colección de germoplasma del género *Cucurbita.* En esta colección se encontraba representada la variabilidad genética del género, incluyendo especies silvestres y variedades cultivadas, tradicionales y modernas, procedentes de las zonas de origen

y diversificación. Históricamente, la especie *C. pepo* ha mostrado un comportamiento altamente susceptible a enfermedades, particularmente a las de etiología viral, haciendo que la resistencia a virus se haya convertido en el principal objetivo de mejora del cultivo de calabacín (Whitaker & Robinson, 1986).

Aunque se ha descrito resistencia parcial o tolerancia a virus en algunas accesiones de *C. pepo* (Walkey et al., 1984; Lebeda & Křistkova, 1996), la gran variabilidad intragénero y la posibilidad de realizar cruzamientos interespecíficos suponen una alternativa para identificar resistencia a virus en otras especies. *Cucurbita moschata* es parcialmente cruzable con *C. pepo* y permite obtener progenie, aunque dependiendo de los genotipos empleados el grado de compatibilidad varía (Paris, 2008; Lira et al., 2009). Se ha descrito también resistencia a enfermedades de etiología viral en algunas accesiones de *C. maxima* (Provvidenti, 1982), sin embargo, en la mejora genética del calabacín la mayoría de los intentos por introgresar la resistencia a virus se ha realizado a partir de cruzamientos con *C. moschata* y especies silvestres (Paris, 2016b).

Las accesiones de *C. pepo* evaluadas en este trabajo representan los principales morfotipos de las subespecies *pepo* y *ovifera* (L.), y procedían de diferentes regiones incluyendo América e Italia, centros de origen y diversificación. Sin embargo, todas las entradas evaluadas resultaron altamente susceptibles al ToLCNDV-ES, incluyendo las accesiones del morfotipo Vegetable Marrow que se han descrito previamente como resistentes a CMV (Walkey et al., 1984). Los resultados obtenidos mediante inoculación controlada confirmaron esta susceptibilidad en otras regiones productoras de calabacín del Mediterráneo, donde este cultivo ha resultado ser el más afectado por el ToLCNDV-ES (Panno et al., 2019; Juárez et al., 2019).

Las accesiones de *C. maxima*, a pesar de proceder de Argentina y África, centro de domesticación y diversificación, respectivamente, y las especies silvestres *C. ecuadorensis*, *C. lundelliana*, *C. foetidissima* y *C. ockeechobeensis*, descritas como

fuente de resistencia a SLCV (McCreight & Kishaba, 1991), también desarrollaron síntomas severos tras la infección con ToLCNDV-ES.

La especie *C. moschata* es originaria de América Central, aunque su cultivo se dispersó por todo el mundo tras la colonización europea. Entre las cucurbitáceas, no genera grandes rendimientos económicos (McCreight, 2016), aunque el cultivo y comercialización de variedades locales aún se mantiene en mercados municipales o regionales de algunos países tropicales en Asia, África y América, y su consumo ha ganado popularidad en los últimos años (Marie-Magdeleine et al., 2011, Paris, 2016a). Algunas accesiones tropicales, como Nigerian Local o MENINA, se han utilizado como fuente de resistencia a potyvirus y cucumovirus (Paris et al., 1988; Pachner et al., 2011), y se han empleado en la mejora de *C. pepo* mediante cruces interespecíficos (Brown et al., 2003; Paris, 2016b).

Acorde a los resultados precedentes, los cribados realizados en este trabajo han permitido identificar dos genotipos de la especie *C. moschata* resistentes al ToLCNDV-ES. Una de las accesiones identificadas es el cultivar 'Large Cheese' (PI 604506), donada al banco de germoplasma nacional de Estados Unidos (USDA-ARS) por la empresa de semillas 'Burpee Company' (Pensilvania, Estados Unidos). Aunque no se encuentra documentado, su origen probablemente es norteamericano, donde este genotipo ha sido tradicionalmente cultivado para uso doméstico, mercados locales y conservas. La otra accesión de *C. moschata* resistente al ToLCNDV-ES (PI 381814) es una variedad local originario de la India. De manera similar, todas las accesiones de cucurbitáceas descritas en otros trabajos con resistencia al ToLCNDV-ES proceden de la India.

La primera vez que se identificó resistencia a uno de los aislados asiáticos del ToLCNDV en cucurbitáceas fue en el género *Luffa* (Islam et al., 2010), en cuatro accesiones procedentes de Nueva Delhi. López et al. (2015) identificaron cinco accesiones de *C. melo* de los grupos momordica (PI 414723, PI 124112 y Kharbuja (PI 614473)) y kachri (WM-7 y WM-9) con resistencia a ToLCNDV-ES y todas originarias de la región norte de la India. Romay et al. (2019) confirmaron la

resistencia al virus en tres de esas accesiones e identificaron tres nuevos genotipos (AM-87, PI179901 e IC-274014) con alto nivel de resistencia también originarios de la India.

Aunque la incidencia del ToLCNDV-ES en el cultivo de pepino (*Cucumis sativus*) no ha sido tan elevada como en calabacín o melón, la presencia de mosca blanca virulífera en explotaciones de este cultivo ha dado lugar a reducciones de cosecha y pérdidas de producción. Ante la demanda de estrategias de control de la virosis, en esta tesis doctoral se ha iniciado un programa de mejora para la resistencia al ToLCNDV-ES en pepino. Las resistencias a enfermedades de etiología viral en pepino se han descrito sólo en un reducido material genético (Wang et al. 1984; Provvidenti, 1987) principalmente en las variedades *sativus* y *hardwickii*, esta última nativa del sur del Himalaya y considerada el ancestro silvestre del pepino cultivado (Staub, 1985; Munshi et al., 2008; Pujol et al., 2019; Crespo et al., 2018). Aunque los cribados realizados en la presente tesis doctoral, no incluían accesiones silvestres de la variedad *hardwickii*, se han identificado fuentes de resistencia al ToLCNDV-ES en la variedad cultivada *sativus*, y al igual que en melón y *C. moschata*, las tres accesiones que han ofrecido mayor resistencia, procedían de la India (CGN23089, CGN23423 y CGN23633).

A partir de los resultados obtenidos, el germoplasma de tipo silvestre y variedades locales de los géneros *Cucumis* y *Cucurbita* procedente de la India debe considerarse como un nicho de resistencia genética al ToLCNDV-ES, de donde es originario el virus y donde se identificó por primera vez infectando cucurbitáceas. Posiblemente, la coexistencia de ambos organismos en la misma región geográfica ha propiciado un proceso de co-evolución entre el virus y la planta en esa área (Dhillon et al., 2012).

El lugar de origen de algunos virus de diferentes especies y géneros a menudo coincide con el centro de origen de sus principales hospedantes (Lovisolo et al., 2003; Hull, 2014). Para que la relación planta-virus se mantenga de manera estable es necesario alcanzar un equilibrio entre ambos organismos. El virus requiere

alcanzar un nivel de replicación y propagación que permita su transmisión, pero sin comprometer el ciclo vital de su hospedante (Lovisolo et al., 2003). Estas interacciones son complejas e implican una presión de selección sobre los genes de resistencia y avirulencia involucrados, repercutiendo directamente en la diversidad de los organismos implicados y convirtiéndose en la fuerza motriz que hace posible su evolución conjunta (Dodds et al., 2006; Charron et al., 2008, Fraile & García-Arenal; 2010; Masri et al., 2015).

La co-evolución entre WMV y cucurbitáceas se ha propuesto con anterioridad para explicar la respuesta diferencial de accesiones de *C. pepo* y *C. maxima* de diversos orígenes según se inoculasen con unos aislados del virus u otros (Krístková & Lebeda, 2000). Se han descrito también procesos de co-evolución planta-patógeno entre los genes de resistencia eIF4E y la proteína de avirulencia VPg de potyvirus (Charron et al., 2008), entre el gen de resistencia *L* del lino (*Linum usitatissimum L.)* y el hongo *Melampsora lini* (Ehrenb.) Desm. (Dodds et al., 2006) y entre los genes de resistencia *Pto* de tomate y *Pseudomonas syringae* pv. tomato (Pst) (Rose et al., 2007).

Al igual que en otros cultivos, la domesticación de las cucurbitáceas dio lugar a una disminución de la diversidad genética en forma de 'cuello de botella', provocando la pérdida de regiones genómicas con genes responsables de características agronómicas de interés, como la resistencia a patógenos (Qi et al., 2013). El hecho de que la mayor parte del germoplasma resistente a ToLCNDV-ES proceda de la India puede deberse a que se trata de accesiones silvestres o landraces que han sufrido un bajo nivel de manipulación y selección por el ser humano.

Los genotipos de melón originarios de la India se han descrito como resistentes a otros begomovirus (Romay et al., 2019) y su diversidad genética ha sido particularmente estudiada por Gonzalo et al. (2019). Estas accesiones no constituyen una colección de tipos landraces tradicionales, en los que sus alelos han sido fijados durante la actividad agrícola, sino que se trata de una población de plantas en las se ha producido intercruzamiento entre melones silvestres y cultivados, mantenido un

alto nivel de diversidad genética. Estas poblaciones difieren del germoplasma silvestre africano, y a partir de ellas se podrían haber originado el resto de las variedades tradicionales cultivadas en la cuenca del Mediterráneo y en Asia mediante domesticación, selección y diversificación divergente (Gonzalo et al., 2019). En pepino se ha descrito una estructura similar, concentrándose la mayor diversidad genética en el germoplasma indio (Qi et al 2013). Por otra parte, en el género *Cucurbita*, la selección por agricultores locales de poblaciones alopátricas durante la domesticación favoreció la evolución divergente dentro de cada especie (Paris, 2016a). Los cultivares de *C. moschata* tipo 'cheese pumkin' se originaron en América del Norte, pero pueden encontrarse variantes de este tipo de calabaza en la India y el sudeste asiático, donde se localiza una de las áreas de diversificación de la especie (Decker-Walters & Walters, 2000).

Todos nuestros resultados y los estudios previos de variabilidad genética realizados en diferentes especies de la familia de las cucurbitáceas, ponen de manifiesto la importancia de mantener y explotar el germoplasma silvestre localizado en los centros de origen y diversificación de las diferentes especies, especialmente para la búsqueda de resistencia a enfermedades. Las accesiones resistentes de *C. moschata* y *C. sativus* identificadas en este trabajo constituyen las primeras referencias de resistencia al ToLCNDV en calabaza y pepino y suponen una valiosa fuente para el desarrollo de cultivares de élite resistentes en programas de mejora genética.

2. Caracterización genética de la resistencia al ToLCNDV-ES en cucurbitáceas

Las resistencias identificadas en el marco de esta tesis doctoral y las proporcionadas en trabajos previos, son directamente aprovechables en programas de mejora frente al ToCLNDV-ES. Las accesiones descritas como resistentes de los grupos momordica y kachri de melón no presentan barreras de cruzabilidad con las variedades cultivadas (Pitrat, 2008) y las entradas de pepino identificadas en nuestros ensayos pertenecen a la variedad cultivada, por lo que pueden utilizarse directamente

para la obtención de descendencia y ser transferidas mediante sucesivas generaciones de retrocruzamiento a los parentales recurrentes comerciales. Como se ha descrito anteriormente, la especie *C. moschata*, a la que pertenecen las dos accesiones resistentes al ToLCNDV-ES, presenta compatibilidad sexual parcial con el calabacín cultivado (*C. pepo*), posibilitando la transferencia de la resistencia. No obstante, para la óptima introgresión de estas resistencias en programas de mejora es necesario su análisis y caracterización, determinando su grado de resistencia y su regulación genética. La construcción de poblaciones segregantes y el empleo de colecciones de marcadores moleculares de tipo SNP han sido fundamentales para abordar el mapeo de la resistencia en cada especie. Los resultados obtenidos en esta tesis han sido las primeras referencias en la caracterización genética de la resistencia al aislado español del ToLCNDV en cucurbitáceas.

Melón

Cuatro de las accesiones resistentes al ToLCNDV-ES de *C. melo* identificadas por López et al. (2015) se cruzaron con la variedad cultivada "Piñonet Piel de Sapo" (PS, *C. melo*, grupo ibericus), la más popular y tradicionalmente apreciada por los consumidores en España, y muy susceptible al virus. Los cuatro híbridos F1 obtenidos fueron asintomáticos tras la infección, sin embargo, la carga viral en las accesiones momordica era mayor que la de los tipos silvestres kachri, implicando diferencias en la configuración genética que regula la resistencia al ToLCNDV-ES en ambos grupos.

Para llevar a cabo el análisis detallado del control genético de la resistencia al ToLCNDV-ES se seleccionó el híbrido obtenido a partir de una de las accesiones silvestres (WM-7). Este genotipo ofrece un elevado nivel de resistencia al virus y aunque procede de una población con elevada variabilidad, ha sido autofecundada en sucesivos ciclos de cultivo en nuestras instalaciones del COMAV, favoreciendo el incremento del número de *loci* en homocigosis. Nuestros análisis han determinado que la resistencia al ToLNDV-ES en esta accesión está regulada por un gen dominante en el cromosoma 11 y dos regiones moduladoras de efecto menor en los

cromosomas 2 y 12. Entre estos tres *loci* existen interacciones epistáticas que sugieren que el gen mayor del cromosoma 11 es parcialmente dominante. Cuando este *locus* se encuentra en homocigosis, es suficiente para conferir resistencia, pero en heterocigosis la acumulación del virus en la planta y el desarrollo de síntomas se ve determinado por el genotipo en las regiones candidatas de los cromosomas 2 y 12, respectivamente.

Romay et al., (2019), confirmaron posteriormente nuestros resultados, mostrando que un gen recesivo y dos dominantes controlan la herencia de la resistencia al ToLCNDV-ES en la accesión IC-274014. Las dos accesiones pertenecen a los grupos WM e IC, estrechamente relacionados genéticamente dentro de la población de genotipos de melón originarios de la India (Gonzalo et al., 2019), lo que sugiere que estas dos entradas podrían compartir genes de resistencia.

La regulación de la resistencia al ToLCNDV también se estudió en *Luffa* (Islam et al., 2010; 2011) y se identificó un gen mayor dominante. En *Solanum habrochaites*, una especie silvestre relacionada con el tomate, se describió una regulación oligogénica controlada por tres genes dominantes que confieren resistencia al ToLCNDV (Rai et al., 2013).

La resistencia a otras virosis en melón también se ha caracterizado. En el genotipo TGR-1551 del grupo acidulus procedente del norte de África, pero introducido desde la India (Gónzalo et al., 2019), la resistencia a WMV está regulada por un gen mayor recesivo en el cromosoma 11 y tres modificadores menores (Pérez de Castro et al., 2019) y a CABYV por un gen dominante y al menos dos genes modificadores (Kassem et al., 2015). Palomares-Rius et al. (2016) también describieron en esta accesión dos regiones involucradas en la resistencia a CSYDV, una responsable de la multiplicación del virus y la otra controlando el desarrollo de síntomas, ambas en el cromosoma 5, mecanismo similar al que hemos identificado en la resistencia al ToLCNDV-ES en WM-7.

Aunque también se han descrito accesiones de *C. melo* con resistencia monogénica dominante o recesiva a enfermedades causadas por virus (Pitrat, 2008;

Fernández-Trujillo et al., 2011; Kassem et al., 2015; McCreight et al., 2015), nuestros resultados confirman que el tipo de herencia de las resistencias a enfermedades víricas en los genotipos exóticos y silvestres de melón siguen principalmente una regulación de carácter poligénico. El manejo de este tipo de resistencias en el proceso de introgresión en cultivares comerciales supone una dificultad añadida frente a las resistencias de carácter monogénico, dependiendo en gran medida del fondo genético vegetal e influenciadas por el ambiente. (Kobayashi et al., 2014; Velasco et al., 2020). Sin embargo, las resistencias oligo o poligénicas son a menudo más durables y difíciles de superar por el patógeno y, particularmente, QTLs de efecto menor pueden incrementar la durabilidad de un gen mayor de resistencia (Palloix et al., 2009; Gallois et al., 2018; Velasco et al., 2020).

Cucurbita

La amenaza generada por el ToLCNDV-ES para los productores de calabacín supone una demanda urgente para transferir la resistencia de las accesiones de *C. moschata* a *C. pepo.* Sin embargo, el estudio de la genética de la resistencia en este género se realizó a partir de las poblaciones segregantes obtenidas del cruce de una accesión resistente de *C. moschata* con otra susceptible de la misma especie, evitando así las distorsiones en la segregación genética que pueden ocurrir tras un cruce interespecífico (Szinay et al., 2010, 2012).

En la caracterización de la genética de la resistencia al ToLCNDV-ES en *C. moschata* hemos identificado un gen mayor de herencia recesiva localizado en el cromosoma 8 del genoma de *C. moschata*, que controla la resistencia en las dos accesiones resistentes al virus. La identificación de un *locus* común de resistencia en ambas accesiones sugiere que los genes incluidos en esta región se encuentran conservados desde antes de la dispersión de la especie desde Centro América a diferentes lugares del mundo. La posterior diversificación de *C. moschata* en la India habría generado nuevos genotipos que perdieron la resistencia al ToLCNDV, conservándose en tipos landraces como es el caso de la accesión PI 381814.

La cruzabilidad parcial entre *C. pepo* y *C. moschata* ha permitido obtener híbridos interespecíficos y generar poblaciones segregantes para el carácter de resistencia al ToLCNDV-ES. Según nuestro conocimiento, esta es la primera vez que se obtienen genotipos descendientes de plantas de calabacín con resistencia a ToLCNDV-ES. Mediante este cruce, fue posible la transferencia del *locus* de resistencia de efecto mayor de *C. mochata* al genoma de calabacín, confirmando un QTL ligado a la resistencia al virus en el cromosoma 17. Sin embargo, la penetrancia del carácter de resistencia entre ambas especies ha resultado incompleta, dando lugar a anomalías en la segregación.

La transferencia genética parcial en cruces interespecíficos entre *C. moschata* y *C. pepo* no es inesperada (Ott et al., 2015). Se han llevado a cabo esfuerzos similares para transferir el gen mayor de resistencia a ZYMV, *Zym-1*, de *C. moschata* a *C. pepo*¸ siendo necesaria la piramidalización de hasta seis genes adicionales para mantener la resistencia en calabacín (Pachner et al., 2015; Capuozzo et al., 2017). El papel que juega el fondo genético en la resistencia a virus vegetales se considera crucial en programas de mejora a resistencias cuando es necesaria la transferencia de genes o QTLs de una especie a otra (Gallois et al., 2018).

En mejora genética, las barreras de incompatibilidad sexual a la hora de realizar cruces interespecíficos han supuesto una gran limitación al abordar programas de mejora. La supresión en la recombinación, las desviaciones en la segregación de los *loci* y la transferencia parcial de las regiones del genoma a la descendencia, son fenómenos frecuentes en este tipo de cruces, sobre todo cuando el distanciamiento filogenético de las especies involucradas se incrementa (Lu et al., 2002; Anderson et al., 2010; Szynay et al., 2010; Barrantes-Santamaría et al., 2020).

Las distorsiones en la segregación de la resistencia a ZYMV en poblaciones procedentes de cruces entre *C. pepo* y *C. mochata* se han asociado a fenómenos de cuasi-ligamiento, en los que *loci* de diferentes cromosomas no segregan de manera independiente o al azar (Formisano et al., 2010), si no que la afinidad entre cromosomas no homólogos o entre regiones de los mismos podría dar lugar a su

herencia como un único grupo de ligamiento integrado (Levi et al., 2003b; Formisano et al., 2010). Estas anomalías también se han descrito en otras cucurbitáceas, y se ha visto una alta prevalencia en poblaciones F_2 derivadas de cruces entre sandía cultivada y la variedad *citroides* (Levi et al., 2003b).

En la mejora genética de *C. pepo* se han obtenido híbridos comerciales con resistencia a enfermedades, introgresadas a partir de *C. moschata*. Formisano et al. (2010) compararon genéticamente híbridos con resistencia a ZYMV, WMV y oidio, e ILs derivadas de los mismos. Los autores observaron una elevada similitud genética entre los genotipos híbridos, pero más baja en las ILs, lo que atribuyen a la presencia de grandes regiones del genoma de *C. moschata* en todos los genotipos con resistencia. Estos autores también sugieren que los genes de resistencia a enfermedades en *C. moschata* podrían encontrarse localizados en las regiones centroméricas y teloméricas del genoma, ya que son regiones en las que no tiene lugar la recombinación genética (Haanstra et al., 1999; Saliba-Colombani et al., 2000) y en los que se han descrito genes de resistencia a enfermedades en otras especies vegetales (Orbach et al., 2000, Nepal et al., 2017). Curiosamente, el QTL ligado a la resistencia al ToLCNDV-ES en *C. moschata* ha sido mapado en la región inicial del cromosoma 8 de *C. moschata* y los QTLs identificados en *C. melo* están localizados al final de los cromosomas 2 y 11 y al comienzo del cromosoma 12.

Para evitar la pérdida de información genética en regiones que contribuyen a la resistencia a enfermedades, es necesario disponer de buenas herramientas moleculares y genómicas. El uso de marcadores moleculares es una herramienta de gran utilidad para estudiar regiones con segregación distorsionada (Barrantes-Santamaría et al., 2020). Cuanto más saturado se encuentre el genoma, más sencilla resulta la monitorización de la transferencia de los genes implicados en la resistencia a virus (Capuozzo et al., 2017).

Pepino

Debido a que el ToLCNDV-ES se identificó inicialmente infectando calabacín, y a que los daños más severos se han registrado en este cultivo y el de melón, la

búsqueda de resistencia en pepino no fue un objetivo prioritario y la caracterización de las resistencias identificadas aún no se ha llevado a cabo con detalle.

Los híbridos F1 obtenidos en el cruce entre las accesiones resistentes y susceptibles de la variedad *sativus* mostraron síntomas y elevada acumulación viral. Estos resultados preliminares sugieren un control de la resistencia recesivo. El modelo de resistencia con herencia recesiva, monogénica o regulada por varios QTLs, ha sido descrito en otras variedades de pepino frente a ZYMV, WMV, CVYV y CMV (Wai & Grumet, 19995; Munshi et al., 2008; Pitrat et al., 2012b; Amano et al., 2013). Nuestro grupo ha desarrollado poblaciones segregantes para llevar a cabo el estudio en profundidad de la regulación genética de la resistencia al ToLCNDV en estas accesiones.

Sintenia entre los loci de resistencia al ToLCNDV de C. melo* y *C. moschata

En los trabajos de esta tesis, hemos identificado dos *loci* de efecto mayor ligados a la resistencia al ToLCNDV-ES, uno en el cromosoma 11 del genoma de *C. melo* y otro en el cromosoma 8 de *C. moschata.* Ambas regiones han resultado sinténicas entre sí, y comparten un grupo de genes ortólogos común.

Las secuencias completas de los genomas de diferentes especies de cucurbitáceas se han comparado en diversos estudios y se han observado relaciones sinténicas entre diferentes regiones cromosómicas (Li et al., 2011; García-Mas et al., 2012; Guo et al., 2013; Yang et al., 2014; Zhu et al., 2016; Pan et al., 2020; Guo et al., 2020b). En cucurbitáceas, estas regiones con sintenia suelen incluir genes de importancia funcional que han mantenido conservada su estructura y agrupación en el genoma evolutivamente (Guo et al., 2020).

Los genes que codifican proteínas NBS-LRR de resistencia a enfermedades en melón, pepino y sandía aparecen agrupados en regiones en las que existe mucha variabilidad (presencia/ausencia de genes) y que son sinténicas entre especies (González et al., 2013; 2014; Morata et al., 2017). Las diferencias en el número de genes NBS-LRR entre unas especies y otras puede deberse a la generación de copias

en *loci* específicos tras la separación de las cucurbitáceas en el proceso de especiación. Aunque se han descrito diferentes grupos de genes NBS-LRR en el genoma de melón sinténicos con pepino y sandía (Morata et al., 2017), el de mayor tamaño lo constituye el grupo del cromosoma 5, que incluye el gen *Vat* de resistencia a áfidos y genes *Pm* de resistencia aoidio. Un segundo grupo se localiza en el cromosoma 9, e incluye los genes *Fom1* y *Prv* de resistencia a *Fusarium oxysporum* f. sp. *melonis* y a *papaya ring spot virus*, respectivamente (Brotman et al., 2005). En el cromosoma 11 de melón no se han descrito grupos de genes de resistencia, sin embargo, el QTL de resistencia identificada al ToLCNDV-ES en esta tesis en *C. melo* y el QTL de resistencia a WMV en la accesión TGR-1551 (Pérez de Castro et al., 2019) se encuentran localizadas en este cromosoma con intervalos que solapan entre sí.

El grupo de genes que se ha identificado en la región sinténica entre *C. moschata* y *C. melo* también es sinténico con una región localizada en el cromosoma 6 de *C. sativus*. La caracterización y cartografía de la resistencia al ToLCNDV en las resistencias identificadas permitirán determinar si este grupo de genes también es responsable de la resistencia al ToLCNDV en pepino.

3. Mecanismos moleculares de resistencia al ToLCNDV en cucurbitáceas

Tras estudiar el tipo de herencia que regula la resistencia al ToLCNDV en las accesiones identificadas de melón y *Cucurbita*, la motivación final de esta tesis doctoral consistió en profundizar en el conocimiento de los mecanismos moleculares que regulan la respuesta a la infección por este geminivirus.

La secuenciación del transcriptoma completo de las accesiones de melón WM-7 y PS en tres estadios diferentes tras la infección con ToLCNDV, ha permitido identificar cambios en la reprogramación transcripcional durante el desarrollo de la enfermedad. Además, hemos observado diferentes perfiles de expresión que afectan a diversas familias génicas.

En la accesión WM-7, la propagación y replicación del virus se inhibe en estadios tempranos tras la inoculación y se mantiene a bajos niveles durante todo el ciclo de vida de la planta. Por el contrario, aunque en la accesión susceptible PS la carga viral al comienzo de la infección es baja y similar a la detectada en WM-7, en estadios posteriores se produce un incremento exponencial de la acumulación viral que conduce al desarrollo de síntomas severos. A nivel transcripcional, el estrés que genera el ToLCNDV en las plantas de PS se ve reflejado en un número de genes con expresión alterada muy superior al identificado en WM-7. Diferencias similares se han observado en estudios transcriptómicos tras la infección por otros geminivirus (Allie et al., 2014; Zaidi et al., 2020), sugiriendo que el virus es capaz de manipular la maquinaria celular del hospedante y producir un desequilibrio que altera el correcto desarrollo de la planta.

En nuestros ensayos, tanto en el genotipo resistente como en el susceptible se produjo una desregulación de genes implicados en la respuesta a estreses. Este tipo de respuesta inmune es genérica, ya que no depende de un factor de avirulencia específico para su activación (Whitham et al., 2006; Ali et al., 2018). Aunque en el genotipo susceptible (PS) la transcripción de estos genes también se ve afectada, la señal de defensa no es efectiva o no alcanza un nivel suficiente para evitar la transcripción, replicación y posterior propagación del virus. Los geminivirus pueden interaccionar con los genes del hospedante, generando una reprogramación transcripcional que favorece la replicación del DNA viral y su posterior transporte a través de los diferentes tejidos de la planta (Ullah et al., 2014). En el genotipo susceptible PS, los procesos de replicación del DNA se encuentran altamente inducidos, al igual que proteínas transmembrana y factores de transcripción que favorecen el incremento de la acumulación viral. Es probable que en los primeros estadios de la infección el ToLCNDV interaccione con factores de *C. melo* implicados en el ciclo celular, incrementado la eficiencia para generar copias del genoma viral. En cambio, estos mismos procesos no se encuentran alterados o se ven reprimidos en la accesión WM-7, resistente al virus. La resistencia al ToLCNDV en un cultivar de tomate también se asoció con la capacidad de replicación del virus en

el hospedante y se identificó un gen DEAD-box helicasa directamente implicado (Sahu et al., 2010; Pandey et al., 2019).

La respuesta de defensa basal puede combinarse con la activación de rutas de defensa específica de manera local y temprana, generalmente inducida por genes R, inhibiendo la propagación viral mediante las reacciones de hipersensibilidad (HR) y muerte celular programada (Ascencio-Ibáñez et al., 2008; Coll et al., 2011). En los genotipos de melón evaluados, la infección por ToLCNDV genera cambios transcripcionales en genes R de diferentes cromosomas, pero también de otras proteínas directamente implicadas en la HR, como receptores de tipo quinasa ricas en cisteína (CRKs), lipoxigenasas o fitohormonas. La regulación hormonal es una de las rutas de defensa que se ven inhibidas durante la infección por geminivirus en plantas (Soitamo et al., 2012; Kumar, 2019). Los cambios transcripcionales que hemos identificado en melón, tanto en la accesión resistente como susceptible, comprometen diferentes genes implicados en la ruta de señalización del ácido jasmónico, que a su vez podrían repercutir en los procesos para desencadenar resistencia temprana.

La activación de la respuesta de defensa suele generar un sobrecoste energético en la planta, que si no se realiza eficientemente puede dificultar su correcto desarrollo fisiológico y suponer un perjuicio mayor que la propia infección viral (Allie et al., 2014). Una estrategia complementaria para inducir resistencia y restringir la infección viral es la activación de proteínas implicadas en las rutas de señalización fotosintéticas (Souza et al., 2019). Los cloroplastos son orgánulos diana durante la infección por virus, juegan un papel crucial en la síntesis y regulación del ácido jasmónico y se han descrito implicados en la respuesta de defensa y desarrollo de síntomas virales (Zhao et al., 2016; Guerrero et al., 2020). En la accesión de melón resistente al ToLCNDV, WM-7, hemos observado una elevada inducción de genes necesarios para llevar a cabo la fotosíntesis. Esta inducción podría determinar la ausencia de síntomas característicos en hojas, como moteados, amarilleamientos y mosaico.

Por otra parte, cambios en proteínas clave en la metilación del DNA viral, como modificaciones en la cromatina, histonas, proteínas Argonauta (AGO) y metiltransferasas, ponen de manifiesto que el silenciamiento génico transcripcional es probablemente uno de los mecanismos responsables de la resistencia al ToLCNDV en la accesión WM-7. La metilación del DNA dependiente de RNA (RdDM) es un mecanismo de regulación epigenética que las plantas han adoptado para combatir la infección por geminivirus (Piedra-Aguilera et al., 2019; Noris et al., 2020). Entre los genes de hortícolas que se han clonado y descrito funcionalmente en la resistencia a geminivirus, los genes *Ty1/Ty3* confieren resistencia a TYLCV en tomate y codifican una RNA polimerasa dependiente de RNA del tipo γ (RDRγ), que lleva a cabo una amplificación del silenciamiento a nivel transcripcional mediante el aumento de las citosinas metiladas en el DNA del virus (Butterbach et al., 2014; Caro et al., 2015; Voorburg et al., 2020). La sobreexpresión de genes de esta familia en el genotipo PS sugiere que estas proteínas pueden ser también determinantes en la infección por ToLCNDV en melón.

Los resultados obtenidos en este trabajo proporcionan por primera vez información a gran escala sobre la reprogramación transcripcional que tiene lugar en melón tras la infección por un begomovirus, comparando entre dos genotipos (resistente y susceptible) y aplicando nuevas tecnologías de secuenciación masiva. Los resultados obtenidos proporcionan información de elevado valor para comprender los procesos implicados en la resistencia identificada en la accesión silvestre de *C. melo*, WM-7. Sin embargo, y a pesar del empleo de técnicas moleculares de alto rendimiento, nuestros esfuerzos no han conseguido identificar genes concretos localizados en la principal región candidata que regula la resistencia al ToLCNDV en melón. En esta región, sólo un gen DNA primasa y varios transportadores transmembrana han mostrado alteración en la expresión tras la infección con el virus. El ortólogo de este gen DNA primasa en *C. moschata* también se localiza en la región ligada a la resistencia al ToLCNDV, por lo que es necesario profundizar en la caracterización de este gen y en su implicación en la resistencia. Para afrontar este objetivo, el mapeo fino mediante poblaciones recombinantes es

una estrategia que se ha empleado en el estudio de genes de resistencia a virus, en cucurbitáceas y otras especies hortícolas (Yang et al., 2014; Pérez de castro et al., 2020). Actualmente, nuestro grupo ha iniciado un ensayo de análisis de poblaciones segregantes con genotipos recombinantes en las tres regiones candidatas de resistencia en *C. melo.* Estos ensayos permitirán acotar el gen o genes que están directamente implicados en la resistencia y que pueden ser activadores de la reprogramación transcripcional que hemos observado en esta tesis.

La combinación de ensayos de mapeo fino con estudios de funcionalidad génica ha propiciado el estudio eficiente de genes de resistencia. El silenciamiento inducido por virus (VIGs) o la edición del genoma empleando la técnica CRISPR/Cas9, son metodologías que permiten anular la expresión de genes candidatos y por tanto evaluar cambios en la respuesta tras la infección con virus. La aplicación de estas técnicas en coordinación con los resultados de pre-mejora obtenidos en esta tesis doctoral, supondría un elevado avance para desentrañar las claves genéticas de la resistencia al ToLCNDV.

Conclusiones

1. En la familia de las cucurbitáceas, los genotipos silvestres y las variedades locales son un reservorio de genes de resistencia al ToLCNDV, y deben ser considerados en los programas de mejora para la resistencia a begomovirus. De hecho, este germoplasma ha proporcionado las primeras fuentes de resistencia al ToLCNDV-ES descritas en el género *Cucurbita* y pepino, dos accesiones de *Cucurbita moschata* y tres de *Cucumis sativus* var. *sativus.* Las accesiones identificadas proceden principalmente de la India, al igual que ocurre con las accesiones resistentes de melón (*Cucumis melo*) descritas en estudios previos a esta tesis doctoral. La co-evolución planta-virus en esta región geográfica es un fenómeno probable.
2. Al igual que para otras virosis, la especie *C. moschata* es la que mayor número de accesiones presenta dentro del género *Cucurbita* con genes de resistencia al ToLCNDV-ES. La resistencia identificada en las accesiones PI 604506 y PI 381814 puede transferirse mediante cruzamientos a *C. pepo,* el cultivo más afectado por esta virosis en el área mediterránea.
3. El estudio de la resistencia a ToLCNDV-ES en *C. melo* ha determinado un nivel de resistencia en los genotipos silvestres del grupo Kachri superior a la del grupo momordica. La accesión con mayor nivel de resistencia del grupo momordica, PI 414723, es multiresistente a diversas virosis que afectan a cucurbitáceas. La resistencia a ToLCNDV-ES de esta entrada se manifiesta con una aparición inicial de síntomas de los que la planta se recupera posteriormente.
4. En la accesión WM-7 del grupo kachri de *C. melo*, un gen mayor parcialmente dominante controla la resistencia a ToLCNDV-ES. La presencia de este *locus* en homocigosis es suficiente para desencadenar la resistencia, pero en heterocigosis dos regiones en los cromosomas 2 y 12 modulan el desarrollo de síntomas y la acumulación viral. Se confirma que el control de la resistencia a ToLCNDV-ES derivada de este melón exótico es poligénica.
5. En las accesiones de *C. moschata* resistentes al ToLCNDV-ES, un *locus* en el cromosoma 8 controla la resistencia y se transmite a la descendencia con

herencia recesiva. Esta resistencia se manifiesta sólo parcialmente en *C. pepo*, lo que sugiere la ocurrencia de genes adicionales o un efecto del fondo genético donante para evitar el desarrollo de la enfermedad.

6. Los *loci* de efecto mayor identificados en *C. melo* y *C. moschata* se encuentran en regiones genómicas sinténicas entre estas dos especies. Estas regiones tienen genes candidatos comunes posiblemente involucrados en un mecanismo de resistencia similar en ambas especies.
7. El análisis global de los transcriptomas de las accesiones de melón WM-7 y PS, ha permitido caracterizar las rutas de expresión génica que dan lugar a la resistencia a ToLCNDV-ES en cucurbitáceas. Los genes implicados en las cascadas de señalización hormonal del ácido jasmónico, fotosíntesis y factores de transcripción de tipo NAC intervienen en la respuesta a la infección del virus en la planta. El silenciamiento a nivel transcripcional mediante metilación del DNA del ToLCNDV-ES es un mecanismo que probablemente interviene en la resistencia identificada en WM-7.
8. Los marcadores moleculares de tipo SNP son de gran utilidad en el mapeo de la resistencia a virus, y combinados con herramientas genómicas y transcriptómicas, son muy eficaces en la mejora genética del cultivo de cucurbitáceas. Los SNPs ligados a la resistencia a ToLCNDV-ES en melón y el género *Cucurbita* identificados en esta tesis doctoral permitirán acelerar la introgresión asistida (MAS) de la resistencia en variedades comerciales de melón, calabaza y calabacín.
9. Los resultados obtenidos en el marco de esta tesis doctoral tienen un impacto directo en el sector agrícola nacional e internacional. Las resistencias evaluadas constituyen un material genético de pre-mejora para la resistencia al ToLCNDV-ES en cucurbitáceas, que ya está siendo explotado tanto para obtener variedades élite comerciales, como para mejorar variedades tradicionales apreciadas por los consumidores.

Referencias generales

Abrahamian, P. E., Sobh, H., & Abou-Jawdah, Y. (2012). First report of Cucurbit chlorotic yellows virus on cucumber in Lebanon. *Plant Disease*, *96*(11), 1704-1704. http://doi: 10.1094/PDIS-05-12-0486-PDN

Abrahamian, P. E., & Abou-Jawdah, Y. (2014). Whitefly-transmitted criniviruses of cucurbits: current status and future prospects. *VirusDisease*, *25*(1), 26-38. https://doi.org/10.1007/s13337-013-0173-9

Abrahamian, P., Sobh, H., Seblani, R., & Abou-Jawdah, Y. (2015). Co-infection of two criniviruses and a begomovirus enhances the disease severity in cucumber. *European Journal of Plant Pathology*, *142*(3), 521-530. https://doi.org/10.1007/s10658-015-0630-y

Adkins, S., Webb, S. E., Achor, D., Roberts, P. D., & Baker, C. A. (2007). Identification and characterization of a novel whitefly-transmitted member of the family Potyviridae isolated from cucurbits in Florida. *Phytopathology*, *97*(2), 145-154. https://doi.org/10.1094/PHYTO-97-2-0145

Aguilar, J. M., Abad, J., & Aranda, M. A. (2006). Resistance to Cucurbit yellow stunting disorder virus in cucumber. *Plant disease*, *90*(5), 583-586. https://doi.org/10.1094/PD-90-0583.

Aguilera, J. G., Hurtado, F. D., Sobrinho, R. R., de Souza Almeida, V., Tavares, S. S., Nick, C., Oliveira Soares, M., Diniz Xavier, C. A., Dias de Freitas, R., Alvarez Gil, M., Zerbini, F. M., & Henriques da Silva, D. J. (2014). Response of tomato (*Solanum* L. section *Lycopersicon* Mill.) germplasm to begomovirus inoculation under controlled and field conditions. *Genetic resources and crop evolution*, *61*(2), 435-450. https://doi.org/10.1007/s10722-013-0048-3

Akano, A. O., Dixon, A. G. O., Mba, C., Barrera, E., & Fregene, M. (2002). Genetic mapping of a dominant gene conferring resistance to cassava mosaic disease. *Theoretical and Applied Genetics*, *105*(4), 521-525. https://doi.org/10.1007/s00122-002-0891-7

Akbergenov, R., Si-Ammour, A., Blevins, T., Amin, I., Kutter, C., Vanderschuren, H., Zhang, P., Gruissem, W., Meins, F., Jr, Hohn, T., & Pooggin, M. M. (2006). Molecular characterization of geminivirus-derived small RNAs in different plant species. *Nucleic acids research*, *34*(2), 462–471. https://doi.org/10.1093/nar/gkj447

Alam, A. M., Somta, P., & Srinives, P. (2014). Identification and confirmation of quantitative trait loci controlling resistance to *mungbean yellow mosaic disease* in mungbean [*Vigna radiata* (L.) Wilczek]. *Molecular breeding*, *34*(3), 1497-1506. https://doi.org/10.1007/s11032-014-0133-0

Al-Musa, A. M., Qusus, S. J., & Mansour, A. N. (1985). Cucumber vein yellowing virus on cucumber in Jordan. *Plant Disease*, *69*(4). https://doi.org/10.1094/PD-69-361c

Alazem, M., & Lin, N. S. (2015). Roles of plant hormones in the regulation of host–virus interactions. *Molecular plant pathology*, *16*(5), 529-540. https://doi.org/10.1111/mpp.12204

Alcaide-Loridan, C., & Jupin, I. (2012). Ubiquitin and plant viruses, let's play together! *Plant physiology*, *160*(1), 72–82. https://doi.org/10.1104/pp.112.201905

Alfaro-Fernández, A., Sánchez-Navarro, J. A., Landeira, M., Font, M. I., Hernández-Llópis, D., & Pallás, V. (2016). Evaluation of PCR and non-radioactive molecular hybridization techniques for the routine diagnosis of *Tomato leaf curl New Delhi virus*, *Tomato yellow leaf curl virus* and *Tomato yellow leaf curl Sardinia virus*. *Journal of Plant Pathology*, 245-254. https://doi.org/10.4454/JPP.V98I2.003

Ali, A. (2020). Epidemiology and evolution of poytviruses infecting cucurbits. En: Awasthi L.P., *Applied Plant Virology* (pp. 405-417). Academic Press. https://doi.org/10.1016/B978-0-12-818654-1.00028-1

Ali, S., Ganai, B. A., Kamili, A. N., Bhat, A. A., Mir, Z. A., Bhat, J. A., Tyagi, A., Islam, S. T., Mushtaq, M., Yadav, P., Rawat, S., & Grover, A. (2018). Pathogenesis-related proteins and peptides as promising tools for engineering plants with multiple stress tolerance. *Microbiological research*, *212-213*, 29–37. https://doi.org/10.1016/j.micres.2018.04.008

Alonso-Prados, J., Fraile, A., & García-Arenal, F. (1997). Impact of cucumber mosaic virus and watermelon mosaic virus 2 infection on melon production in Central Spain. *Journal of Plant Pathology*, *79*(2), 131-134. https://doi.org/10.2307/41997879

Amano, M., Mochizuki, A., Kawagoe, Y., Iwahori, K., Niwa, K., Svoboda, J., Maena, T., & Imura, Y. (2013). High-resolution mapping of *zym*, a recessive gene for *Zucchini yellow mosaic virus* resistance in cucumber. *Theoretical and applied genetics*, *126*(12), 2983-2993. https://doi.org/10.1007/s00122-013-2187-5

Anabestani, A., Behjatnia, S. A. A., Izadpanah, K., Tabein, S., & Accotto, G. P. (2017). Seed transmission of beet curly top virus and beet curly top Iran virus in a local cultivar of petunia in Iran. *Viruses*, *9*(10), 299. https://doi.org/10.3390/v9100299

Anagnostou, K., Jahn, M., & Perl-Treves, R. (2000). Inheritance and linkage analysis of resistance to zucchini yellow mosaic virus, watermelon mosaic virus, papaya ringspot virus and powdery mildew in melon. *Euphytica*, *116*(3), 265-270. https://doi.org/10.1023/A:1004005716806

Anbinder, I., Reuveni, M., Azari, R., Paran, I., Nahon, S., Shlomo, H., Chen, L., Lapidot, M., & Levin, I. (2009). Molecular dissection of Tomato leaf curl virus resistance in tomato line TY172 derived from Solanum peruvianum. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik*, *119*(3), 519–530. https://doi.org/10.1007/s00122-009-1060-z

Anderson, L. K., Covey, P. A., Larsen, L. R., Bedinger, P., & Stack, S. M. (2010). Structural differences in chromosomes distinguish species in the tomato clade. *Cytogenetic and genome research*, *129*(1-3), 24-34. https://doi.org/10.1159/000313850

Arbelaez, J. D., Moreno, L. T., Singh, N., Tung, C. W., Maron, L. G., Ospina, Y., Martinez, C. P., Grenier, C., Lorieux, M., & McCouch, S. (2015). Development and GBS-genotyping of introgression lines (ILs) using two wild species of rice, *O. meridionalis* and *O. rufipogon*, in a common recurrent parent, *O. sativa* cv. Curinga. *Molecular breeding: new strategies in plant improvement*, *35*(2), 81. https://doi.org/10.1007/s11032-015-0276-7

Ascencio-Ibáñez, J. T., Sozzani, R., Lee, T. J., Chu, T. M., Wolfinger, R. D., Cella, R., & Hanley-Bowdoin, L. (2008). Global analysis of *Arabidopsis* gene expression uncovers a complex array of changes impacting pathogen response and cell cycle during geminivirus infection. *Plant physiology*, *148*(1), 436–454. https://doi.org/10.1104/pp.108.121038

Ashkani, S., Rafii, M. Y., Shabanimofrad, M., Miah, G., Sahebi, M., Azizi, P., Tanweer, F. A., Akhtar, M. S., & Nasehi, A. (2015). Molecular Breeding Strategy and Challenges Towards Improvement of Blast Disease Resistance in Rice Crop. *Frontiers in plant science*, *6*, 886. https://doi.org/10.3389/fpls.2015.00886

Ashwathappa, K. V., Venkataravanappa, V., Reddy, C. L., & Reddy, M. K. (2020). Association of *Tomato leaf curl New Delhi virus* with mosaic and leaf curl disease of C*hrysanthemum* and its whitefly cryptic species. *Indian Phytopathology*, 1-10. https://doi.org/10.1007/s42360-020-00214-1.

Akhtar, K. P., Akram, A., Ullah, N., Saleem, M. Y., & Saeed, M. (2019). Evaluation of *Solanum* species for resistance to *Tomato leaf curl New Delhi virus* using chip grafting assay. *Scientia Horticulturae*, *256*, 108646. https://doi.org/10.1016/j.scienta.2019.108646

Baena-González, E., Rolland, F., Thevelein, J. M., & Sheen, J. (2007). A central integrator of transcription networks in plant stress and energy signalling. *Nature*, *448*(7156), 938-942. https://doi.org/10.1038/nature06069

Baloglu, M. C. (2018). Genomics of Cucurbits. En: Rout, G. R., & Peter, K.V. (Eds.) *Genetic Engineering of Horticultural Crops* (pp. 413-432). Academic Press. https://doi.org/10.1016/B978-0-12-810439-2.00017-9.

Bandaranayake, W. M. E. K., Wickramarachchi, W. A. R. T., Wickramasinghe, H. A. M., Rajapakshe, R. G. A. S., & Dissanayake, D. M. K. K. (2014). Molecular detection and characterization of begomoviruses associated with cucurbitaceae vegetables in Sri Lanka. *Journal of the National Science Foundation of Sri Lanka*, *42*(3). https://doi.org/10.4038/jnsfsr.v42i3.7400

Barbieri, M., Acciarri, N., Sabatini, E., Sardo, L., Accotto, G. P., & Pecchioni, N. (2010). Introgression of resistance to two Mediterranean virus species causing tomato yellow leaf curl into a valuable traditional tomato variety. *Journal of Plant Pathology*, 485-493.

Barbosa, G. D. S., LIMA, J. A. D. A., QUEIRÓZ, M. A. D., Dias, R. D. C. S., & Lima, C. S. (2016). Identification and effects of mixed Infection of Potyvirus isolates with Cucumber mosaic virus in cucurbits. *Revista Caatinga*, *29*(4), 1028-1035. https://doi.org/10.1590/1983-21252016v29n429rc

Bari, R., & Jones, J. D. (2009). Role of plant hormones in plant defence responses. *Plant molecular biology*, *69*(4), 473-488. https://doi.org/10.1007/s11103-008-9435-0

Barrantes-Santamaría, W., Granell, A., & Monforte, A. J. (2020). Mapping of a gene involved in distorted segregation in interspecific tomato populations. *Agronomía Mesoamericana*, *31*(2), 419-431. https://doi.org/10.15517/AM.V31I2.39485

Barrera-Redondo, J., Ibarra-Laclette, E., Vázquez-Lobo, A., Gutiérrez-Guerrero, Y. T., de la Vega, G. S., Piñero, D., Montes-Hernández, S., Lira-Saade, R., & Eguiarte, L. E. (2019). The genome of Cucurbita argyrosperma (silver-seed gourd) reveals faster rates of protein-coding gene and long noncoding RNA turnover and neofunctionalization within Cucurbita. *Molecular plant*, *12*(4), 506-520. https://doi.org/10.1016/j.molp.2018.12.023

Barrera-Pacheco, A., Joaquin-Ramos, A. D. J., Torres-Pacheco, I., Gonzalez-Chavira, M. M., Perez-Perez, M. C. I., Guevara-Olvera, L., & Guevara-Gonzalez, R. G. (2008). Analysis of transcriptional expression induced in *Capsicum chinense* BG-3821 under conditions of biotic and abiotic stress. *Agrociencia*, *42*(1), 95-106. Retrieved August 30, 2020, from: http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1405-31952008000100010&lng=en&tlng=.

Bhattacharjee, P., Das, R., Mandal, A., & Kundu, P. (2017). Functional characterization of tomato membrane-bound NAC transcription factors. *Plant molecular biology*, *93*(4-5), 511-532. https://doi.org/10.1007/s11103-016-0579-z

Bertin, S., Luigi, M., Parrella, G., Giorgini, M., Davino, S., & Tomassoli, L. (2018). Survey of the distribution of *Bemisia tabaci* (Hemiptera: *Aleyrodidae*) in Lazio region (Central Italy): a threat for the northward expansion of *Tomato leaf curl New Delhi virus* (*Begomovirus*: *Geminiviridae*) infection. *Phytoparasitica*, *46*(2), 171-182. https://doi.org/10.1007/s12600-018-0649-7

Bhatt, B. S., Chahwala, F. D., Rathore, S., Singh, B., & Singh, A. K. (2019). Biology and Molecular Epidemiology of Begomovirus Infection on Cucurbit Crops. En: Ansar, M., & Ghatak. A., (Eds.) *The Vegetable Pathosystem* (pp. 385-408). Apple Academic Press. https://doi.org/10.1201/9780429022999-14.

Bian, X. Y., Thomas, M. R., Rasheed, M. S., Saeed, M., Hanson, P., De Barro, P. J., & Rezaian, M. A. (2007). A recessive allele (tgr-1) conditioning tomato resistance to geminivirus infection is associated with impaired viral movement. *Phytopathology*, 97(8), 930-937. https://doi.org/10.1094/PHYTO-97-8-0930

Bisognin, D. A. (2002). Origin and evolution of cultivated cucurbits. *Ciência Rural*, *32*(4), 715-723. https://doi.org/10.1590/S0103-84782002000400028

Blanca, J. M., Cañizares, J., Ziarsolo, P., Esteras, C., Mir, G., Nuez, F., Garcia-Mas, J., & Picó, M. B. (2011). Melon transcriptome characterization: Simple sequence repeats and single nucleotide polymorphisms discovery for high throughput genotyping across the species. *The Plant Genome*, *4*(2), 118-131. https://doi.org/10.3835/plantgenome2011.01.0003

Blanca, J., Esteras, C., Ziarsolo, P., Pérez, D., Collado, C., de Pablos, R. R., Ballester, A., Roig, C., Cañizares, J., & Picó, B. (2012). Transcriptome sequencing for SNP discovery across *Cucumis melo*. *BMC genomics*, *13*(1), 280. https://doi.org/10.1186/1471-2164-13-280

Bonato, O., Lurette, A., Vidal, C., & Fargues, J. (2007). Modelling temperature-dependent bionomics of Bemisia tabaci (Q-biotype). *Physiological Entomology*, *32*(1), 50-55. https://doi.org/10.1111/j.1365-3032.2006.00540.x

Boubourakas, I. N., Hatziloukas, E., Antignus, Y., & Katis, N. I. (2004). Etiology of leaf chlorosis and deterioration of the fruit interior of watermelon plants. *Journal of phytopathology*, *152*(10), 580-588. https://doi.org/10.1111/j.1439-0434.2004.00900.x

Boyhan, G., Norton, J. D., Jacobsen, B. J., & Abrahams, B. R. (1992). Evaluation of watermelon and related germplasm for resistance to zucchini yellow mosaic virus. *HortScience*, *27*(11), 1173a-1173. https://doi.org/10.21273/HORTSCI.27.11.1173a

Branham, S. E., Levi, A., Farnham, M. W., & Wechter, W. P. (2017). A GBS-SNP-based linkage map and quantitative trait loci (QTL) associated with resistance to Fusarium oxysporum f. sp. niveum race 2 identified in Citrullus lanatus var. citroides. *Theoretical and applied genetics*, *130*(2), 319-330. https://doi.org/10.1007/s00122-016-2813-0

Brotman, Y., Kovalski, I., Dogimont, C., Pitrat, M., Portnoy, V., Katzir, N., & Perl-Treves, R. (2005). Molecular markers linked to papaya ring spot virus resistance and Fusarium race 2 resistance in melon. *Theoretical and Applied Genetics*, *110*(2), 337-345. https://doi.org/10.1007/s00122-004-1845-z

Brotman, Y., Normantovich, M., Goldenberg, Z., Zvirin, Z., Kovalski, I., Stovbun, N., Doniger, T., Bolger, A. M., Troadec, C., Bendahmane, A., Cohen, R., Katzir, N., Pitrat, M., Dogimont, C., & Perl-Treves, R. (2013). Dual resistance of melon to Fusarium oxysporum races 0 and 2 and to Papaya ring-spot virus is controlled by a pair of head-to-head-oriented NB-LRR genes of unusual architecture. *Molecular plant*, *6*(1), 235-238. https://doi.org/10.1093/mp/sss121.

Brown, R. N., Bolanos-Herrera, A., Myers, J. R., & Jahn, M. M. (2003). Inheritance of resistance to four cucurbit viruses in Cucurbita moschata. *Euphytica*, *129*(3), 253-258. https://doi.org/10.1023/A:1022224327064

Brown, J. K., Zerbini, F. M., Navas-Castillo, J., Moriones, E., Ramos-Sobrinho, R., Silva, J. C., Fiallo-Olivé, E., Briddon, R. W., Hernández-Zepeda, C., Idris, A., Malathi, V. G., Martin, D. P., Rivera-Bustamante, R., Ueda, S., & Varsani, A. (2015). Revision of Begomovirus taxonomy based on pairwise sequence comparisons. *Archives of virology*, *160*(6), 1593–1619. https://doi.org/10.1007/s00705-015-2398-y

Brustolini, O., Machado, J., Condori-Apfata, J. A., Coco, D., Deguchi, M., Loriato, V., Pereira, W. A., Alfenas-Zerbini, P., Zerbini, F. M., Inoue-Nagata, A. K., Santos, A. A., Chory, J., Silva, F. F., & Fontes, E. (2015). Sustained NIK-mediated antiviral signalling confers broad-spectrum tolerance to begomoviruses in cultivated plants. *Plant biotechnology journal*, *13*(9), 1300–1311. https://doi.org/10.1111/pbi.12349

Bruzzone, C. M., & Steer, C. J. (2015). High-resolution melting analysis of single nucleotide polymorphisms. In *Molecular Typing of Blood Cell Antigens* (pp. 5-27). Humana Press, New York, NY. https://doi.org/10.1007/978-1-4939-2690-9_2

Butterbach, P., Verlaan, M. G., Dullemans, A., Lohuis, D., Visser, R. G., Bai, Y., & Kormelink, R. (2014). Tomato yellow leaf curl virus resistance by Ty-1 involves increased cytosine methylation of viral genomes and is compromised by cucumber

mosaic virus infection. *Proceedings of the National Academy of Sciences*, *111*(35), 12942-12947. https://doi.org/10.1073/pnas.1400894111

CABI, 2020. Invasive Species Compendium. Wallingford, UK: CAB International. www.cabi.org/isc.

Cantú-Iris, M., Pastor-Palacios, G., Mauricio-Castillo, J. A., Bañuelos-Hernández, B., Avalos-Calleros, J. A., Juárez-Reyes, A., Rivera-Bustamante, R., & Argüello-Astorga, G. R. (2019). Analysis of a new begomovirus unveils a composite element conserved in the CP gene promoters of several Geminiviridae genera: Clues to comprehend the complex regulation of late genes. *PloS one*, *14*(1), e0210485. https://doi.org/10.1371/journal.pone.0210485

Capuozzo, C., Formisano, G., Iovieno, P., Andolfo, G., Tomassoli, L., Barbella, M. M., Picó, B., Paris, H. P., & Ercolano, M. R. (2017). Inheritance analysis and identification of SNP markers associated with ZYMV resistance in *Cucurbita pepo*. *Molecular Breeding*, *37*(8), 99. https://doi.org/10.1007/s11032-017-0698-5

Cardoso, A. I. I., Pavan, M. A., Sakate, R. K., & Fattori, K. (2010). Inheritance of cucumber tolerance to Zucchini yellow mosaic virus. *Journal of Plant Pathology*, *92*, 245-248. https://doi.org/10.2307/41998792

Caro, M., Verlaan, M. G., Julián, O., Finkers, R., Wolters, A. A., Hutton, S. F., Scott, J. W., Kormelink, R., Visser, R., Díez, M. J., Pérez-de-Castro, A., & Bai, Y. (2015). Assessing the genetic variation of *Ty-1* and *Ty-3* alleles conferring resistance to *tomato yellow leaf curl virus* in a broad tomato germplasm. *Molecular breeding: new strategies in plant improvement*, *35*(6), 132. https://doi.org/10.1007/s11032-015-0329-y

Carr, J. P., Lewsey, M. G., & Palukaitis, P. (2010). Signaling in induced resistance. *Advances in virus research*, *76*, 57–121. https://doi.org/10.1016/S0065-3527(10)76003-6

Castanera, R., Ruggieri, V., Pujol, M., Garcia-Mas, J., & Casacuberta, J. M. (2019). An improved melon reference genome with single-molecule sequencing uncovers a recent burst of transposable elements with potential impact on genes. *Frontiers in plant science*, *10*. doi: 10.3389/fpls.2019.01815

Castro, R. M., Moreira, L., Rojas, M. R., Gilbertson, R. L., Hernández, E., Mora, F., & Ramírez, P. (2013). Occurrence of Squash yellow mild mottle virus and Pepper golden mosaic virus in Potential New Hosts in Costa Rica. *The plant pathology journal*, *29*(3), 285–293. https://doi.org/10.5423/PPJ.OA.12.2012.0182

Catalá, M. S., Gomariz, J., Comelles, J. C., & Sánchez, E. (2009). Estado actual de la mejora en las principales enfermedades de melón. *Phytoma España: La revista profesional de sanidad vegetal*, (205), 20-25.

Chakraborty, S., & Newton, A. C. (2011). Climate change, plant diseases and food security: an overview. *Plant Pathology*, 60(1): 2–14. https://doi.org/10.1111/j.1365-3059.2010.02411.x

Chakraborty, N., & Basak, J. (2018). Comparative transcriptome profiling of a resistant vs. susceptible Vigna mungo cultivar in response to Mungbean yellow mosaic India

virus infection reveals new insight into MYMIV resistance. *Current Plant Biology*, *15*, 8-24. https://doi.org/10.1016/j.cpb.2018.11.001

Chandrasekaran, J., Brumin, M., Wolf, D., Leibman, D., Klap, C., Pearlsman, M., Sherman, A., Arazi, T., & Gal-On, A. (2016). Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. *Molecular plant pathology*, *17*(7), 1140–1153. https://doi.org/10.1111/mpp.12375

Chang, H. H., Chien, R. C., Tsai, W. H., Jan, F. J. (2009) Molecular and biological characterization of a mechanically transmissible *Tomato leaf curl New Delhi virus* infecting oriental melon plants. Poster presented at the APS 2009 Annual Meeting (Portland, US, 2009-08-01).

Chang, H. H., Ku, H. M., Tsai, W. S., Chien, R. C., & Jan, F. J. (2010). Identification and characterization of a mechanical transmissible begomovirus causing leaf curl on oriental melon. *European journal of plant pathology*, *127*(2), 219-228. https://doi.org/10.1007/s10658-010-9586-0

Charron, C., Nicolaï, M., Gallois, J. L., Robaglia, C., Moury, B., Palloix, A., & Caranta, C. (2008). Natural variation and functional analyses provide evidence for co-evolution between plant eIF4E and potyviral VPg. *The Plant Journal*, *54*(1), 56-68. https://doi.org/doi:10.1111/j.1365-313X.2008.03407.x

Chellappan, P., Vanitharani, R., & Fauquet, C. M. (2005). MicroRNA-binding viral protein interferes with Arabidopsis development. *Proceedings of the National Academy of Sciences*, *102*(29), 10381-10386. https://doi.org/10.1073/pnas.0504439102.

Chen, T., Lv, Y., Zhao, T., Li, N., Yang, Y., Yu, W., He, X., & Zhang, B. (2013). Comparative transcriptome profiling of a resistant vs. susceptible tomato (*Solanum lycopersicum*) cultivar in response to infection by tomato yellow leaf curl virus. *PloS one*, *8*(11), e80816. https://doi.org/10.1371/journal.pone.0080816

Chen, J. Y., Huang, J. Q., Li, N. Y., Ma, X. F., Wang, J. L., Liu, C., Liu, Y. F., Liang, Y., Bao, Y. M., & Dai, X. F. (2015). Genome-wide analysis of the gene families of resistance gene analogues in cotton and their response to Verticillium wilt. *BMC plant biology*, *15*, 148. https://doi.org/10.1186/s12870-015-0508-3

Chen, Q., Yu, G., Wang, X., Meng, X., & Lv, C. (2020). Genetics and Resistance Mechanism of the Cucumber (*Cucumis sativus* L.) Against Powdery Mildew. *Journal of Plant Growth Regulation*, 1-7. https://doi.org/10.1007/s00344-020-10075-7

Cheng, Y. H., Huang, C. H., Chang, C. J., & Jan, F. J. (2019). Identification and characterisation of watermelon green mottle mosaic virus as a new cucurbit-infecting tobamovirus. *Annals of Applied Biology*, *174*(1), 31-39. https://doi.org/10.1111/aab.12467

Choi, Y., Bowman, J. W., & Jung, J. U. (2018). Autophagy during viral infection-a double-edged sword. *Nature Reviews Microbiology*, *16*(6), 341-354. https://doi.org/10.1038/s41579-018-0003-6

Chomicki, G., & Renner, S. S. (2015). Watermelon origin solved with molecular phylogenetics including L innaean material: another example of museomics. *New Phytologist*, *205*(2), 526-532.

https://doi.org/10.1111/nph.13163

Chomicki, G., Schaefer, H., & Renner, S. S. (2020). Origin and domestication of Cucurbitaceae crops: insights from phylogenies, genomics and archaeology. *New Phytologist*, *226*(5), 1240-1255. https://doi.org/10.1111/nph.16015

Chikh-Ali, M., Natsuaki, T., & Karasev, A. V. (2019). Molecular diversity of main cucurbit viruses in Syria. *Journal of Plant Pathology*, *101*(4), 1067-1075. https://doi.org/10.1007/s42161-019-00337-z

Chung, H. Y., & Sunter, G. (2014). Interaction between the transcription factor AtTIFY4B and begomovirus AL2 protein impacts pathogenicity. *Plant molecular biology*, *86*(1-2), 185-200. https://doi.org/10.1007/s11103-014-0222-9

Cohen, S., & Nitzany, F. E. (1960). A whitefly transmitted virus of cucurbits in Israel. *Phytopathologia Mediterranea*, 44-46.

Cohen, R., Hanan, A., & Paris, H. S. (2003). Single-gene resistance to powdery mildew in zucchini squash (*Cucurbita pepo*). *Euphytica*, *130*(3), 433-441. https://doi.org/10.1023/A:1023082612420

Coll, N. S., Epple, P., & Dangl, J. L. (2011). Programmed cell death in the plant immune system. *Cell death and differentiation*, *18*(8), 1247–1256. https://doi.org/10.1038/cdd.2011.37

Coudriet, D. L., Kishaba., A. N., & Bohn, G. W. (1981). Inheritance of resistance to *muskmelon necrotic spot virus* in a melon aphid-resistant breeding line of muskmelon. *Journal of the American Society for Horticultural Science*, *106*, 789-791.

Crespo, O., Janssen, D., Robles, C., & Ruiz, L. (2018). Resistance to *Cucumber green mottle mosaic virus* in *Cucumis sativus*. *Euphytica*, *214*(11), 201. https://doi.org/10.1007/s10681-018-2286-0.

Czosnek, H., Eybishtz, A., Sade, D., Gorovits, R., Sobol, I., Bejarano, E., Rosas-Díaz, T., & Lozano-Durán, R. (2013). Discovering host genes involved in the infection by the Tomato Yellow Leaf Curl Virus complex and in the establishment of resistance to the virus using Tobacco Rattle Virus-based post transcriptional gene silencing. *Viruses*, *5*(3), 998–1022. https://doi.org/10.3390/v5030998

Dangl, J. L., & Jones, J. D. (2001) Plant pathogens and integrated defence responses to infection. *Nature*, *411*(6839), 826-833.https://doi.org/10.1038/35081161

Danin-Poleg, Y., Paris, H. S., Cohen, S., Rabinowitch, H. D., & Karchi, Z. (1997). Oligogenic inheritance of resistance to zucchini yellow mosaic virus in melons. *Euphytica*, *93*(3), 331-337. https://doi.org/10.1023/A:1002944432083

Daryono, B. S., Somowiyarjo, S., & Natsuaki, K. T. (2005). Screening for resistance to *Kyuri green mottle mosaic virus* in various melons. *Plant breeding*, *124*(5), 487-490. https://doi.org/10.1111/j.1439-0523.2005.01121.x

Daryono, B. S., Wakui, K., & Natsuaki, K. T. (2010). Linkage analysis and mapping of SCAR markers linked to CMV-B2 resistance gene in melon. *SABRAO Journal of Breeding and Genetics 2010*, *42*(1), 35-45.

Davino, S., Miozzi, L., Panno, S., Rubio, L., Davino, M., & Accotto, G. P. (2012). Recombination profiles between Tomato yellow leaf curl virus and Tomato yellow leaf curl Sardinia virus in laboratory and field conditions: evolutionary and taxonomic implications. *Journal of General Virology*, *93*(12), 2712-2717. https://doi.org/10.1099/vir.0.045773-0

De Barro, P. J., Liu, S. S., Boykin, L. M., & Dinsdale, A. B. (2011). Bemisia tabaci: a statement of species status. *Annual review of entomology*, *56*, 1-19. https://doi.org/10.1146/annurev-ento-112408-085504

de Ronde, D., Butterbach, P., & Kormelink, R. (2014). Dominant resistance against plant viruses. *Frontiers in plant science*, *5*, 307. https://doi.org/10.3389/fpls.2014.00307

Decker-Walters, D. S., & Walters, T. W. (2000). Squash. En: Kiple, K. F., & Ornelas, K. C. (Eds.), *The Cambridge world history of food. Cambridge University Press, Cambridge, UK*, (pp. 335-351). https://doi.org/10.1017/CHOL9780521402149.034

Dempewolf, H., Eastwood, R. J., Guarino, L., Khoury, C. K., Müller, J. V., & Toll, J. (2014). Adapting agriculture to climate change: a global initiative to collect, conserve, and use crop wild relatives. *Agroecology and Sustainable Food Systems*, *38*(4), 369-377.

Dempewolf, H., Baute, G., Anderson, J., Kilian, B., Smith, C., & Guarino, L. (2017). Past and future use of wild relatives in crop breeding. *Crop Science*, *57*(3), 1070-1082. https://doi.org/10.2135/

Desbiez, C., Costa, C., Wipf-Scheibel, C., Girard, M., & Lecoq, H. (2007). Serological and molecular variability of watermelon mosaic virus (genus Potyvirus). *Archives of virology*, *152*(4), 775-781. https://doi.org/10.1007/s00705-006-0899-4

Desbiez, C., Verdin, E., Tepfer, M., Wipf-Scheibel, C., Millot, P., Dafalla, G., & Lecoq, H. (2016). Characterization of a new cucurbit-infecting ipomovirus from Sudan. *Archives of virology*, *161*(10), 2913-2915. https://doi.org/10.1007/s00705-016-2981-x

Desbiez, C., Millot, P., Wipf-Scheibel, C., Blancard, D., Chesneau, T., & Lecoq, H. (2016b). First report of Pepo aphid-borne yellows virus in cucurbits in Tanzania and Mayotte. *New Disease Reports*, *33*, 20. http://dx.doi.org/10.5197/j.2044-0588.2016.033.020

Dhillon, N. P. S., Ranjana, R., Singh, K., Eduardo, I., Monforte, A. J., Pitrat, M., Dhillon, N. K., & Singh, P. P. (2007). Diversity among landraces of Indian snapmelon (Cucumis melo var. momordica). *Genetic Resources and Crop Evolution*, *54*(6), 1267-1283. https://doi.org/10.1007/s10722-006-9108-2

Dhillon, N. P., Monforte, A. J., Pitrat, M., Sudhakar, P., Singh, P. K., Reitsma, K. R., Garcia-Mas, J., Sharma, A., & McCreight, J. D. (2012). Melon landraces of India: contributions and importance. *Plant breeding reviews*, *35*, 85-150.

Dhillon, N. P., Sanguansil, S., Srimat, S., Laenoi, S., Schafleitner, R., Pitrat, M., & McCreight, J. D. (2019). Inheritance of resistance to cucurbit powdery mildew in bitter gourd. *HortScience*, *54*(6), 1013-1016. https://journals.ashs.org/hortsci/view/journals/hortsci/54/6/article-p1013.xml

Dhole, V. J., & Reddy, K. S. (2012). Genetic analysis of resistance to *mungbean yellow mosaic virus* in mungbean (*Vigna radiata*). *Plant breeding*, *131*(3), 414-417. https://doi.org/10.1111/j.1439-0523.2012.01964.x

Diaz, A., Fergany, M., Formisano, G., Ziarsolo, P., Blanca, J., Fei, Z., Z., Staub, J. E., Zalapa, J. E., Cuevas, H. E., Dace, G., Oliver, M., Boissot, N., Dogimont, C., Pitrat, M., Hofstede, R., van Koert, P., Harel-Beja, R., Tzuri, G., Portnoy, V., Cohen, S., Schaffer, A., Katzir, N., Xu, Y., Zhang, H., Fukino, N., Matsumoto, S., Garcia-Mas, J., & Oliver, M. (2011). A consensus linkage map for molecular markers and quantitative trait loci associated with economically important traits in melon (*Cucumis melo* L.). *BMC plant biology*, *11*(1), 1-14. https://doi.org/10.1186/1471-2229-11-111

Díaz-Pendón, J.A., Fernández-Muñoz, R., Gómez-Guillamón, M. L., & Moriones, E. (2005). Inheritance of resistance to Watermelon mosaic virus in Cucumis melo that impairs virus accumulation, symptom expression, and aphid transmission. *Phytopathology*, *95*(7), 840-846. https://doi.org/10.1094/PHYTO-95-0840

Dodds, P. N., Lawrence, G. J., Catanzariti, A. M., Teh, T., Wang, C. I., Ayliffe, M. A., Kobe. B., & Ellis, J. G. (2006). Direct protein interaction underlies gene-for-gene specificity and coevolution of the flax resistance genes and flax rust avirulence genes. *Proceedings of the National Academy of Sciences*, *103*(23), 8888-8893. https://doi.org/10.1073/pnas.0602577103

Dogimont, C., Bussemakers, A., Martin, J., Slama, S., Lecoq, H., & Pitrat, M. (1997). Two complementary recessive genes conferring resistance to Cucurbit Aphid Borne Yellows Luteovirus in an Indian melon line (Cucumis melo L.). *Euphytica*, *96*(3), 391-395. https://doi.org/10.1023/A:1003069703294

Doolittle, S. P. (1916). A new infectious mosaic disease of cucumber. *Phytopathology*, *6*(2), 145-147.

Domingo-Calap, M. L., Moreno, A. B., Díaz Pendón, J. A., Moreno, A., Fereres, A., & López-Moya, J. J. (2020). Assessing the impact on virus transmission and insect vector behavior of a viral mixed infection in melon. *Phytopathology*, *110*(1), 174-186. https://doi.org/10.1094/PHYTO-04-19-0126-FI

Dong, J., Xu, J., Xu, X., Xu, Q., & Chen, X. (2019). Inheritance and Quantitative Trait Locus Mapping of Fusarium Wilt Resistance in Cucumber. *Frontiers in plant science*, *10*, 1425. doi: 10.3389/fpls.2019.01425

Dou, J., Zhao, S., Lu, X., He, N., Zhang, L., Ali, A., Kuang, H., & Liu, W. (2018). Genetic mapping reveals a candidate gene (ClFS1) for fruit shape in watermelon (Citrullus

lanatus L.). *Theoretical and applied genetics*, *131*(4), 947-958. https://doi.org/10.1007/s00122-018-3050-5

EFSA PLH *Panel* (EFSA Panel on Plant Health) (2013). Scientific Opinion on the risks to plant health posed by Bemisia tabaci species complex and viruses it transmits for the EU territory. *EFSA Journal* 2013;*11*(*4*):3162, 302 pp. https://doi.org/10.2903/j.efsa.2013.3162

Egel, D. S., & Martyn, R. D. (2007). Fusarium wilt of watermelon and other cucurbits. *The Plant Health Instructor*, *10*, 1094. https://doi.org/10.1094/PHI-I-2007-0122-01. Updated 2013.

Ekbic, E., Fidan, H., Yildiz, M., & Kazim, A. B. A. K. (2010). Screening of Turkish melon accessions for resistance to ZYMV, WMV and CMV. *Notulae Scientia Biologicae*, *2*(1), 55-57. https://doi.org/10.15835/nsb213555

Elkabetz, M., Paris, H. S., Burger, Y., Hanan, A., & Cohen, R. (2016). Two genes for resistance to *Fusarium oxysporum* f. sp. *radicis-cucumerinum* in melon (*Cucumis melo*, *Cucurbitaceae*). *Scientia horticulturae*, *201*, 57-60. https://doi.org/10.1016/j.scienta.2016.01.029.

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS one*, *6*(5), e19379. https://doi.org/10.1371/journal.pone.0019379

Endl, J., Achigan-Dako, E. G., Pandey, A. K., Monforte, A. J., Pico, B., & Schaefer, H. (2018). Repeated domestication of melon (Cucumis melo) in Africa and Asia and a new close relative from India. *American journal of botany*, *105*(10), 1662-1671. https://doi.org/10.1002/ajb2.1172

EPPO 2020. European and Mediterranean Plant Protection Organization. https://www.eppo.int/

EPPO Reporting Service First report of Tomato leaf curl New Delhi virus in Portugal no. 08 – 2019a Num. article: 2019/164 https://gd.eppo.int/reporting/article-6594

EPPO Reporting Service First report of Tomato leaf curl New Delhi virus in Estonia no. 07 – 2019b Num. article: 2019b/146. https://gd.eppo.int/reporting/article-6576

Espino de Paz, A. I., & Otazo Gonzalez, H. C., (2018). Virus de Nueva Delhi (ToLCNDV) en cucurbitáceas. Agrocabildo publicaciones, Información técnica. http://www.agrocabildo.org/publicaciones_detalle.asp?id=664

Espino de Paz, A. I., Botella-Guillén, M., Otazo-González, H. C., Alfaro-Fernández, A., Font-San-Ambrosio, I., Galipienso, L., & Rubio, L. (2019). First Report of Tomato leaf curl New Delhi virus Infecting Cucurbits in the Canary Islands. *Plant Disease*, *103*(7), 1798-1798. https://doi.org/10.1094/PDIS-01-19-0054-PDN

Essafi, A., Díaz-Pendón, J. A., Moriones, E., Monforte, A. J., Garcia-Mas, J., & Martín-Hernández, A. M. (2009). Dissection of the oligogenic resistance to Cucumber

mosaic virus in the melon accession PI 161375. *Theoretical and applied genetics*, *118*(2), 275-284. https://doi.org/10.1007/s00122-008-0897-x

Esteras, C., Gómez, P., Monforte, A. J., Blanca, J., Vicente-Dólera, N., Roig, C., Nuez, F., & Picó, B. (2012). High-throughput SNP genotyping in Cucurbita pepo for map construction and quantitative trait loci mapping. *BMC genomics*, *13*(1), 80. https://doi.org/10.1186/1471-2164-13-80

Eybishtz, A., Peretz, Y., Sade, D., Akad, F., & Czosnek, H. (2009). Silencing of a single gene in tomato plants resistant to Tomato yellow leaf curl virus renders them susceptible to the virus. *Plant molecular biology*, *71*(1-2), 157–171. https://doi.org/10.1007/s11103-009-9515-9

Eybishtz, A., Peretz, Y., Sade, D., Gorovits, R., & Czosnek, H. (2010). Tomato yellow leaf curl virus infection of a resistant tomato line with a silenced sucrose transporter gene LeHT1 results in inhibition of growth, enhanced virus spread, and necrosis. *Planta*, *231*(3), 537–548. https://doi.org/10.1007/s00425-009-1072-6

Fazeli, R., Heydarnejad, J., Massumi, H., Shaabanian, M., & Varsani, A. (2009). Genetic diversity and distribution of tomato-infecting begomoviruses in Iran. *Virus Genes*, *38*(2), 311-319. https://doi.org/10.1007/s11262-008-0310-5

Fadhila, C., Lal, A., Vo, T. T., Ho, P. T., Hidayat, S. H., Lee, J., Kil, E. J., & Lee, S. (2020). The threat of seed-transmissible *pepper yellow leaf curl Indonesia virus* in chili pepper. *Microbial Pathogenesis*, 104132. https://doi.org/10.1016/j.micpath.2020.104132

Feng, S., Zhang, J., Mu, Z., Wang, Y., Wen, C., Wu, T., Yu, C., Li, Z., & Wang, H. (2020). Recent progress on the molecular breeding of *Cucumis sativus* L. in China. *Theoretical and Applied Genetics*, *133*(5), 1777-1790. https://doi.org/10.1007/s00122-019-03484-0

Fiallo-Olivé, E., Pan, L. L., Liu, S. S., & Navas-Castillo, J. (2020). Transmission of begomoviruses and other whitefly-borne viruses: dependence on the vector species. *Phytopathology*, *110*(1), 10-17. https://doi.org/10.1094/PHYTO-07-19-0273-FI

FAOSTAT (2017 y 2018). http://www.fao.org/faostat/en/#data

Fernandez-Trujillo, J. P., Picó, B., Garcia-Mas, J., Alvarez, J. M., Monforte, A. J. (2011). Breeding for fruit quality in melon. En: Jenks, M. A., & Bebeli, P. J. (Eds.) Breeding for fruit quality (pp. 261-278). John Wiley & Sons Inc, Hoboken. https://doi.org/10.1002/9780470959350.ch12

Ferriol, M. & Picó, B. (2008). Pumpkin and Winter Squash. En: Prohens, J. and Nuez, F. (Eds.), *Vegetables I. Handbook of Plant Breeding*, (Vol. 1, pp. 317-349), Springer New York, NY. https://doi.org/10.1007/978-0-387-30443-4_10

Fondong, V. N. (2013). Geminivirus protein structure and function. *Molecular plant pathology*, *14*(6), 635-649. https://doi.org/10.1111/mpp.12032

Fondong, V. N. (2017). The search for resistance to cassava mosaic geminiviruses: how much we have accomplished, and what lies ahead. *Frontiers in plant science*, *8*, 408. https://doi.org/10.3389/fpls.2017.00408

Font-San Ambrosio, M. I. & Alfaro-Fernández, A. (2014). Sintomatología del virus del rizado del tomate de Nueva Delhi (Tomato leaf curl New Delhi virus, ToLCNDV) en los cultivos españoles. *Phytoma España: La revista profesional de sanidad vegetal*, (257), 36-41.

Font-San Ambrosio, M. I., & Alfaro-Fernández, A. (2015). "El virus de Nueva Delhi" (*Tomato leaf curl New Delhi virus*, ToLCNDV) amplía su gama de hospedantes en los cultivos españoles. *Phytoma España: La revista profesional de sanidad vegetal*, *272*, 25-32.

Formisano, G., Paris, H. S., Frusciante, L., & Ercolano, M. R. (2010). Commercial Cucurbita pepo squash hybrids carrying disease resistance introgressed from *Cucurbita moschata* have high genetic similarity. *Plant Genetic Resources*, *8*(3), 198. https://doi.org/10.1017/S1479262110000183

Fortes, I. M., Sánchez-Campos, S., Fiallo-Olivé, E., Díaz-Pendón, J. A., Navas-Castillo, J., & Moriones, E. (2016). A novel strain of *tomato leaf curl New Delhi virus* has spread to the Mediterranean basin. *Viruses*, *8*(11), 307. https://doi.org/10.3390/v8110307

Fraile, A., & García-Arenal, F. (2010). The coevolution of plants and viruses: resistance and pathogenicity. *Advances in virus research*, *76*, 1–32. https://doi.org/10.1016/S0065-3527(10)76001-2

Freitag, J. H. (1956). Beetle Transmission, Host Range, and Properties of Squash Mosaic Virus. *Phytopathology*, *46*(2).

Gabriel, S., Ziaugra, L., & Tabbaa, D. (2009). SNP genotyping using the Sequenom MassARRAY iPLEX platform. *Current protocols in human genetics*, *60*(1), 2-12. https://doi.org/10.1002/0471142905.hg0212s60

Gallois, J. L., Moury, B., & German-Retana, S. (2018). Role of the Genetic Background in Resistance to Plant Viruses. *International journal of molecular sciences*, *19*(10), 2856. https://doi.org/10.3390/ijms19102856

Gangadhara Rao, P., Behera, T. K., Gaikwad, A. B., Munshi, A. D., Jat, G. S., & Boopalakrishnan, G. (2018). Mapping and QTL analysis of gynoecy and earliness in bitter gourd (Momordica charantia L.) using genotyping-by-sequencing (GBS) technology. *Frontiers in plant science*, *9*, 1555. https://doi.org/10.3389/fpls.2018.01555.

Gaikwad, K. A., Sharma, A., & Cheema, D. S. (2010). Molecular detection and characterization of leaf curl virus infecting tomato in Punjab, India. In *III International Symposium on Tomato Diseases 914* (pp. 153-156). https://doi.org/10.17660/ActaHortic.2011.914.27

García-Arenal, F., & Zerbini, F. M. (2019). Life on the edge: geminiviruses at the interface between crops and wild plant hosts. *Annual review of virology*, *6*, 411-433. https://doi.org/10.1146/annurev-virology-092818-015536

Garcia-Mas, J. & Puigdomènech, P. (2016). Melon Genome Sequence. *Biotechnology in Agriculture and Forestry*, *70*, 15-29. https://doi.org/10.1007/978-3-662-48535-4_2.

Garcia-Mas, J., Benjak, A., Sanseverino, W., Bourgeois, M., Mir, G., González, V. M., Hénaff, E., Câmara, F., Cozzuto, L., Lowy, E., Alioto, T., Capella-Gutiérrez, S., Blanca, J., Cañizares, J., Ziarsolo, P., Gonzalez-Ibeas, D., Rodríguez-Moreno, L., Droege, M., Du, L., Alvarez-Tejado, M., Lorente-Galdos, B., Melé, M., Yang, L., Weng, Y., Navarro, A., Marques-Bonet, T., Aranda, M.A., Nuez, F., Picó, B., Gabaldón, T., Roma, G., Guigó, R., Casacuberta, J.M., Arús, P., & Puigdomènech, P. (2012). The genome of melon (Cucumis melo L.). *Proceedings of the National Academy of Sciences of the United States of America*, *109*(29), 11872–11877. https://doi.org/10.1073/pnas.1205415109

García-Neria, M. A., & Rivera-Bustamante, R. F. (2011). Characterization of Geminivirus resistance in an accession of *Capsicum chinense* Jacq. *Molecular Plant-Microbe Interactions*, *24*(2), 172-182. https://doi.org/ 10.1094/MPMI-06-10-0126.

Gilbert-Albertini, F., Lecoq, H., Pitrat, M., & Nicolet, J. L. (1993). Resistance of *Cucurbita moschata* to *watermelon mosaic virus type* 2 and its genetic relation to resistance to *zucchini yellow mosaic virus*. *Euphytica*, *69*(3), 231-237. https://doi.org/10.1007/BF00022369

Gilbert, R. Z., Kyle, M. M., Munger, H. M., & Gray, S. M. (1994). Inheritance of resistance to Watermelon mosaic virus in Cucumis melo L. *HortScience*, *29*(2), 107-110. https://doi.org/10.21273/HORTSCI.29.2.107

Gill, U., Scott, J. W., Shekasteband, R., Ogundiwin, E., Schuit, C., Francis, D. M., Sim, S. C., Smith, H., & Hutton, S. F. (2019). *Ty-6*, a major begomovirus resistance gene on chromosome 10, is effective against *Tomato yellow leaf curl virus* and *Tomato mottle virus*. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik*, *132*(5), 1543–1554. https://doi.org/10.1007/s00122-019-03298-0

Giner, A., Pascual, L., Bourgeois, M., Gyetvai, G., Rios, P., Picó, B., Troadec, C., Bendahmane, A., Garcia-Mas, J., & Martín-Hernández, A. M. (2017). A mutation in the melon Vacuolar Protein Sorting 41prevents systemic infection of Cucumber mosaic virus. *Scientific reports*, *7*(1), 10471. https://doi.org/10.1038/s41598-017-10783-3

Giordano, L. B., Silva-Lobo, V. L., Santana, F. M., Fonseca, M. E. N., & Boiteux, L. S. (2005). Inheritance of resistance to the bipartite *Tomato chlorotic mottle begomovirus* derived from *Lycopersicon esculentum* cv. 'Tyking'. *Euphytica*, *143*(1-2), 27-33. https://doi.org/10.1007/s10681-005-1685-1.

Glick, E., Zrachya, A., Levy, Y., Mett, A., Gidoni, D., Belausov, E., Citovsky, V., & Gafni, Y. (2008). Interaction with host SGS3 is required for suppression of RNA silencing by *tomato yellow leaf curl virus* V2 protein. *Proceedings of the National Academy of Sciences*, *105*(1), 157-161. https://doi.org/10.1073/pnas.0709036105

Gnanasekaran, P., KishoreKumar, R., Bhattacharyya, D., Vinoth Kumar, R., & Chakraborty, S. (2019). Multifaceted role of geminivirus associated betasatellite in pathogenesis. *Molecular plant pathology*, *20*(7), 1019-1033. https://doi.org/10.1111/mpp.12800

Gómez, P., Rodríguez-Hernández, A. M., Moury, B., & Aranda, M. A. (2009). Genetic resistance for the sustainable control of plant virus diseases: breeding, mechanisms and durability. *European journal of plant pathology*, *125*(1), 1-22. https://doi.org/10.1007/s10658-009-9468-5

Gómez, J., Serrano, Y., Pérez, A., Porcel, E., Gómez, R., & Aguilar, M. I. (2014). *Fusarium solani* f. sp. *cucurbitae*, affecting melon in Almería province, Spain. *Australasian plant disease notes*, *9*(1), 136. https://doi.org/10.1007/s10658-009-9468-5

Gómez-Aix, C., Pascual, L., Cañizares, J., Sánchez-Pina, M. A., & Aranda, M. A. (2016). Transcriptomic profiling of Melon necrotic spot virus-infected melon plants revealed virus strain and plant cultivar-specific alterations. *BMC genomics*, *17*(1), 429. https://doi.org/10.1186/s12864-016-2772-5

González, V. M., Aventín, N., Centeno, E., Puigdomènech, P. (2013). High presence/absence gene variability in defense-related gene clusters of *Cucumis melo*. *BMC Genomics*, *14*, 782. https://doi.org/10.1186/1471-2164-14-782

González, V. M., Aventín, N., Centeno, E., & Puigdomènech, P. (2014). Interspecific and intraspecific gene variability in a 1-Mb region containing the highest density of NBS-LRR genes found in the melon genome. *BMC genomics*, *15*(1), 1131. https://doi.org/10.1186/1471-2164-15-1131

González, V., Armijos, E., & Garcés-Claver, A. (2020a). Fungal Endophytes as Biocontrol Agents against the Main Soil-Borne Diseases of Melon and Watermelon in Spain. *Agronomy*, *10*(6), 820. https://doi.org/10.3390/agronomy10060820

González, V., García-Martínez, S., Ruiz, J. J., Flores-León, A., Picó, B., & Garcés-Claver, A. (2020b). First Report of *Neocosmospora falciformis* Causing Wilt and Root Rot of Muskmelon in Spain. *Plant Disease*, *104*(4), 1256. https://doi.org/10.1094/PDIS-09-19-2013-PDN

Gonzalez-Ibeas, D., Cañizares, J., & Aranda, M. A. (2012). Microarray analysis shows that recessive resistance to *Watermelon mosaic virus* in melon is associated with the induction of defense response genes. *Molecular plant-microbe interactions*, *25*(1), 107-118. https://doi.org/10.1094/MPMI-07-11-0193

Gonzalo M.J., & Monforte A.J. (2016) Genetic Mapping of Complex Traits in Cucurbits. En: Grumet R., Katzir N., Garcia-Mas J. (Eds.) *Genetics and Genomics of Cucurbitaceae. Plant Genetics and Genomics: Crops and Models*, (Vol 20, pp 269-290). Springer, Cham. https://doi.org/10.1007/7397_2016_9

Gonzalo, M. J., Díaz, A., Dhillon, N. P., Reddy, U. K., Picó, B., & Monforte, A. J. (2019). Re-evaluation of the role of Indian germplasm as center of melon diversification based on genotyping-by-sequencing analysis. *BMC genomics*, *20*(1), 448. https://doi.org/10.1186/s12864-019-5784-0

Gorovits, R., & Czosnek, H. (2017). The involvement of heat shock proteins in the establishment of Tomato yellow leaf curl virus infection. *Frontiers in plant science*, *8*, 355. https://doi.org/10.3389/fpls.2017.00355

Gorovits, R., Moshe, A., Amrani, L., Kleinberger, R., Anfoka, G., & Czosnek, H. (2017). The six *Tomato yellow leaf curl virus* genes expressed individually in tomato induce different levels of plant stress response attenuation. *Cell Stress and Chaperones*, *22*(3), 345-355. https://doi.org/10.1007/s12192-017-0766-0

Gray, S., Cilia, M., & Ghanim, M. (2014). Circulative, "nonpropagative" virus transmission: an orchestra of virus-, insect-, and plant-derived instruments. *Advances in virus research*, *89*, 141–199. https://doi.org/10.1016/B978-0-12-800172-1.00004-5

Grumet, R., Kabelka, E., McQueen, S., Wai, T., & Humphrey, R. (2000). Characterization of sources of resistance to the watermelon strain of Papaya ringspot virus in cucumber: allelism and co-segregation with other potyvirus resistances. *Theoretical and Applied Genetics*, *101*(3), 463-472. https://doi.org/10.1007/s001220051504

Grumet, R., Garcia-Mas, J., & Katzir, N. (2017). Cucurbit genetics and genomics: a look to the future. In *Genetics and Genomics of Cucurbitaceae* (pp. 409-415). Springer, Cham. https://doi.org/10.1007/7397_2017_1

Guerrero, J., Regedanz, E., Lu, L., Ruan, J., Bisaro, D. M., & Sunter, G. (2020). Manipulation of the Plant Host by the Geminivirus AC2/C2 Protein, a Central Player in the Infection Cycle. *Frontiers in plant science*, *11*, 591. https://doi.org/10.3389/fpls.2020.00591

Guevara-Olvera, L., Ruíz-Nito, M. L., Rangel-Cano, R. M., Torres-Pacheco, I., Rivera-Bustamante, R. F., Muñoz-Sánchez, C. I., González-Chavirad, M.M., Cruz-Hernandeza. A., & Guevara-González, R. G. (2012). Expression of a germin-like protein gene (*CchGLP*) from a geminivirus-resistant pepper (*Capsicum chinense* Jacq.) enhances tolerance to geminivirus infection in transgenic tobacco. *Physiological and Molecular Plant Pathology*, *78*, 45-50. https://doi.org/10.1016/j.pmpp.2012.01.005

Guiu-Aragonés, C., Monforte, A. J., Saladié, M., Corrêa, R. X., Garcia-Mas, J., & Martín-Hernández, A. M. (2014). The complex resistance to cucumber mosaic cucumovirus (CMV) in the melon accession PI161375 is governed by one gene and at least two quantitative trait loci. *Molecular Breeding*, *34*(2), 351-362. https://doi.org/10.1007/s11032-014-0038-y

Guner, N. (2004). *Papaya ringspot virus* watermelon strain and *Zucchini yellowmosaic* virus resistance in watermelon. North Carolina State University, Raleigh, PhD Thesis, (under the direction of Dr. Todd C. Wehner).https://repository.lib.ncsu.edu/bitstream/handle/1840.16/3271/etd.pdf?sequence=1&isAllowed=y

Guner, N., Pesic-VanEsbroeck, Z., Rivera-Burgos, L. A., & Wehner, T. C. (2018). Inheritance of resistance to Papaya ringspot virus-watermelon strain in watermelon. *HortScience*, *53*(5), 624-627. https://journals.ashs.org/hortsci/view/journals/hortsci/53/5/article-p624.xml

Guner, N., Rivera-Burgos, L. A., & Wehner, T. C. (2018). Inheritance of resistance to Zucchini yellow mosaic virus in watermelon. *HortScience*, *53*(8), 1115-1118. https://doi.org/10.21273/HORTSCI13169-18

Guo, S., Zhang, J., Sun, H., Salse, J., Lucas, W. J., Zhang, H., Zheng, Y., Mao, L., Ren, Y., Wang, Z., Min, J., Guo, X., Murat, F., Ham, B. K., Zhang, Z., Gao, S., Huang, M., Xu, Y., Zhong, S., Bombarely, A., Mueller, L. A., Zhao, H., He, H., Zhang, Y., Zhang, Z., Huang, S., Tan, T., Pang, E., Lin, K., Hu, Q., Kuang, H., Ni, P., Wang, B., Liu, J., Kou, Q., Hou, W., Zou, X., Jiang, J., Gong, G., Klee, K., Schoof, H., Huang, Y., Hu, X., Dong, S., Liang, D., Wang, J., Wu, K., Xia, Y., Zhao, X., Zheng, Z., Xing, M., Liang, X., Huang, B., Lv, T., Wang, J., Yin, Y., Yi, H., Li, R., Wu, M., Levi, A., Zhang, X., Giovannoni, J. J., Wang, J., Li, Y., Fei, Z., & Xu, Y. (2013). The draft genome of watermelon (Citrullus lanatus) and resequencing of 20 diverse accessions. *Nature genetics*, *45*(1), 51-58. https://doi.org/10.1038/ng.2470

Guo, W. L., Chen, B. H., Guo, Y. Y., Yang, H. L., Mu, J. Y., Wang, Y. L., Li, X. Z., & Zhou, J. G. (2019a). Improved Powdery Mildew Resistance of Transgenic *Nicotiana benthamiana* Overexpressing the *Cucurbita moschata CmSGT1* Gene. *Frontiers in plant science*, *10*, 955. https://doi.org/10.3389/fpls.2019.00955

Guo, S.; Zhao, S.; Sun, H.; Wang, X.; Wu, S.; Lin, T.; Ren, Y.; Gao, L.; Deng, Y.; Zhang, J.; Lu X, Zhang H, Shang J, Gong G1, Wen C1, He N2, Tian S1, Li M1, Liu J2, Wang Y1, Zhu Y2, Jarret R5, Levi A6, Zhang X1, Huang S7,8, Fei Z9,10, Liu W11, Xu Y (2019b) Resequencing of 414 cultivated and wild watermelon accessions identifies selection for fruit quality traits. *Nature Genetics*, *51*, 1616–1623. https://doi.org/10.1038/s41588-019-0518-4

Guo, Q., Shu, Y. N., Liu, C., Chi, Y., Liu, Y. Q., & Wang, X. W. (2019c). Transovarial transmission of tomato yellow leaf curl virus by seven species of the Bemisia tabaci complex indigenous to China: Not all whiteflies are the same. *Virology*, *531*, 240-247. https://doi.org/10.1016/j.virol.2019.03.009

Guo, S., Sun, H., Xu, Y., & Fei, Z. (2020a). *Citrullus lanatus*. *Trends in genetics: TIG*, *36*(6), 456. https://doi.org/10.1016/j.tig.2020.01.010.

Guo, Y., Gao, M., Liang, X., Xu, M., Liu, X., Zhang, Y., Liu, X., Liu, J., Gao, Y., Qu, S., & Luan, F. (2020b). Quantitative Trait Loci for Seed Size Variation in Cucurbits - A Review. *Frontiers in plant science*, *11*, 304. https://doi.org/10.3389/fpls.2020.00304

Gyoutoku, Y., Okazaki, S., Furuta, A., Etoh, T., Mizobe, M., Kuno, K., Hayashida, S., & Okuda, M. (2009). Chlorotic yellows disease of melon caused by Cucurbit chlorotic yellows virus, a new crinivirus. *Japanese Journal of Phytopathology (Japan)*, *75*(2), 109-111. https://doi.org/10.3186/jjphytopath.75.109

Haanstra, J. P. W., Wye, C., Verbakel, H., Meijer-Dekens, F., Van den Berg, P., Odinot, P., van Heusden, A. W., Tanksley, S., Lindhout, P., & Peleman, J. (1999). An integrated high-density RFLP-AFLP map of tomato based on two Lycopersicon

esculentum× L. pennellii F2 populations. *Theoretical and Applied Genetics*, *99*(1-2), 254-271. https://doi.org/10.1007/s001220051231

Haider, M.S., Tahir, M., Latif, S., & Briddon, R.W. (2006). First report of *Tomato leaf curl New Delhi virus* infecting *Eclipta prostrata* in Pakistan. *Plant Patholology*., 53, 285. https://doi.org/10.1111/j.1365-3059.2005.01278.x.

Hameed, A., Tahir, M. N., Amin, I., & Mansoor, S. (2017). First report of *tomato leaf curl New Delhi virus* and a tomato yellow leaf curl Thailand betasatellite causing severe leaf curl disease of potato in Pakistan. *Plant Disease*, *101*(6), 1065-1065. https://doi.org/10.1094/PDIS-09-16-1335-PDN

Hamed, K., Menzel, W., Dafalla, G., Gadelseed, A. M. A., & Winter, S. (2011). First report of Cucurbit chlorotic yellows virus infecting muskmelon and cucumber in Sudan. *Plant disease*, *95*(10), 1321-1321. https://doi.org/10.1094/PDIS-04-11-0349

Hanley-Bowdoin, L., Bejarano, E. R., Robertson, D., & Mansoor, S. (2013). Geminiviruses: masters at redirecting and reprogramming plant processes. *Nature Reviews Microbiology*, *11*(11), 777-788. https://doi.org/10.1038/nrmicro3117

Hanson, P., Green, S. K., & Kuo, G. (2006). Ty-2, a gene on chromosome 11 conditioning geminivirus resistance in tomato. *Tomato Genet Coop Rep*, *56*, 17-18.

Hanssen, I. M., Lapidot, M., & Thomma, B. P. (2010). Emerging viral diseases of tomato crops. *Molecular plant-microbe interactions*, *23*(5), 539-548. https://doi.org/10.1094/MPMI-23-5-0539

Hao, N., Han, D., Huang, K., Du, Y., Yang, J., Zhang, J., Wen, C., & Wu, T. (2020). Genome-based breeding approaches in major vegetable crops. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik*, *133*(5), 1739–1752. https://doi.org/10.1007/s00122-019-03477-z

Hasegawa, D. K., Chen, W., Zheng, Y., Kaur, N., Wintermantel, W. M., Simmons, A. M., Fei, Z., & Ling, K. S. (2018). Comparative transcriptome analysis reveals networks of genes activated in the whitefly, *Bemisia tabaci* when fed on tomato plants infected with *Tomato yellow leaf curl virus*. *Virology*, *513*, 52-64. https://doi.org/10.1016/j.virol.2017.10.008

Hashimoto, M., Neriya, Y., Yamaji, Y., & Namba, S. (2016). Recessive resistance to plant viruses: potential resistance genes beyond translation initiation factors. *Frontiers in Microbiology*, *7*, 1695. https://doi.org/10.3389/fmicb.2016.01695 Crossref,

Haxim, Y., Ismayil, A., Jia, Q., Wang, Y., Zheng, X., Chen, T., Qian, L., Liu, N., Wang, Y., Han, S., Cheng, J., Qi, Y., Hong, Y., & Liu, Y. (2017). Autophagy functions as an antiviral mechanism against geminiviruses in plants. *eLife*, *6*, e23897. https://doi.org/10.7554/eLife.23897

Heath M. C. (2000). Hypersensitive response-related death. *Plant molecular biology*, *44*(3), 321–334. https://doi.org/10.1023/a:1026592509060

Herrington, M. E., Prytz, S., Wright, R. M., Walker, I. O., Brown, P., Persley, D. M., & Greber, R. S. (2001). 'Dulong QHI' and 'Redlands Trailblazer', PRSV-W-, ZYMV-

, andWMV-resistantwinter squash cultivars. *HortScience*, 36(4), 811-812. https://doi.org/10.21273/HORTSCI.36.4.811

Hesketh, E. L., Saunders, K., Fisher, C., Potze, J., Stanley, J., Lomonossoff, G. P., & Ranson, N. A. (2018). The 3.3 Å structure of a plant geminivirus using cryo-EM. *Nature communications*, *9*(1), 1-10. https://doi.org/10.1038/s41467-018-04793-6

Hong, C., Weiping, K., Junfen, L., & Jiping, L. (2015). Analysis of powdery mildew resistance in wild melon MLO mutants. *Horticultural Plant Journal*, *1*(3), 165-171. https://doi.org/10.16420/j.issn.2095-9885.2015-0036.

Howlader, J., Hong, Y., Natarajan, S, Sumi, K.R, Kim, H.T, Park, J.I & Nou I.S (2020). Development of powdery mildew race 5-specific SNP markers in *Cucumis melo* L. using whole-genome resequencing. *Horticulture, Environment, and Biotechnology*. *61*, 347–357. https://doi.org/10.1007/s13580-019-00217-6

Huang, Y., Li, M. Y., Wu, P., Xu, Z. S., Que, F., Wang, F., & Xiong, A. S. (2016). Members of WRKY Group III transcription factors are important in TYLCV defense signaling pathway in tomato (*Solanum lycopersicum*). *BMC genomics*, *17*(1), 1-18. https://doi.org/10.1186/s12864-016-3123-2

Huang, Y., Li, T., Xu, Z. S., Wang, F., & Xiong, A. S. (2017). Six NAC transcription factors involved in response to TYLCV infection in resistant and susceptible tomato cultivars. *Plant Physiology and Biochemistry*, *120*, 61-74. doi:10.1016/j.plaphy.2017.09.020

Huang, S., Li, R., Zhang, Z., Li, L., Gu, X., Fan, W., Lucas, W. J., Wang, X., Xie, B., Ni, P., Ren, Y., Zhu, H., Li, J., Lin, K., Jin, W., Fei, Z., Li, G., Staub, J., Kilian, A., van der Vossen, E. A., Wu, Y., Guo, J., He, J., Jia, Z., Ren, Y., Tian, G., Lu, Y., Ruan, J., Qian, W., Wang, M., Huang, Q., Li, B., Xuan, Z., Cao, J., Asan, Wu, Z., Zhang, J., Cai, Q., Bai, Y., Zhao, B., Han, Y., Li, Y., Li, X., Wang, S., Shi, Q., Liu, S., Cho, W. K., Kim, J. Y., Xu, Y., Heller-Uszynska, K., Miao, H., Cheng, Z., Zhang, S., Wu, J., Yang, Y., Kang, H., Li, M., Liang, H., Ren, X., Shi, Z., Wen, M., Jian, M., Yang, H., Zhang, G., Yang, Z., Chen, R., Liu, S., Li, J., Ma, L., Liu, H., Zhou, Y., Zhao, J., Fang, X., Li, G., Fang, L., Li, Y., Liu, D., Zheng, H., Zhang, Y., Qin, N., Li, Z., Yang, G., Yang, S., Bolund, L., Kristiansen, K., Zheng, H., Li, S., Zhang, X., Yang, H., Wang, J., Sun, R., Zhang, B., Jiang, S., Wang, J., Du, Y., & Li, S. (2009). The genome of the cucumber, *Cucumis sativus*. L. *Nature Genetics*, *41*(12), 1275-1281. https://doi.org/10.1038/ng.475

Hull, R. (2002). Architecture and Assembly of Virus Particles. En: Hull, R. (Ed.), *Plant Virology (Fourth Edition)*, Academic Press, https://doi.org/10.1016/B978-012361160-4/50056-6.

Hull, R. (2014). Origins and evolution of plant viruses. En: Hull R. (Ed.), *Plant Virology*, (pp. 423-476). https://doi.org/10.1016/B978-0-12-384871-0.00008-X

Hulsmans, S., Rodriguez, M., Coninck, B. De, & Rolland, F. (2016). The *SnRK1* Energy Sensor in Plant Biotic Interactions. *Trends in Plant Science*, *21* (8), 648–661. https://doi.org/10.1016/J.TPLANTS.2016.04.008

Hussain, M., Mansoor, S., Iram, S., Zafar, Y., & Briddon, R.W. (2000) First report of Tomato leaf curl New Delhi virus affecting chilli pepper in Pakistan. *New Disease Reports* 9, 20. http://www.ndrs.org.uk/article.php?id=009020

Hussain, M., Mansoor, S., Iram, S., Zafar, Y., & Briddon, R. W. (2004). First report of Tomato leaf curl New Delhi virus affecting chilli pepper in Pakistan. *Plant pathology*, *53*(6). https://doi.org/10.1111/j.1365-3059.2004.01073.x

Hussain, M., Mansoor, S., Iram, S., Fatima, A. N., & Zafar, Y. (2005). The nuclear shuttle protein of *Tomato leaf curl New Delhi virus* is a pathogenicity determinant. *Journal of Virology*, *79*(7), 4434-4439. https://doi.org/10.1128/JVI.79.7.4434-4439.2005

Hussain, Z., Lata, S., Kumar, P., Kumar, S., & Tomar, B. S. (2019) Recessive behavior of Ty-3 gene for ToLCNDV disease resistance in Tomato (*Solanum lycopersicum* L.). Congress communication. Accessed: https://www.researchgate.net/profile/Kumar_Sumit3/publication/340476078_Recessive_behavior_of_Ty-3_gene_for_ToLCNDV_disease_resistance/links/5e8bf9c2a6fdcca789fbec50/Recessive-behavior-of-Ty-3-gene-for-ToLCNDV-disease-resistance.pdf

Hutton, S. F., Scott, J. W., & Schuster, D. J. (2012). Recessive Resistance to Tomato yellow leaf curl virus from the Tomato Cultivar Tyking Is Located in the Same Region as Ty-5 on Chromosome 4, *HortScience horts*, *47*(3), 324-327.

Hutton, S. F., Scott, J. W., Shekasteband, R., Levin, I., & Lapidot, M. (2013). Combinations of *Ty* resistance genes generally provide more effective control against begomoviruses than do single genes. In *IV International Symposium on Tomato Diseases 1069* (pp. 59-64).

Hutton, S. F., & Scott, J. W. (2014). *Ty-6*, a major begomovirus resistance gene located on chromosome 10. *Reports of Tomato Genetic Cooperative*, 64, 14–18.

Ibaba, J. D., Sivparsad, B. J., Laing, M. D., & Gubba, A. (2017). Use of a chimeric transgene construct to confer broad resistance in zucchini (*Cucurbita pepo* L.) plants against cucurbit-infecting potyviruses occurring in KwaZulu-Natal, South Africa. *South African Journal of Plant and Soil*, *34*(3), 235-237. DOI: 10.1080/02571862.2016.1245791

Idris, A. M., Mills-Lujan, K., Martin, K., & Brown, J. K. (2008). Melon chlorotic leaf curl virus: characterization and differential reassortment with closest relatives reveal adaptive virulence in the squash leaf curl virus clade and host shifting by the host-restricted bean calico mosaic virus. *Journal of virology*, *82*(4), 1959–1967. https://doi.org/10.1128/JVI.01992-07

Islam, S., Munshi, A. D., Mandal, B., Kumar, R., & Behera, T. K. (2010). Genetics of resistance in *Luffa cylindrica* Roem. against Tomato leaf curl New Delhi virus. *Euphytica*, *174*(1), 83-89. https://doi.org/10.1007/s10681-010-0138-7

Islam, S., Anilabh Das, M., Verma, M., Arya, L., Mandal, B., Tusar Kanti, B., Kumar, R. & Sanjay Kumar, L. (2011). Screening of Luffa cylindrica Roem. for resistance against *Tomato leaf curl New Delhi virus*, inheritance of resistance, and identification of SRAP markers linked to the single dominant resistance gene. *The*

Journal of Horticultural Science and Biotechnology, *86*(6), 661-667. https://doi.org/10.1080/14620316.2011.11512819

Ito, T., Sharma, P., Kittipakorn, K., & Ikegami, M. (2008). Complete nucleotide sequence of a new isolate of tomato leaf curl New Delhi virus infecting cucumber, bottle gourd and muskmelon in Thailand. *Archives of Virology*, *153*(3), 611-613. https://doi.org/10.1007/s00705-007-0029-y

Jackel, J. N., Storer, J. M., Coursey, T., & Bisaro, D. M. (2016). Arabidopsis RNA polymerases IV and V are required to establish H3K9 methylation, but not cytosine methylation, on geminivirus chromatin. *Journal of virology*, *90*(16), 7529-7540. DOI: 10.1128/JVI.00656-16

Jacquemond, M. (2012). Cucumber mosaic virus. En : Loebenstein, G., & Lecoq, H. (Eds.), *Advances in virus research* (Vol. 84, pp. 439-504). Academic Press. https://doi.org/10.1016/B978-0-12-394314-9.00013-0.

Jaganathan D, Ramasamy K, Sellamuthu G, Jayabalan S and Venkataraman G (2018) CRISPR for Crop Improvement: An Update Review. *Frontiers in Plant Scice*, *9*:985. https://doi.org/10.3389/fpls.2018.00985

Jagger, I. C. (1916). Experiments with the cucumber mosaic disease. *Phytopathology*, *6*(2), 149-151.

Jamil, N., Rehman, A., Hamza, M., Hafeez, A., Ismail, H., Zubair, M., Mansoor, S., & Amin, I. (2017). First report of *Tomato leaf curl New Delhi virus*, a bipartite begomovirus, infecting soybean (*Glycine max*). *Plant Disease*, *101*(5), 845. https://doi.org/10.1094/PDIS-09-16-1267-PDN

Janssen D. & Riuz L. (2014). ToLCNDV: un nuevo virus de cucurbitáceas en España transmitido por mosca blanca. *Almería en VERDE*, *116*, 12-15.

Janssen D., Ruiz L., Simón A. & García C. (2014). Control en invernadero del virus de Nueva Delhi de la hoja rizada del tomate. *Vida Rural*, *378*, 32-36.

Janssen, D., Simon, A., Crespo, O., & Ruiz, L. (2017). Genetic population structure of Bemisia tabaci in Spain associated with Tomato leaf curl New Delhi virus. *Plant Protect. Sci*, *53*, 25-31. https://doi.org/10.17221/62/2016-PPS

Jat, G. G., Kumar, P., Perveen, S., & Tomar, B. S. (2019). qPCR analysis of Ty-2 and Ty-3 gene pyramided lines of tomato for resistance to tomato leaf curl New Delhi virus (ToLCNDV). *Indian Journal of Agricultural Sciences*, *89*(10), 1719-22.

Jeevalatha, A., Siddappa, S., Kumar, A., Kaundal, P., Guleria, A., Sharma, S., Nagesh, M., & Singh, B. P. (2017). An insight into differentially regulated genes in resistant and susceptible genotypes of potato in response to tomato leaf curl New Delhi virus-[potato] infection. *Virus research*, *232*, 22–33. https://doi.org/10.1016/j.virusres.2017.01.015

Jeske H. (2009) Geminiviruses. En: de Villiers, E. M., Hausen, H. (Eds.). *TT Viruses. Current Topics in Microbiology and Immunology*, (Vol. 331, pp. 185-226) Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-70972-5_11

Ji, Y., Scott, J. W., Schuster, D. J., and Maxwell, D. P. (2009). Molecular mapping of *Ty-4*, a new *Tomato yellow leaf curl virus* resistance locus on chromosome 3 of tomato. *J. Am. Soc. Hortic. Sci.* 134, 281–288

Ji, Y., Schuster, D. J., & Scott, J. W. (2007). *Ty-3*, a begomovirus resistance locus near the *Tomato yellow leaf curl virus* resistance locus *Ty-1* on chromosome 6 of tomato. *Molecular Breeding*, *20*(3), 271-284. https://doi.org/10.1007/s11032-007-9089-7

Jones, R. A., & Naidu, R. A. (2019). Global dimensions of plant virus diseases: Current status and future perspectives. *Annual review of virology*, *6*, 387-409. https://doi.org/10.1146/annurev-virology-092818-015606

Jones, P., Sattar, M. H. A., & Al Kaff, N. (1988). incidence of virus disease in watermelon and sweetmelon crops in the Peoples Democratic Republic of Yemen and its impact on cropping policy. *Annals of applied biology*. *17*, 203-207.

Juárez, M., Legua, P., Mengual, C. M., Kassem, M. A., Sempere, R. N., Gómez, P., Truniger, V. & Aranda, M. A. (2013). Relative incidence, spatial distribution and genetic diversity of cucurbit viruses in eastern Spain. *Annals of Applied Biology*, *162*(3), 362-370. https://doi.org/10.1111/aab.12029

Juárez, M., Tovar, R., Fiallo-Olivé, E., Aranda, M. A., Gosálvez, B., Castillo, P., Moriones, E., & Navas-Castillo, J. (2014). First Detection of *Tomato leaf curl New Delhi virus* Infecting Zucchini in Spain. *Plant disease*, *98*(6), 857. https://doi.org/10.1094/PDIS-10-13-1050-PDN

Juárez, M., Rabadán, M. P., Martínez, L. D., Tayahi, M., Grande-Pérez, A., & Gomez Lopez, P. (2019). Natural hosts and genetic diversity of the emerging tomato leaf curl New Delhi virus in Spain. *Frontiers in microbiology*, *10*, 140. https://doi.org/10.3389/fmicb.2019.00140

Jung, J., Kim, H. J., Lee, J. M., Oh, C. S., Lee, H. J., & Yeam, I. (2015). Gene-based molecular marker system for multiple disease resistances in tomato against Tomato yellow leaf curl virus, late blight, and verticillium wilt. *Euphytica*, *205*(2), 599-613. https://doi.org/10.1007/s10681-015-1442-z

Junta de Andalucía. Consejeria de Agricultura, pesca y desarrollo rural. (2018). Delegación territorial de Almeria- Fichas fitopatológicas. Tomato Leaf Curl New Delhi Virus ToLCNDV.http://www.juntadeandalucia.es/export/cdn-micrositios/documents/71753/321091/Tomato+Leaf+Curl+New+Delhi+Virus+ToLCNDV/045af4ab-0f7f-4f36-900a-8419c078109b

Jyothsna, P., Haq, Q. M., Singh, P., Sumiya, K. V., Praveen, S., Rawat, R., Briddon, R. W., & Malathi, V. G. (2013). Infection of *tomato leaf curl New Delhi virus* (ToLCNDV), a bipartite begomovirus with betasatellites, results in enhanced level of helper virus components and antagonistic interaction between DNA B and betasatellites. *Applied microbiology and biotechnology*, *97*(12), 5457–5471. https://doi.org/10.1007/s00253-012-4685-9

Kabelka, E., & Grumet, R. (1997). Inheritance of resistance to the Moroccan watermelon mosaic virus in the cucumber line TMG-1 and cosegregation with zucchini yellow mosaic virus resistance. *Euphytica*, *95*(2), 237-242. https://doi.org/10.1023/A:1002987203987

Kabelka, E., Ullah, Z., & Grumet, R. (1997). Multiple alleles for zucchini yellow mosaic virus resistance at the zym locus in cucumber. *Theoretical and applied genetics*, *95*(5-6), 997-1004. https://doi.org/10.1007/s001220050653

Kadirvel, P., De la Peña, R., Schafleitner, R., Huang, S., Geethanjali, S., Kenyon, L., Tsai, W., & Hanson, P. (2013). Mapping of QTLs in tomato line FLA456 associated with resistance to a virus causing *tomato yellow leaf curl disease*. *Euphytica*, *190*(2), 297-308. https://doi.org/10.1007/s10681-012-0848-0

Kaldis, A., Berbati, M., Melita, O., Reppa, C., Holeva, M., Otten, P., & Voloudakis, A. (2018). Exogenously applied dsRNA molecules deriving from the Zucchini yellow mosaic virus (ZYMV) genome move systemically and protect cucurbits against ZYMV. *Molecular plant pathology*, *19*(4), 883–895. https://doi.org/10.1111/mpp.12572

Karchi Z, Cohen S, Govers A (1975) Inheritance of resistance to Cucumber Mosaic Virus in melons. *Phytopathology*, 65, 479–481.

Kassem, M. A., Gosalvez, B., Garzo, E., Fereres, A., Gómez-Guillamón, M. L., & Aranda, M. A. (2015). Resistance to Cucurbit aphid-borne yellows virus in Melon Accession TGR-1551. *Phytopathology*, *105*(10), 1389-1396. https://doi.org/10.1094/PHYTO-02-15-0041-R

Kaur, N., Chen, W., Fei, Z., & Wintermantel, W. M. (2019). Differences in gene expression in whitefly associated with CYSDV-infected and virus-free melon, and comparison with expression in whiteflies fed on ToCV- and TYLCV-infected tomato. *BMC genomics*, *20*(1), 654. https://doi.org/10.1186/s12864-019-5999-0

Kawazu, Y., Shimomura, K., Maeda, S., Yamato, Y., Ueda, S., Okuda, S., Okuda, M., & Sugiyama, M. (2018). QTL mapping for resistance to Cucurbit chlorotic yellows virus in melon (Cucumis melo L.). Euphytica. 214. https://doi.org/10.1007/s10681-018-2321-1.

Khan, M. S., Raj, S. K., Singh, R. (2005). First report of *Tomato leaf curl New Delhi virus* infecting chilli in India. *New Disease Reports*, *11*, 41. https://doi.org/10.1111/j.1365-3059.2006.01324.x

Khan, M. S., Ji, S. H., & Chun, S. C. (2012). Begomoviruses and their emerging threats in South Korea: a review. *The Plant Pathology Journal*, *28*(2), 123-136. https://doi.org/10.5423/PPJ.RW.11.2011.0219

Kheireddine, A., Sifres, A., Sáez, C., Picó, B., and López, C. (2019). First report of tomato leaf curl New Delhi virus infecting cucurbit plants in Algeria. *Plant Disease.* 103:3291. https://doi.org/10.1094/PDIS-05-19-1118-PDN

Kheireddine, A., Sáez, C., Sifres, A., Picó, B., & López, C. (2020). First Report of Cucurbit Chlorotic Yellows Virus Infecting Cucumber and Zucchini in Algeria. *Plant Disease*, *104*(4), 1264-1264. 10.1094/PDIS-10-19-2091-PDN.

Khoury, C.K., Carver, D., Kates, H.R., Achicanoy H.A., Van Zonneveld, M., Thomas E., Heinitz, C., Jarret, R., Labate, J.A., Reitsma, K., Nabhan, G.P. & Greene, S.L. (2020) Distributions, conservation status, and abiotic stress tolerance potential of

wild cucurbits (*Cucurbita* L.). *Plants, People, Planet*. 2: 269– 283. https://doi.org/10.1002/ppp3.10085

Kil, E. J., Kim, S., Lee, Y. J., Byun, H. S., Park, J., Seo, H., Kim, C. S., Shim, J. K., Lee, J. H., Kim, J. K., Lee, K. Y., Choi, H. S., & Lee, S. (2016). *Tomato yellow leaf curl virus* (TYLCV-IL): a seed-transmissible geminivirus in tomatoes. *Scientific reports*, *6*, 19013. https://doi.org/10.1038/srep19013

Kil, E. J., Park, J., Choi, H. S., Kim, C. S., & Lee, S. (2017). Seed Transmission of *Tomato yellow leaf curl virus* in White Soybean (*Glycine max*). *The plant pathology journal*, *33*(4), 424–428. https://doi.org/10.5423/PPJ.NT.02.2017.0043

Kil, E., Park, J., Choi, E., Byun, H., Lee, K., An, C., Lee, J., Lee, G., Choi, H., Kim, C., Kim, J., & Lee, S. (2018). Seed transmission of *Tomato yellow leaf curl virus* in sweet pepper (*Capsicum annuum*). *European journal of plant pathology*, *150*, 759-764. https://doi.org/10.1007/s10658-017-1304-8

Kil, E. J., Vo, T. T. B., Fadhila, C., Ho, P. T., Lal, A., Troiano, E., Parrella, G. & Lee, S. (2020). Seed Transmission of *Tomato Leaf Curl New Delhi Virus* from Zucchini Squash in Italy. *Plants*, *9*(5), 563. https://doi.org/10.3390/plants9050563

Kim, Kwang-Hwan & Ahn, Sung-Gyu & Jihyun, Hwang & Choi, Young-Mi & Shik, Moon & Park, Younghoon. (2013). Inheritance of Resistance to Powdery Mildew in the Watermelon and Development of a Molecular Marker for Selecting Resistant Plants. *Horticulture, Environment, and Biotechnology*. *54*(2), 134-140. 54. https://doi.org/10.1007/s13580-013-0156-1.

Kim, K. H., Hwang, J. H., Han, D. Y., Park, M., Kim, S., Choi, D., Kim, Y., Lee, G. P., Kim, S. T., & Park, Y. H. (2015a). Major Quantitative Trait Loci and Putative Candidate Genes for Powdery Mildew Resistance and Fruit-Related Traits Revealed by an Intraspecific Genetic Map for Watermelon (*Citrullus lanatus* var. *lanatus*). *PloS one*, *10*(12), e0145665. https://doi.org/10.1371/journal.pone.0145665

Kim, J., Kil, E. J., Kim, S., Seo, H., Byun, H. S., Park, J. Chung, M. N., Kwak, H. R., Kim, M. K., Kim, C. S., Yang, J. W., Lee, K. Y., Choi, H. S., & Lee, S. (2015b). Seed transmission of *Sweet potato leaf curl virus* in sweet potato (*Ipomoea batatas*). *Plant Pathology*, *64*(6), 1284-1291. https://doi.org/10.1111/ppa.12366

Kim, M., Park, Y., Lee, J., & Sim, S. C. (2020). Development of molecular markers for *Ty-2* and *Ty-3* selection in tomato breeding. *Scientia Horticulturae*, *265*, 109230. https://doi.org/10.1016/j.scienta.2020.109230

Kobayashi, K., Sekine, K. T., & Nishiguchi, M. (2014). Breakdown of plant virus resistance: can we predict and extend the durability of virus resistance? *Journal of general plant pathology*, *80*(4), 327-336. https://doi.org/10.1007/s10327-014-0527-1

Köklü, G. & Yilmaz, Ö. (2006). Occurrence of cucurbit viruses on field-grown melon and watermelon in the Thrace region of Turkey. *Phytoprotection*, *87* (3), 123–130. https://doi.org/10.7202/015854ar

Kone, N., Asare-Bediako, E., Silue, S., Kone, D., Koita, O., Menzel, W., & Winter, S. (2017). Influence of planting date on incidence and severity of viral disease on cucurbits

under field condition. *Annals of Agricultural Sciences*, *62*(1), 99-104. https://doi.org/10.1016/j.aoas.2017.05.005.

Kong, L., Liu, Y., Wang, X., & Chang, C. (2020). Insight into the role of epigenetic processes in abiotic and biotic stress response in wheat and barley. *International Journal of Molecular Sciences*, *21*(4), 1480. https://doi.org/10.3390/ijms21041480

Kothandaraman, S. V., Devadason, A., & Ganesan, M. V. (2016). Seed-borne nature of a begomovirus, Mung bean yellow mosaic virus in black gram. *Applied microbiology and biotechnology*, *100*(4), 1925-1933. https://doi.org/10.1007/s00253-015-7188-7

Kousik, C. S., Adkins, S., Turechek, W. W., & Roberts, P. D. (2009). Sources of resistance in US plant introductions to watermelon vine decline caused by *Squash vein yellowing virus*. *HortScience*, *44*(2), 256-262. https://doi.org/10.21273/HORTSCI.44.2.256

Kousik, C. S., Adkins, S., Turechek, W. W., Webster, C. G., Webb, S. E., Baker, C. A., Stansly, P. A. & Roberts, P. D. (2012a). Progress and challenges in managing watermelon vine decline caused by whitefly-transmitted Squash Vein Yellowing Virus (SqVYV). *Israel Journal of Plant Sciences*, *60*(4), 435-445. https://doi.org/10.1560/IJPS.60.4.435

Kousik, C. S., Adkins, S., Turechek, W. W., Webster, C. G., & Roberts, P. D. (2012b). 392291-VDR, a Watermelon Germplasm Line with Resistance to Squash vein yellowing virus-caused Watermelon Vine Decline. *HortScience*, *47*(12), 1805-1807. https://doi.org/10.21273/HORTSCI.47.12.1805

Křistkova, E., & Lebeda, A. (2000). Resistance in *Cucurbita pepo* and *Cucurbita maxima* germplasm to watermelon mosaic potyvirus-2. *Plant Genetic Resources Newsletter*, *121*, 47-52.

Kumar, N. (2006). Breeding of horticultural crops: principles and practices. Delhi: New India Publishing, New Delhi. 173-177.

Kumar, N., Kulwal, P., Gaur, A., Tyagi, A., Khurana, J. Khurana, P., Balyan, H., & Gupta, P. (2006). QTL analysis for grain weight in common wheat. *Euphytica*. *151*. 135-144. https://doi.org/10.1007/s10681-006-9133-4.

Kumar, A., Tiwari, K. L., Datta, D., & Singh, M. (2014). Marker assisted gene pyramiding for enhanced *Tomato leaf curl virus disease resistance* in tomato cultivars. *Biologia plantarum*, *58*(4), 792-797. https://doi.org/10.1007/s10535-014-0449-y

Kumar, R., & Tyagi, S. (2018). Breeding for Disease Resistance: A Journey from Conventional to Molecular Approach. *Global Hi-Tech Horticulture*

Kumar R. V. (2019a). Plant Antiviral Immunity Against Geminiviruses and Viral Counter-Defense for Survival. *Frontiers in microbiology*, *10*, 1460. https://doi.org/10.3389/fmicb.2019.01460

Kumar, A., Jindal, S. K., Dhaliwal, M. S., Sharma, A., Kaur, S., & Jain, S. (2019). Gene pyramiding for elite tomato genotypes against ToLCV (*Begomovirus* spp.), late

blight (*Phytophthora infestans*) and RKN (*Meloidogyne* spp.) for northern India farmers. *Physiology and molecular biology of plants: an international journal of functional plant biology*, *25*(5), 1197–1209. https://doi.org/10.1007/s12298-019-00700-5

Kundu, A., & Pal, A. (2012). Identification and characterization of elite inbred lines with MYMIV-resistance in *Vigna mungo*. *Field Crops Research*, *135*, 116-125. https://doi.org/10.1016/j.fcr.2012.07.006

Kundu, A., Paul, S., Dey, A., & Pal, A. (2017). High throughput sequencing reveals modulation of microRNAs in Vigna mungo upon Mungbean Yellow Mosaic India Virus inoculation highlighting stress regulation. *Plant Science*, *257*, 96-105. https://doi.org/10.1016/j.plantsci.2017.01.016

Kuon, J. E., Qi, W., Schläpfer, P., Hirsch-Hoffmann, M., von Bieberstein, P. R., Patrignani, A., Poveda, L., Grob, S., Keller, M., Shimizu-Inatsugi, R., Grossniklaus, U., Vanderschuren, H., & Gruissem, W. (2019). Haplotype-resolved genomes of geminivirus-resistant and geminivirus-susceptible African cassava cultivars. *BMC biology*, *17*(1), 75. https://doi.org/10.1186/s12915-019-0697-6

Kuria, P., Ilyas, M., Ateka, E., Miano, D., Onguso, J., Carrington, J. C., & Taylor, N. J. (2017). Differential response of cassava genotypes to infection by cassava mosaic geminiviruses. *Virus research*, *227*, 69-81. https://doi.org/10.1016/j.virusres.2016.09.022

Kushwaha, N., Singh, A. K., Basu, S., & Chakraborty, S. (2015). Differential response of diverse solanaceous hosts to *tomato leaf curl New Delhi virus* infection indicates coordinated action of NBS-LRR and RNAi-mediated host defense. *Archives of Virology*, *160*(6), 1499-1509. https://doi.org/10.1007/s00705-015-2399-x

Kushwaha, N. K., Bhardwaj, M., & Chakraborty, S. (2017). The replication initiator protein of a geminivirus interacts with host monoubiquitination machinery and stimulates transcription of the viral genome. *PLoS pathogens*, *13*(8), e1006587. https://doi.org/10.1371/journal.ppat.1006587

Lapidot, M., & Friedmann, M. (2002). Breeding for resistance to whitefly-transmitted geminiviruses. *Annals of Applied Biology*, *140*(2), 109-127. https://doi.org/10.1111/j.1744-7348.2002.tb00163.x

Lapidot, M., Karniel, U., Gelbart, D., Fogel, D., Evenor, D., Kutsher, Y., Makhbash, Z., Nahon, S., Shlomo, H., Chen, L., Reuveni, M., & Levin, I. (2015). A Novel Route Controlling Begomovirus Resistance by the Messenger RNA Surveillance Factor Pelota. *PLoS genetics*, *11*(10), e1005538. https://doi.org/10.1371/journal.pgen.1005538

Lazarowitz, S. G., & Shepherd, R. J. (1992). Geminiviruses: genome structure and gene function. *Critical Reviews in Plant Sciences*, *11*(4), 327-349. https://doi.org/10.1080/07352689209382350

Lebeda, A., & Křístková, E. (1996). Resistance in *Cucurbita pepo and Cucurbita maxima* germplasms to cucumber mosaic virus. *Genetic Resources and Crop Evolution*, *43*(5), 461-469.

Lebeda, A., Křístková, E., Sedláková, B., McCreight, J. D., & Coffey, M. D. (2016). Cucurbit powdery mildews: methodology for objective determination and denomination of races. *European journal of plant pathology*, *144*(2), 399–410. https://doi.org/10.1007/s10658-015-0776-7

Lecoq, H., Bourdin, D., WIPF-SCHEIBEL, C., Bon, M., Lot, H., Lemaire, O., & Herrbach, E. (1992). A new yellowing disease of cucurbits caused by a luteovirus, cucurbit aphid-borne yellows virus. *Plant Pathology*, *41*(6), 749-761. https://doi.org/10.1111/j.1365-3059.1992.tb02559.x

Lecoq, H., Wisler, G., & Pitrat, M. (1998). Cucurbit viruses: the classics and the emerging. En: McCreight, J.D. (Ed.), *Cucurbitaceae* (Vol. 98, pp. 126-142) ASHS Press, Alexandria, Virginia, USA.

Lecoq, H., & Desbiez, C. (2012). Viruses of cucurbit crops in the Mediterranean region: an ever-changing picture. In *Advances in virus research* (Vol. 84, pp. 67-126). Academic Press. https://doi.org/10.1016/B978-0-12-394314-9.00003-8.

Lecoq, H., & Katis, N. (2014). Control of cucurbit viruses. En: Loebenstein, G., &, Katis, N. (Eds.), *Advances in virus research* (Vol. 90, pp. 255-296). Academic Press. http://dx.doi.org/10.1016/B978-0-12-801246-8.00005-6

Lee, E. S., Kim, J., Hong, J. P., Kim, D. S., Kim, M., Huh, Y. C., & Lee, H. E. (2018). Development of HRM markers based on SNPs identified from next generation resequencing of susceptible and resistant parents to gummy stem blight in watermelon. *Korean Society of Breeding Science*, *50*(4), 424-433. https://doi.org/10.9787/KJBS.2018.50.4.424

Lee, C. H., Zheng, Y. X., Chan, C. H., Ku, H. M., Chang, C. J., & Jan, F. J. (2020). A single amino acid substitution in the movement protein enables the mechanical transmission of a geminivirus. *Molecular plant pathology*, *21*(4), 571–588. https://doi.org/10.1111/mpp.12917

Lefebvre, V., Boissot, N., & Gallois, J. L. (2020). Host plant resistance to pests and pathogens, the genetic leverage in integrated pest and disease management. En: Gullino, M., Albajes, R., & Nicot, P. (Eds.) Integrated Pest and Disease Management in Greenhouse Crops. *Plant Pathology in the 21st Century*, (Vol 9. Pp 259-283). Springer, Cham. https://doi.org/10.1007/978-3-030-22304-5_9

Levi, A., Thomas, C. E., Newman, M., Zhang, X., Xu, Y., & Wehner, T. C. (2003b). Massive preferential segregation and nonrandom assortment of linkagegroups produce quasi-linkage in an F2 mapping population of watermelon. *HortScience*, *38*, 782.

Levi, A., Simmons, A. M., Massey, L., Coffey, J., Wechter, W. P., Jarret, R. L., Tadmor, Y., NImmakayala, P., & Reddy, U. K. (2017). Genetic diversity in the desert watermelon Citrullus colocynthis and its relationship with Citrullus species as determined by high-frequency oligonucleotides-targeting active gene markers. *Journal of the American Society for Horticultural Science*, *142*(1), 47-56. https://doi.org/10.21273/JASHS03834-16

Levy, A. & Czosnek, H. (2003) The DNA-B of the non-phloem-limited *Bean dwarf mosaic virus* (BDMV) is able to move the phloem-limited *Abutilon mosaic virus* (AbMV) out of the phloem, but DNA-B of AbMV is unable to confine BDMV to the

phloem. *Plant Molecular Biology*, *53*, 789–803. https://doi.org/10.1023/B:PLAN.0000023662.25756.43

Li, B., & Dewey, C. N. (2011). RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC bioinformatics*, *12*(1), 323. https://doi.org/10.1186/1471-2105-12-323

Li, R., Zheng, Y., Fei, Z., & Ling, K. S. (2015). Complete Genome Sequence of an Emerging Melon Necrotic Spot Virus Isolate Infecting Greenhouse Cucumber in North America. *Genome announcements*, *3*(4), e00775-15. https://doi.org/10.1128/genomeA.00775-15

Li, B., Zhao, Y., Zhu, Q., Zhang, Z., Fan, C., Amanullah, S., Gao, P., & Luan, F. (2017a). Mapping of powdery mildew resistance genes in melon (*Cucumis melo* L.) by bulked segregant analysis. *Scientia Horticulturae*, *220*, 160-167. https://doi.org/10.1016/j.scienta.2017.04.001

Li, X., An, M., Xia, Z., Bai, X., & Wu, Y. (2017b). Transcriptome analysis of watermelon (*Citrullus lanatus*) fruits in response *to Cucumber green mottle mosaic virus* (CGMMV) infection. *Scientific reports*, *7*(1), 1-12. https://doi.org/10.1038/s41598-017-17140-4

Li, F., Wang, Y., & Zhou, X. (2017c). SGS3 cooperates with RDR6 in triggering geminivirus-induced gene silencing and in suppressing geminivirus infection in Nicotiana benthamiana. *Viruses*, *9*(9), 247. https://doi.org/10.3390/v9090247

Li, F., Zhao, N., Li, Z., Xu, X., Wang, Y., Yang, X., Liu, S. S., Wang, A., & Zhou, X. (2017d). A calmodulin-like protein suppresses RNA silencing and promotes geminivirus infection by degrading SGS3 via the autophagy pathway in *Nicotiana benthamiana*. *PLoS pathogens*, *13*(2), e1006213. https://doi.org/10.1371/journal.ppat.1006213

Li, Q., Li, H., Huang, W., Xu, Y., Zhou, Q., Wang, S., Ruan, J., & Zhang, Z. (2019a). A chromosome-scale genome assembly of cucumber (*Cucumis sativus* L.). *GigaScience*, *8*(6), giz072. https://doi.org/10.1093/gigascience/giz072

Liu, L., Sun, T., Liu, X., Guo, Y., Huang, X., Gao, P., & Wang, X. (2019b). Genetic analysis and mapping of a striped rind gene (st3) in melon (*Cucumis melo* L.). *Euphytica*, *215*(2), 20. https://doi.org/10.1007/s10681-019-2353-1

Li, F., Zhang, M., Zhang, C., & Zhou, X. (2020). Nuclear autophagy degrades a geminivirus nuclear protein to restrict viral infection in Solanaceous plants. *New Phytologist*, *225*(4), 1746-1761. https://doi.org/10.1111/nph.16268

Lira, R., Téllez, O., & Dávila, P. (2009). The effects of climate change on the geographic distribution of Mexican wild relatives of domesticated *Cucurbitaceae*. *Genetic Resources and Crop Evolution*, *56*(5), 691-703. https://doi.org/10.1007/s10722-008-9394-y

Loebenstein, G. (2009). Local Lesions and Induced Resistance. *Advances in Virus Research*, 75, 73–117. https://doi.org/10.1016/S0065-3527(09)07503-4

Loebenstein, G. & Katis, N. (2014). Control of plant virus diseases seed-propagated crops. Preface. En: Loebenstein, G. & Katis, N., (Eds.), *Advances in Virus Research*. Academic Press, (Vol. 90, pp xi). https://doi.org/10.1016/B978-0-12-801246-8.09985-6.

López, C., Ferriol, M., & Picó, M. B. (2015) Mechanical transmission of *Tomato leaf curl New Delhi virus* to cucurbit germplasm: selection of tolerance sources in *Cucumis melo*. Euphytica 204:279–691. http:// doi:10.1007/s10681-015-1371-x

Louro, D., Quinot, A., Neto, E., Fernandes, J. E., Marian, D., Vecchiati, M., Caciagli, P., & Vaira, A. M. (2003). Occurrence of Cucumber vein yellowing virus in cucurbitaceous species in southern Portugal. *Plant Pathology, 53*(2), 241. http:// doi:10.1111/j.0032-0862.2004.00996.x

Lovisolo, O., Hull, R., & Rosler, O. (2003). Coevolution of viruses with hosts and vectors and possible paleontology. *Advances in Virus Research*, 62, 325-379. http:// doi:10.1016/s0065-3527(03)62006-3

Lu, H., Romero-Severson, J., & Bernardo, R. (2002). Chromosomal regions associated with segregation distortion in maize. *Theoretical and applied genetics*, *105*(4), 622-628. https://doi.org/10.1007/s00122-002-0970-9

Luan, F., Delannay, I., & Staub, J. E. (2008). Chinese melon (Cucumis melo L.) diversity analyses provide strategies for germplasm curation, genetic improvement, and evidentiary support of domestication patterns. *Euphytica*, *164*(2), 445-461. https://doi.org/10.1007/s10681-008-9699-0

Luigi, M., Bertin, S., Manglli, A., Troiano, E., Davino, S., Tomassoli, L., & Parrella, G. (2019). First report of *Tomato Leaf Curl New Delhi Virus* causing Yellow Leaf Curl of pepper in Europe. *Plant Disease*, *103*(11), 2970-2970. https://doi.org/10.1094/PDIS-06-19-1159-PDN

Luna, A. P., & Lozano-Durán, R. (2020). Geminivirus-Encoded Proteins: Not All Positional Homologs Are Made Equal. *Frontiers in Microbiology*, *11*, 878. https://doi.org/10.3389/fmicb.2020.00878

Luna, A. P., Rodríguez Negrete, E. A., Morilla, G., Castillo-Garriga, A., & Rodriguez-Bejarano, E. (2019). Study of the functional domains of the PTGS suppressor V2 from geminivirus Beet curly top virus (BCTV). Contribuciones a congresos. https://riuma.uma.es/xmlui/bitstream/handle/10630/18133/Poster_MPMI_2019_1172019.pdf?sequence=2&isAllowed=y

Luria, N., Smith, E., Sela, N., Koren, A., Lachman, O., & Dombrovsky, A. (2019). Insights into a watermelon virome contribute to monitoring distribution of whitefly-borne viruses. *Phytobiomes Journal*, *3*(1), 61-70. https://doi.org/10.1094/PBIOMES-07-18-0034-R

Lv, S. Z., Ma, D. H., Huo, Z. R., Shen, W. Y., Li, S. J., & Chen, Z. W. (1994). A new variety Jinchun NO.4 of cucumber with high yield and disease resistance. China Veg 2:1–3, (in Chinese).

Maina, S., Edwards, O. R., de Almeida, L., Ximenes, A., & Jones, R. A. (2017). Analysis of an RNA-seq strand-specific library from an east timorese cucumber sample

reveals a complete cucurbit aphid-borne yellows virus genome. *Genome Announcements*, *5*(19). https://doi.org/10.1128/genomeA.00320-17.

Maine E. M. (2000). A conserved mechanism for post-transcriptional gene silencing?. *Genome biology*, *1*(3), REVIEWS1018. https://doi.org/10.1186/gb-2000-1-3-reviews1018

Maiti, S., Basak, J., Kundagrami, S., Kundu, A., & Pal, A. (2011). Molecular marker-assisted genotyping of *mungbean yellow mosaic India virus* resistant germplasms of mungbean and urdbean. *Molecular biotechnology*, *47*(2), 95-104. https://doi.org/10.1007/s12033-010-9314-1

Maiti, S., Paul, S., & Pal, A. (2012). Isolation, characterization, and structure analysis of a non-TIR-NBS-LRR encoding candidate gene from MYMIV-resistant Vigna mungo. *Molecular biotechnology*, *52*(3), 217-233. https://doi.org/10.1007/s12033-011-9488-1

Malathi, V. G., Renukadevi, P., Chakraborty, S., Biswas, K. K., Roy, A., Sivalingam, P. N., Venkataravanappa V. & Mandal, B. (2017). Begomoviruses and their satellites occurring in India: distribution, diversity and pathogenesis. In *A century of plant virology in India* (pp. 75-177). Springer, Singapore. https://doi.org/10.1007/978-981-10-5672-7_5

Maluf, W. R., Silva, I. S., & Moura, W. M. (1985). Inheritance of watermelon mosaic virus-1 (WMV-1) resistance in squash Cucurbita maxima Duch. *Revista Brasileira de Genética*, *8*(1), 175-182.

Maluf, W. R., Pereira, J. J., & Figueira, A. R. (1997). Inheritance of resistance to the papaya ringspot virus-watermelon strain from two different accessions of winter squash *Cucurbita maxima* Duch. *Euphytica*, *94*(2), 163-168. https://doi.org/10.1023/A:1002998216465

Mandal, B., Mandal, S., Sohrab, S. S., Pun, K. B., & Varma, A. (2004). A new yellow mosaic disease of chayote in India. *Plant pathology*, *53*(6). https://doi.org/10.1111/j.1365-3059.2004.01075.x

Mandal, A., Sarkar, D., Kundu, S., & Kundu, P. (2015). Mechanism of regulation of tomato *TRN1* gene expression in late infection with *tomato leaf curl New Delhi virus* (ToLCNDV). *Plant Science*, *241*, 221-237. https://doi.org/10.1016/j.plantsci.2015.10.008.

Mandal, A., Mishra, A. K., Dulani, P., Muthamilarasan, M., Shweta, S., & Prasad, M. (2018). Identification, characterization, expression profiling, and virus-induced gene silencing of armadillo repeat-containing proteins in tomato suggest their involvement in *tomato leaf curl New Delhi virus* resistance. *Functional & integrative genomics*, *18*(2), 101–111. https://doi.org/10.1007/s10142-017-0578-4

Mandoulakani, B. A., Rahmanpour, S., Shaaf, S., Khoei, S. G., Rastgou, M., & Rafezi, R. (2015). Towards the identification of retrotransposon-based and ISSR molecular markers associated with populations resistant to ZYMV in melon. *South African Journal of Botany*, *100*, 141-147. https://doi.org/10.1016/j.sajb.2015.05.027

Manivannan, K., Renukadevi, P., Malathi, V. G., Karthikeyan, G., & Balakrishnan, N. (2019). A new seed-transmissible begomovirus in bitter gourd (Momordica charantia L.). *Microbial pathogenesis*, *128*, 82-89. https://doi.org/10.1016/j.micpath.2018.12.036

Mansoor, S., Khan, S. H., Hussain, M., Mushtaq, N., Zafar, Y., & Malik, K. A. (2000). Evidence that watermelon leaf curl disease in Pakistan is associated with Tomato leaf curl virus-India, a bipartite begomovirus. *Plant Disease*, *84*(1), 102-102. https://doi.org/10.1094/PDIS.2000.84.1.102A

MAPA (2017 y 2019). Estadísticas agrarias. Superficies y producciones anuales de cultivos:

- https://www.mapa.gob.es/es/estadistica/temas/estadisticas-agrarias/agricultura/superficies-producciones-anuales-cultivos/
- https://www.mapa.gob.es/es/agricultura/temas/producciones-agricolas/frutas-y-hortalizas/Informacion_subsectorial.aspx

Masri, L., Branca, A., Sheppard, A. E., Papkou, A., Laehnemann, D., Guenther, P. S., Prahl, S., Saebelfeld, M., Hollensteiner, J., Liesegang, H., Brzuszkiewicz, E., Daniel, R., Michiels, N. K., Schulte, R. D., Kurtz, J., Rosenstiel, P., Telschow, A., Bornberg-Bauer, E., & Schulenburg, H. (2015). Host-Pathogen Coevolution: The Selective Advantage of Bacillus thuringiensis Virulence and Its Cry Toxin Genes. *PLoS biology*, *13*(6), e1002169. https://doi.org/10.1371/journal.pbio.1002169

Marie-Magdeleine, C., Mahieu, M., & Archimède, H. (2011). Pumpkin (*Cucurbita moschata* Duchesne ex Poir.) Seeds as an Anthelmintic Agent?. En: Preedy, V. R., Watson, R. R., & Patel, V. B., *Nuts and Seeds in Health and Disease Prevention* (pp. 933-939). Academic Press. https://doi.org/10.1016/B978-0-12-375688-6.10110-0

Marone, D., Russo, M. A., Laidò, G., De Leonardis, A. M., & Mastrangelo, A. M. (2013). Plant nucleotide binding site–leucine-rich repeat (NBS-LRR) genes: active guardians in host defense responses. *International journal of molecular sciences*, *14*(4), 7302-7326. https://doi.org/10.3390/ijms14047302

Martin, G. B., Bogdanove, A. J., & Sessa, G. (2003). Understanding the functions of plant disease resistance proteins. *Annual review of plant biology*, *54*(1), 23-61. https://doi.org/10.1146/annurev.arplant.54.031902.135035

Maruthi, M. N., Rekha, A. R., Cork, A., Colvin, J., Alam, S. N., & Kader, K. A. (2005). First report of Tomato leaf curl New Delhi virus infecting tomato in Bangladesh. *Plant disease*, *89*(9), 1011-1011. https://doi.org/10.1094/PD-89-1011C

Maruthi, M.N., Rekha, A.R., & Muniyappa, V. (2007). Pumpkin yellow vein mosaic disease is caused by two distinct begomoviruses: complete viral sequences and comparative transmission by an indigenous *Bemisia tabaci* and the introduced B-biotype. *Eppo Bulletin*, *37*, 412-419. https://doi.org/10.1111/J.1365-2338.2007.01127.X

Mascarell-Creus, A., Cañizares, J., Vilarrasa-Blasi, J., Mora-García, S., Blanca, J., Gonzalez-Ibeas, D., Saladié, M., Roig, C., Deleu, W., Picó-Silvent, B., López-Bigas, N., Aranda, M.A., Garcia-Mas, J., Nuez, F., Puigdomènech, P., & Caño-Delgado, A.I., (2009). An oligo-based microarray offers novel transcriptomic approaches

for the analysis of pathogen resistance and fruit quality traits in melon (*Cucumis melo* L.). *BMC Genomics*, *10*, 467. https://doi.org/10.1186/1471-2164-10-467

Mccreight, James & Kishaba, Albert. (1991). Reaction of Cucurbit Species to Squash Leaf Curl Virus and Sweetpotato Whitefly. *Journal of the American Society for Horticultural Science*, *116*(1), 137-141. https://doi.org/10.21273/JASHS.116.1.137.

McCreight, J. D., Bohn, G. W., & Kishaba, A. N. (1992). Pedigree of PI 414723 melon. *Cucurbit Genetics Cooperative Report*, *15*, 51-52.

McCreight, J. D., Liu, H. Y., & Turini, T. A. (2008). Genetic resistance to Cucurbit leaf crumple virus in melon. *HortScience*, *43*(1), 122-126. https://doi.org/10.21273/HORTSCI.43.1.122

McCreight, J. D., & Wintermantel, W. M. (2011). Genetic resistance in melon PI 313970 to Cucurbit yellow stunting disorder virus. *HortScience*, *46*(12), 1582-1587. https://doi.org/10.21273/HORTSCI.46.12.1582

McCreight, J. D., Wintermantel, W. M., Natwick, E. T., Sinclair, J. W., Crosby, K. M., & Gómez-Guillamón, M. L. (2015). Recessive resistance to CYSDV in melon TGR 1551. In *V International Symposium on Cucurbits 1151* (pp. 101-108). DOI: 10.17660/ActaHortic.2017.1151.17

McCreight, J.D. (2016). Cultivation and Uses of Cucurbits. En: R. Grumet, N. Katzir y J. Garcia-Mas (Eds.), *Genetics and Genomics of Cucurbitaceae. Plant Genetics and Genomics: Crops and Models*, (vol 20, pp. 1-12). España: Springer, Cham. https://doi.org/10.1007/7397_2016_2

McPhail-Medina, R., Wessel-Beaver, L., & Rodrigues, J. C. V. (2012). Inheritance of resistance to papaya ringspot virus in tropical pumpkin is controlled by at least two genes. In *Cucurbitaceae*. Proceedings of the Xth EUCARPIA meeting on genetics and breeding of Cucurbitaceae (eds. Sari, Solmaz and Aras) Antalya (Turkey) (pp. 697-701).

Mejía-Teniente, L., Joaquin-Ramos, A. D. J., Torres-Pacheco, I., Rivera-Bustamante, R. F., Guevara-Olvera, L., Rico-García, E., & Guevara-Gonzalez, R. G. (2015). Silencing of a germin-like protein gene (*CchGLP*) in geminivirus-resistant pepper (*Capsicum chinense* Jacq.) BG-3821 increases susceptibility to single and mixed infections by geminiviruses PHYVV and PepGMV. *Viruses*, *7*(12), 6141-6151. https://doi.org/10.3390/v7122930

Mejía, L., Teni, R. E., Garcia, B. E., Fulladolsa, A. C., Méndez, L., Melgar, S., & Maxwell, D. P. (2010). Preliminary observations on the effectiveness of five introgressions for resistance to begomoviruses in tomatoes. *TGC Report*, *60*, 41-53.

Melgarejo, P., García-Jiménez, J., Jordá, M. C., López, M. M., M., Andrés, F. & Duran-Vila, N. (2010). Patógenos de plantas descritos en España. 2a edición. Ministerio de Medio Ambiente y Medio Rural y Marino. España.

Messelink, G. J., Calvo, F. J., Marín, F., & Janssen, D. (2020). Cucurbits. En; Gullino, M. L., Albajes, R., & Nicot P. C. (Eds.), *Integrated Pest and Disease Management in*

Greenhouse Crops (Vol. 9, pp. 537-566). Springer International Publishing. https://doi.org/10.1007/978-3-030-22304-5_19

Miozzi, L., Napoli, C., Sardo, L., & Accotto, G. P. (2014). Transcriptomics of the interaction between the monopartite phloem-limited geminivirus tomato yellow leaf curl Sardinia virus and Solanum lycopersicum highlights a role for plant hormones, autophagy and plant immune system fine tuning during infection. *PloS one*, *9*(2). https://doi.org/10.1371/journal.pone.0089951

Miras, M., Juárez, M., & Aranda, M. A. (2019). Resistance to the Emerging *Moroccan Watermelon Mosaic Virus* in Squash. *Phytopathology*, *109*(5), 895-903. https://doi.org/10.1094/PHYTO-10-18-0395-R

Mizutani, T., Daryono, B. S., Ikegami, M., & Natsuaki, K. T. (2011). First report of Tomato leaf curl New Delhi virus infecting cucumber in Central Java, Indonesia. *Plant Disease*, *95*(11), 1485-1485. https://doi.org/10.1094/PDIS-03-11-0196

Mnari-Hattab, M., Zammouri, S., Belkadhi, M. S., Doña, D. B., Ben Nahia, E., & Hajlaoui, M. R. (2015). First report of *Tomato leaf curl New Delhi virus* infecting cucurbits in Tunisia. *New Disease Reports*, *31*(21), 2044-0588. https://doi.org/ 10.5197/j.2044-0588.2015.031.021

Moffett, P. (2009). Mechanisms of recognition in dominant *R* gene mediated resistance. *Advances in Virus Research*. *75*, 1–33. https://doi.org/10.1016/S0065-3527(09)07501-0

Moore, B. J., & McGuire, J. M. (1968). Translocation of tobacco ringspot virus in cucumber and zinnia. *Journal of the Arkansas Academy of Science*, *22*(1), 67-71.

Monaghan, J., & Zipfel, C. (2012). Plant pattern recognition receptor complexes at the plasma membrane. *Current opinion in plant biology*, *15*(4), 349-357. https://doi.org/10.1016/j.pbi.2012.05.006

Montero-Pau, J., Esteras, C., Blanca, J., Ziarsolo, P., Cañizares, J., & Picó, B. (2016). Genetics and Genomics of Cucurbita spp. En: Grumet R., Katzir N., Garcia-Mas J. (Eds.), *Genetics and Genomics of Cucurbitaceae. Plant Genetics and Genomics: Crops and Models.* (Vol 20. pp. 211-227). Springer, Cham. https://doi.org/10.1007/7397_2016_7

Montero-Pau, J., Blanca, J., Esteras, C., Martínez-Pérez, E. M., Gómez, P., Monforte, A. J., Cañizares, J., & Picó, B. (2017). An SNP-based saturated genetic map and QTL analysis of fruit-related traits in Zucchini using Genotyping-by-sequencing. *BMC genomics*, *18*(1), 94. https://doi.org/10.1186/s12864-016-3439-y

Montero-Pau, J., Blanca, J., Bombarely, A., Ziarsolo, P., Esteras, C., Martí-Gómez, C., Ferriol, M., Gómez, P., Jamilena, M., Mueller, L., Picó, B., & Cañizares, J. (2018). De novo assembly of the zucchini genome reveals a whole-genome duplication associated with the origin of the Cucurbita genus. *Plant biotechnology journal*, *16*(6), 1161–1171. https://doi.org/10.1111/pbi.12860

Montes-Garcia, C.E., Garza-Ortega, S., & Brown, J.K. (1998). Inheritance of the resistance to squash leaf curl virus in *Cucurbita pepo* L. En: McCreight, J.D.

(Ed.) *Cucurbitaceae '98: Evaluation and Enhancement of Cucurbit Germplasm.* A.S.H.S., Alexandria, Virginia, (pp. 328–330).

Monserrat, A. & Lacasa, A. (2014). Medidas para la prevención y control del virus Nueva Delhi 'ToLCNDV'. *Agrícola Vergel, 371*, pp. 20-25.

Morales, M., Orjeda, G., Nieto, C., Van Leeuwen, H., Monfort, A., Charpentier, M., Caboche, M., Arús, P., Puigdomènech, P., Aranda, M. A., Dogimont, C., Bendahmane, A., & Garcia-Mas, J. (2005). A physical map covering the *nsv* locus that confers resistance to *Melon necrotic spot virus in melon* (*Cucumis melo* L.). *Theoretical and applied genetics*, *111*(5), 914-922. https://doi.org/10.1007/s00122-005-0019-y

Morata, J., Puigdomènech, P. Variability among Cucurbitaceae species (melon, cucumber and watermelon) in a genomic region containing a cluster of NBS-LRR genes. BMC Genomics 18, 138 (2017). https://doi.org/10.1186/s12864-017-3529-5

Moreno, A. B., & López-Moya, J. J. (2020). When viruses play team sports: Mixed infections in plants. *Phytopathology*, *110*(1), 29-48. https://doi.org/10.1094/PHYTO-07-19-0250-FI

Morgil, H., Gercek, Y. C., & Tulum, I. (2020). Single Nucleotide Polymorphisms (SNPs) in Plant Genetics and Breeding. En: Çalışkan, M., Erol, O., & Öz, G.C. *The Recent Topics in Genetic Polymorphisms*. IntechOpen. https://doi.org/10.5772/intechopen.91886.

Moriones, E., Praveen, S., & Chakraborty, S. (2017). Tomato Leaf Curl New Delhi Virus: An Emerging Virus Complex Threatening Vegetable and Fiber Crops. *Viruses*, *9*(10), 264. https://doi.org/10.3390/v9100264

Mourrain, P., Béclin, C., Elmayan, T., Feuerbach, F., Godon, C., Morel, J. B., Jouette, D., Lacombe, A. M., Nikic, S., Picault, N., Rémoué, K., Sanial, M., Vo, T. A., & Vaucheret, H. (2000). Arabidopsis SGS2 and SGS3 genes are required for posttranscriptional gene silencing and natural virus resistance. *Cell*, *101*(5), 533–542. https://doi.org/10.1016/s0092-8674(00)80863-6

Munger, H. M., & Provvidenti, R. (1987). Inheritance of resistance to zucchini yellow mosaic virus in *Cucurbita moschata*. *Cucurbit Genet Coop Rep*, *10*, 80-81.

Munshi, A. D., Panda, B., Mandal, B., Bisht, I. S., Rao, E. S., & Kumar, R. (2008). Genetics of resistance to Cucumber mosaic virus in Cucumis sativus var. hardwickii R. Alef. *Euphytica*, *164*(2), 501-507. https://doi.org/10.1007/s10681-008-9741-2

Mushtaq, R., Shahzad, K., Mansoor, S., Shah, Z. H., Alsamadany, H., Mujtaba, T., Al-Zahrani, Y., Alzahrani, H., Ahmed, Z., & Bashir, A. (2018). Exploration of *cotton leaf curl virus* resistance genes and their screening in *Gossypium arboreum* by targeting resistance gene analogues. *AoB PLANTS*, *10*(6), ply067. https://doi.org/10.1093/aobpla/ply067

Mushtaq, M., Sakina, A., Wani, S. H., Shikari, A. B., Tripathi, P., Zaid, A., Galla, A., Abdelrahman, M., Sharma, M., Singh, A. K., & Salgotra, R. K. (2019). Harnessing Genome Editing Techniques to Engineer Disease Resistance in Plants. *Frontiers in plant science*, *10*, 550. https://doi.org/10.3389/fpls.2019.00550

Mushtaq, R., Shahzad, K., Shah, Z. H., Alsamadany, H., Alzahrani, H., Alzahrani, Y., Mujtaba, T., Ahmed, Z., Mansoor, S., & Bashir, A. (2020). Isolation of biotic stress resistance genes from cotton (*Gossypium arboreum*) and their analysis in model plant tobacco (*Nicotiana tabacum*) for resistance against *cotton leaf curl disease* complex. *Journal of virological methods*, *276*, 113760. https://doi.org/10.1016/j.jviromet.2019.113760

Naegele R.P., Wehner T.C. (2016) Genetic Resources of Cucumber. En: Grumet R., Katzir N., Garcia-Mas J. (Eds.) *Genetics and Genomics of Cucurbitaceae. Plant Genetics and Genomics: Crops and Models*, (Vol 20, pp. 61-86). Springer, Cham. https://doi.org/10.1007/7397_2016_15

Nagendran, K., Kumar, S. M., Manoranjitham, S. K., & Karthikeyan, G. (2014). Molecular detection and characterization of Tomato leaf curl New Delhi virus causing mosaic disease on bitter gourd in Tamil Nadu, India. *Trends in Biosciences*, *7*(23), 3925-3931.

Nagesh, G. C., Rao, E. S., Pitchaimuthu, M., Varalakshmi, B., Lakshmana Reddy, D. C., Samuel, D. K., Rekha, A., & Krishna Reddy, M. (2018). Genetic analysis of resistance to watermelon bud necrosis orthotospovirus in watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai]. *Plant Breeding*, *137*(5), 814-822. https://doi.org/10.1111/pbr.12639

Naqvi, A. R., Haq, Q. M., & Mukherjee, S. K. (2010). MicroRNA profiling of *tomato leaf curl new delhi virus* (ToLCNDV) infected tomato leaves indicates that deregulation of mir159/319 and mir172 might be linked with leaf curl disease. *Virology journal*, *7*(1), 281. https://doi.org/10.1186/1743-422X-7-281

Naqvi, A. R., Choudhury, N. R., Mukherjee, S. K., & Haq, Q. M. R. (2011b). In silico analysis reveals that several tomato microRNA/microRNA∗ sequences exhibit propensity to bind to *tomato leaf curl virus* (ToLCV) associated genomes and most of their encoded open reading frames (ORFs). *Plant physiology and biochemistry*, *49*(1), 13-17. https://doi.org/10.1016/j.plaphy.2010.09.013

Naqvi, R. Z., Zaidi, S. S., Akhtar, K. P., Strickler, S., Woldemariam, M., Mishra, B., Mukhtar, M. S., Scheffler, B. E., Scheffler, J. A., Jander, G., Mueller, L. A., Asif, M., & Mansoor, S. (2017). Transcriptomics reveals multiple resistance mechanisms against *cotton leaf curl disease* in a naturally immune cotton species, *Gossypium arboreum*. *Scientific reports*, 7(1), 15880. https://doi.org/10.1038/s41598-017-15963-9

Naqvi, R. Z., Zaidi, S. S., Mukhtar, M. S., Amin, I., Mishra, B., Strickler, S., Mueller, L. A., Asif, M., & Mansoor, S. (2019). Transcriptomic analysis of cultivated cotton *Gossypium hirsutum* provides insights into host responses upon whitefly-mediated transmission of *cotton leaf curl disease*. PloS one, 14(2), e0210011. https://doi.org/10.1371/journal.pone.0210011

Navas-Castillo, J., Fiallo-Olivé, E., & Sánchez-Campos, S. (2011). Emerging virus diseases transmitted by whiteflies. *Annual review of phytopathology*, *49*, 219-248. https://doi.org/10.1146/annurev-phyto-072910-095235

Nepal, M. P., Andersen, E. J., Neupane, S., & Benson, B. V. (2017). Comparative genomics of non-TNL disease resistance genes from six plant species. *Genes*, *8*(10), 249. https://doi.org/10.3390/genes8100249

Ngwepe, R. M., Mashilo, J., & Shimelis, H. (2019). Progress in genetic improvement of citron watermelon (*Citrullus lanatus* var. *citroides*): a review. *Genetic Resources and Crop Evolution*, *66*(3), 735-758. https://doi.org/10.1007/s10722-018-0724-4

Nieto, C., Morales, M., Orjeda, G., Clepet, C., Monfort, A., Sturbois, B., Puigdomènech, P., Pitrat, M., Caboche, M., Dogimont, C., Garcia-Mas, J., Aranda, M. A., & Bendahmane, A. (2006). An eIF4E allele confers resistance to an uncapped and non-polyadenylated RNA virus in melon. *The Plant journal: for cell and molecular biology*, *48*(3), 452–462. https://doi.org/10.1111/j.1365-313X.2006.02885.x

Nieto, C., Piron, F., Dalmais, M., Marco, C. F., Moriones, E., Gómez-Guillamón, M. L., Truniger, V., Gómez, P., Garcia-Mas, J., Aranda, M. A., & Bendahmane, A. (2007). EcoTILLING for the identification of allelic variants of melon eIF4E, a factor that controls virus susceptibility. *BMC plant biology*, *7*, 34. https://doi.org/10.1186/1471-2229-7-34

Nimmakayala, P., Tomason, Y. R., Abburi, V. L., Alvarado, A., Saminathan, T., Vajja, V. G., Salazar, G., Panicker, G. K., Levi, A., Wechter, W. P., McCreight, J. D., Korol, A. B., Ronin, Y., Garcia-Mas, J., & Reddy, U. K. (2016). Genome-Wide Differentiation of Various Melon Horticultural Groups for Use in GWAS for Fruit Firmness and Construction of a High Resolution Genetic Map. *Frontiers in plant science*, *7*, 1437. https://doi.org/10.3389/fpls.2016.01437

Noris, E., & Catoni, M. (2020). Role of methylation during geminivirus infection. En: Poltronieri, P., & Hong, Y. (Eds.), *Applied Plant Biotechnology for Improving Resistance to Biotic Stress* (pp. 291-305). Academic Press. https://doi.org/10.1016/B978-0-12-816030-5.00013-6

Observatorio de Precios y Mercados. Junta de Andalucía, 2019. https://www.juntadeandalucia.es/agriculturaypesca/observatorio/servlet/FrontController

Okogbenin, E., Egesi, C. N., Olasanmi, B., Ogundapo, O., Kahya, S., Hurtado, P., Marin, J., Akinbo, O., Mba, C., Gomez, H., de Vicente, C., Baiyeri, S., Uguru, M., Ewa, F., & M. Fregene (2012). Molecular marker analysis and validation of resistance to cassava mosaic disease in elite cassava genotypes in Nigeria. *Crop Science*, *52*(6), 2576-2586. https://doi.org/10.2135/cropsci2011.11.0586

Orbach, M. J., Farrall, L., Sweigard, J. A., Chumley, F. G., & Valent, B. (2000). A telomeric avirulence gene determines efficacy for the rice blast resistance gene Pi-ta. *The Plant Cell*, *12*(11), 2019-2032. https://doi.org/10.1105/tpc.12.11.2019

Orfanidou, C. G., Malandraki, I., Beris, D., Kektsidou, O., Vassilakos, N., Varveri, C., Katis N.I., & Maliogka, V. I. (2019). First report of *tomato leaf curl New Delhi virus* in zucchini crops in Greece. *Journal of Plant Pathology*, *101*(3), 799-799. https://doi.org/10.1007/s42161-019-00265-y

Osipowski, P., Pawełkowicz, M., Wojcieszek, M., Skarzyńska, A., Przybecki, Z., & Pląder, W. (2020). A high-quality cucumber genome assembly enhances computational comparative genomics. *Molecular Genetics and Genomics*, *295*(1), 177-193. https://doi.org/10.1007/s00438-019-01614-3

Ott, J., Wang, J., & Leal, S. M. (2015). Genetic linkage analysis in the age of whole-genome sequencing. *Nature Reviews Genetics*, *16*(5), 275-284. https://doi.org/10.1038/nrg3908

Oumouloud, A., El-Otmani, M., Chikh-Rouhou, H., Claver, A. G., Torres, R. G., Perl-Treves, R., & Alvarez, J. M. (2013). Breeding melon for resistance to Fusarium wilt: recent developments. *Euphytica*, *192*(2), 155-169. https://doi.org/10.1007/s10681-013-0904-4

Ozaslan, M., Aytekin, T., Bas, B., Kilic, H., Afacan, I. D., & Dag, D. S. (2006). Virus Diseases of Cucurbits in Gaziantep, Turkey. *Plant Pathology Journal*, *5*(1), 24-27. https://doi.org/10.3923/ppj.2006.24.27

Pachner, M., & Lelley, T. (2004). Different genes for resistance to *zucchini yellow mosaic virus* (ZYMV) in *Cucurbita moschata*. In *Progress in cucurbit genetics and breeding research. Proceedings of Cucurbitaceae 2004*, Olomouc (Czech Republic) Palacky University (pp. 237-243).

Pachner, M., Paris, H. S., & Lelley, T. (2011). Genes for resistance to zucchini yellow mosaic in tropical pumpkin. *Journal of Heredity*, *102*(3), 330-335. https://doi.org/10.1093/jhered/esr006

Pachner, M., Paris, H. S., Winkler, J., & Lelley, T. (2015). Phenotypic and marker-assisted pyramiding of genes for resistance to zucchini yellow mosaic virus in oilseed pumpkin (Cucurbita pepo). *Plant Breeding*, *134*(1), 121-128. https://doi.org/10.1111/pbr.12219

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1995). Tomato leaf curl geminivirus from India has a bipartite genome and coat protein is not essential for infectivity. *Journal of General Virology*, *76*(1), 25-35. https://doi.org/10.1099/0022-1317-76-1-25

Padidam, M., Beachy, R. N., & Fauquet, C. M. (1996). The role of AV2 ("precoat") and coat protein in viral replication and movement in tomato leaf curl geminivirus. *Virology*, *224*(2), 390-404. https://doi.org/10.1006/viro.1996.0546

Palloix, A., Ayme, V., & Moury, B. (2009). Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. *New Phytologist*, *183*(1), 190-199. https://doi.org/10.1111/j.1469-8137.2009.02827.x

Palomares-Ríus, F. J., Viruel, M. A., Yuste-Lisbona, F. J., López-Sesé, A. I., & Gómez-Guillamón, M. L. (2011). Simple sequence repeat markers linked to QTL for resistance to Watermelon mosaic virus in melon. *Theoretical and applied genetics*, *123*(7), 1207. https://doi.org/10.1007/s00122-011-1660-2

Palomares-Ríus, F. J., Garcés-Claver, A., & Gómez-Guillamón, M. L. (2016). Detection of two QTLs associated with resistance to cucurbit yellow stunting disorder virus in

Melon Line TGR 1551. In *XIth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae* (pp. 334-337).

Pan, Y., Wen, C., Han, Y., Wang, Y., Li, Y., Li, S., Cheng, X., & Weng, Y. (2020). QTL for horticulturally important traits associated with pleiotropic andromonoecy and carpel number loci, and a paracentric inversion in cucumber. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik*, *133*(7), 2271–2290. https://doi.org/10.1007/s00122-020-03596-y

Pandey, S., Muthamilarasan, M., Sharma, N., Chaudhry, V., Dulani, P., Shweta, S., Jha, S., Mathur, S., & Prasad, M. (2019). Characterization of DEAD-box family of RNA helicases in tomato provides insights into their roles in biotic and abiotic stresses. *Environmental and Experimental Botany*, *158*, 107-116. https://doi.org/10.1016/j.envexpbot.2018.11.018

Pandey, S., Prasad, A., Sharma, N., & Prasad, M. (2020). Linking the plant stress responses with RNA helicases. *Plant Science*, *299*, 110607. https://doi.org/10.1016/j.plantsci.2020.110607.

Panno, S., Iacono, G., Davino, M., Marchione, S., Zappardo, V., Bella, P., Tomassoli, L., Accotto, G.P. & Davino, S. (2016). First report of Tomato leaf curl New Delhi virus affecting zucchini squash in an important horticultural area of southern Italy. *New Disease Reports*, *33*(6), 2044-0588. http://dx.doi.org/10.5197/j.2044-0588.2016.033.006

Panno, S., Caruso, A. G., Troiano, E., Luigi, M., Manglli, A., Vatrano, T., Iacono, G., Marchione, S., Bertin, S., Tomassoli, L., Parrella, G. and Davino, S. (2019). Emergence of tomato leaf curl New Delhi virus in Italy: estimation of incidence and genetic diversity. *Plant Pathology*, 6*8*, 601-608. http://dx.doi.org/10.1111/ppa.12978

Paris, H. S., Cohen, S., Burger, Y., & Yoseph, R. (1988). Single-gene resistance to zucchini yellow mosaic virus in Cucurbita moschata. *Euphytica*, *37*(1), 27-29.

Paris, H.S. 2008. Summer Squash. En: Prohens-Tomás, J., Nuez, F., Carena M. J., (Eds.), *Vegetables I. Handbook of Plant Breeding*. (Vol I, p. 351-379). Springer, New York. https://doi.org/10.1007/978-0-387-30443-4_11

Paris, H. S. (2015). Origin and emergence of the sweet dessert watermelon, *Citrullus lanatus*. *Annals of Botany*, *116*(2), 133-148. https://doi.org/10.1093/aob/mcv077

Paris, H. S. (2016a). Genetic Resources of Pumpkins and Squash, *Cucurbita* spp. En: Grumet R., Katzir, N., & Garcia-Mas, J., (Eds.), *Genetics and genomics of Cucurbitaceae* (Vol. 20, 111-154). Springer, Cham. https://doi.org/10.1007/7397_2016_3

Paris, H. S. (2016b). Germplasm enhancement of *Cucurbita pepo* (pumpkin, squash, gourd: *Cucurbitaceae*): progress and challenges. *Euphytica*, *208*(3), 415-438. https://doi.org/10.1007/s10681-015-1605-y

Park, G., Kim, J., Jin, B., Yang, H. B., Park, S. W., Kang, S. C., Chung, S. M., & Park, Y. (2018). Genome-wide sequence variation in watermelon inbred Lines and its implication for marker-assisted breeding. *Horticultural Science and Technology*, *36*(2), 280-291. https://doi.org/10.12972/kjhst.20180028

Parrella, G., Troiano, E., Formisano, G., Accotto, G. P., & Giorgini, M. (2018). First report of *tomato leaf curl New Delhi virus* associated with severe mosaic of pumpkin in Italy. *Plant Disease*, *102*(2), 459. https://doi.org/10.1094/PDIS-07-17-0940-PDN

Parrella, G., Troiano, E., Lee, S., & Kil, E. J. (2020). *Tomato Leaf Curl New Delhi Virus* Found Associated with Eggplant Yellowing Disease in Italy. *Plant Disease*, *104*(7), 2034. https://doi.org/10.1094/PDIS-12-19-2635-PDN

Pascual, L., Yan, J., Pujol, M., Monforte, A. J., Picó, B., & Martín-Hernández, A. M. (2019). *CmVPS41* Is a General Gatekeeper for Resistance to *Cucumber Mosaic Virus* Phloem Entry in Melon. *Frontiers in plant science*, *10*, 1219. https://doi.org/10.3389/fpls.2019.01219

Pereira, L., Ruggieri, V., Pérez, S., Alexiou, K. G., Fernández, M., Jahrmann, T., Pujol, M. & Garcia-Mas J. (2018). QTL mapping of melon fruit quality traits using a high-density GBS-based genetic map. *BMC Plant Biol. 18*(324). https://doi.org/10.1186/s12870-018-1537-5.

Pérez-de-Castro, A., Esteras, C., Alfaro-Fernández, A., Daròs, J. A., Monforte, A. J., Picó, B., & Gómez-Guillamón, M. L. (2019). Fine mapping of wmv 1551, a resistance gene to Watermelon mosaic virus in melon. *Molecular Breeding*, *39*(7), 93. https://doi.org/10.1007/s11032-019-0998-z

Pérez-de-Castro, A., Esteras, C., Alfaro-Fernández, A., Daròs, J. A., Monforte, A. J., Picó, B., & Gómez-Guillamón, M. L. (2019). Fine mapping of *wmv 1551*, a resistance gene to *Watermelon mosaic virus* in melon. *Molecular Breeding*, *39*(7), 93. https://doi.org/10.1007/s11032-019-0998-z

Pérez-Padilla, V., Fortes, I. M., Romero-Rodríguez, B., Arroyo-Mateos, M., Castillo, A. G., Moyano, C., De León, L., & Moriones, E. (2020). Revisiting Seed Transmission of the Type Strain of *Tomato yellow leaf curl virus* in Tomato Plants. *Phytopathology*, *110*(1), 121–129. https://doi.org/10.1094/PHYTO-07-19-0232-FI

Perkel, J. (2008). SNP genotyping: six technologies that keyed a revolution. *Nature Methods*, *5*(5), 447-453. https://doi.org/10.1038/nmeth0508-447

Perotto, M. C., Pozzi, E. A., Celli, M. G., Luciani, C. E., Mitidieri, M. S., & Conci, V. C. (2018). Identification and characterization of a new potyvirus infecting cucurbits. *Archives of virology*, *163*(3), 719-724. https://doi.org/10.1007/s00705-017-3660-2

Perpiñá, G., Esteras, C., Gibon, Y., Monforte, A. J., & Picò, B. (2016). A new genomic library of melon introgression lines in a cantaloupe genetic background for dissecting desirable agronomical traits. *BMC Plant Biology*, *16*(1), 164. https://doi.org/10.1186/s12870-016-0842-0

Phaneendra, C., Rao, K. R. S. S., Jain, R. K., & Mandal, B. (2012). *Tomato leaf curl New Delhi virus* is associated with pumpkin leaf curl: a new disease in northern India. *Indian Journal of Virology*, *23*(1), 42-45. https://doi.org/10.1007/s13337-011-0054-z

Picó, B., Villar, C., Nuez, F. (2003). Screening Cucumis sativus landraces for resistance to cucumber vein yellowing virus. *Plant Breed.* 122, 426–430. https://doi.org/10.1046/j.1439-0523.2003.00882.x

Piedra-Aguilera, Á., Jiao, C., Luna, A. P., Villanueva, F., Dabad, M., Esteve-Codina, A., Díaz-Pendón, J. A., Fei, Z., Bejarano, E. R., & Castillo, A. G. (2019). Integrated single-base resolution maps of transcriptome, sRNAome and methylome of *Tomato yellow leaf curl virus* (TYLCV) in tomato. *Scientific reports*, *9*(1), 2863. https://doi.org/10.1038/s41598-019-39239-6

Pitrat, M., & Lecoq, H. (1980). Inheritance of resistance to cucumber mosaic virus transmission by *Aphis gossypii* in *Cucumis melo*. *Phytopathology*, *70*(10), 958-961. https://doi.org/10.1094/Phyto-70-958

Pitrat, M., Lecoq, H., & Risser, G. (1982). *Vat* and *Fn*, two linked genes in muskmelon. *Cucurbit Genetics Coop. Rpt*, *5*, 29-30.

Pitrat, M., & Lecoq, H. (1984). Inheritance of *zucchini yellow mosaic virus* resistance in *Cucumis melo* L. *Euphytica*, *33*(1), 57-61. https://doi.org/10.1007/BF00022750

Pitrat, M. (2008). Melon. En: Prohens J., Nuez F. (Eds.), *Vegetables I. Handbook of Plant Breeding*, (Vol 1. Pp. 283-315). Springer, New York, NY. https://doi.org/10.1007/978-0-387-30443-4_9

Pitrat, M. (2012a). Vegetable crops in the Mediterranean Basin with an overview of virus resistance. In: Loebenstein, G. & Lecoq, H. (Eds.) *Advances in virus research, Viruses and Virus Diseases of Vegetables in the Mediterranean Basin*, (Vol. 84, pp. 1-29). Academic Press. https://doi.org/10.1016/B978-0-12-394314-9.00001-4.

Pitrat, M., Wipf-Scheibel, C., Besombes, D., Desbiez, C., & Lecoq, H. (2012b). Resistance of melon to *Cucumber Vein Yellowing Virus* (CVYV). *Cucurbitaceae 2012, Proceedings of the Xth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae* (eds. Sari, Solmaz, Aras) Antalya (Turkey) pp 157–164.

Pitrat M. (2016a) Disease Resistance in Melon and Its Modification by Molecular Breeding Techniques. En: Ezura H., Ariizumi T., Garcia-Mas J., Rose J. (Eds.) *Functional Genomics and Biotechnology in Solanaceae and Cucurbitaceae Crops. Biotechnology in Agriculture and Forestry*, (Vol 70, pp. 175-197). Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-662-48535-4_11

Pitrat, M. (2016b). Melon genetic resources: phenotypic diversity and horticultural taxonomy. En: Grumet R., Katzir, N., & Garcia-Mas, J., (Eds.), *Genetics and genomics of Cucurbitaceae* (Vol. 20, pp. 25-59). Cham, Switzerland: Springer Nature. https://doi.org/10.1007/7397_2016_10

Poland, J. A., & Rife, T. W. (2012). Genotyping-by-sequencing for plant breeding and genetics. *The Plant Genome*, *5*(3), 92-102. https://doi.org/10.3835/plantgenome2012.05.0005

Pozzi, E., Perotto, M. C., Bertin, S., Manglli, A., Luciani, C., Conci, V. C., & Tomassoli, L. (2020). Molecular variability of watermelon mosaic virus isolates from

Argentina. *European Journal of Plant Pathology*, *156*, 1-9. https://doi.org/10.1007/s10658-020-01965-3

Praveen, S., Singh, P., Sharma, P. K. and Mishra, A. K. Cloning and Sequencing of AC4 from Jatropha begomovirus. Unpublished JOURNAL Submitted to NCBI (01-MAY-2008).

Pradhan, B., Naqvi, A. R., Saraf, S., Mukherjee, S. K., & Dey, N. (2015). Prediction and characterization of *Tomato leaf curl New Delhi virus* (ToLCNDV) responsive novel microRNAs in Solanum lycopersicum. *Virus research*, *195*, 183-195. https://doi.org/10.1016/j.virusres.2014.09.001

Pradhan, B., Van Tien, V., Dey, N., & Mukherjee, S. K. (2017). Molecular Biology of Geminivirus DNA Replication. *Avid Science*, https://avidscience.com/book/viral-replication/

Prasanna, H. C., Sinha, D. P., Rai, G. K., Krishna, R., Kashyap, S. P., Singh, N. K., Singh, M., & Malathi, V. G. (2015). Pyramiding T y-2 and T y-3 genes for resistance to monopartite and bipartite tomato leaf curl viruses of I ndia. *Plant pathology*, *64*(2), 256-264. https://doi.org/10.1111/ppa.12267

Pratap, D., Kashikar, A. R., & Mukherjee, S. K. (2011). Molecular characterization and infectivity of a Tomato leaf curl New Delhi virus variant associated with newly emerging yellow mosaic disease of eggplant in India. *Virology journal*, *8*(1), 305. http://www.virologyj.com/content/8/1/305 https://doi.org/10.1186/1743-422X-8-305

Provvidenti, R. (1982). Sources of resistance and tolerance to viruses in accessions of Cucurbita maxima. *Cucurbit Genetenic Cooperative Reports*, *5*, 46-47.

Provvidenti, R. (1986a). Viral diseases of cucurbits and sources of resistance. ASPAC, Food & Fertilizer Technol. Center, Tech. *Bulletin*, *93*, 1-16.

Provvidenti, R. (1986b). Reactions of accessions of *Citrullus colocynthis* from Nigéria to *Zucchini yellow mosaic virus* and other cucurbit viruses. *Cucurbit Genetic Cooperative Report*, *9*, 82-83.

Provvidenti, R. (1987). Inheritance of resistance to a strain of *zucchini yellow mosiac virus* in cucumber. *HortScience*, *22*(1), 102-103.

Provvidenti, R. (1991). Inheritance of resistance to the Florida strain of zucchini yellow mosaic virus in watermelon. *HortScience*, *26*(4), 407-408. https://doi.org/10.21273/HORTSCI.26.4.407

Pujol, M., Alexiou, K. G., Fontaine, A. S., Mayor, P., Miras, M., Jahrmann, T., Garcia-Mas, J., & Aranda, M. A. (2019). Mapping Cucumber Vein Yellowing Virus Resistance in Cucumber (*Cucumis sativus* L.) by Using BSA-seq Analysis. *Frontiers in plant science*, *10*, 1583. https://doi.org/10.3389/fpls.2019.01583

Qi, J., Liu, X., Shen, D., Miao, H., Xie, B., Li, X., Zeng, P., Wang, S., Shang, Y., Gu, X., Du, Y., Li, Y., Lin, T., Yuan, J., Yang, X., Chen, J., Chen, H., Xiong, X., Huang, K., Fei, Z., Mao, L., Tian, L., Städler, T., Renner, S. S., Kamoun, S., Lucas, W. J., Zhang, Z & Huang, S. (2013). A genomic variation map provides insights into the

genetic basis of cucumber domestication and diversity. *Nature genetics*, *45*(12), 1510–1515. https://doi.org/10.1038/ng.2801

Rabbi, I. Y., Hamblin, M. T., Kumar, P. L., Gedil, M. A., Ikpan, A. S., Jannink, J. L., & Kulakow, P. A. (2014). High-resolution mapping of resistance to cassava mosaic geminiviruses in cassava using genotyping-by-sequencing and its implications for breeding. *Virus research*, *186*, 87-96. https://doi.org/10.1016/j.virusres.2013.12.028

Rahman, M., Ali, A.; Qaiser khan, A., Abbas, A., Rahmat, Z., Sarfraz, Z., Khalid, A., Gul, M., Munir, A., Atifiqbal, M., Scheffler, J., Scheffler, B., (2019)Use of genetic and genomic approaches for combating cotton leaf curl disease complex in Pakistan. CottonGen, https://www.cottongen.org/node/1287525

Rai, N. K., Sahu, P. P., Gupta, S., Reddy, M. K., Ravishankar, K. V., Singh, M., Sadashiva, A. T., & Prasad, M. (2013). Identification and validation of an ISSR marker linked to *Tomato leaf curl New Delhi virus* resistant gene in a core set of tomato accessions. *Vegetable Science*, *40*(1), 1-6.

Raj, S. K., Khan, M. S., Snehi, S. K., & Roy, R. K. (2007). Yellow vein netting of Bimili jute (*Hibiscus cannabinus* L.) in India caused by a strain of *Tomato leaf curl New Delhi virus* containing DNA β. *Australasian Plant Disease Notes*, *2*(1), 45-47. https://doi.org/10.1071/DN07021

Raj, S. K., Snehi, S. K., Khan, M. S., Singh, R., & Khan, A. A. (2008). Molecular evidence for association of *Tomato leaf curl New Delhi virus* with leaf curl disease of papaya (*Carica papaya* L.) in India. *Australasian Plant Disease Notes*, *3*(1), 152-155. https://doi.org/10.1007/BF03211275

Rajeshwari, R., & Reddy, M. K. (2014). Biological Characterisation of *Tomato Leaf Curl New Delhi Virus* Infecting Bottle Gourd (*Lagenaria siceraria*) from Karnataka. *EDITORIAL COMMITTEE*, *48*(3), 387-393.

Ram, C., Berwal, M. K., Gora, J. S., Kumar, R., Haldhar, S. M., Gurjar, K., & Singh, D. (2019). Genomic and biotechnological interventions for crop improvement in cucurbitaceous crops: A review. *Journal of Agriculture and Ecology*, *7*, 1-15. http://krishi.icar.gov.in/jspui/handle/123456789/24049

Ramesh, S. V., Sahu, P. P., Prasad, M., Praveen, S., & Pappu, H. R. (2017). Geminiviruses and Plant Hosts: A Closer Examination of the Molecular Arms Race. *Viruses*, *9*(9), 256. https://doi.org/10.3390/v9090256.

Rao, G. P., & Reddy, M. G. (2020). Overview of yield losses due to plant viruses. En: Awasthi, L.P. (Ed.), *Applied Plant Virology* (Chapter 38, pp. 531-562). Academic Press. https://doi.org/10.1016/B978-0-12-818654-1.00038-4

Reingold, V., Lachman, O., Sela, N., Luria, N., & Dombrovsky, A. (2016). Watermelon fruit rot disease in Israel is caused by a distinct Squash vein yellowing virus (SqVYV) strain. *Plant Disease*, *100*(6), 1176-1183. https://doi.org/10.1094/PDIS-09-15-1040-RE

Renner, S. S., Chomicki, G., & Greuter, W. (2014). Proposal to conserve the name Momordica lanata (*Citrullus lanatus*)(watermelon, *Cucurbitaceae*), with a

conserved type, against *Citrullus battich*. *Taxon*, *63*(4), 941-942. https://doi.org/10.12705/634.29

Reyes, M. I., Flores-Vergara, M. A., Guerra-Peraza, O., Rajabu, C., Desai, J., Hiromoto-Ruiz, Y. H., Ndunguru, J., Hanley-Bowdoin, L., Kjemtrup, S., Ascencio-Ibáñez, J. T., & Robertson, D. (2017). A VIGS screen identifies immunity in the Arabidopsis Pla-1 accession to viruses in two different genera of the Geminiviridae. *The Plant journal: for cell and molecular biology*, *92*(5), 796–807. https://doi.org/10.1111/tpj.13716

Rhee, S. J., Kwon, T., Seo, M., Jang, Y. J., Sim, T. Y., Cho, S., Han, S. W., & Lee, G. P. (2017). De novo-based transcriptome profiling of male-sterile and fertile watermelon lines. *PloS one*, *12*(11), e0187147. https://doi.org/10.1371/journal.pone.0187147

Risser G., Pitrat M., Lecoq H., Rode J. C. (1981). Varietal susceptibility of melon to muskmelon yellow stunt virus (MYSV) and to its transmission by *Aphis gossypii*. Inheritance of the wilting reaction. *Agronomie* 1 835–838. https://doi.org/10.1051/agro:19811002

Riyaz, S.U., Haranivasan, G., Rajamuthuramalingam, M.T., Immunael Jesse, D.M. & Kathiravan, K. First report on *Tomato leaf curl New Delhi virus* infecting *Catharanthus roseus* in Tamil Nadu-India. JOURNAL Unpublished. Submit to NCBI 2011.

Robinson, R. W., Weeden, N. F., & Provvidenti, R. (1988). Inheritance of resistance to zucchini yellow mosaic virus in the interspecific cross Cucurbita maxima× C. ecuadorensis. *Report, Cucurbit Genetics Cooperative, USA*, (11), 74-75.

Rodríguez, E., Téllez, M. M., & Janssen, D. (2019). Whitefly Control Strategies against *Tomato Leaf Curl New Delhi Virus* in Greenhouse Zucchini. *International journal of environmental research and public health*, *16*(15), 2673. https://doi.org/10.3390/ijerph16152673

Rojas, M. R., Macedo, M. A., Maliano, M. R., Soto-Aguilar, M., Souza, J. O., Briddon, R. W., Kenyon, L., Rivera Bustamante, R. F., Murilo Zerbini, F., Adkins, S., Legg, J. P., Kvarnheden, A., Wintermantel W. M., Sudarshana M.R., Peterschmitt, M., Lapidot, M., Martin, D. P., Moriones, E., Inoue-Nagata, A.K., & Gilberson, R. L. (2018). World management of geminiviruses. *Annual review of phytopathology*, *56*, 637-677. https://doi.org/10.1146/annurev-phyto-080615-100327

Román, B., Gómez, P., Picó, B., López, C., & Janssen, D. (2019). Candidate gene analysis of *Tomato leaf curl New Delhi virus* resistance in *Cucumis melo*. *Scientia Horticulturae*, *243*, 12-20. https://doi.org/10.1016/j.scienta.2018.07.005

Román, B., Gomez, P., Pico, B., Die, J.V. (2020). The NBS-LRR Gene Class is a Small Family in *Cucurbita pepo*. *Preprints*, 2020010048. https://doi.org/10.20944/preprints202001.0048.v1.

Romay, G., Pitrat, M., Lecoq, H., Wipf-Scheibel, C., Millot, P., Girardot, G., & Desbiez, C. (2019). Resistance against melon chlorotic mosaic virus and *tomato leaf curl New*

Delhi virus in melon. *Plant disease*, *103*(11), 2913-2919. https://doi.org/10.1094/PDIS-02-19-0298-RE

Roossinck, M. J., Martin, D. P., & Roumagnac, P. (2015). Plant virus metagenomics: advances in virus discovery. *Phytopathology*, *105*(6), 716-727. https://doi.org/10.1094/PHYTO-12-14-0356-RVW

Rosas-Díaz, T., Macho, A. P., Beuzón, C. R., Lozano-Durán, R., & Bejarano, E. R. (2016). The C2 Protein from the Geminivirus Tomato Yellow Leaf Curl Sardinia Virus Decreases Sensitivity to Jasmonates and Suppresses Jasmonate-Mediated Defences. *Plants (Basel, Switzerland)*, *5*(1), 8. https://doi.org/10.3390/plants5010008

Rosas-Díaz, T., Zhang, D., & Lozano-Durán, R. (2017). No evidence of seed transmissibility of Tomato yellow leaf curl virus in Nicotiana benthamiana. *Journal of Zhejiang University. Science. B*, *18*(5), 437–440. https://doi.org/10.1631/jzus.B1600463

Rose, L. E., Michelmore, R. W., & Langley, C. H. (2007). Natural variation in the Pto disease resistance gene within species of wild tomato (*Lycopersicon*). II. Population genetics of Pto. *Genetics*, *175*(3), 1307-1319. https://doi.org/10.1534/genetics.106.063602

Rosen, R., Kanakala, S., Kliot, A., Cathrin Pakkianathan, B., Farich, B. A., Santana-Magal, N., Elimelech, M., Kontsedalov, S., Lebedev, G., Cilia, M., & Ghanim, M. (2015). Persistent, circulative transmission of begomoviruses by whitefly vectors. *Current opinion in virology*, *15*, 1–8. https://doi.org/10.1016/j.coviro.2015.06.008

Roy, A., Spoorthi, P., Panwar, G., Bag, M. K., Prasad, T. V., Kumar, G., Gangopadhyay, K. K., & Dutta, M. (2013). Molecular Evidence for Occurrence of Tomato leaf curl New Delhi virus in Ash Gourd (Benincasa hispida) Germplasm Showing a Severe Yellow Stunt Disease in India. *Indian journal of virology: an official organ of Indian Virological Society*, *24*(1), 74–77. https://doi.org/10.1007/s13337-012-0115-y

Ruíz, M. L., Simón, A., Velasco, L., García, M. C., & Janssen, D. (2015). First report of *Tomato leaf curl New Delhi virus* infecting tomato in Spain. *Plant Disease*, *99*(6), 894-894. https://doi.org/10.1094/PDIS-10-14-1072-PDN

Ruíz, L., Simon, A., Velasco, L., & Janssen, D. (2017). Biological characterization of *Tomato leaf curl New Delhi virus* from Spain. *Plant Pathology*, *66*(3), 376-382. https://doi.org/10.1111/ppa.12587

Rybicki, E. P. (2015). A Top Ten list for economically important plant viruses. *Archives of virology*, *160*(1), 17-20. https://doi.org/10.1007/s00705-014-2295-9

Sade, D., Eybishtz, A., Gorovits, R., Sobol, I., & Czosnek, H. (2012). A developmentally regulated lipocalin-like gene is overexpressed in Tomato yellow leaf curl virus-resistant tomato plants upon virus inoculation, and its silencing abolishes resistance. *Plant molecular biology*, *80*(3), 273–287. https://doi.org/10.1007/s11103-012-9946-6

Sagar, V., Karmakar, P., Devi, J., Gupta, N., & Meena, B. R. (2020). Begomovirus Menace and Its Management in Vegetable Crops. En: Rakshit A., Singh H., Singh A.,

Singh U., Fraceto L. (Eds.), *Frontiers in Stress Management for Durable Agriculture* (pp. 493-521). Springer, Singapore. https://doi.org/10.1007/978-981-15-1322-0_26

Saha, D., Rana, R. S., Sureja, A. K., Verma, M., Arya, L., & Munshi, A. D. (2013). Cloning and characterization of NBS-LRR encoding resistance gene candidates from *Tomato Leaf Curl New Delhi Virus* resistant genotype of *Luffa cylindrica* Roem. *Physiological and molecular plant pathology*, *81*, 107-117. https://doi.org/10.1016/j.pmpp.2012.11.007

Saha, A., Saha, B., & Saha, D. (2014). Molecular detection and partial characterization of a begomovirus causing leaf curl disease of potato in sub-Himalayan West Bengal, India. *Journal of environmental biology*, *35*(3), 601.

Sahu, P. P., Rai, N. K., Chakraborty, S., Singh, M., Chandrappa, P. H., Ramesh, B., Chattopadhyay, D., & Prasad, M. (2010). Tomato cultivar tolerant to *Tomato leaf curl New Delhi virus* infection induces virus-specific short interfering RNA accumulation and defence-associated host gene expression. *Molecular plant pathology*, *11*(4), 531–544. https://doi.org/10.1111/j.1364-3703.2010.00630.x

Sahu, P. P., Sharma, N., Puranik, S., Muthamilarasan, M., & Prasad, M. (2014a). Involvement of host regulatory pathways during geminivirus infection: a novel platform for generating durable resistance. *Functional & integrative genomics*, *14*(1), 47-58. https://doi.org/10.1007/s10142-013-0346-z

Sahu, P. P., Sharma, N., Puranik, S., & Prasad, M. (2014b). Post-transcriptional and epigenetic arms of RNA silencing: a defense machinery of naturally tolerant tomato plant against *Tomato leaf curl New Delhi virus*. *Plant molecular biology reporter*, *32*(5), 1015-1029. https://doi.org/10.1007/s11105-014-0708-2

Sahu, P. P., Sharma, N., Puranik, S., Chakraborty, S., & Prasad, M. (2016). Tomato 26S Proteasome subunit RPT4a regulates ToLCNDV transcription and activates hypersensitive response in tomato. *Scientific reports*, *6*(1), 1-12. https://doi.org/10.1038/srep27078

Sai, C. B., Nagarajan, P., Raveendran, M., Rabindran, R., & Senthil, N. (2017). Understanding the inheritance of *mungbean yellow mosaic virus* (MYMV) resistance in mungbean (*Vigna radiata* L. Wilczek). *Molecular breeding*, *37*(5), 1-15. https://doi.org/10.1007/s11032-017-0650-8

Saïdou, A. A., Thuillet, A. C., Couderc, M., Mariac, C., & Vigouroux, Y. (2014). Association studies including genotype by environment interactions: prospects and limits. *BMC genetics*, *15*(1), 3. https://doi.org/10.1186/1471-2156-15-3

Saliba-Colombani, V., Causse, M., Gervais, L., & Philouze, J. (2000). Efficiency of RFLP, RAPD, and AFLP markers for the construction of an intraspecific map of the tomato genome. *Genome*, *43*(1), 29–40. https://doi.org/10.1139/g99-096

Samretwanich, K., Chiemsombat, P., Kittipakorn, K., & Ikegami, M. (2000). Tomato leaf curl geminivirus associated with cucumber yellow leaf disease in Thailand. *Journal of Phytopathology*, *148*(11/12), 615-617. https://doi.org/10.1023/A:1008962402329

Sánchez-Durán, M. A., Dallas, M. B., Ascencio-Ibañez, J. T., Reyes, M. I., Arroyo-Mateos, M., Ruiz-Albert, J., Hanley-Bowdoin, L., & Bejarano, E. R. (2011). Interaction between geminivirus replication protein and the SUMO-conjugating enzyme is required for viral infection. *Journal of virology*, *85*(19), 9789–9800. https://doi.org/10.1128/JVI.02566-10

Sangeetha, B., Malathi, V. G., Alice, D., Suganthy, M., & Renukadevi, P. (2018). A distinct seed-transmissible strain of tomato leaf curl New Delhi virus infecting Chayote in India. *Virus research*, *258*, 81-91. https://doi.org/10.1016/j.virusres.2018.10.009

Santos, A. A., Lopes, K. V., Apfata, J. A., & Fontes, E. P. (2010). NSP-interacting kinase, NIK: a transducer of plant defence signalling. *Journal of Experimental Botany*, *61*(14), 3839-3845. https://doi.org/10.1093/jxb/erq219

Sarowar, S., Kim, Y. J., Kim, K. D., Hwang, B. K., Ok, S. H., & Shin, J. S. (2009). Overexpression of lipid transfer protein (LTP) genes enhances resistance to plant pathogens and LTP functions in long-distance systemic signaling in tobacco. *Plant cell reports*, *28*(3), 419-427. https://doi.org/10.1007/s00299-008-0653-3

Sayed, S. S., Sajjad, K., Anupam, V., Adel, M. A., Adeel, G. C., Ghazi, A. D., et al. (2013). Characterization of tomato leaf curl New Delhi virus infecting cucurbits: evidence for sap transmission in a host specific manner. Afr. J. Biotechnol. 12, 5000–5009. https://doi.org/doi: 10.5897/AJB2013.12012

Schmitt-Keichinger, C. (2019). Manipulating cellular factors to combat viruses: a case study from the plant eukaryotic translation initiation factors eIF4. *Frontiers in Microbiology*, *10*, 17. https://doi.org/10.3389/fmicb.2019.00017

Scott, J. W., Hutton, S. F., & Freeman, J. H. (2015). Fla. 8638B and Fla. 8624 tomato breeding lines with begomovirus resistance genes *ty-5* plus *Ty-6* and *Ty-6*, respectively. *HortScience*, *50*(9), 1405-1407. https://doi.org/10.21273/HORTSCI.50.9.1405

Scussel, S., Claverie, S., Hoareau, M., Simiand, C., Reynaud, B., Moustache, R., Lefeuvre, P., Delatte, H. & Lett, J. M. (2018). First report of *Tomato leaf curl New Delhi virus* and the whitefly *Bemisia tabaci* Asia1 species on tomato in the Seychelles. https://doi.org/10.5197/j.2044-0588.2018.038.002

Sebastian, P., Schaefer, H., Telford, I. R., & Renner, S. S. (2010). Cucumber (*Cucumis sativus*) and melon (*C. melo*) have numerous wild relatives in Asia and Australia, and the sister species of melon is from Australia. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(32), 14269–14273. https://doi.org/10.1073/pnas.1005338107

Sevik, M. A. (2017). The Infection Process and Symptom Expression of Zucchini Yellow Mosaic Virus Isolates from Squashes in Cucurbit Crops. *Fresenius Environmental Bulletin*, *26*, 5372-5376.

Shang, J., Kong, S., Li, N., Wang, J., Zhou, D., Li, N., & Ma, S. (2020). Genetic mapping and localization of major QTL for bitterness in melon (*Cucumis melo* L.). *Scientia Horticulturae*, *266*, 109286. https://doi.org/10.1016/j.scienta.2020.109286

Sharma, S., Kang, S. S., & Sharma, A. (2015). First report of mixed infection of *Zucchini yellow mosaic virus* and *Tomato leaf curl New Delhi virus* in bittergourd in India. *Journal of Plant Pathology*, *97*(2). http://dx.doi.org/10.4454/JPP.V97I2.045

Sharma, A., Katoch, V., & Rana, C. (2016). Important Diseases of Cucurbitaceous Crops and Their Management. En: Pessarakli, M. (Eds.), *Handbook of cucurbits growth, cultural practices, and physiology*, (pp. 301-324) CRC press.

Sharma, N., & Prasad, M. (2017). An insight into plant–Tomato leaf curl New Delhi virus interaction. *The Nucleus*, *60*, 1-14. https://doi.org/10.1007/s13237-017-0224-5.

Shen, W., Reyes, M. I., and Hanley-Bowdoin, L. (2009). *Arabidopsis* protein kinases GRIK1 and GRIK2 specifically activate *SnRK1* by phosphorylating its activation loop. *Plant Physiol.* 150, 996–1005. https://doi.org/10.1104/pp.108.132787

Shen, Q., Bao, M., & Zhou, X. (2012). A plant kinase plays roles in defense response against geminivirus by phosphorylation of a viral pathogenesis protein. *Plant signaling & behavior*, *7*(7), 888–892. https://doi.org/10.4161/psb.20646

Shen, Q., Hu, T., Bao, M., Cao, L., Zhang, H., Song, F., Xie, Q., & Zhou, X. (2016). Tobacco RING E3 ligase NtRFP1 mediates ubiquitination and proteasomal degradation of a geminivirus-encoded βC1. *Molecular plant*, *9*(6), 911-925. https://doi.org/10.1016/j.molp.2016.03.008

Shi, L., Yang, Y., Xie, Q., Miao, H., Bo, K., Song, Z., Wang, Y., Xie, B., Zhang, S., & Gu, X. (2018). Inheritance and QTL mapping of cucumber mosaic virus resistance in cucumber (Cucumis Sativus L.). *PloS one*, *13*(7), e0200571. https://doi.org/10.1371/journal.pone.0200571

Shih, S. L., Tsai, W. S., Lee, L. M., & Kenyon, L. (2013). Molecular characterization of begomoviruses infecting *Sauropus androgynus* in Thailand. *Journal of phytopathology*, *161*(2), 78-85. https://doi.org/10.1111/jph.12028

Singh, D., Leskovar, D. I., Sharma, S. P., Sarao, N. K., & Vashisht, V. K. (2020). Genetic diversity and interrelationship among Indian and exotic melons based on fruit morphology, quality components and microsatellite markers. *Physiology and Molecular Biology of Plants*, 1-18. https://doi.org/10.1007/s12298-020-00814-1

Sifres Cuerda, A. G., Sáez-Sánchez, C., Ferriol Molina, M., Selmani, E. A., Riado, J., Picó Sirvent, M. B., & López Del Rincón, C. (2018). First report of *tomato leaf curl New Delhi virus* infecting zucchini in Morocco. *Plant Disease*, *102*(5), 1045-1045. https://doi.org/10.1094/PDIS-10-17-1600-PDN

Simón, A., Ruiz, L., Velasco, L., & Janssen, D. (2018). Absolute quantification of Tomato leaf curl New Delhi virus Spain strain, ToLCNDV-ES: Virus accumulation in a host-specific manner. *Plant disease*, *102*(1), 165-171. https://doi.org/10.1094/PDIS-06-17-0840-RE

Simons, A., Jarret, R-B., Cantrell, C., & Levi A. (2019). *Citrullus ecirrhosus*: Wild source of resistance against *Bemisia tabaci* (Hemiptera: Aleyrodidae) for cultivated watermelon. *Journal of Economic Entomology*, *112*(5), 2425-2432. https://doi.org/10.1093/jee/toz069.

Sivalingam, P. N., Sumiya, K. V., Malathi, V. G., & Unit, P. V. (2011). Carrot as a new host for a begomovirus: Yellow mosaic disease of carrot reported in India. *New Disease Reports*, *23*(34), 2044-0588. http://dx.doi.org/10.5197/j.2044-0588.2011.023.034

Sivalingam, P. N., & Varma, A. (2012). Role of betasatellite in the pathogenesis of a bipartite begomovirus affecting tomato in India. *Archives of virology*, *157*(6), 1081-1092. https://doi.org/10.1007/s00705-012-1261-7

Słomnicka, R., Olczak-Woltman, H., Korzeniewska, A., Gozdowski, D., Niemirowicz-Szczytt, K., & Bartoszewski, G. (2018). Genetic mapping of *psl* locus and quantitative trait loci for angular leaf spot resistance in cucumber (*Cucumis sativus* L.). *Molecular breeding: new strategies in plant improvement*, *38*(9), 111. https://doi.org/10.1007/s11032-018-0866-2

Snehi, S. K., Parihar, S. S., Gupta, G., Singh, V., & Purvia, A. S. (2016). Molecular detection and identification of begomovirus isolate on tomato from Central Region of India. *Journal of Plant Pathology & Microbiology*, *7*(389), 2. https://doi.org/10.4172/2157-7471.1000389

Sohrab, S. S., Mandal, B., Pant, R. P., & Varma, A. (2003). First report of association of *Tomato leaf curl virus-New Delhi* with yellow mosaic disease of Luffa cylindrica in India. *Plant disease*, *87*(9), 1148-1148. https://doi.org/10.1094/PDIS.2003.87.9.1148A

Sohrab, S. S., Mandal, B., Ali, A., & Varma, A. (2010). Chlorotic curly stunt: a severe begomovirus disease of bottle gourd in northern India. *Indian Journal of Virology*, *21*(1), 56-63. https://doi.org/10.1007/s13337-010-0002-3

Sohrab, S. S., Karim, S., Varma, A., Azhar, E. I., Mandal, B., Abuzenadah, A. M., & Chaudhary, A. G. (2013). Factors affecting sap transmission of *Tomato leaf curl New Delhi* begomovirus infecting sponge gourd in India. *Phytoparasitica*, *41*(5), 591-592. https://doi.org/10.1007/s12600-013-0319-8

Soitamo, A. J., Jada, B., & Lehto, K. (2012). Expression of geminiviral AC2 RNA silencing suppressor changes sugar and jasmonate responsive gene expression in transgenic tobacco plants. *BMC plant biology*, *12*, 204. https://doi.org/10.1186/1471-2229-12-204

Song, M., Wei, Q., Wang, J., Fu, W., Qin, X., Lu, X., Cheng, F., Yang, K., Zhang, L., Yu, X., Li, J., Chen, J., & Lou, Q. (2018). Fine Mapping of *CsVYL*, Conferring Virescent Leaf Through the Regulation of Chloroplast Development in Cucumber. *Frontiers in plant science*, *9*, 432. https://doi.org/10.3389/fpls.2018.00432

Song, M., Zhang, M., Cheng, F., Wei, Q., Wang, J., Davoudi, M., Chen, J., & Lou, Q. (2020). An irregularly striped rind mutant reveals new insight into the function of PG1β in cucumber (*Cucumis sativus* L.). *Theoretical and Applied Genetics*, *133*(2), 371-382. https://doi.org/10.1007/s00122-019-03468-0

Soto-Burgos, J., & Bassham, D. C. (2017). *SnRK1* activates autophagy via the TOR signaling pathway in *Arabidopsis thaliana*. *PloS one*, *12*(8), e0182591. https://doi.org/10.1371/journal.pone.0182591

Souza, P., Garcia-Ruiz, H., & Carvalho, F. (2019). What proteomics can reveal about plant-virus interactions? Photosynthesis-related proteins on the spotlight. *Theoretical and experimental plant physiology*, *31*(1), 227–248. https://doi.org/10.1007/s40626-019-00142-0

Spadaro, D., Agustí, N., Ortega, S. F., & Ruiz, M. A. H. (2020). Diagnostics and identification of diseases, insects and mites. In *Integrated Pest and Disease Management in Greenhouse Crops* (pp. 231-258). Springer, Cham. https://doi.org/10.1007/978-3-030-22304-5_8

Srivastava, K. M., Hallan, V., Raizada, R. K., Chandra, G., Singh, B. P., & Sane, P. V. (1995). Molecular cloning of Indian tomato leaf curl vims genome following a simple method of concentrating the supercoiled replicative form of viral DNA. *Journal of Virological Methods*, *51*(2-3), 297-304. https://doi.org/10.1016/0166-0934(94)00122-W

Srivastava, A., Manisha, M., Saritha, R. K., Jat, S. L., Gosavy, G. U., & Pritam, K. (2015). Natural epiphytotic screening of chilli germplasm lines against leaf curl virus complex. *International Journal of Tropical Agriculture*, *33*(4), 3581-3586.

Srivastava, A., Kumar, S., Jaidi, M., Raj, S. K., & Shukla, S. K. (2016). First report of *tomato leaf curl New Delhi virus* on opium poppy (*Papaver somniferum*) in India. *Plant Disease*, *100*(1), 232. https://doi.org/10.1094/PDIS-08-15-0883-PDN

Stansly, P. A., & Naranjo, S. E. (Eds.). (2010). *Bemisia: bionomics and management of a global pest*. Springer Science & Business Media. https://doi.org/10.1007/978-90-481-2460-2

Staub, J. E. (1985). Use of *Cucumis sativus* var. *hardwickii* germplasm in backcrosses with *Cucumis sativus* var. *sativus*. *HortScience*, *20*, 436-438.

Strange, E. B., Guner, N., Pesic-VanEsbroeck, Z., & Wehner, T. C. (2002). Screening the Watermelon Germplasm Collection for Resistance to Papaya Ringspot Virus Type-W. *Crop Science*, *42*(4), 1324-1330. https://doi.org/10.2135/cropsci2002.1324

Su, T., Li, X., Yang, M., Shao, Q., Zhao, Y., Ma, C., & Wang, P. (2020). Autophagy: An Intracellular Degradation Pathway Regulating Plant Survival and Stress Response. *Frontiers in Plant Science*, *11*, 164. https://doi.org/10.3389/fpls.2020.00164

Šubr, Z., Predajňa, L., Šoltys, K., Bokor, B., Budiš, J., & Glasa, M. (2020). Comparative Transcriptome Analysis of Two Cucumber Cultivars with Different Sensitivity to *Cucumber Mosaic Virus* Infection. *Pathogens (Basel, Switzerland)*, *9*(2), 145. https://doi.org/10.3390/pathogens9020145

Sugiyama, M. (2013). The present status of breeding and germplasm collection for resistance to viral diseases of cucurbits in Japan. *Journal of the Japanese Society for Horticultural Science*, *82*(3), 193-202. https://doi.org/10.2503/jjshs1.82.193

Sugiyama, M., Kawazu, Y., Fukino, N., Yoshioka, Y., Shimomura, K., Sakata, Y., & Okuda, M. (2015). Mapping of quantitative trait loci for Melon yellow spot virus resistance in cucumber (Cucumis sativus L.). *Euphytica*, *205*(2), 615-625. https://doi.org/10.1007/s10681-015-1444-x

Sun, J., Zhang, Z., Zong, X., Huang, S., Li, Z., & Han, Y. (2013). A high-resolution cucumber cytogenetic map integrated with the genome assembly. *BMC genomics*, *14*(1), 461. https://doi.org/10.1186/1471-2164-14-461

Sun, W. J., Lv, W. J., Li, L. N., Yin, G., Hang, X., Xue, Y., Chen, J., & Shi, Z. (2016). Eugenol confers resistance to *Tomato yellow leaf curl virus* (TYLCV) by regulating the expression of *SlPer1* in tomato plants. *New biotechnology*, *33*(3), 345–354. https://doi.org/10.1016/j.nbt.2016.01.001

Sun, H., Wu, S., Zhang, G., Jiao, C., Guo, S., Ren, Y., Zhang, J., Zhang, H., Gong, G., Jia, Z., Zhang, F., Tian, J., Lucas, W. J., Doyle, J. J., Li, H., Fei, Z., & Xu, Y. (2017a). Karyotype Stability and Unbiased Fractionation in the Paleo-Allotetraploid Cucurbita Genomes. *Molecular plant*, *10*(10), 1293–1306. https://doi.org/10.1016/j.molp.2017.09.003

Sun, X., Wang, Z., Gu, Q., Li, H., Han, W., & Shi, Y. (2017b). Transcriptome analysis of Cucumis sativus infected by *Cucurbit chlorotic yellows virus*. *Virology journal*, *14*(1), 1-8. https://doi.org/10.1186/s12985-017-0690-z

Sun, Y., Fan, M., & He, Y. (2019). Transcriptome Analysis of Watermelon Leaves Reveals Candidate Genes Responsive to *Cucumber green mottle mosaic virus* Infection. *International journal of molecular sciences*, *20*(3), 610. https://doi.org/10.3390/ijms20030610

Sundararaj, D., Denison, M. I. J., Gunasekaran, D., Uma, M. R. S., Thangavelu, R. M., & Kathiravan, K. (2020). First Report of *Tomato Leaf Curl New Delhi Virus* Infecting *Crossandra infundibuliformis* in India. *Plant Disease*, *104*(3), 999. https://doi.org/10.1094/PDIS-08-19-1764-PDN

Supakitthanakorn, S., Akarapisan, A., & Ruangwong, O. U. (2018). First record of melon yellow spot virus in pumpkin and its occurrence in cucurbitaceous crops in Thailand. *Australasian Plant Disease Notes*, *13*(1), 32. https://doi.org/10.1007/s13314-018-0314-5

Suruthi, V., Nakkeeran, S., Renukadevi, P., Malathi, V. G., & Rajasree, V. (2018). Evidence of seed transmission of *dolichos yellow mosaic virus*, a begomovirus infecting lablab-bean in India. *VirusDisease*, *29*(4), 506-512. https://doi.org/10.1007/s13337-018-0494-9

Syller, J. (2012). Facilitative and antagonistic interactions between plant viruses in mixed infections. *Molecular Plant Pathology*, *13*(2), 204–216. https://doi.org/10.1111/j.1364-3703.2011.00734.x

Szinay, D. (2010). *The development of FISH tools for genetic, phylogenetic and breeding studies in tomato (Solanum lycopersicum)*. Doctoral thesis under supervion of Prof. Dr. Visser R.G.F. (WU) Accessed: http://library.wur.nl/WebQuery/wurpubs/fulltext/137490

Szinay, D., Wijnker, E., van den Berg, R., Visser, R. G., de Jong, H., & Bai, Y. (2012). Chromosome evolution in Solanum traced by cross-species BAC-FISH. *New Phytologist*, *195*(3), 688-698. https://doi.org/10.1111/j.1469-8137.2012.04195.x

Taheri, S., Abdullah, T. L., Jain, S. M., Sahebi, M., & Azizi, P. (2017). TILLING, high-resolution melting (HRM), and next-generation sequencing (NGS) techniques in plant mutation breeding. *Molecular breeding*, *37*(3), 40. https://doi.org/10.1007/s11032-017-0643-7

Tahir, M., & Haider, M. S. (2005). First report of *Tomato leaf curl New Delhi virus* infecting bitter gourd in Pakistan. *Plant pathology*, *54*(6), 807-807. https://doi.org/10.1111/j.1365-3059.2005.01215.x

Tatineni, S., Stewart, L. R., Sanfaçon, H., Wang, X., Navas-Castillo, J., & Hajimorad, M. R. (2020). Fundamental Aspects of Plant Viruses−An Overview on Focus Issue Articles. *Phytopathology*, *110*(1), 6-9. https://doi.org/10.1094/PHYTO-10-19-0404-FI

Téllez, M., Simon, A., Rodriguez, E., & Janssen, D. (2017). Control of tomato leaf curl New Delhi virus in zucchini using the predatory mite Amblyseius swirskii. *Biological Control*, *114*, 106-113. https://doi.org/10.1016/j.biocontrol.2017.08.008

Thakur, H., Sharma, S., & Thakur, M. (2019). Recent trends in muskmelon (C ucumis melo L.) research: an overview. *The Journal of Horticultural Science and Biotechnology*, *94*(4), 533-547. https://doi.org/10.1080/14620316.2018.1561214

Tian, G., Yang, Y., Zhang, S., Miao, H., Lu, H., Wang, Y., Xie, B., & Gu, X. (2015). Genetic analysis and gene mapping of *papaya ring spot virus* resistance in cucumber. *Molecular Breeding*, *35*(4), 110. https://doi.org/10.1007/s11032-015-0279-4.

Tian, G., Miao, H., Yang, Y., Zhou, J., Lu, H., Wang, Y., Xie, B., Zhang, Gu, & Gu, X. (2016). Genetic analysis and fine mapping of Watermelon mosaic virus resistance gene in cucumber. *Molecular Breeding*, *36*(9), 131. https://doi:10.1007/s11032-016-0524-5.

Tiwari, A. K., Sharma, P. K., Khan, M. S., Snehi, S. K., Raj, S. K., & Rao, G. P. (2010). Molecular detection and identification of *Tomato leaf curl New Delhi virus* isolate causing yellow mosaic disease in Bitter gourd (*Momordica charantia*), a medicinally important plant in India. *Medicinal Plants-International Journal of Phytomedicines and Related Industries*, *2*(2), 117-123. https://doi.org/10.5958/j.0975-4261.2.2.018

Trisciuzzi, N., Silletti, M. R., Gallitelli, D., & Spanò, R. (2018). First detection of *tomato leaf curl New Delhi virus* in melon and zucchini squash in southern Italy. *Journal of Plant Pathology*, *100*(1), 149-149. https://doi.org/10.1007/s42161-018-0038-6

Tu, Y. C., Tsai, W. S., Wei, J. Y., Chang, K. Y., Tien, C. C., Hsiao, H. Y., & Fu, S. F. (2017). The C2 protein of *tomato leaf curl Taiwan virus* is a pathogenicity determinant that interferes with expression of host genes encoding chromomethylases. *Physiologia plantarum*, *161*(4), 515-531. https://doi.org/10.1111/ppl.12615

Ullah, R., Akhtar, K. P., Moffett, P., Mansoor, S., Briddon, R. W., & Saeed, M. (2014). An analysis of the resistance of *Gossypium arboreum* to *cotton leaf curl disease* by grafting. *European journal of plant pathology*, *139*(4), 837-847. https://doi.org/10.1007/s10658-014-0437-2

Usharani, K. S., Surendranath, B., Paul-Khurana, S. M., Garg, I. D., & Malathi, V. G. (2004). Potato leaf curl-a new disease of potato in northern India caused by a strain of tomato leaf curl New Delhi virus. *Plant pathology*, *53*(2). https://doi.org/10.1111/j.0032-0862.2004.00959.x

Vafaei, S. H., & Mahmoodi, M. (2017). Presence of Recombinant Strain of *Cucurbit Aphid Borne Yellows Virus* in Iran. *Iranian journal of biotechnology*, *15*(4), 289–295. https://doi.org/10.15171/ijb.1541

Van Loon, L. C., & Van Strien, E. A. (1999). The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiological and molecular plant pathology*, *55*, 85-97. https://doi.org/10.1006/pmpp.1999.0213

Varma, A., Mandal, B., & Singh, M. K. (2011). Global emergence and spread of whitefly (Bemisia tabaci) transmitted geminiviruses. En: Thompson W. (Eds.), *The whitefly, Bemisia tabaci (Homoptera: Aleyrodidae) interaction with geminivirus-infected host plants* (pp. 205-292). Springer, Dordrecht. https://doi.org/10.1007/978-94-007-1524-0_10

Varma, A., Mandal, B., & Singh, M. K. (2013). Emergence of begomoviruses: a major threat to vegetable production in Southeast Asia. En: Holmer, R., Linwattana, G., Nath, P., & Keatinge, J. D. H. (Eds.), *Proceedings SEAVEG 2012, Chiang Mai, Thailand, 24-26 January 2012. High value vegetables in Southeast Asia: production, supply and demand*, (pp. 88-96). AVRDC-WorldVegetableCenter.

Velasco, L., Ruiz, L., Galipienso, L., Rubio, L., & Janssen, D. (2020). A Historical Account of Viruses in Intensive Horticultural Crops in the Spanish Mediterranean Arc: New Challenges for a Sustainable Agriculture. *Agronomy*, *10*(6), 860. https://doi.org/10.3390/agronomy10060860

Venkataravanappa, V., Reddy, C. L., Jalali, S., & Reddy, M. K. (2012). Molecular characterization of distinct bipartite begomovirus infecting bhendi (Abelmoschus esculentus L.) in India. *Virus Genes*, *44*(3), 522-535. https://doi.org/10.1007/s11262-012-0732-y

Venkataravanappa, V., Narasimha Reddy, L. R. C., Saha, S., Subbanna, S. K., & Manem, K. R. (2017). Detection and characterization of *tomato leaf curl New Delhi virus* association with mosaic disease of ivy gourd (*Coccinia grandis* (L.) Voigt) in North India. *Archives of Biological Sciences*, *70*(2). 339-347. https://doi.org/10.2298/ABS170616051V.

Verchot J. (2016). Plant Virus Infection and the Ubiquitin Proteasome Machinery: Arms Race along the Endoplasmic Reticulum. *Viruses*, *8*(11), 314. https://doi.org/10.3390/v8110314

Verlaan, M. G., Szinay, D., Hutton, S. F., de Jong, H., Kormelink, R., Visser, R. G., Scott, J. W., & Bai, Y. (2011). Chromosomal rearrangements between tomato and *Solanum chilense* hamper mapping and breeding of the TYLCV resistance gene Ty-1. *The Plant journal: for cell and molecular biology*, *68*(6), 1093–1103. https://doi.org/10.1111/j.1365-313X.2011.04762.x

Verlaan, M. G., Hutton, S. F., Ibrahem, R. M., Kormelink, R., Visser, R. G., Scott, J. W., Edwards, J. D., & Bai, Y. (2013). The Tomato Yellow Leaf Curl Virus resistance

genes Ty-1 and Ty-3 are allelic and code for DFDGD-class RNA-dependent RNA polymerases. *PLoS genetics*, *9*(3), e1003399. https://doi.org/10.1371/journal.pgen.1003399

Vidavski, F. S. (2007). Exploitation of resistance genes found in wild tomato species to produce resistant cultivars; pile up of resistant genes. En: Czosnek, H. (Eds.), *Tomato yellow leaf curl virus disease* (pp. 363-372). Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-4769-5_21

Vidavski, F., Czosnek, H., Gazit, S., Levy, D., & Lapidot, M. (2008). Pyramiding of genes conferring resistance to *Tomato yellow leaf curl virus* from different wild tomato species. *Plant Breeding*, *127*(6), 625-631. https://doi.org/10.1111/j.1439-0523.2008.01556.x

Vilmorin & Cie. (2010). Squash leaf curl virus (SLCV) resistance in cucurbits. http://patents.justia.com/patent/8609928

Vinutha, T., Kumar, G., Garg, V., Canto, T., Palukaitis, P., Ramesh, S. V., & Praveen, S. (2018). Tomato geminivirus encoded RNAi suppressor protein, AC4 interacts with host AGO4 and precludes viral DNA methylation. *Gene*, *678*, 184-195. https://doi.org/10.1016/j.gene.2018.08.009

Voinnet, O., Pinto, Y. M., & Baulcombe, D. C. (1999). Suppression of gene silencing: a general strategy used by diverse DNA and RNA viruses of plants. *Proceedings of the National Academy of Sciences*, *96*(24), 14147-14152. https://doi.org/10.1073/pnas.96.24.14147

Voorburg, C. M., Yan, Z., Bergua-Vidal, M., Wolters, A. A., Bai, Y., & Kormelink, R. (2020). *Ty-1*, a universal resistance gene against geminiviruses that is compromised by co-replication of a betasatellite. *Molecular plant pathology*, *21*(2), 160–172. https://doi.org/10.1111/mpp.12885

Wai, T., & Grumet, R. (1995). Inheritance of resistance to *watermelon mosaic virus* in the cucumber line TMG-1: tissue-specific expression and relationship to zucchini yellow mosaic virus resistance. *Theoretical and Applied Genetics*, *91*(4), 699-706.

Walkey, D. G. A., & Pink, D. A. C. (1984). Resistance in vegetable marrow and other *Cucurbita* spp. to two British strains of cucumber mosaic virus. *The Journal of Agricultural Science*, *102*(1), 197-205.

Wang, Y. J., Provvidenti, R., & Robinson, R. W. (1984). Inheritance of resistance to watermelon mosaic virus 1 in cucumber. *HortScience* 19:587-588.

Wang, Y., Gaba, V., Yang, J., Palukaitis, P., & Gal-On, A. (2002). Characterization of synergy between Cucumber mosaic virus and potyviruses in cucurbit hosts. *Phytopathology*, *92*(1), 51-58. https://doi.org/10.1094/PHYTO.2002.92.1.51

Wang, J., Hu, Z., Zhao, T., Yang, Y., Chen, T., Yang, M., Yu, W., & Zhang, B. (2015). Genome-wide analysis of bHLH transcription factor and involvement in the infection by yellow leaf curl virus in tomato (Solanum lycopersicum). *BMC genomics*, *16*(1), 39. https://doi.org/10.1186/s12864-015-1249-2

Wang, Y., Zhu, P., Zhou, Q., Zhou, X., Guo, Z., Cheng, L., Zhu, L. He, X. Zhu, Y., & Hu, Y. (2019a). Detection of disease in *Cucurbita maxima* Duch. ex Lam. caused by a mixed infection of *Zucchini yellow mosaic virus*, *Watermelon mosaic virus*, and *Cucumber mosaic virus* in Southeast China using a novel small RNA sequencing method. *PeerJ*, *7*, e7930. https://doi.org/10.7717/peerj.7930

Wang, Y., Wu, Y., Gong, Q., Ismayil, A., Yuan, Y., Lian, B., Jia, Q., Han, M., Deng, H., Hong, Y., Hanley-Bowdoin, L., Qi, Y., & Liu, Y. (2019b). Geminiviral V2 protein suppresses transcriptional gene silencing through interaction with AGO4. *Journal of virology*, *93*(6). https://doi.org/10.1128/JVI.01675-18

Wang, Y., Bo, K., Gu, X., Pan, J., Li, Y., Chen, J., Wen, C., Ren, Z., Ren, H., Chen, X., Grumet, R., & Weng, Y. (2020). Molecularly tagged genes and quantitative trait loci in cucumber with recommendations for QTL nomenclature. *Horticulture research*, *7*(1), 1-20. https://doi.org/10.1038/s41438-019-0226-3

Wechter, W. P., Kousik, C., McMillan, M., & Levi, A. (2012). Identification of resistance to Fusarium oxysporum f. sp. niveum race 2 in Citrullus lanatus var. citroides plant introductions. *HortScience*, *47*(3), 334-338. https://doi.org/10.21273/HORTSCI.47.3.334

Wechter, W. P., McMillan, M. M., Farnham, M. W., & Levi, A. (2016). Watermelon germplasm lines USVL246-FR2 and USVL252-FR2 tolerant to Fusarium oxysporum f. sp. niveum race 2. *HortScience*, *51*(8), 1065-1067. https://doi.org/10.21273/HORTSCI.51.8.1065

Weeden, N.F., Robinson, R.W. & Ignart, F. (1984). Linkage between an isozyme locus and one of the genes controlling resistance to watermelon mosaic virus 2 in *Cucurbita ecuadorensis*. Cucurbit Genetic Cooperative. Rep.7: 86–87.

Wege, C., & Pohl, D. (2007). Abutilon mosaic virus DNA B component supports mechanical virus transmission, but does not counteract begomoviral phloem limitation in transgenic plants. *Virology*, *365*(1), 173-186. https://doi.org/10.1016/j.virol.2007.03.041

Wei, J., He, Y. Z., Guo, Q., Guo, T., Liu, Y. Q., Zhou, X. P., Liu, S. S., & Wang, X. W. (2017). Vector development and vitellogenin determine the transovarial transmission of begomoviruses. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(26), 6746–6751. https://doi.org/10.1073/pnas.1701720114

Weng, Y.**,** (2016). The Cucumber Genome. In: Grumet R., Katzir N., Garcia-Mas J. (Eds.) *Genetics and Genomics of Cucurbitaceae. Plant Genetics and Genomics: Crops and Models*, (Vol. 20, pp. 183-197). Springer, Cham DOI https://doi.org/10.1007/7397_2016_6

Weng, Y., & Wehner, T. C. (2017). Cucumber gene catalog 2017. *Cucurbit Genet. Coop. Rep*, *40*, 17-54.

Whitaker, T., & Robinson, R. (1986). Squash breeding. En: Basset, M. J. (Ed.) *Breeding vegetable crops*. *Westport: Avi*, 209-242. Westport, Connecticut: AVI Publishing Company, Inc.

Whitham, S. A., Yang, C., & Goodin, M. M. (2006). Global impact: elucidating plant responses to viral infection. *Molecular plant-microbe interactions: MPMI*, *19*(11), 1207–1215. https://doi.org/10.1094/MPMI-19-1207

Whitham, S. A., & Hajimorad, M. R. (2016). Plant genetic resistance to viruses. En: Wang, A., & Zhou, X. (Eds.), *Current Research Topics in Plant Virology*, (pp. 87-111). Springer International Publishing, Basel, Switzerland. https://doi.org/10.1007/978-3-319-32919-2_4

Widana Gamage, S. M., Rotenberg, D., Schneweis, D. J., Tsai, C. W., & Dietzgen, R. G. (2018). Transcriptome-wide responses of adult melon thrips (*Thrips palmi*) associated with capsicum chlorosis virus infection. *PloS one*, *13*(12), e0208538. https://doi.org/10.1371/journal.pone.0208538

Wilkinson, M. (2001). Broadening the Genetic Base of Crop Production. *Heredity*, *87*(6), 710-710. https://doi.org/10.1046/j.1365-2540.2001.0810c.x

Wilisiani, F., Mashiko, T., Wang, W. Q., Suzuki, T., Hartono, S., Neriya, Y., Nishigawa, H., & Natsuaki, T. (2019). New recombinant of Tomato leaf curl New Delhi virus infecting melon in Indonesia. *Journal of General Plant Pathology*, *85*(4), 306-310. https://doi.org/10.1007/s10327-019-00849-7.

Wintermantel, W. M., Gilbertson, R. L., Natwick, E. T., & McCreight, J. D. (2017). Emergence and epidemiology of cucurbit yellow stunting disorder virus in the American Desert southwest, and development of host plant resistance in melon. *Virus research*, *241*, 213-219. https://doi.org/10.1016/j.virusres.2017.06.004

Wintermantel, W. M., Jenkins Hladky, L. L., Fashing, P., Ando, K., & McCreight, J. D. (2019). First report of Cucurbit chlorotic yellows virus infecting melon in the New World. *Plant disease*, *103*(4), 778-778. https://doi.org/10.1094/PDIS-08-18-1390-PDN

Wright, A. A., Cross, A. R., & Harper, S. J. (2020). A bushel of viruses: Identification of seventeen novel putative viruses by RNA-seq in six apple trees. *Plos one*, *15*(1), e0227669. https://doi.org/10.1371/journal.pone.0227669

Wu, S., Wang, X., Reddy, U., Sun, H., Bao, K., Gao, L., Mao, L., Patel, T., Ortiz, C., Abburi, V. L., Nimmakayala, P., Branham, S., Wechter, P., Massey, L., Ling, K. S., Kousik, C., Hammar, S. A., Tadmor, Y., Portnoy, V., Gur, A., Katzir, N., Davis, A., Hernandez, A. G., Wright, C.L., McGregor, C., Jarret, R., Zhang, X., Xu, Y., Wehner, T. C., Grumet, R., Levi, A., & Fei, Z. (2019). Genome of 'Charleston Gray', the principal American watermelon cultivar, and genetic characterization of 1,365 accessions in the US National Plant Germplasm System watermelon collection. *Plant biotechnology journal*, *17*(12), 2246-2258. https://doi.org/10.1111/pbi.13136

Xia, C., Li, S., Hou, W., Fan, Z., Xiao, H., Lu, M., Sano, T., & Zhang, Z. (2017). Global Transcriptomic Changes Induced by Infection of Cucumber (*Cucumis sativus* L.) with Mild and Severe Variants of Hop Stunt Viroid. *Frontiers in microbiology*, *8*, 2427. https://doi.org/10.3389/fmicb.2017.02427

Xiao, Y., Rouzine, I. M., Bianco, S., Acevedo, A., Goldstein, E. F., Farkov, M., Brodsky, L., & Andino, R. (2016). RNA recombination enhances adaptability and is required

for virus spread and virulence. *Cell host & microbe*, *19*(4), 493-503. https://doi.org/10.1016/j.chom.2016.03.009

Yadav, R. K., Shukla, R. K., & Chattopadhyay, D. (2009). Soybean cultivar resistant to *Mungbean Yellow Mosaic India Virus* infection induces viral RNA degradation earlier than the susceptible cultivar. *Virus research*, *144*(1-2), 89-95. https://doi.org/10.1016/j.virusres.2009.04.011

Yadav, R. K., & Chattopadhyay, D. (2014). Differential soybean gene expression during early phase of infection with *Mungbean yellow mosaic India virus*. *Molecular biology reports*, *41*(8), 5123-5134. https://doi.org/10.1007/s11033-014-3378-0

Yadav, C. B., Bhareti, P., Muthamilarasan, M., Mukherjee, M., Khan, Y., Rathi, P., & Prasad, M. (2015). Genome-wide SNP identification and characterization in two soybean cultivars with contrasting *mungbean yellow mosaic India virus* disease resistance traits. *PloS one*, *10*(4), e0123897. https://doi.org/10.1371/journal.pone.0123897

Yamaguchi, H., Ohnishi, J., Saito, A., Ohyama, A., Nunome, T., Miyatake, K., & Fukuoka, H. (2018). An NB-LRR gene, TYNBS1, is responsible for resistance mediated by the Ty-2 Begomovirus resistance locus of tomato. *Theoretical and Applied Genetics*, *131*(6), 1345-1362. https://doi.org/10.1007/s00122-018-3082-x

Yan, Z., Pérez-de-Castro, A., Díez, M. J., Hutton, S. F., Visser, R., Wolters, A. A., Bai, Y., & Li, J. (2018). Resistance to Tomato Yellow Leaf Curl Virus in Tomato Germplasm. *Frontiers in plant science*, *9*, 1198. https://doi.org/10.3389/fpls.2018.01198

Yang, L., Koo, D. H., Li, Y., Zhang, X., Luan, F., Havey, M. J., Jiang, J., & Weng, Y. (2012). Chromosome rearrangements during domestication of cucumber as revealed by high-density genetic mapping and draft genome assembly. *The Plant journal: for cell and molecular biology*, *71*(6), 895–906. https://doi.org/10.1111/j.1365-313X.2012.05017.x

Yang, X., Caro, M., Hutton, S. F., Scott, J. W., Guo, Y., Wang, X., Rashid, M. H., Szinay, D., de Jong, H., Visser, R. G., Bai, Y., & Du, Y. (2014). Fine mapping of the *tomato yellow leaf curl virus* resistance gene *Ty-2* on chromosome 11 of tomato. *Molecular breeding: new strategies in plant improvement*, *34*(2), 749–760. https://doi.org/10.1007/s11032-014-0072-9

Yazdani-Khameneh, S., Golnaraghi, A. R., & Rakhshandehroo, F. (2013). Report of a new begomovirus on melon in Iran. *New Disease Reports*, *28*, 17. http://dx.doi.org/10.5197/j.2044-0588.2013.028.017

Yeam, I. (2016). Current advances and prospectus of viral resistance in horticultural crops. *Horticulture, Environment, and Biotechnology*, *57*(2), 113-122. https://doi.org/10.1007/s13580-016-0105-x

Yoshioka, K., Hanada, K., Harada, T., Minobe, Y., & Oosawa, K. (1993). Virus resistance in transgenic melons that express coat protein gene of cucumber mosaic virus and in these progenies. *Japanese Journal of Breeding*, *43*, 629-634. http://dx.doi.org/10.1270/jsbbs1951.43.629.

You, W. J., Feng, Y. R., Shen, Y. H., Chen, Y. R., Chen, T. Y., & Fu, S. F. (2019). Silencing of NbCMT3s has pleiotropic effects on development by interfering with autophagy-related genes in Nicotiana benthamiana. *Plant and Cell Physiology*, *60*(5), 1120-1135. https://doi.org/10.1093/pcp/pcz034

Yu, T. A., Chiang, C. H., Wu, H. W., Li, C. M., Yang, C. F., Chen, J. H., Chen, Y. W., & Yeh, S. D. (2011). Generation of transgenic watermelon resistant to Zucchini yellow mosaic virus and Papaya ringspot virus type W. Plant cell reports, 30(3), 359–371. https://doi.org/10.1007/s00299-010-0951-4

Zaffalon, V., Mukherjee, S. K., Reddy, V. S., Thompson, J. R., & Tepfer, M. (2012). A survey of geminiviruses and associated satellite DNAs in the cotton-growing areas of northwestern India. *Archives of virology*, *157*(3), 483-495. https://doi.org/10.1007/s00705-011-1201-y

Zaidi, S. S. E. A., Shafiq, M., Amin, I., Scheffler, B. E., Scheffler, J. A., Briddon, R. W., & Mansoor, S. (2016). Frequent occurrence of *Tomato leaf curl New Delhi virus* in cotton leaf curl disease affected cotton in Pakistan. *Plos one*, *11*(5), e0155520. https://doi.org/10.1371/journal.pone.0155520

Zaidi, S. S., Shakir, S., Farooq, M., Amin, I., & Mansoor, S. (2017a). First report of a novel strain of *Tomato yellow leaf curl virus* causing yellow leaf curl disease on cluster bean in Pakistan. *Plant Disease*, *101*(6), 1071-1071. https://doi.org/10.1094/PDIS-12-16-1859-PDN

Zaidi, S. S. E. A., Martin, D. P., Amin, I., Farooq, M., & Mansoor, S. (2017b). *Tomato leaf curl New Delhi virus*: a widespread bipartite begomovirus in the territory of monopartite begomoviruses. *Molecular plant pathology*, *18*(7), 901-911. https://doi.org/10.1111/mpp.12481

Zaidi, S. S., Naqvi, R. Z., Asif, M., Strickler, S., Shakir, S., Shafiq, M., Khan, A. M., Amin, I., Mishra, B., Mukhtar, M. S., Scheffler, B. E., Scheffler, J. A., Mueller, L. A., & Mansoor, S. (2020). Molecular insight into *cotton leaf curl geminivirus* disease resistance in cultivated cotton (*Gossypium hirsutum*). *Plant biotechnology journal*, *18*(3), 691–706. https://doi.org/10.1111/pbi.13236

Zamir, D., Ekstein-Michelson, I., Zakay, Y., Navot, N., Zeidan, M., Sarfatti, M., Eshed, Y., Harel, E., Pleban, T., van-Oss, H., Kedar, N., Rabinowitch, H. D., & Czosnek, H. (1994). Mapping and introgression of a tomato yellow leaf curl virus tolerance gene, TY-1. *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik*, *88*(2), 141–146. https://doi.org/10.1007/BF00225889

Zamir, D., Ekstein-Michelson, I., Zakay, Y., Navot, N., Zeidan, M., Sarfatti, M., Eshed, Y. , Harel, E., Pleban, T. , van-Oss, H., Kedar, N., Rabinowitch, H. D., & Czosnek, H. (1994). Mapping and introgression of a tomato yellow leaf curl virus tolerance gene, *Ty-1*. *Theoretical and Applied Genetics*, *88*(2), 141-146. https://doi.org/10.1007/BF00225889

Zhan, B., Cao, M., Wang, K., Wang, X., & Zhou, X. (2019). Detection and characterization of *Cucumis melo cryptic virus*, *Cucumis melo amalgavirus* 1, and *melon necrotic spot virus* in *Cucumis melo*. *Viruses*, *11*(1), 81. https://doi.org/10.3390/v11010081

Zhang, W. W., Pan, J. S., He, H. L., Zhang, C., Li, Z., Zhao, J. L., Yuan, X. J., Zhu, L. H., Huang, S. W., & Cai, R. (2012). Construction of a high density integrated genetic map for cucumber (Cucumis sativus L.). *TAG. Theoretical and applied genetics. Theoretische und angewandte Genetik*, *124*(2), 249–259. https://doi.org/10.1007/s00122-011-1701-x

Zhang, Y., Xia, R., Kuang, H., & Meyers, B. C. (2016). The Diversification of Plant NBS-LRR Defense Genes Directs the Evolution of MicroRNAs That Target Them. *Molecular biology and evolution*, *33*(10), 2692–2705. https://doi.org/10.1093/molbev/msw154

Zhang, J., Yang, J., Zhang, L., Luo, J., Zhao, H., Zhang, J., & Wen, C. (2020). A new Snp genotyping technology target Snp-seq and its application in genetic analysis of cucumber varieties. *Scientific reports*, *10*(1), 1-11. https://doi.org/10.1038/s41598-020-62518-6

Zhao, J., Zhang, X., Hong, Y., & Liu, Y. (2016). Chloroplast in Plant-Virus Interaction. *Frontiers in microbiology*, *7*, 1565. https://doi.org/10.3389/fmicb.2016.01565

Zhao, G., Lian, Q., Zhang, Z., Fu, Q., He, Y., Ma, S., Ruggieri, V., Monforte, A. J., Wang, P., Julca, I., Wang, H., Liu, J., Xu, Y., Wang, R., Ji, J., Xu, Z., Kong, W., Zhong, Y., Shang, J., Pereira, L., Argyris, J., Zhang, J., Mayobre, C., Pujol, M., Oren, E., Ou, D., Wang, J., Sun, D., Zhao, S., Zhu, Y., Li, N., Katzir, N., Gur, A., Dogimont, C., Schaefer, H., Fan, W., Bendahmane, A., Fei, Z., Pitrat, M., Gabaldón, T., Lin, T., Garcia-Mas, J., Xu, Y., & Huang, S. (2019). A comprehensive genome variation map of melon identifies multiple domestication events and loci influencing agronomic traits. *Nature Genetics*, *51*(11), 1607-1615. https://doi.org/10.1038/s41588-019-0522-8

Zhao, Y., Yang, X., Zhou, G., & Zhang, T. (2020). Engineering plant virus resistance: from RNA silencing to genome editing strategies. *Plant Biotechnology Journal*, *18*(2), 328. doi:10.1111/pbi.13278

Zheng, Y., Wu, S., Bai, Y., Sun, H., Jiao, C., Guo, S., & Xu, Y. (2019). Cucurbit Genomics Database (CuGenDB): a central portal for comparative and functional genomics of cucurbit crops. *Nucleic acids research*, *47*(D1), D1128-D1136. https://doi.org/10.1093/nar/gky944

Zhong, Y. J., Zhou, Y. Y., Li, J. X., Yu, T., Wu, T. Q., Luo, J. N., Luo, S-B., & Huang, H. X. (2017). A high-density linkage map and QTL mapping of fruit-related traits in pumpkin (*Cucurbita moschata* Duch.). *Scientific reports*, *7*(1), 1-12. https://doi.org/10.1038/s41598-017-13216-3

Zhou X. (2013). Advances in understanding begomovirus satellites. *Annual review of phytopathology*, *51*, 357–381. https://doi.org/10.1146/annurev-phyto-082712-102234

Zhou, X. P. (2013). Advances in understanding begomovirus satellites. *Annual Review of Phytopathology*, *51*, 357-381. https://doi.org/10.1146/annurev-phyto-082712-102234

Zhu, W. Y., Huang, L., Chen, L., Yang, J. T., Wu, J. N., Qu, M. L., Yao, D. Q., Guo, C. L., Lian, H. L., He, H. L., Pan, J. S., & Cai, R. (2016). A High-Density Genetic Linkage Map for Cucumber (Cucumis sativus L.): Based on Specific Length Amplified Fragment (SLAF) Sequencing and QTL Analysis of Fruit Traits in Cucumber. *Frontiers in plant science*, *7*, 437. https://doi.org/10.3389/fpls.2016.00437

Zhu, Q., Gao, P., Wan, Y., Cui, H., Fan, C., Liu, S., & Luan, F. (2018). Comparative transcriptome profiling of genes and pathways related to resistance against powdery mildew in two contrasting melon genotypes. *Scientia horticulturae*, *227*,169-180. https://doi.org/10.1016/j.scienta.2017.09.033

Zorzatto, C., Machado, J. P., Lopes, K. V., Nascimento, K. J., Pereira, W. A., Brustolini, O. J., Reis, P. A., Calil, I. P., Deguchi, M., Sachetto-Martins, G., Gouveia, B. C., Loriato, V. A., Silva, M. A., Silva, F. F., Santos, A. A., Chory, J., & Fontes, E. P. (2015). NIK1-mediated translation suppression functions as a plant antiviral immunity mechanism. *Nature*, *520*(7549), 679–682. https://doi.org/10.1038/nature141