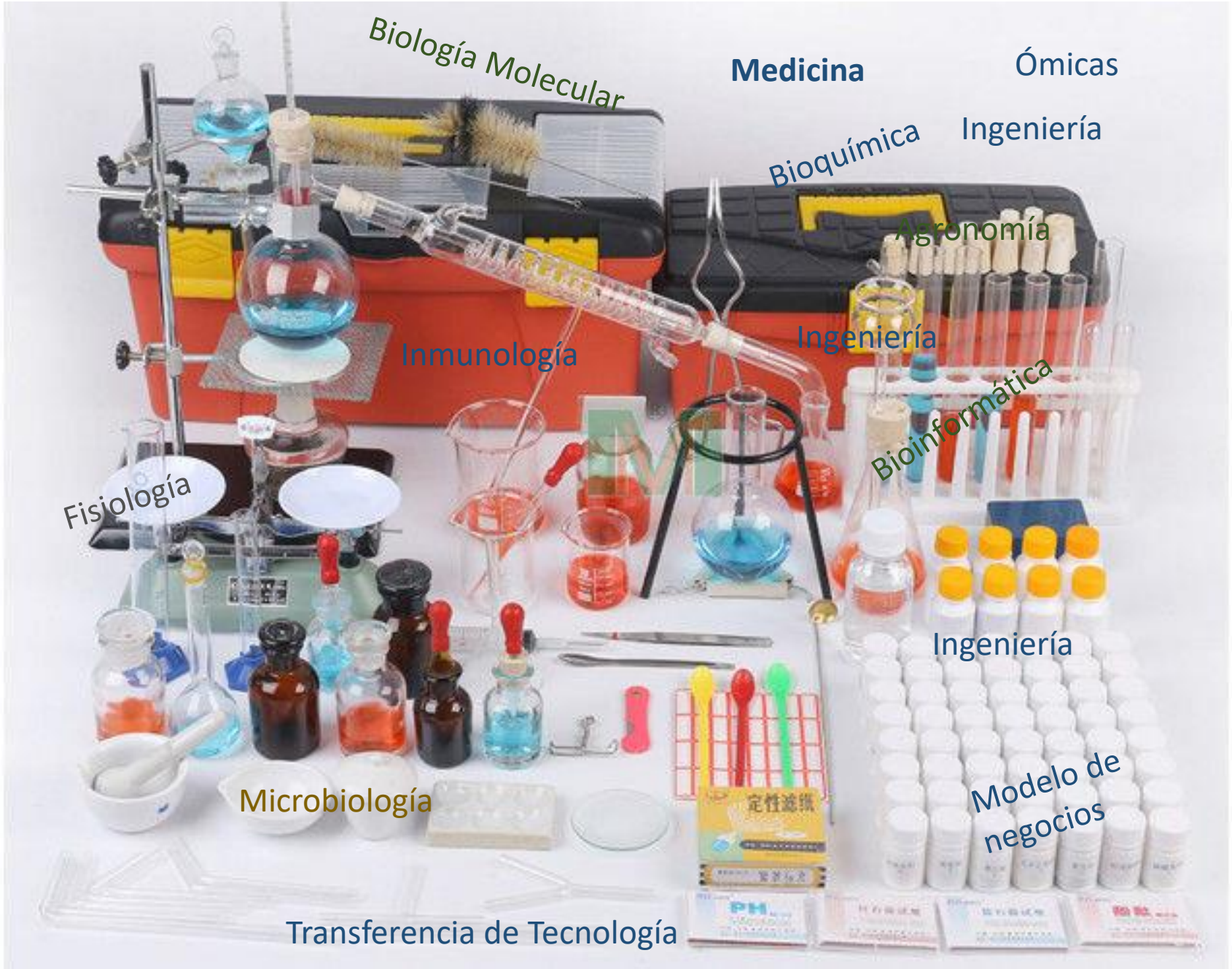
Cinvestav

Simposio: Tendencias de la Fitosanidad
UNIVERSIDAD AUTÓNOMA CHAPINGO

El uso de la Biotecnología en la protección vegetal

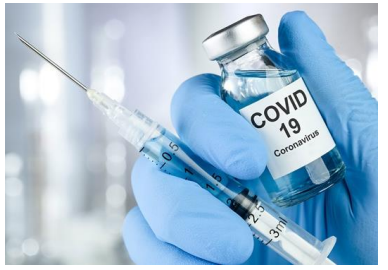
Beatriz Xoconostle

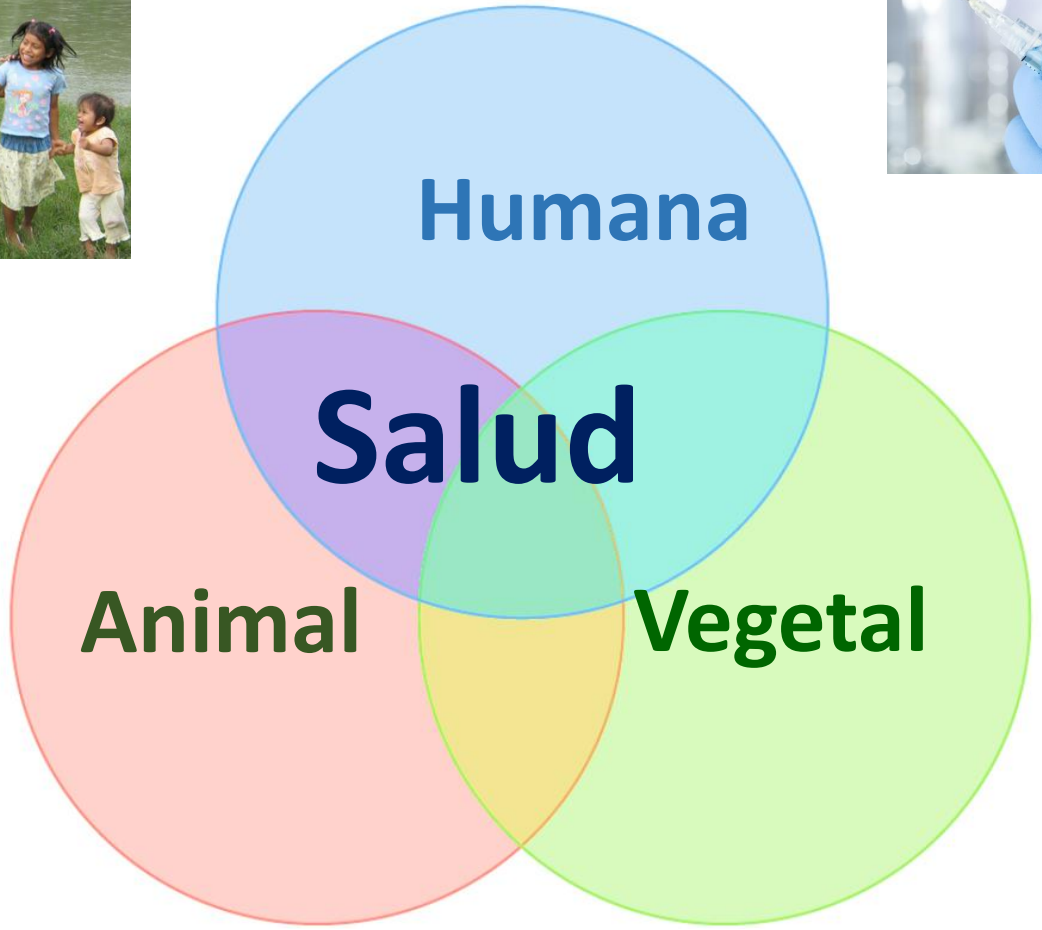

Biología Molecular
Medicina
Ómicas
Bioquímica
Ingeniería
Agronomía
Inmunología
Ingeniería
Bioinformática
Fisiología
Ingeniería
Microbiología
Modelo de negocios
Transferencia de Tecnología

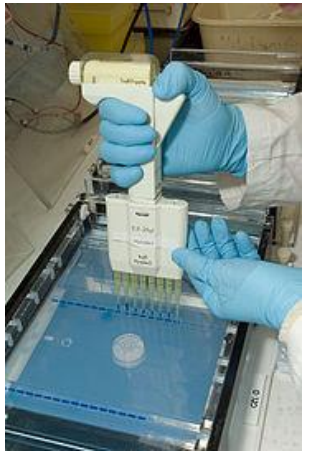
OBJETIVOS DE DESARROLLO SOSTENIBLE

Producido en colaboración con **TROLLBÄCK + COMPANY** | TheGlobalGoals@trollback.com | +1.212.529.1010
Para cualquier duda sobre la utilización, por favor comuniquese con: dpicampaigns@un.org

México con Salud







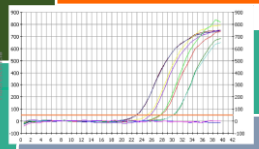
FAO, 2012.

Caracterización de enfermedades emergentes

Cinvestav

SENASICA

Identificación de plantas enfermas



CINVESTAV/SENASICA

Aislamiento de ácidos nucleicos

Métodos de detección

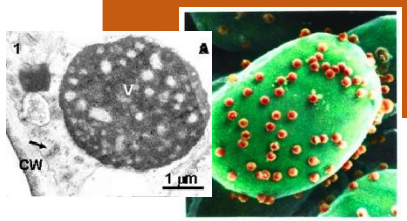
CINVESTAV/SENASICA

Secuenciación masiva

Bioinfor-mática

XIUHCOATL

ID de organismos asociados

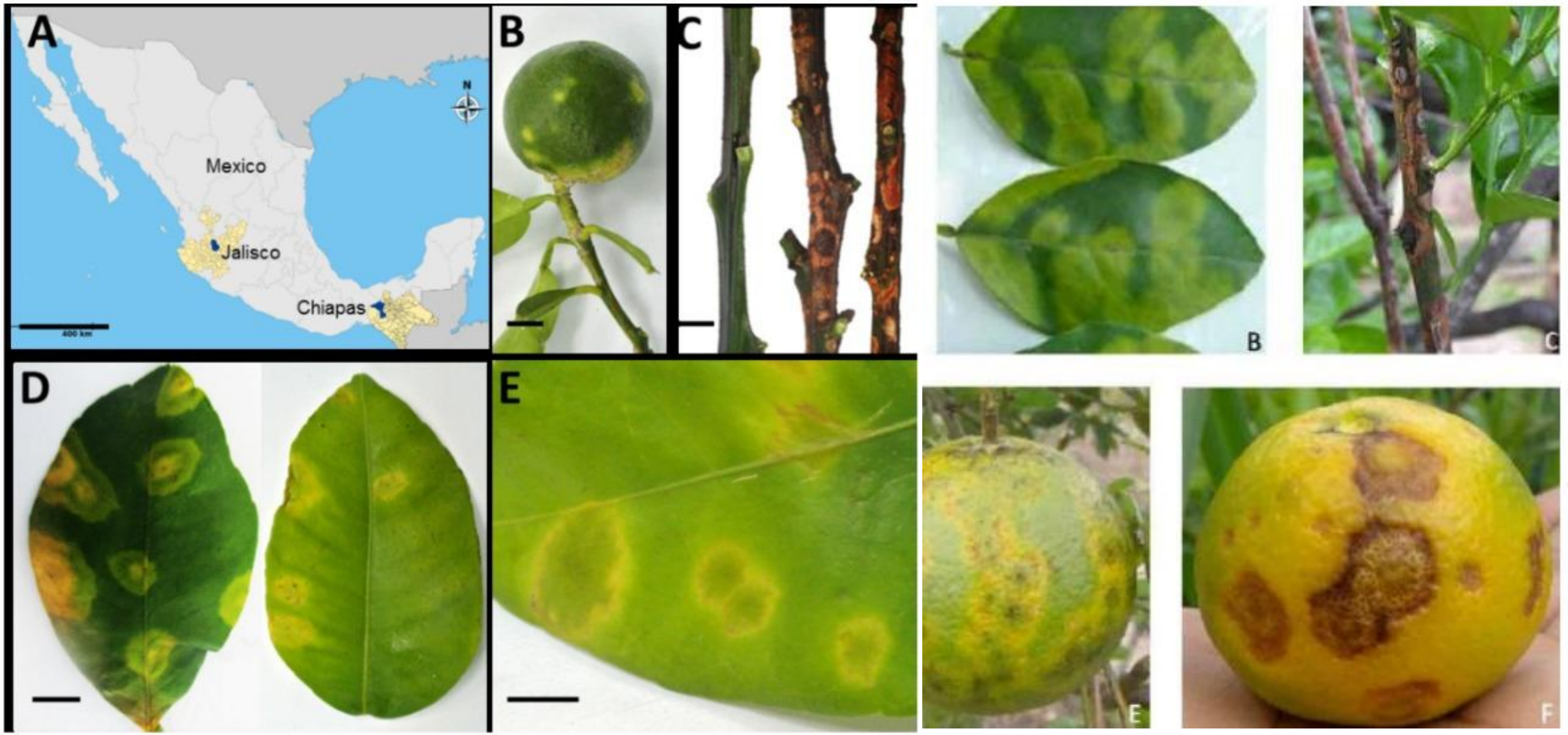


Control o erradicación

Enfermedades de cítricos

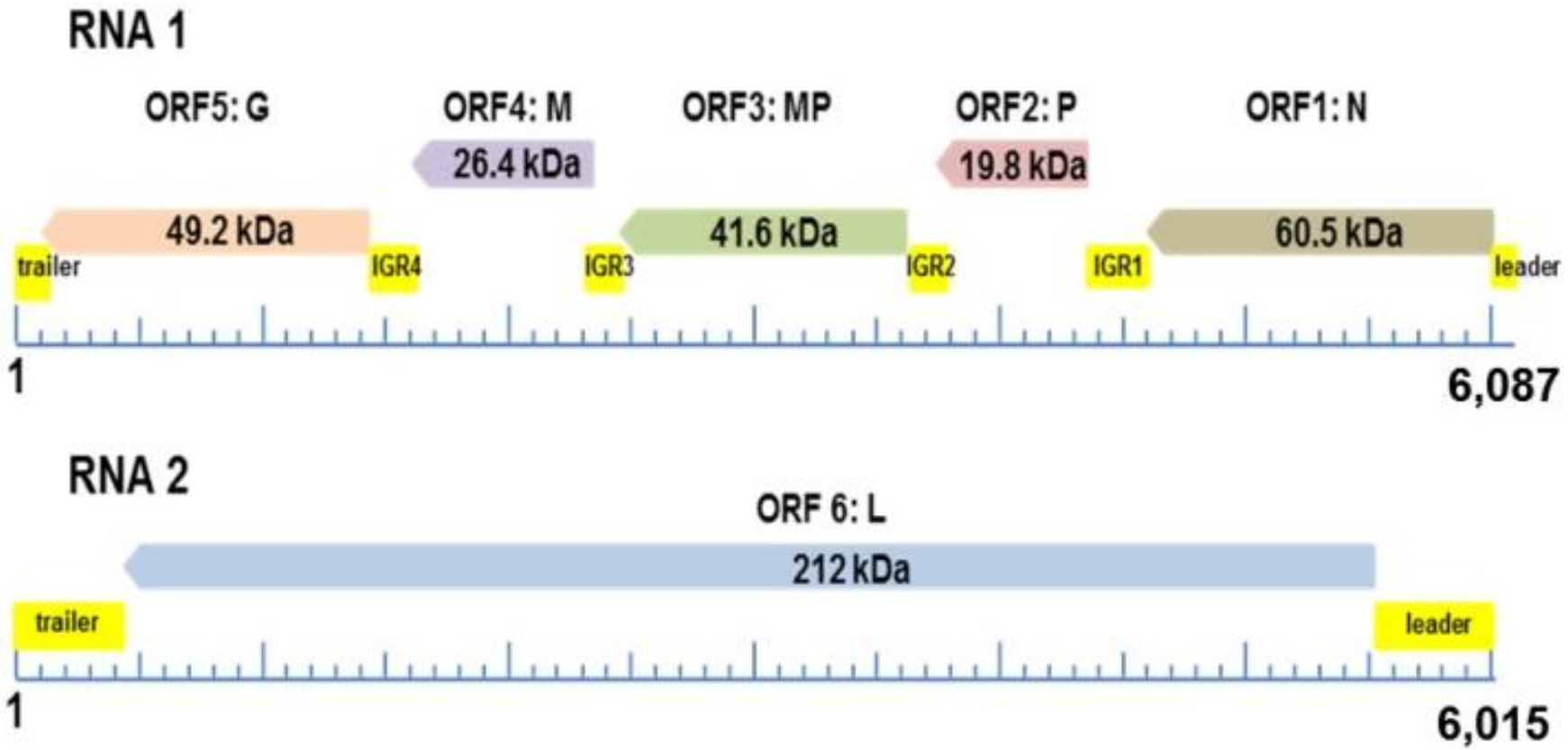
- **Leprosis** / *Brevipalpus spp.*
- **Tristeza de los cítricos** / *Toxoptera citricida*
- **HLB** / *Diaphorina citri*

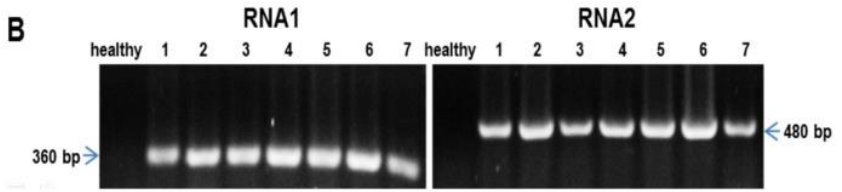
Citrus leprosis



Cruz-Jaramillo et al., 2014; SENASICA. 2019. Leprosis de los cítricos/

Genoma de CNSV

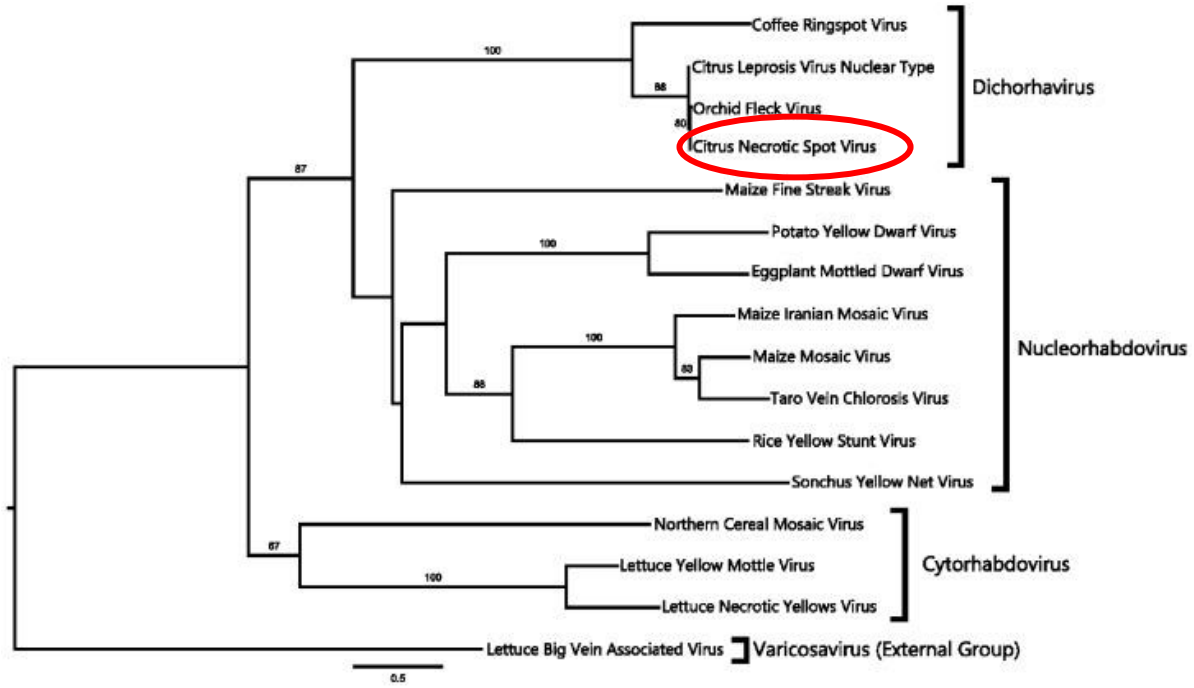




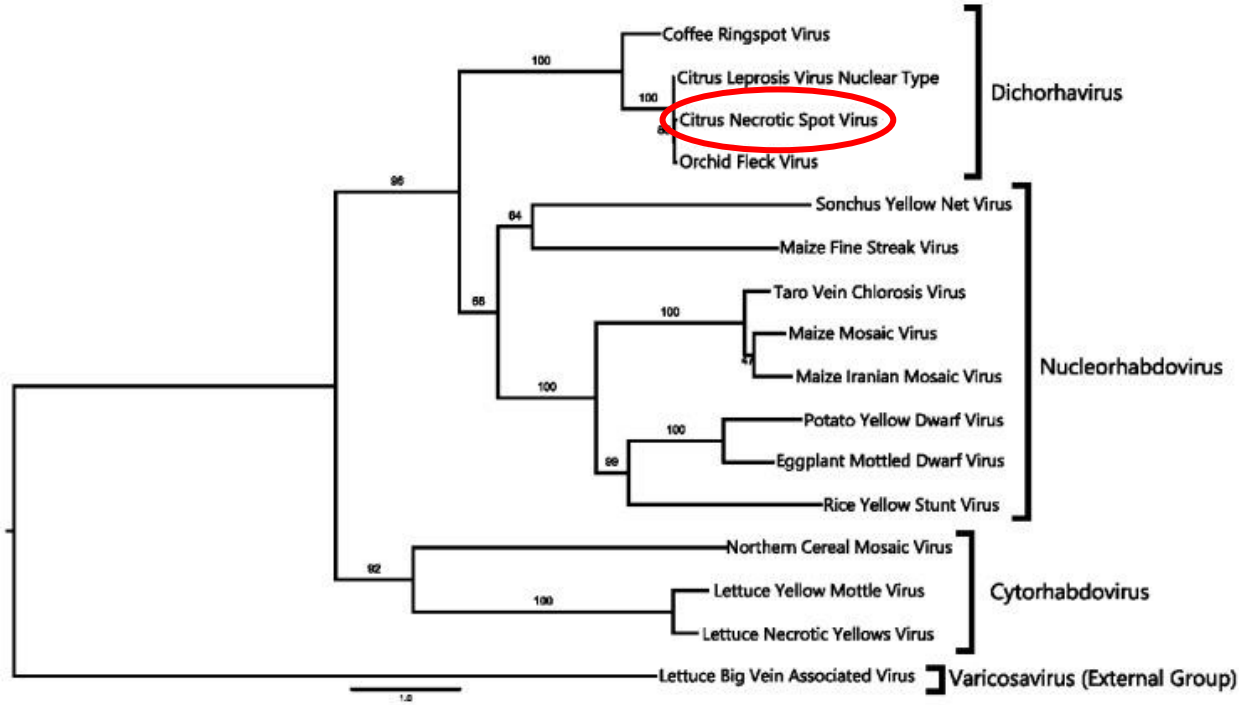
Cruz-Jaramillo et al., 2014.

Filogenia de CNSV

Nucleocápside



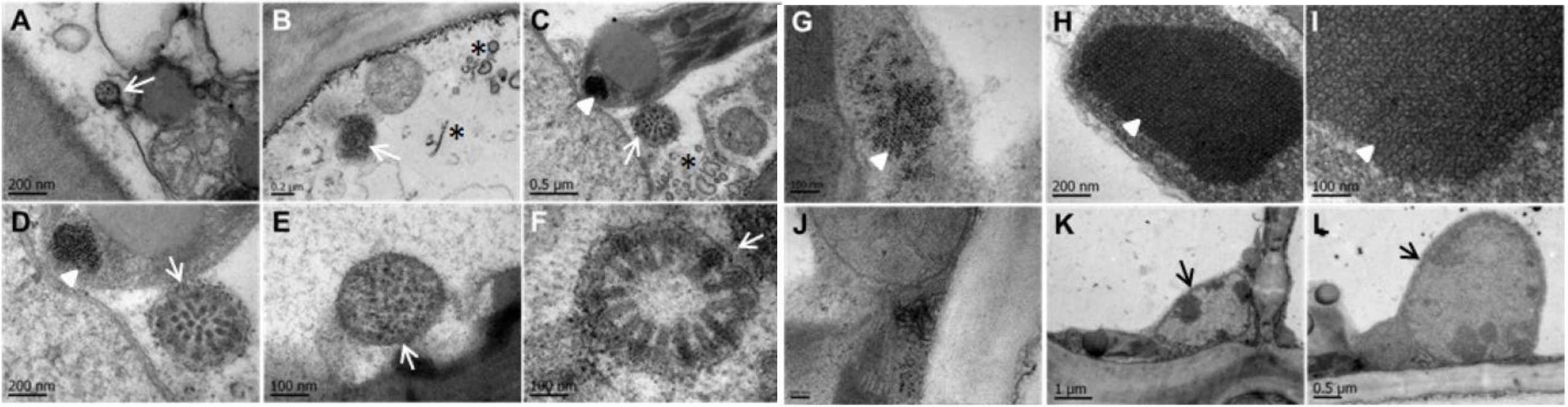
RNA Polimerasa RNA-dependiente



Cruz-Jaramillo et al., 2014.

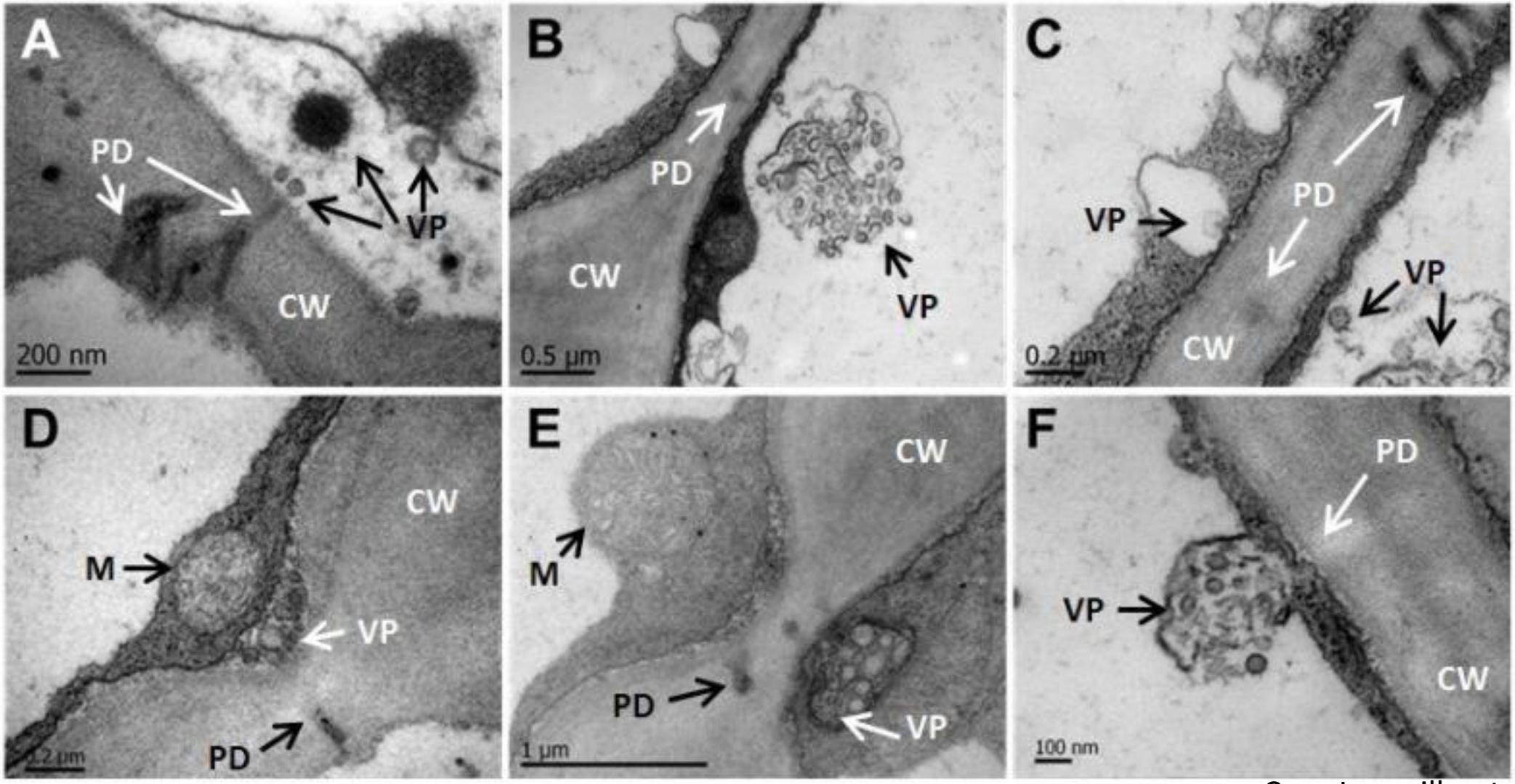
Cinvestav

Los viriones están en el citoplasma de células infectadas



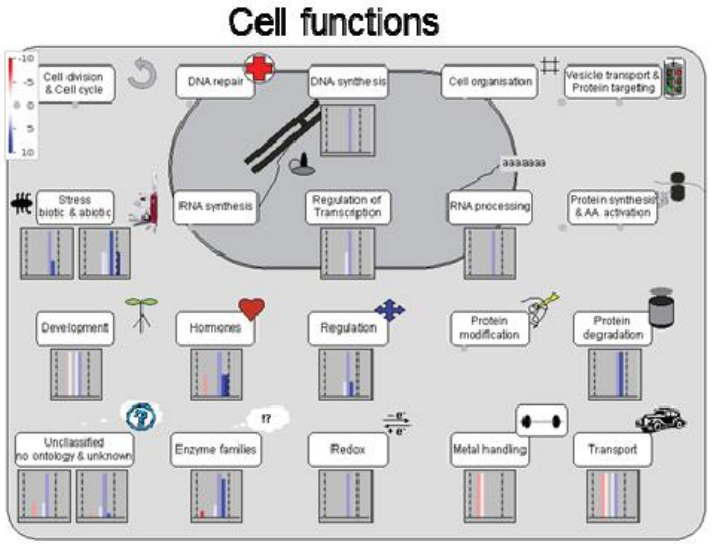
Cruz-Jaramillo et al., 2014.

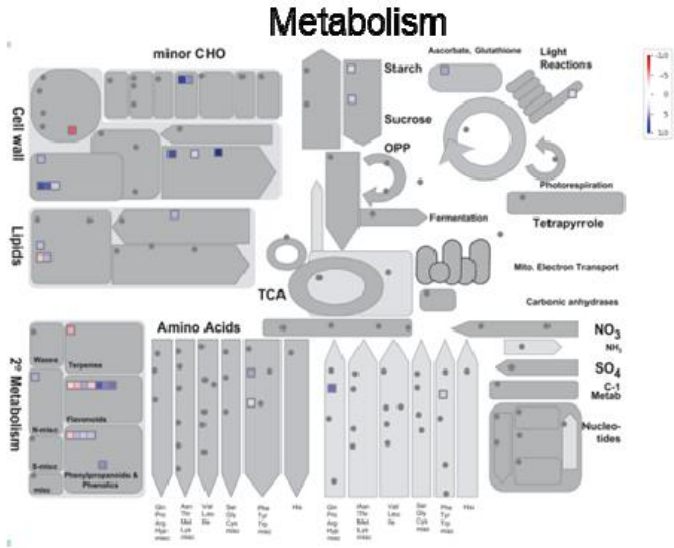
CNSV interactúa con plasmodesmos

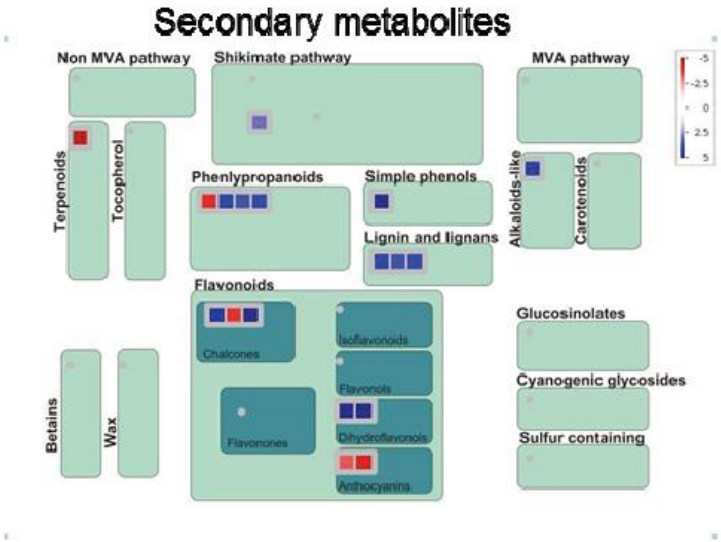


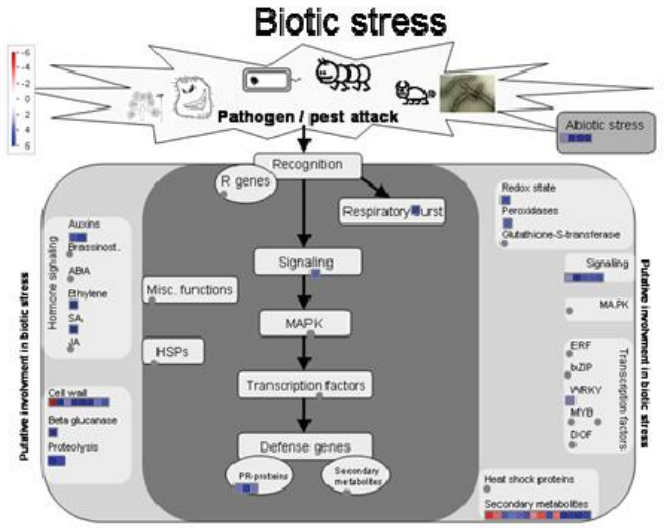
Cruz-Jaramillo *et al.,* 2014.

CNSV induce la síntesis de metabolitos secundarios



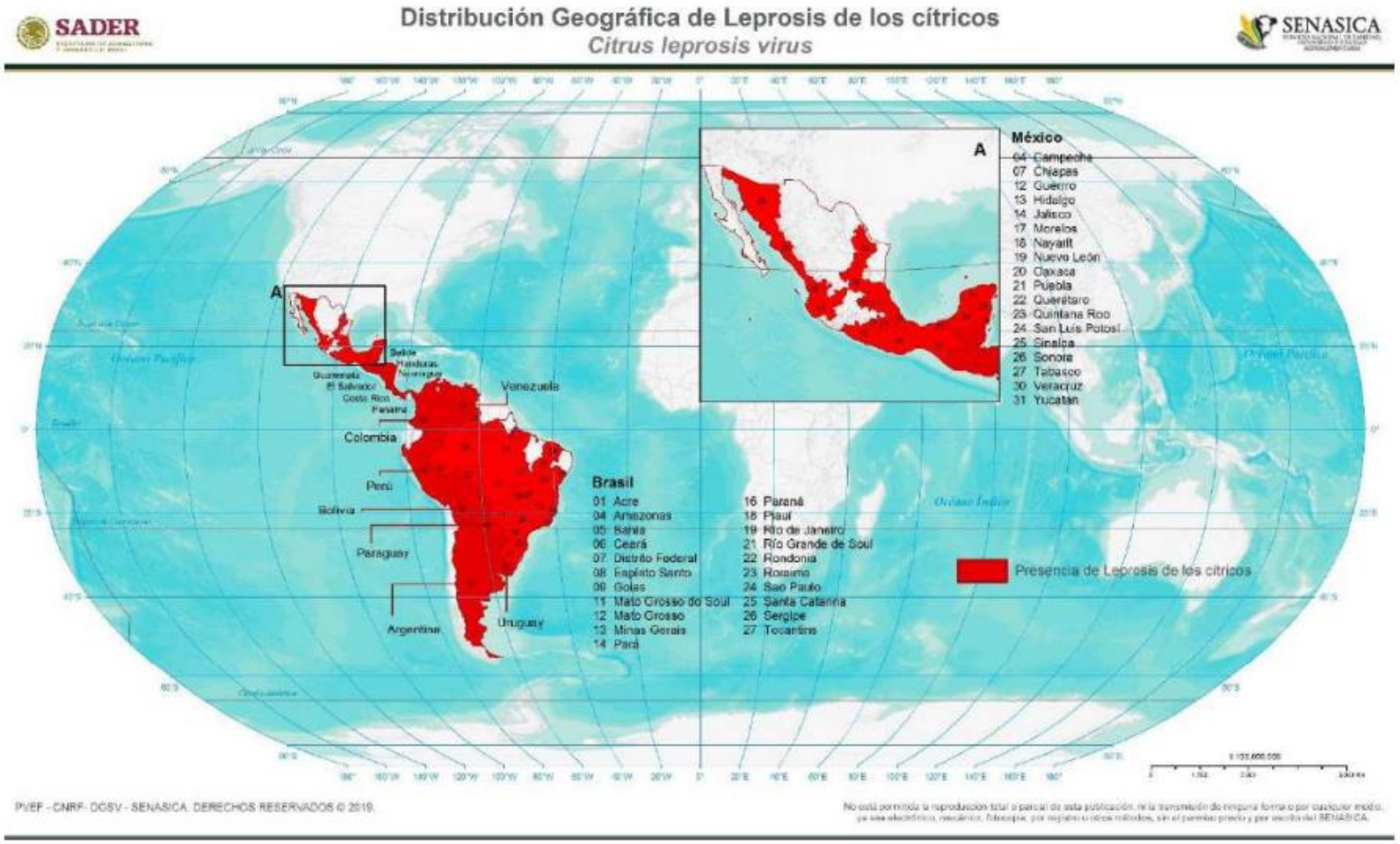






Cruz-Jaramillo *et al.*, 2014, Ramírez-Pool, 2022.

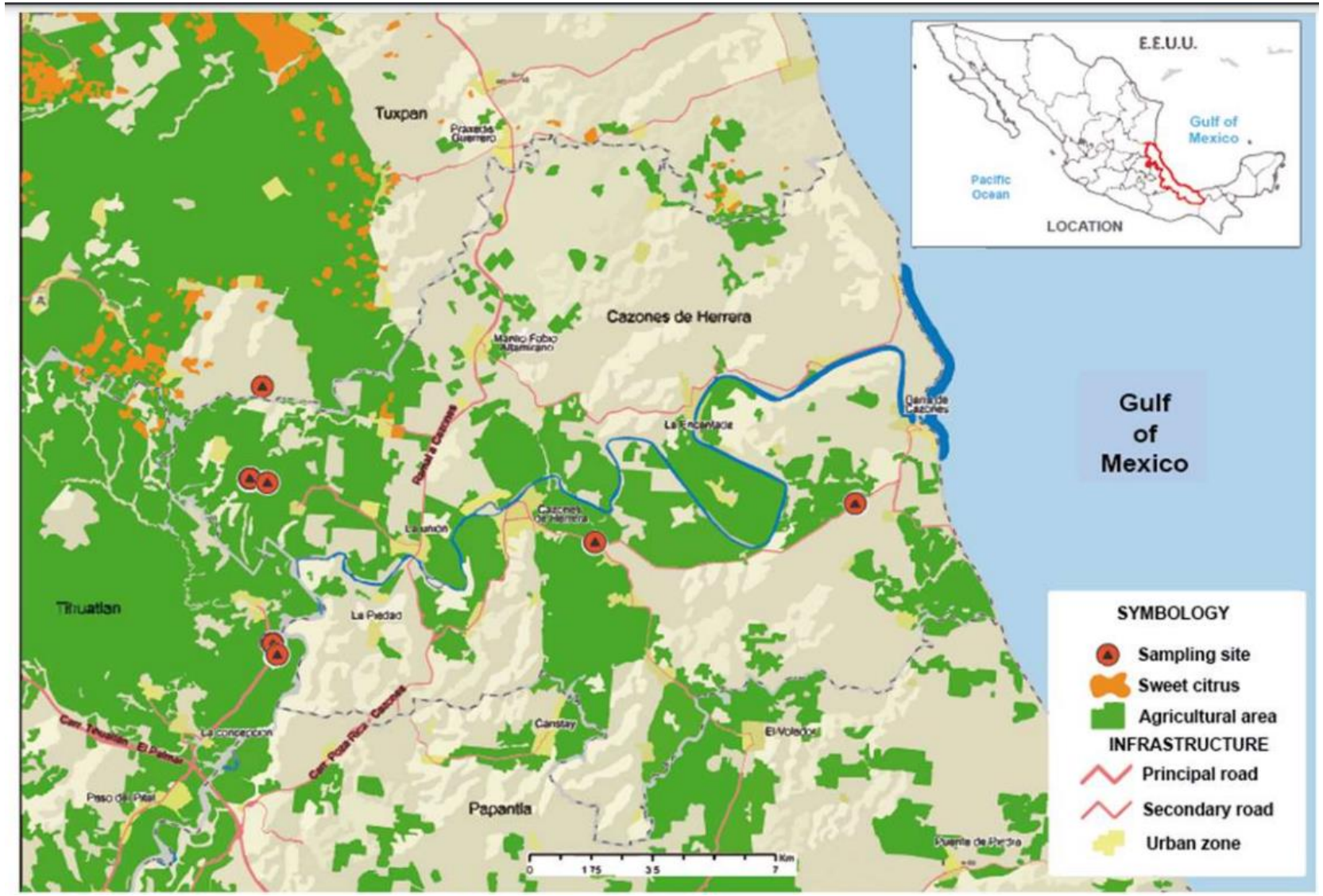
SENASICA monitorea al vector y plantas sintomáticas



Cruz-Jaramillo et al., 2014; SENASICA. 2019. Leprosis de los cítricos/

Cinvestav

Virus de la Tristeza de los cítricos

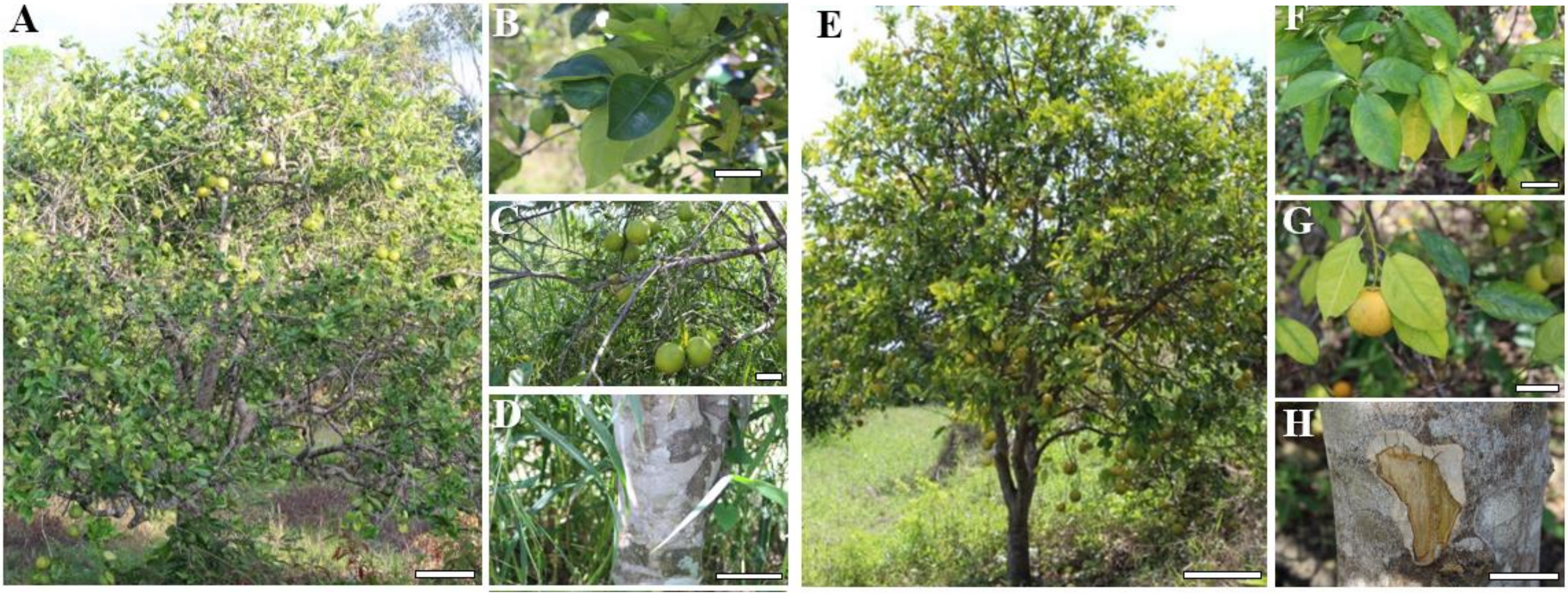


Ramírez-Pool et al., 2021, 2022.

Naranja dulce infectada con CTV en Veracruz, México.

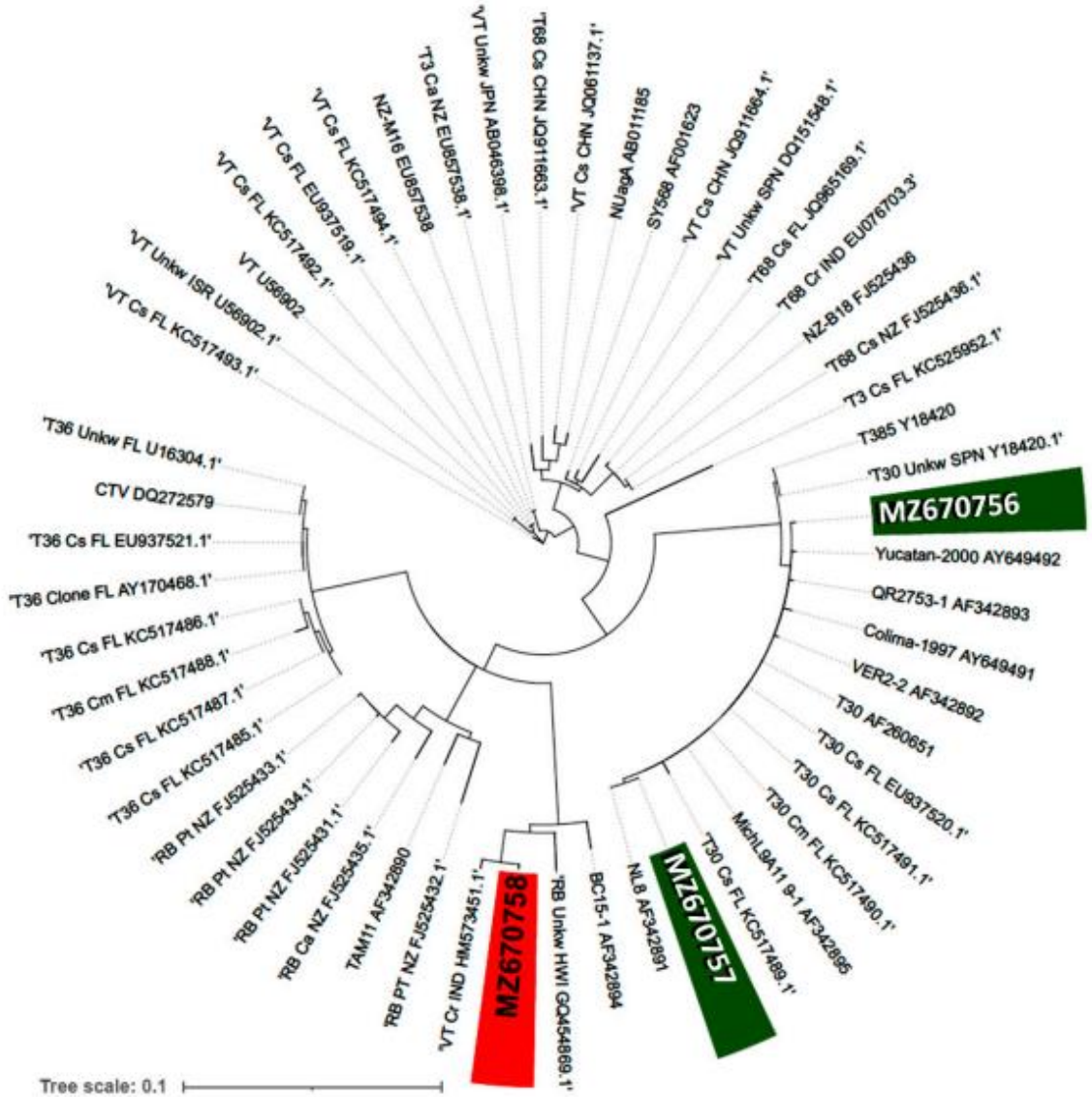
CTV atenuada

CTV severa



Ramírez-Pool et al., 2021, 2022.

Filogenia de aislados de CTV basados en CP



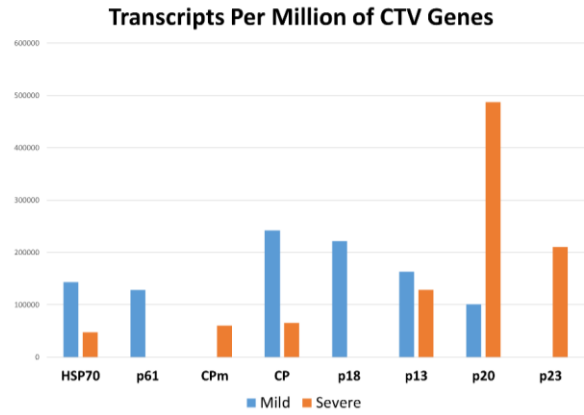
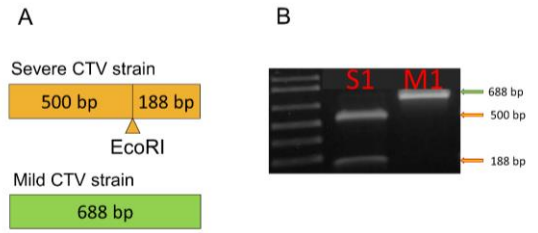


Figure S5. Comparison of viral ORF transcripts per million reads obtained in plants infected with either mild (blue) or severe (orange) CTV strains. Gene description: HSP70H, Hsp70-homolog; p61, 61-kDa protein; CPM, minor capsid protein; CP, major capsid protein; p18, 18-kDa protein; p13, 13-kDa protein; p20, 20-kDa protein/RNA silencing suppressor; p23, RNA silencing suppressor.

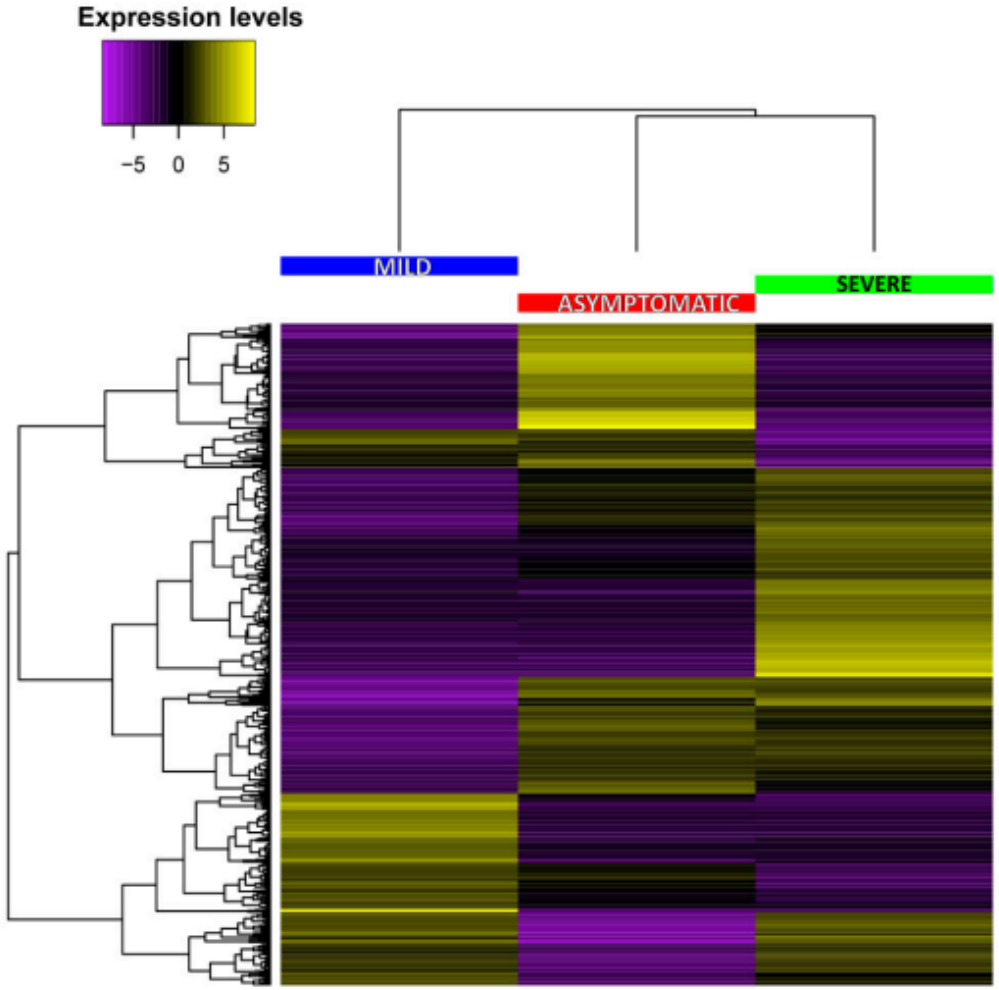


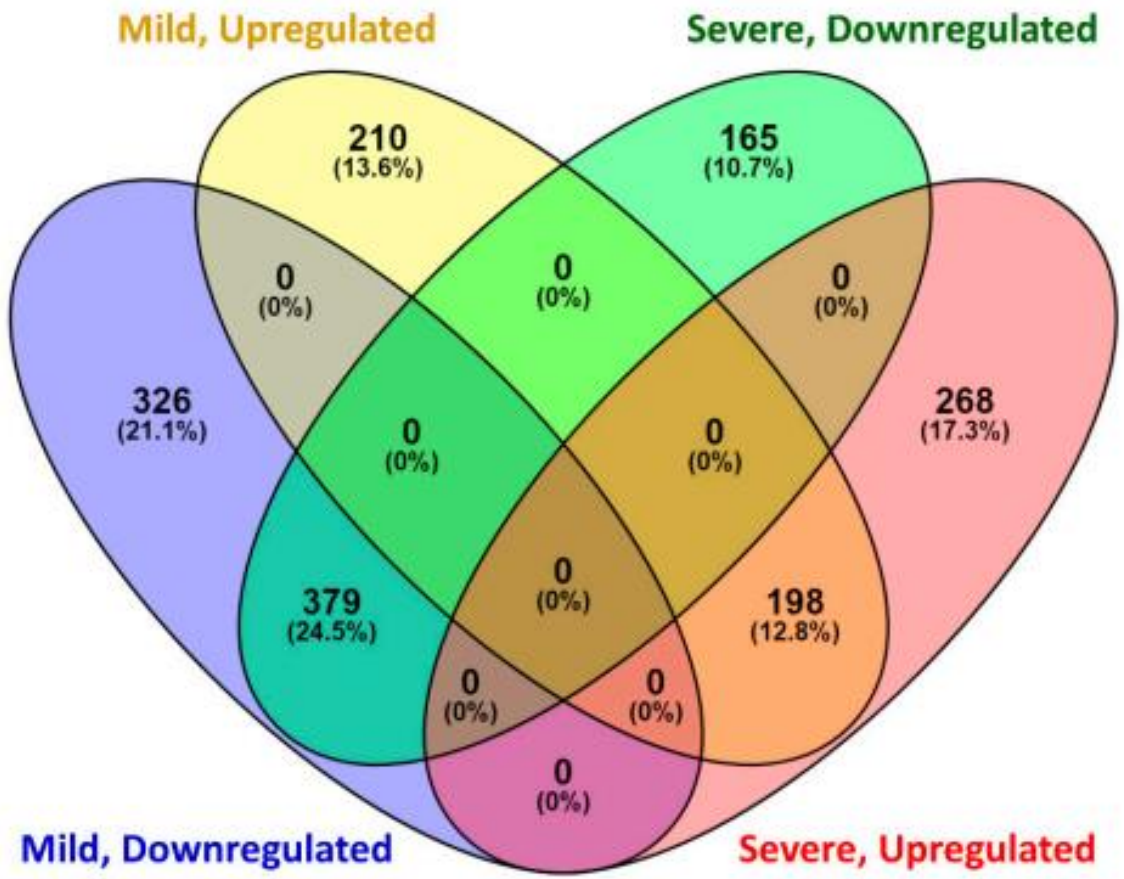
Identification of severe or mild CTV strain by digestion with EcoRI restriction enzyme.

Ramírez-Pool et al., 2021, 2022.

Cinvestav

Expresión de genes en *C. sinensis con* CTV

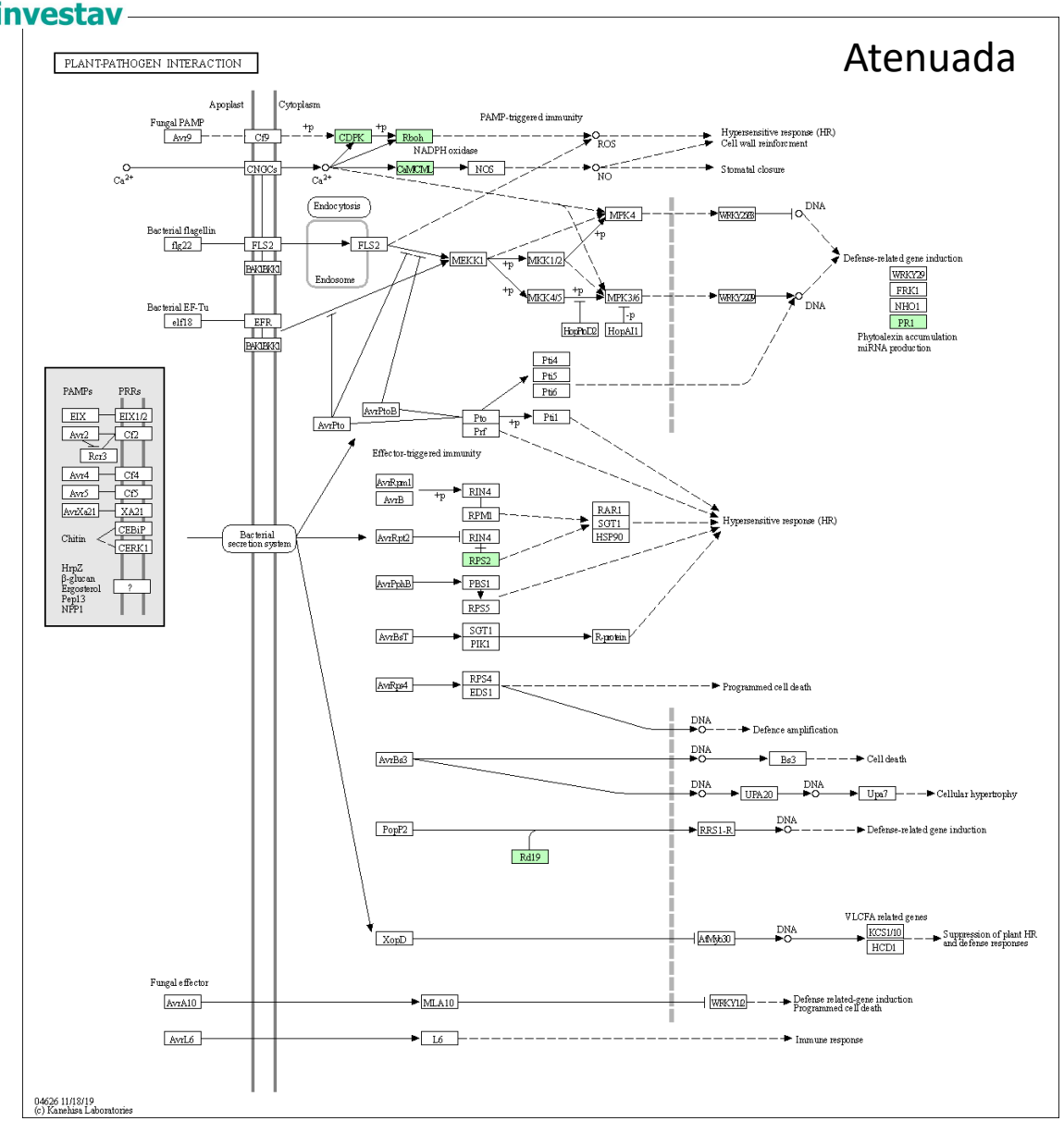


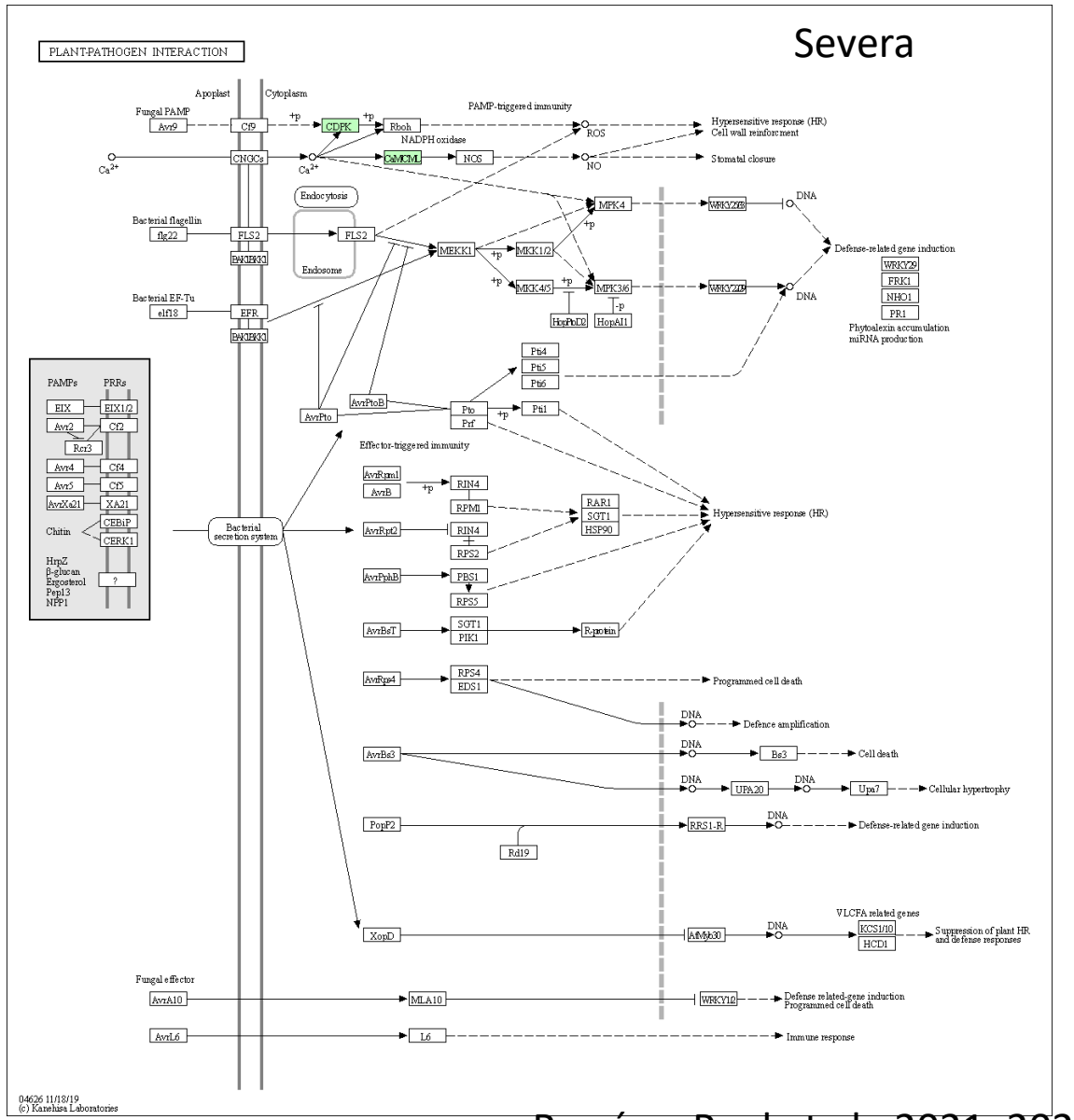


Ramírez-Pool et al., 2021, 2022.

Transcritos sobreregulados en la interacción planta-patógeno

Cinvestav





Annotation was calculated with KEGG Blast Koala

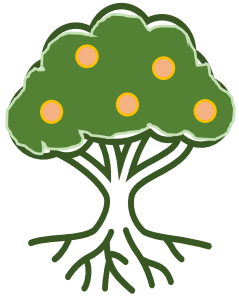
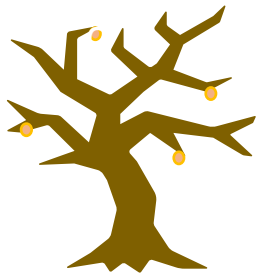
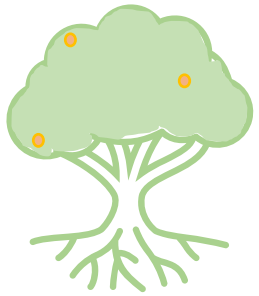
Ramírez-Pool et al., 2021, 2022.

Presencia de los viroides Exocortis y Hop stunt

Table 1. Number of reads of *Citrus exocortis viroid* and *Hop stunt viroid* in asymptomatic and CTV-infected orange trees.

Condition	TPM *	FPKM **
	Citrus exocortis viroid	
Asymptomatic	1,000,000	4,528,301.89
CTV severe isolate	1,000,000	4,244,325.52
CTV mild isolate	0	0
	Hop stunt viroid	
Asymptomatic	1,000,000	5,813,953.49
CTV severe isolate	1,000,000	5,639,097.74
CTV mild isolate	0	0

* The normalized expression profile is shown as TPM (transcripts per million). ** FPKM: fragments per kilobases of contigs per million mapped reads.

Asymptomatic	Severe	Mild
		
------	CTV severe	CTV mild
CEVd	CEVd	-----
HSVd	HSVd	-----

Ramírez-Pool et al., 2021, 2022.

Cítricos produciendo antimicrobianos para mitigar HLB

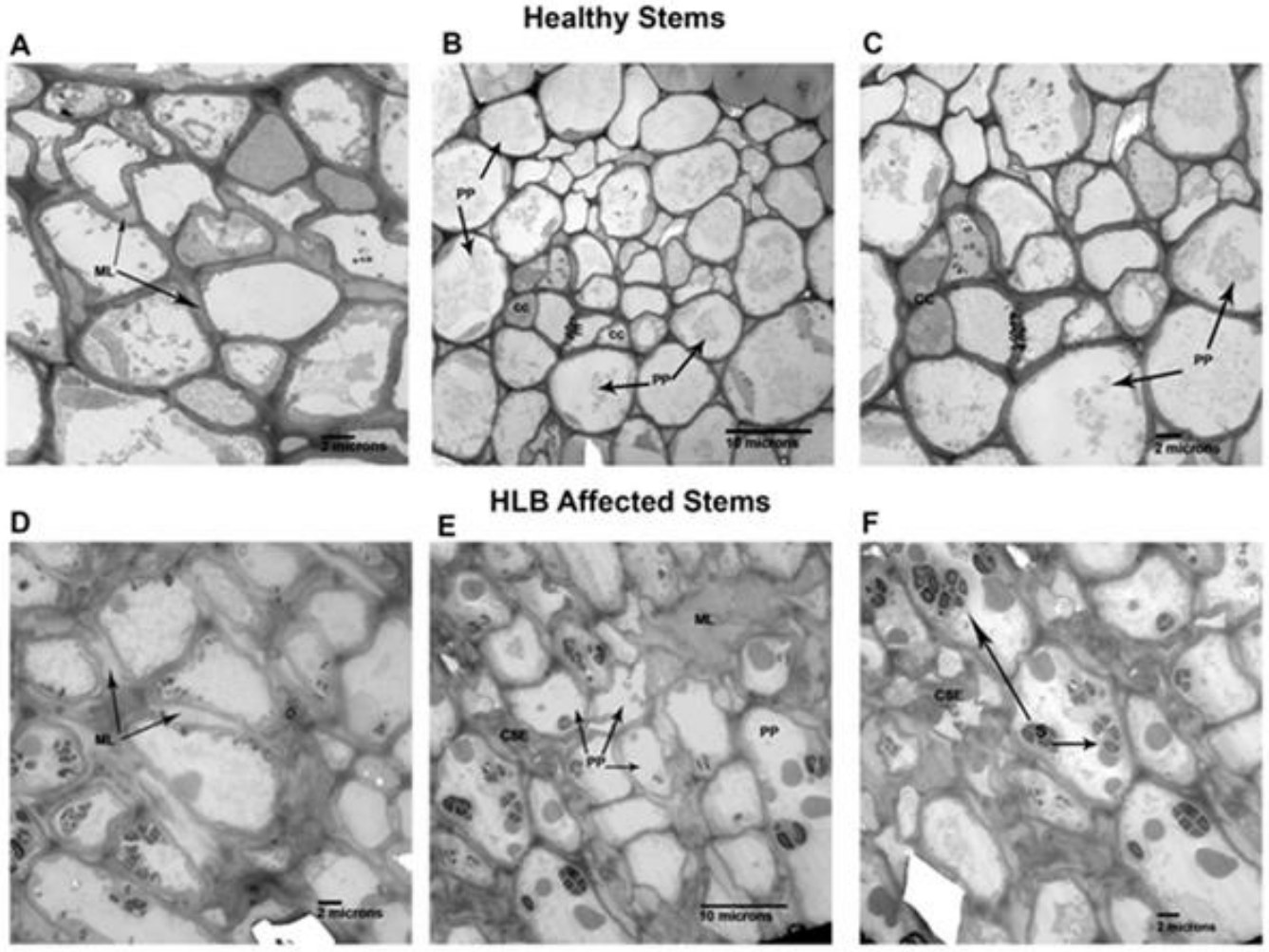
Cinvestav

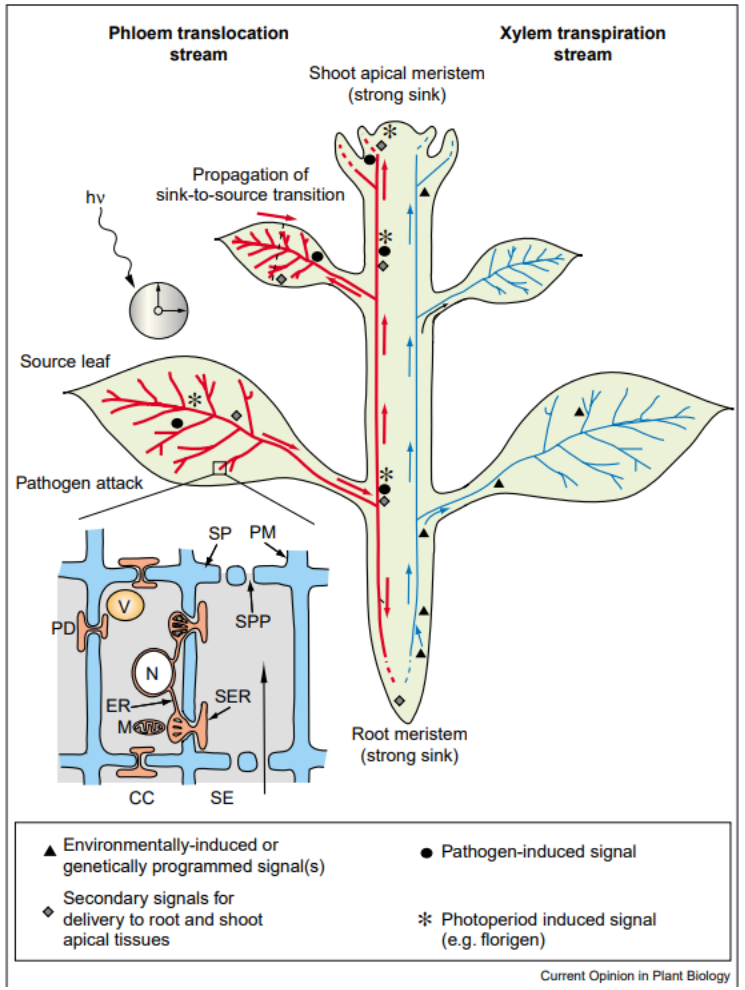
Cinvestav

HLB

www.senasica.gob.mx

Candidatus Liberibacter asiaticus está en tejido vacular.

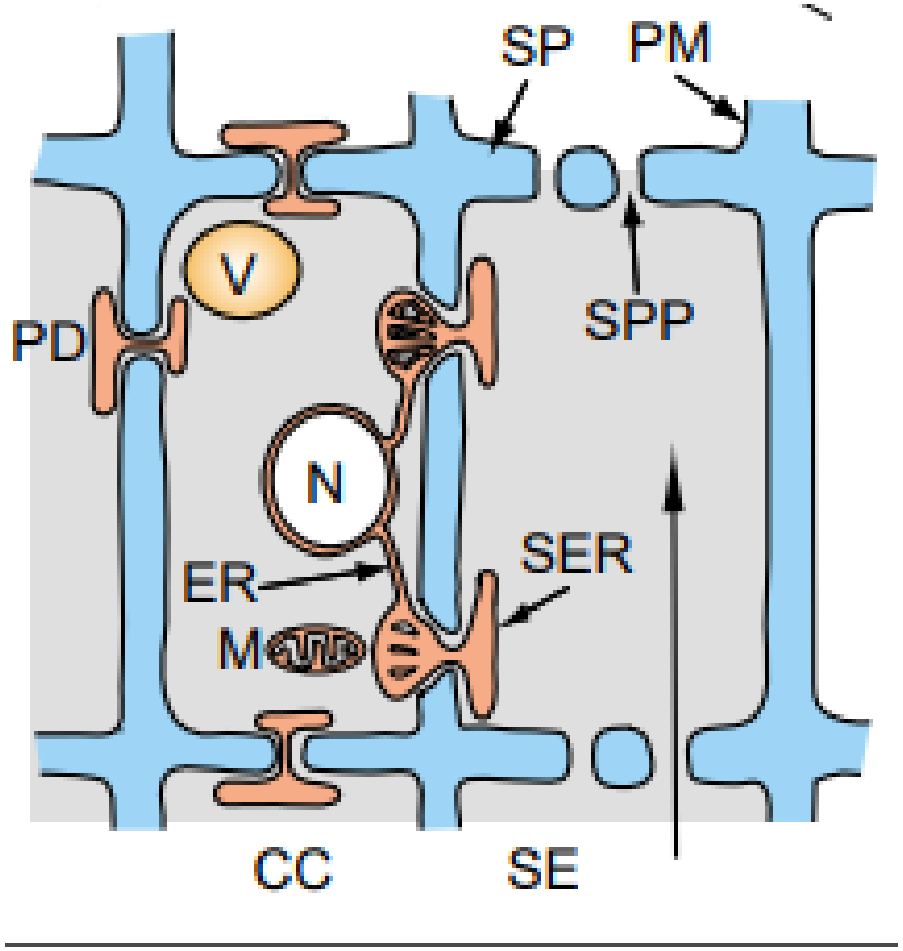


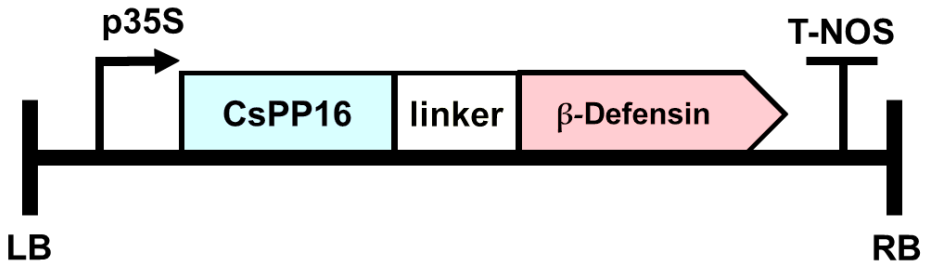


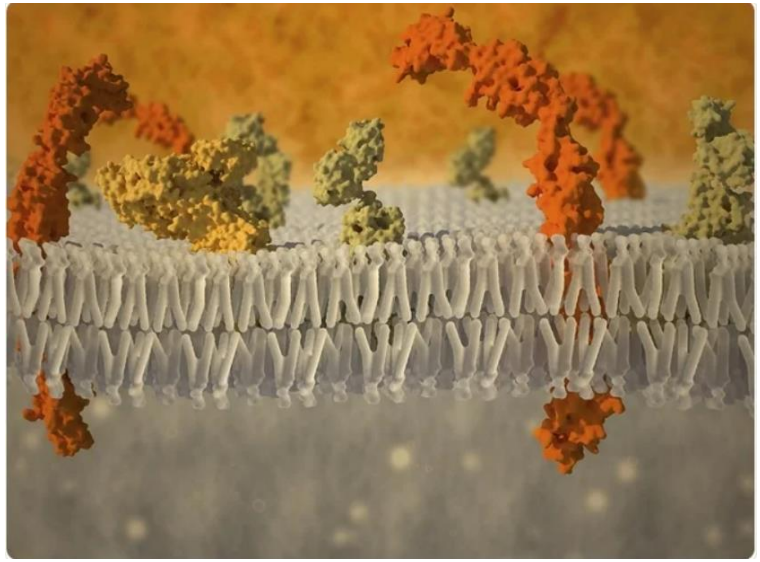
Aritua V, Achor D, Gmitter FG, Albrigo G, Wang N (2013) Transcriptional and Microscopic Analyses of Citrus Stem and Root Responses to Candidatus Liberibacter asiaticus Infection. PLOS ONE 8(9): e73742. https://doi.org/10.1371/journal.pone.0073742
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0073742

Ruiz-Medrano et al., 2005, 2009, 2015.

AMPs no se detectan en el proteoma de la savia del floema en cucurbitáceas

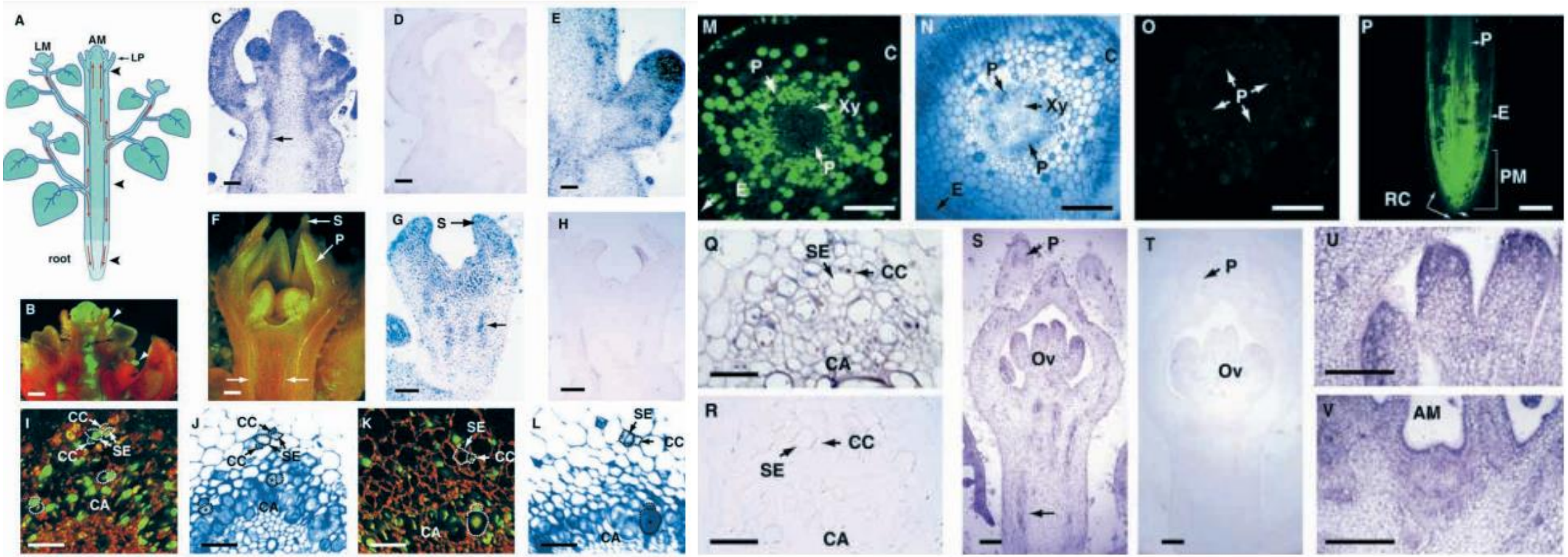






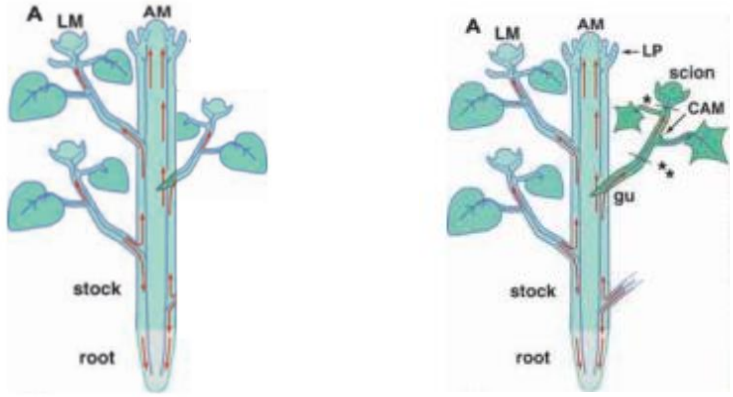
Ham et al., 2011; Ruiz-Medrano et al., 2005; www.news-medical.net/

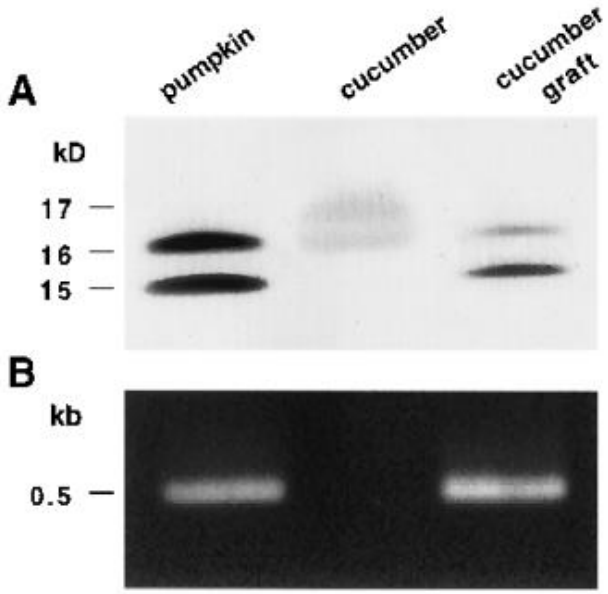
Las proteínas supracelulares viajan a tejidos distantes a cumplir con su función

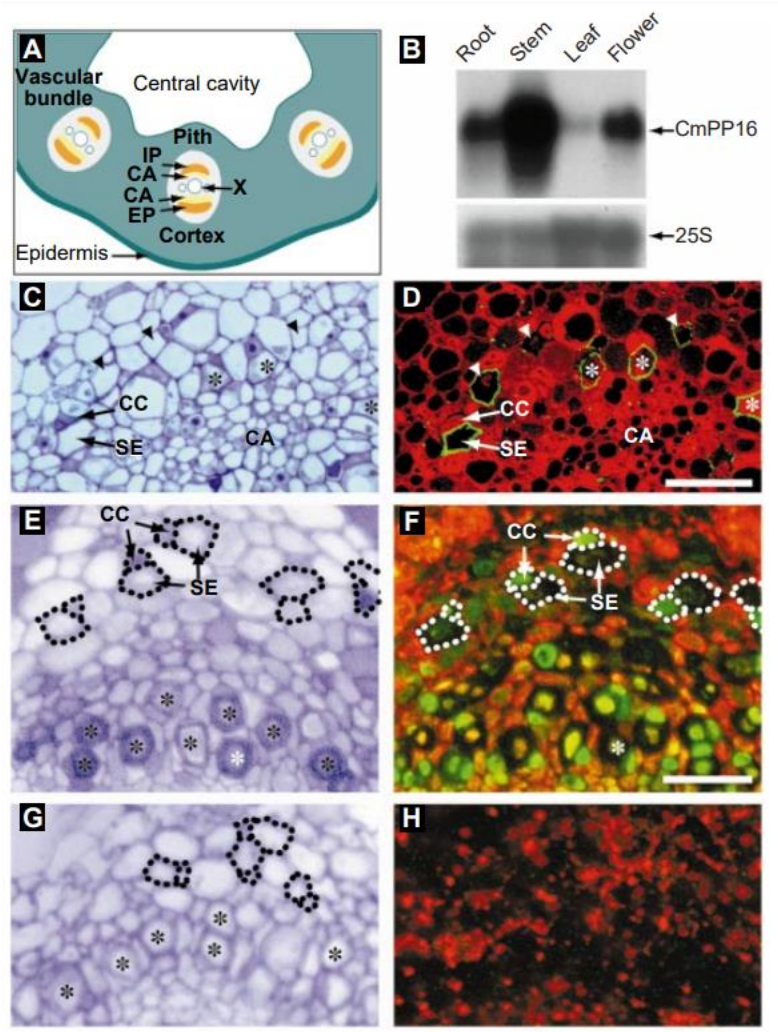


Lucas et al, 1997; Xoconostle-Cazares et al., 1999, 2002; Ruiz Medrano et al., 2000, 2005, 2010, Toscano-Morales et al., 2015. 2016, 2017; Montero-Tavera et al., 2008; Ramírez-Ortega et al., 2016, 2017.

CmPP16 es una proteína supracelular en la savia del floema

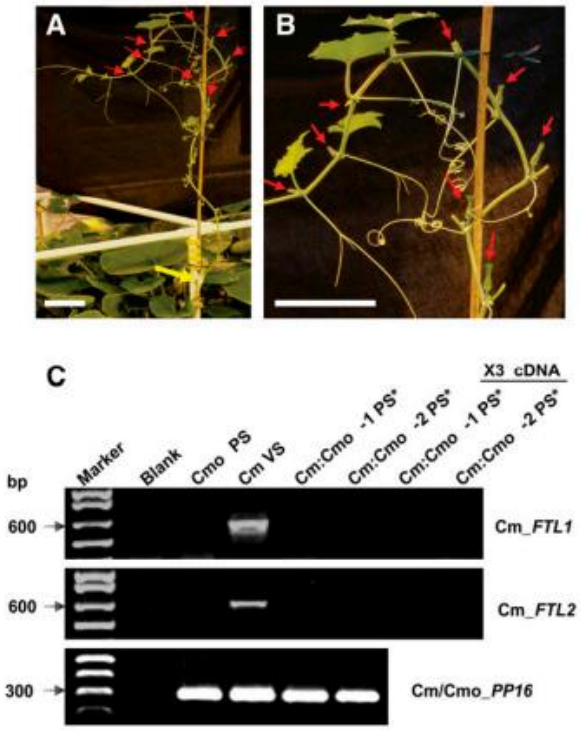


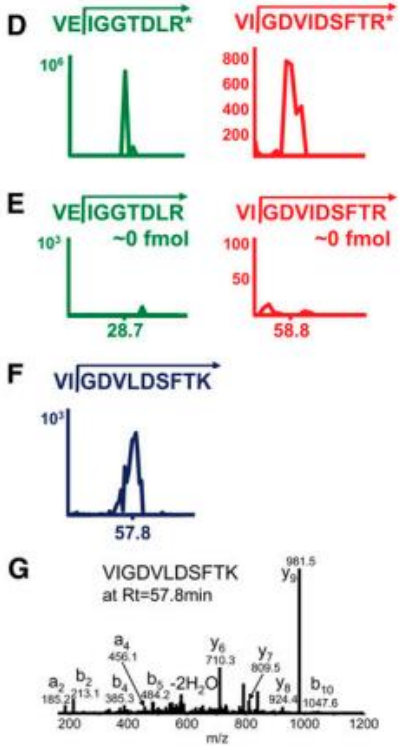


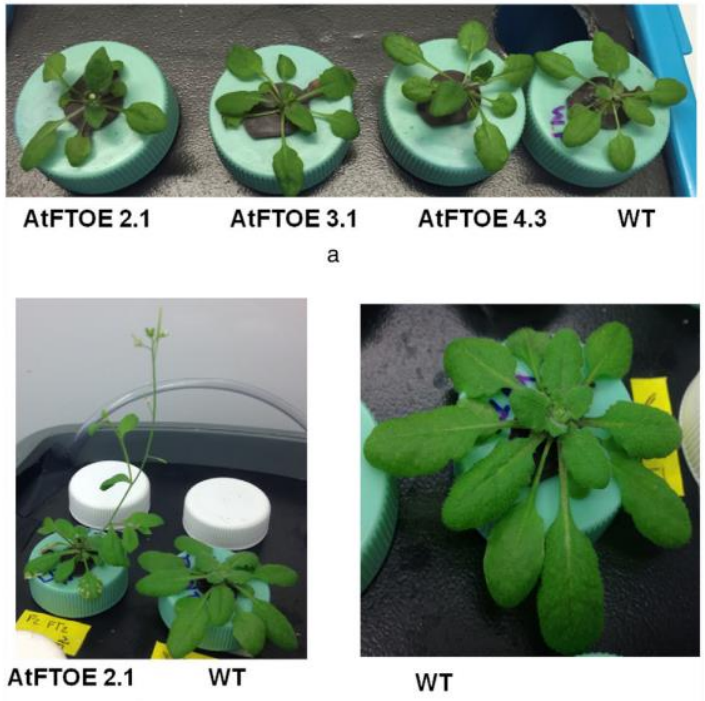


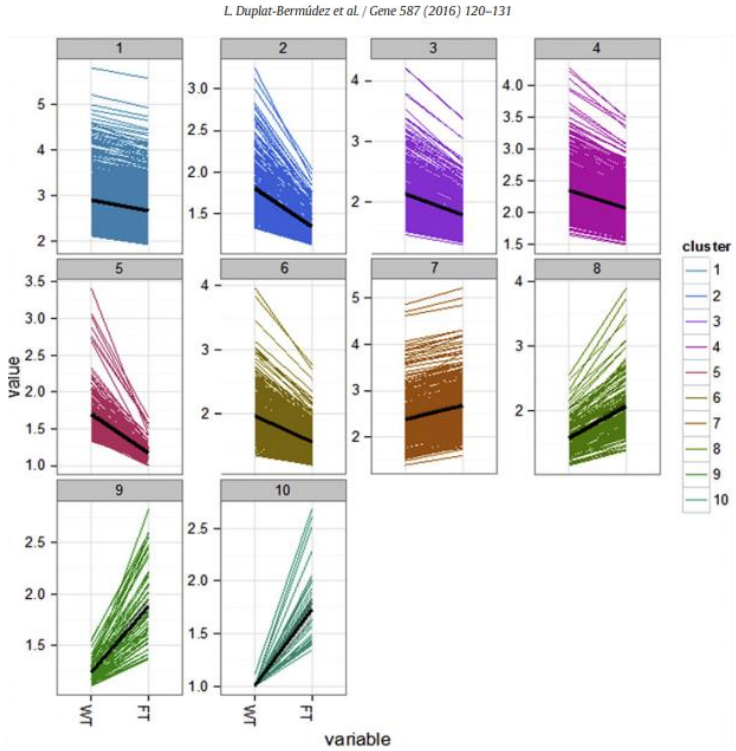
Xoconostle-Cazares et al., 1999, 2002; Ruiz Medrano et al., 2000, 2005, 2010

Otras proteínas supracelulares inducen floración



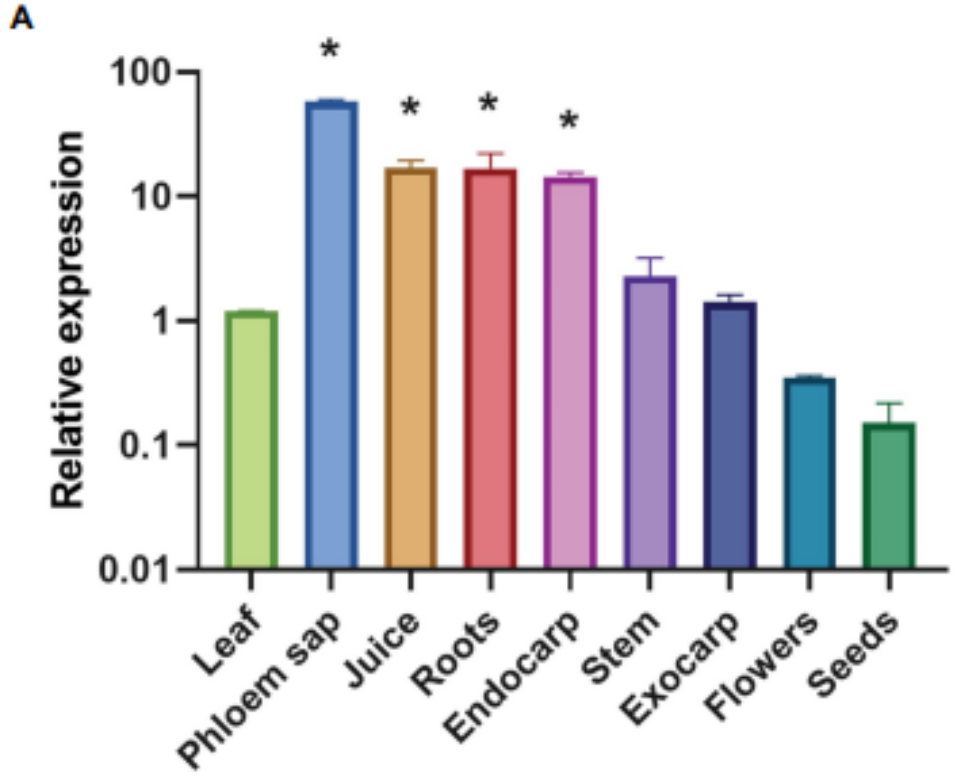


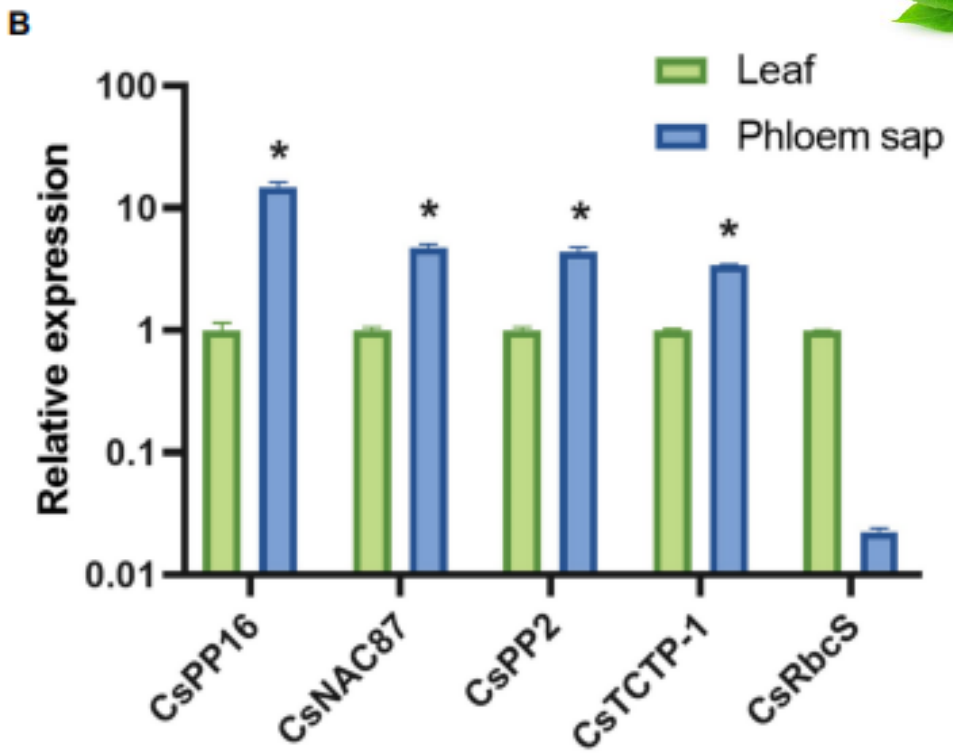




Lin et al. , 2008: Duplat et al., 2016.

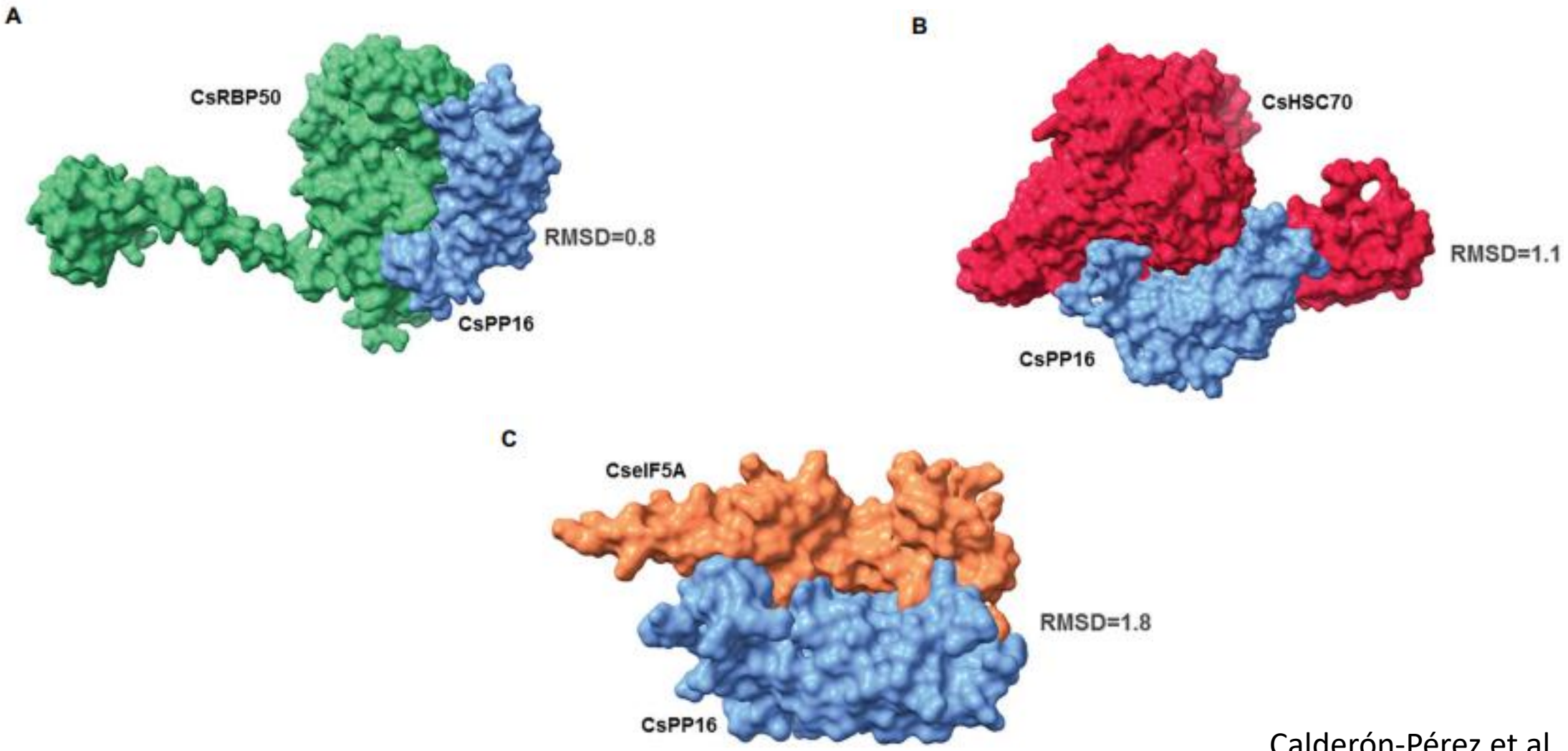
CsPP16 mRNA se acumulan en la savia del floema





Calderón-Pérez et al., 2022; Jiménez-López et al., 2022.

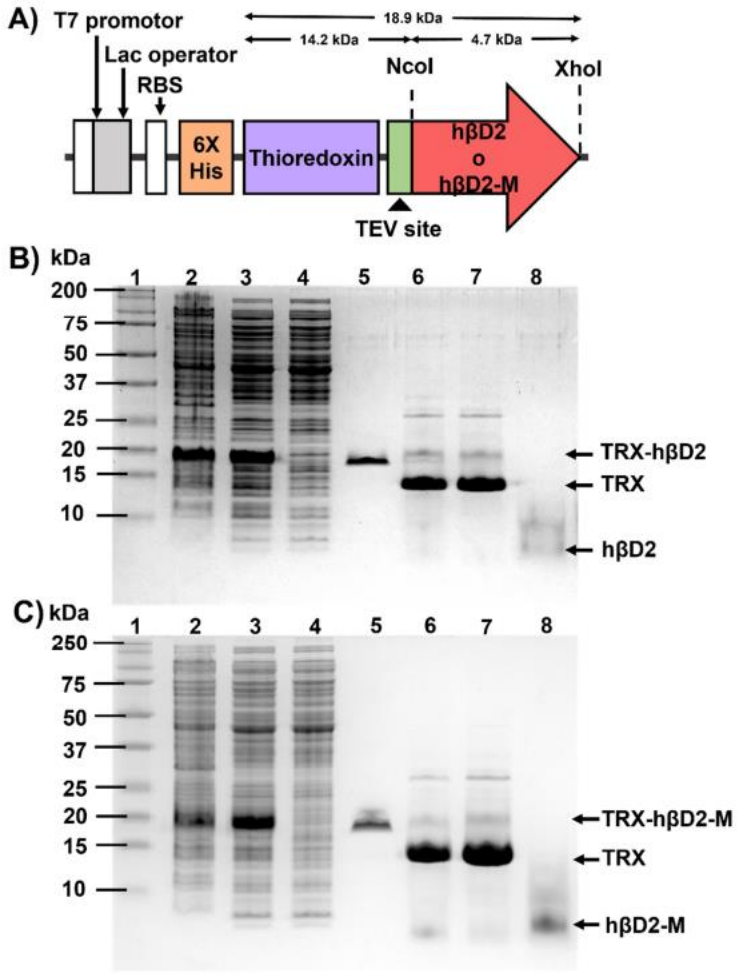
CsPP16 interactúa con proteínas supracelulares

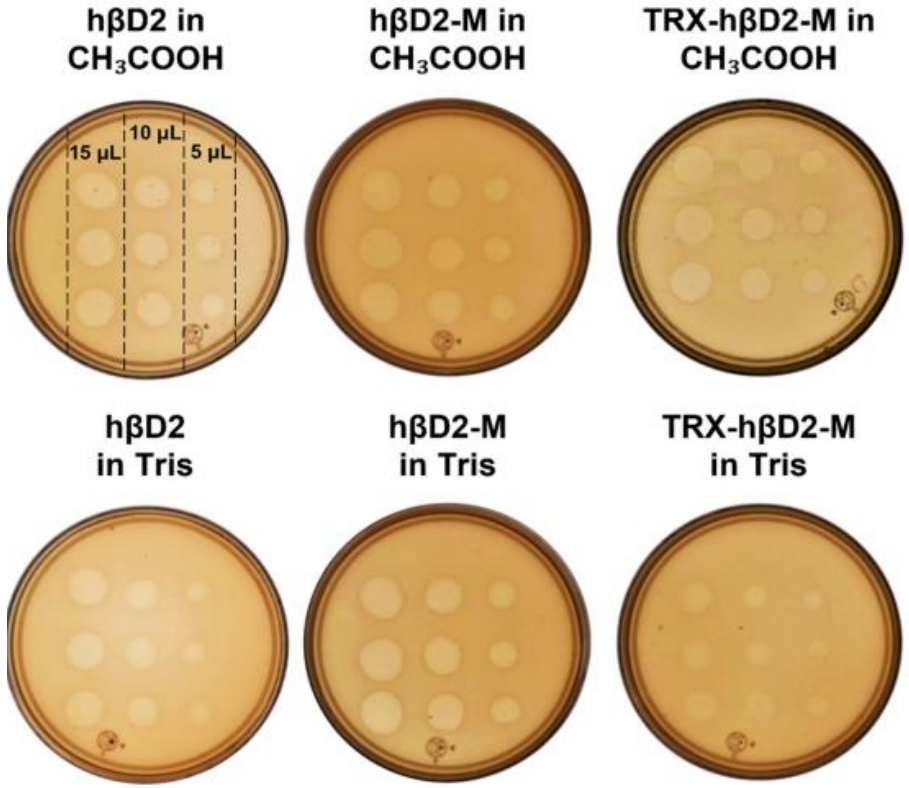


Calderón-Pérez et al., 2022

Proteínas antimicrobianas

hβD2, hβD2-M and Trx- hβD2-M



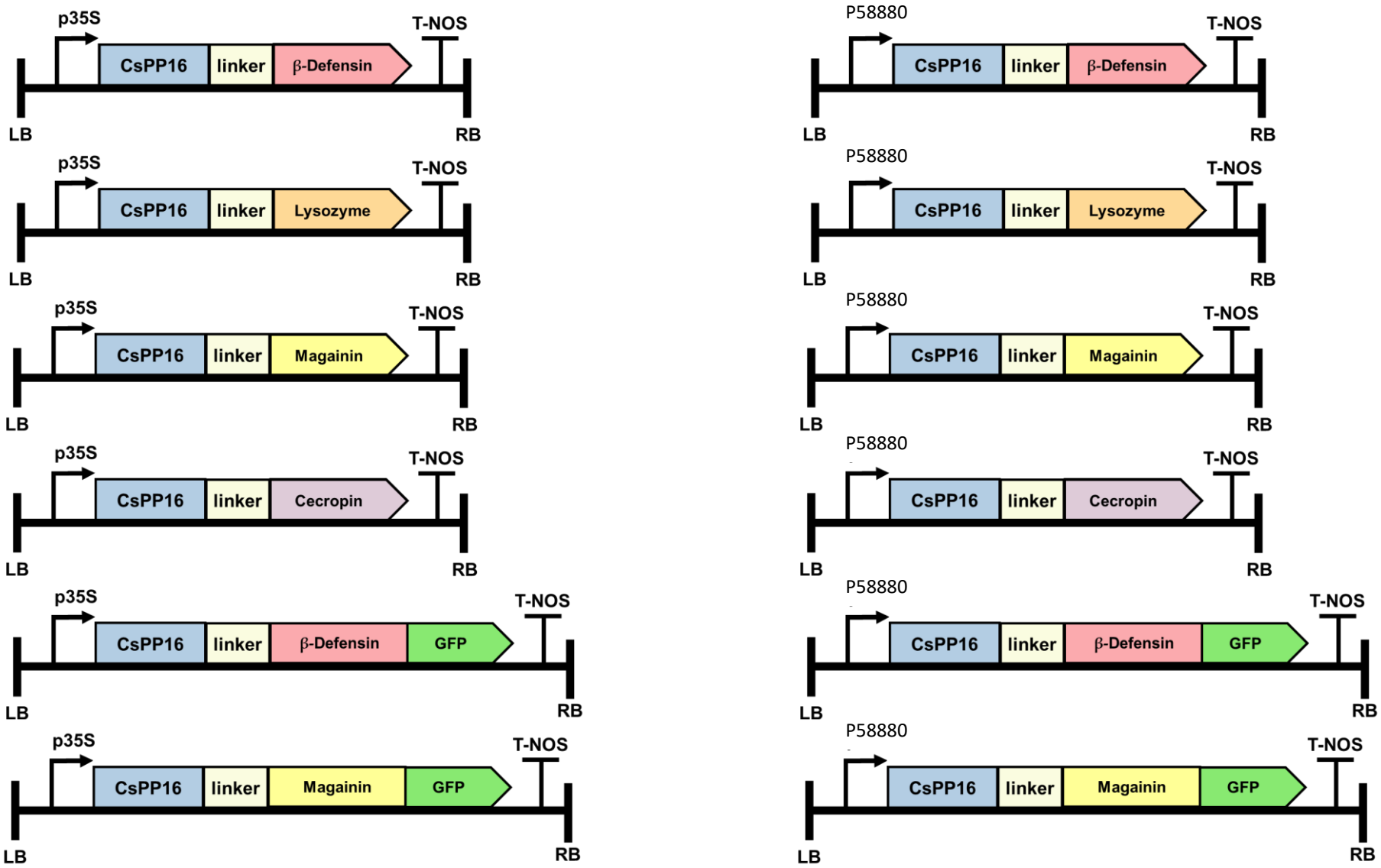


igure 2. Antimicrobial activity of AMPs by the plaque diffusion method. Different volumes of the ample of interest were deposited on each plate in triplicate at a concentration of 50 µM.

Clavibacter michiganensis michiganensis

Marcelino-Pérez et al., 2021

Genes sintéticos para movilizar proteínas al floema



Ruiz-Medrano et al., 2015; Calderón-Pérez et al., 2022

Métodos de transformación de cítricos

Biolistics

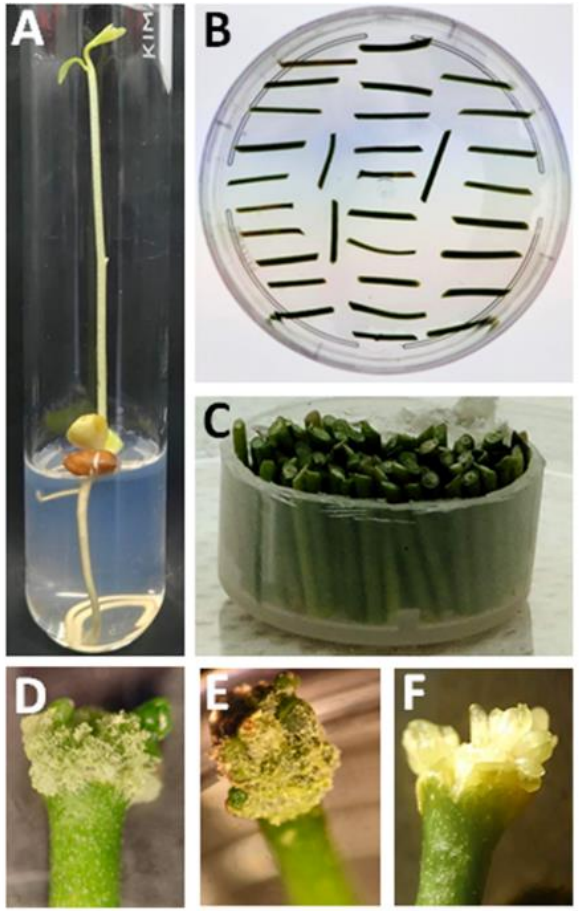


FIGURE 1 | Bombardment and regeneration of Citrus explants. (A) Germinated seedlings *in vitro*; (B) Cut of epicotyls. (C) Arrangement of ep... for bombardment. (D,E) Callus in apical regions of the explants produced in the dark after transformation. (F) Photosynthetic somatic embryo... dominance were produced after light exposure.

Agrobacterium

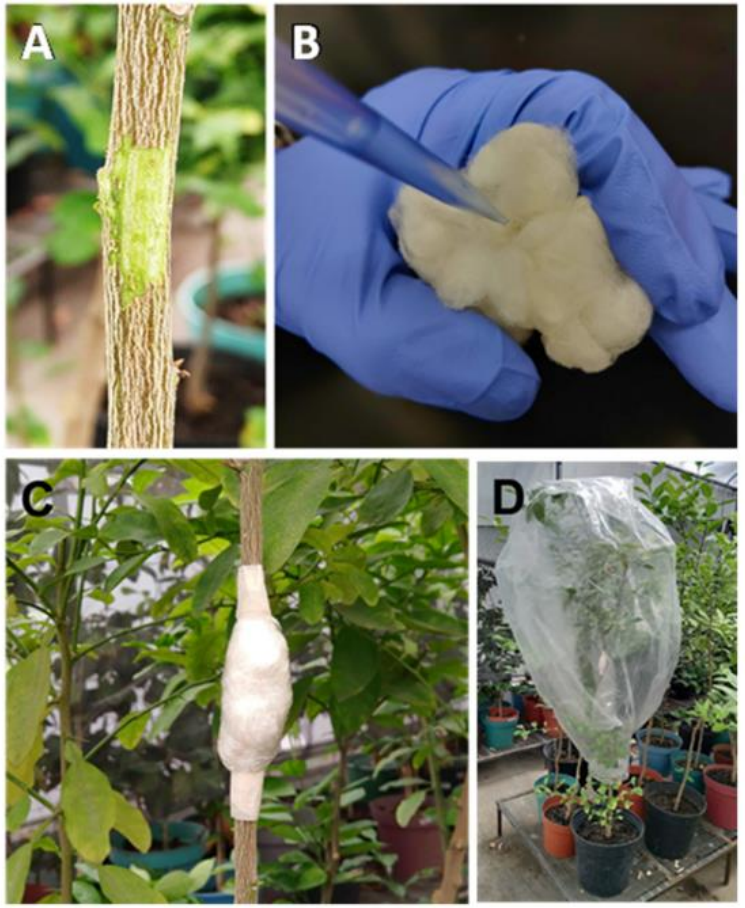
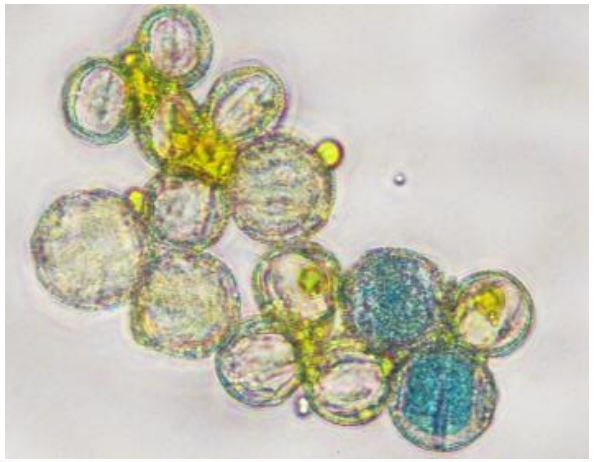
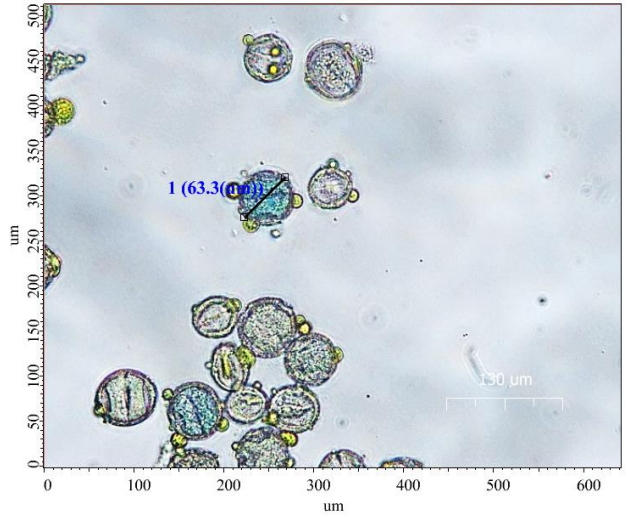


FIGURE 4 | Localized expression procedure. (A) Exposition of photosynthetic tissue by scraping made with a scalpel. (B) Soaking of a cotton swab with *Agrobacterium* culture. (C) Wrapping of plant tissue. (D) Treated plant, covered with plastic.

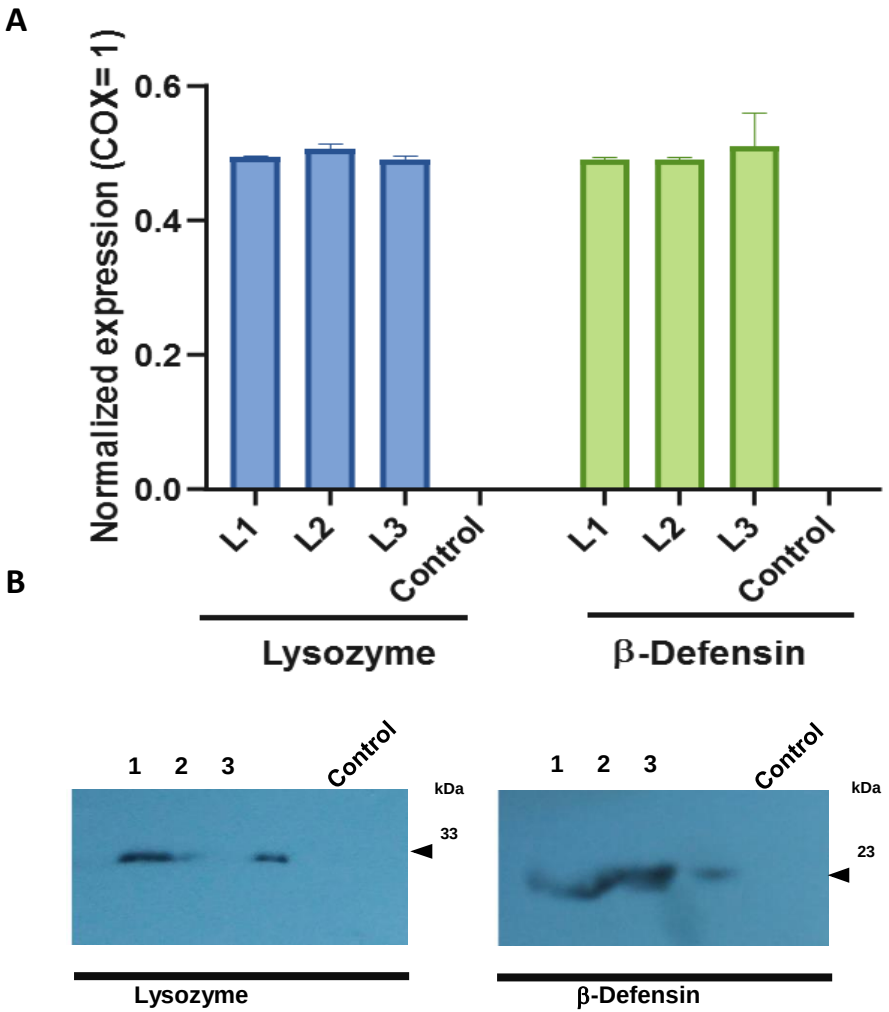
Pollen magnetofection

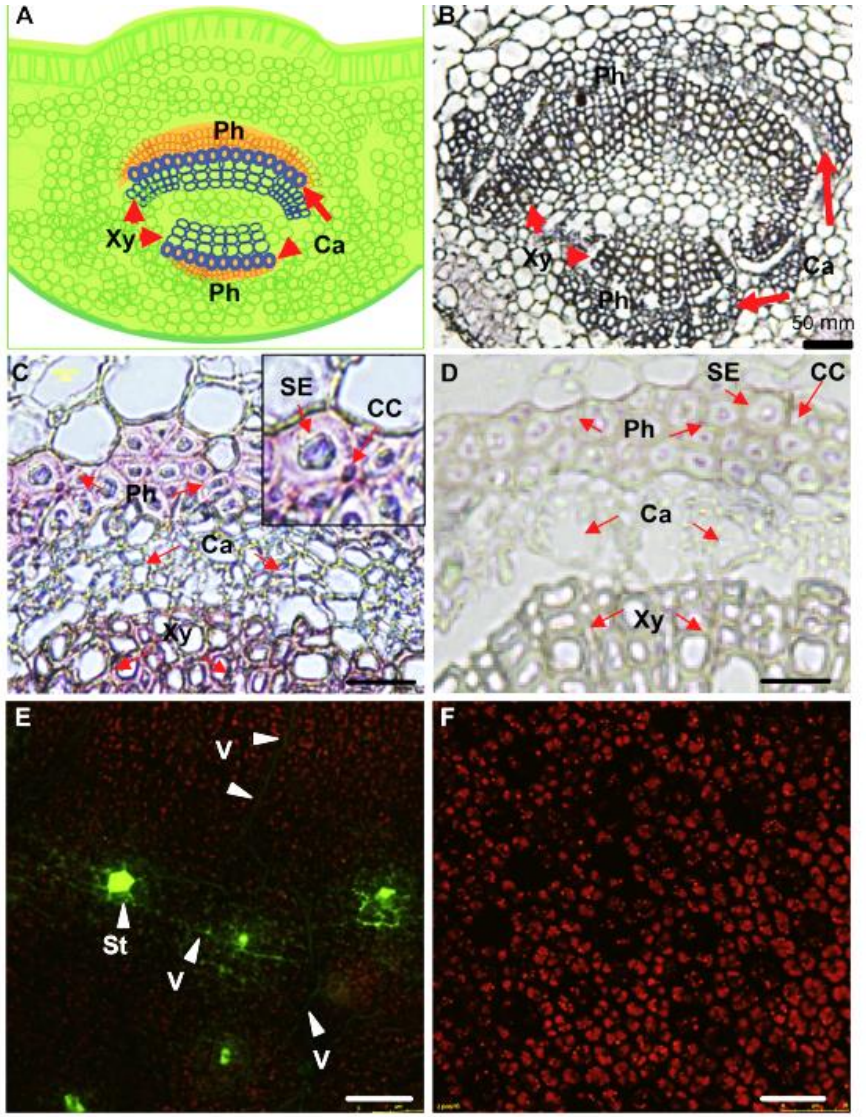




Guerra-Lupián et al., 2018; Conti et al, 2021; Calderón Pérez et al., 2022

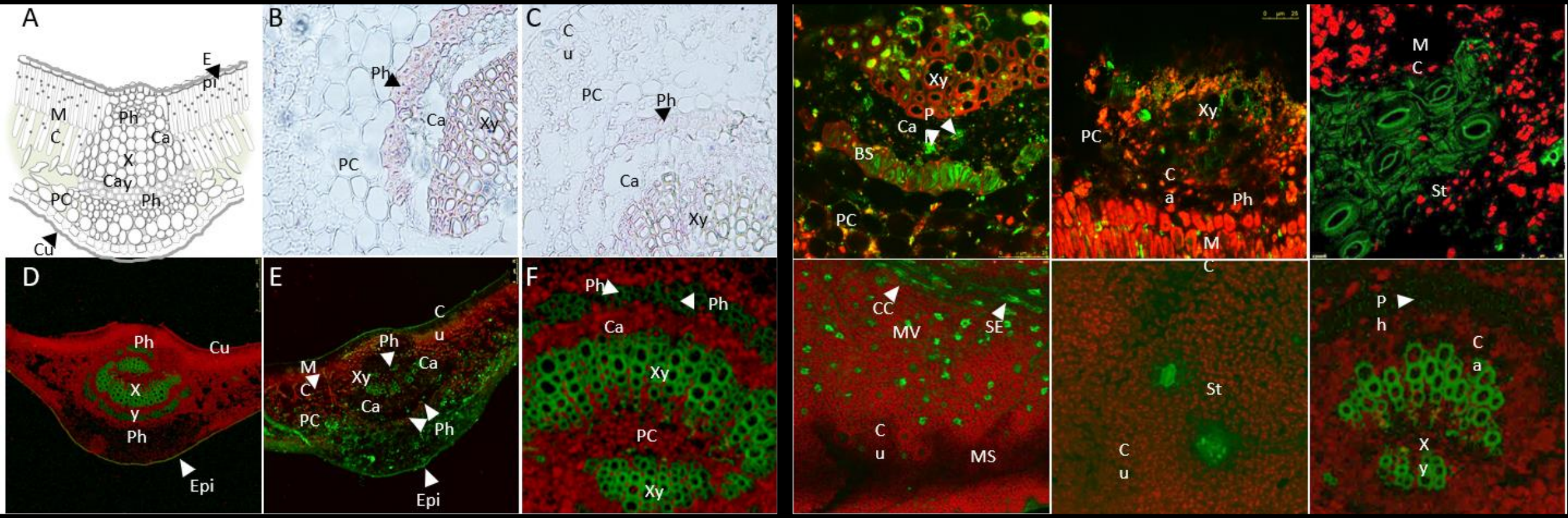
Detección de transcritos en plantas





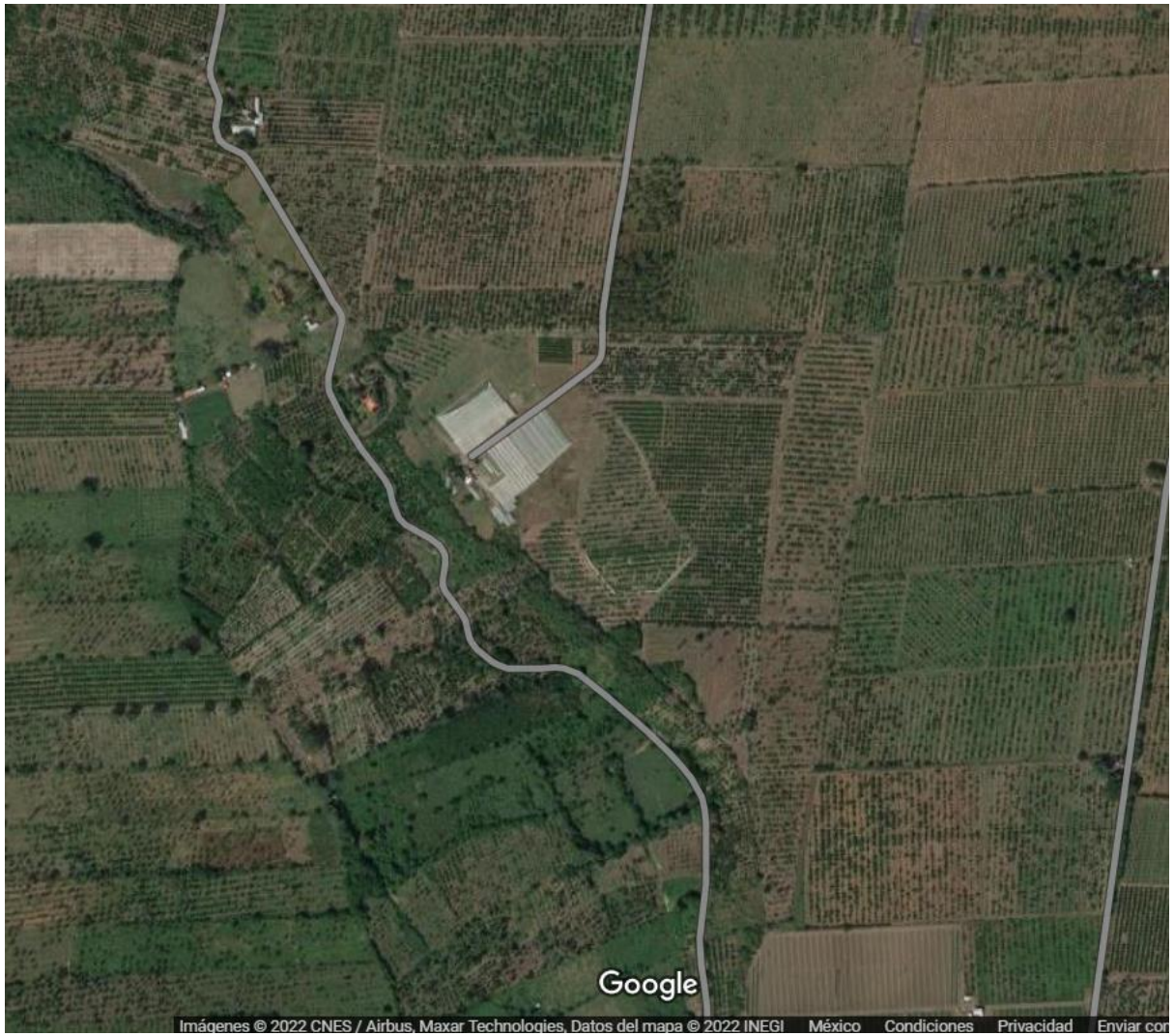
Calderón Pérez et al., 2022

CsPP16-AMP se acumula en tejido vascular



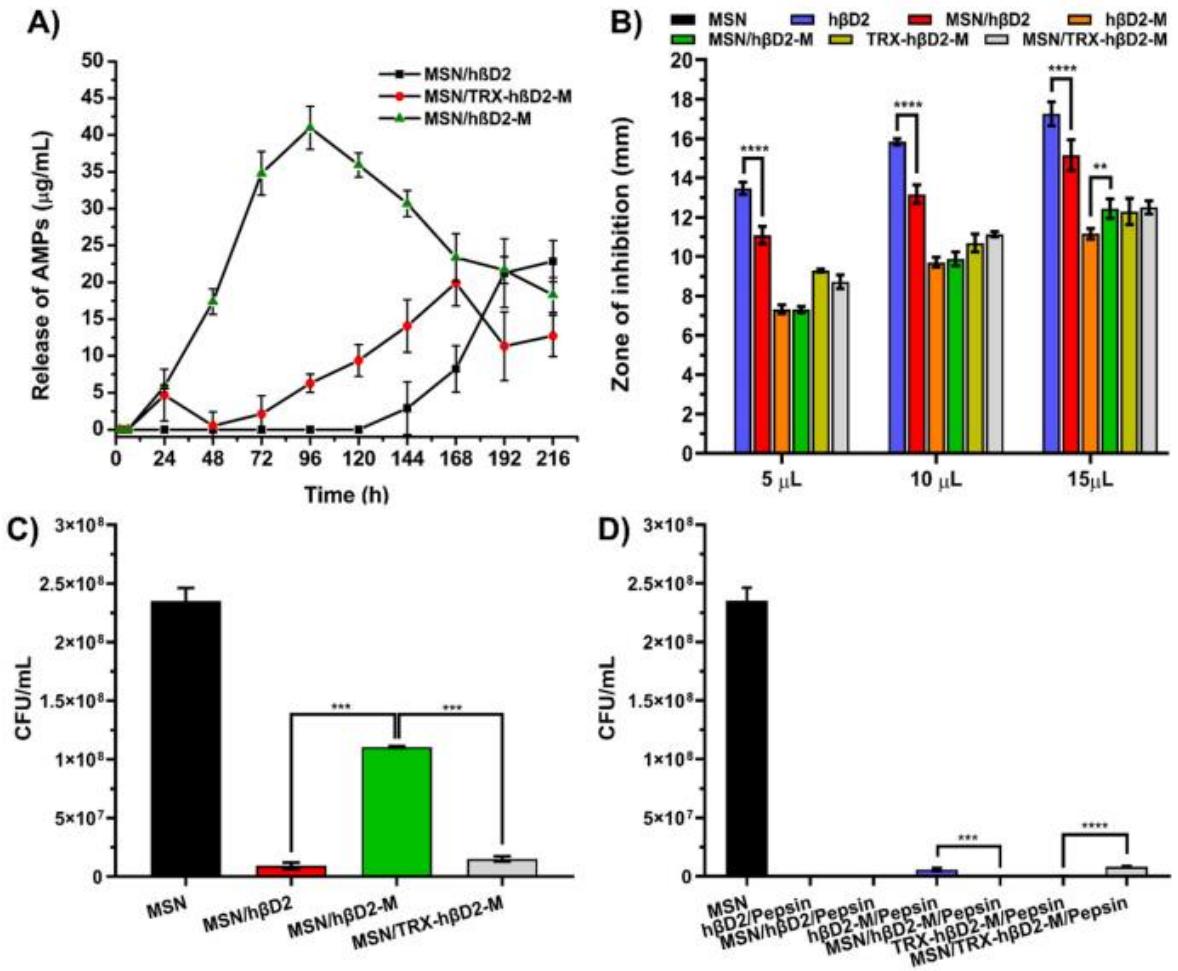
Calderón Pérez et al., 2022

Ensayos a cielo abierto en Colima



Guerra-Lupián et al., 2017; Calderón Pérez et al., 2022

AMPs en nanopartículas de quitosano



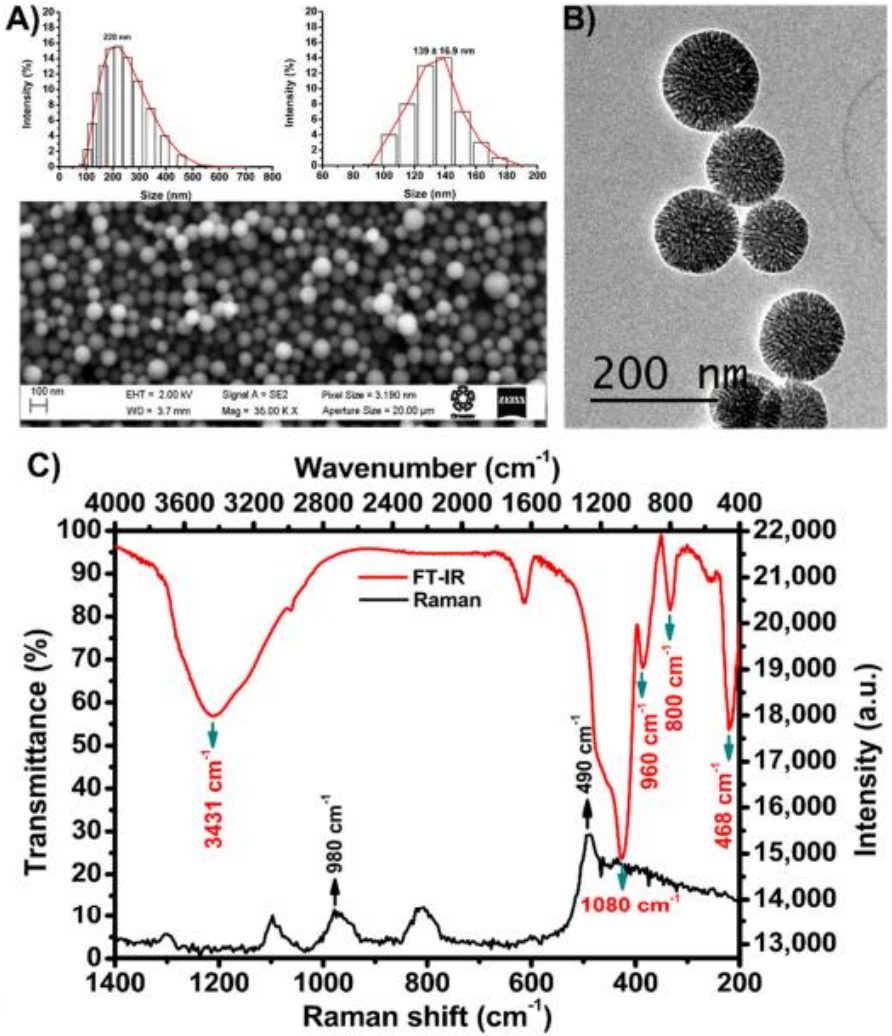
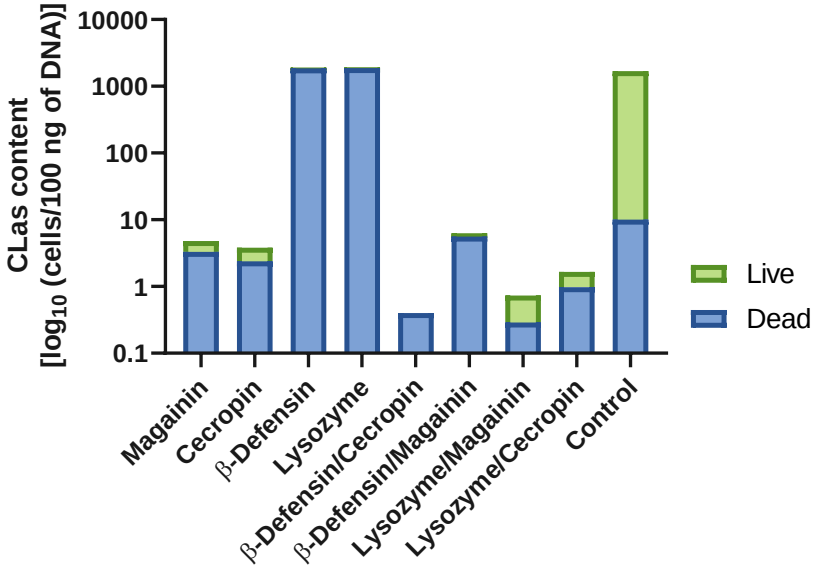
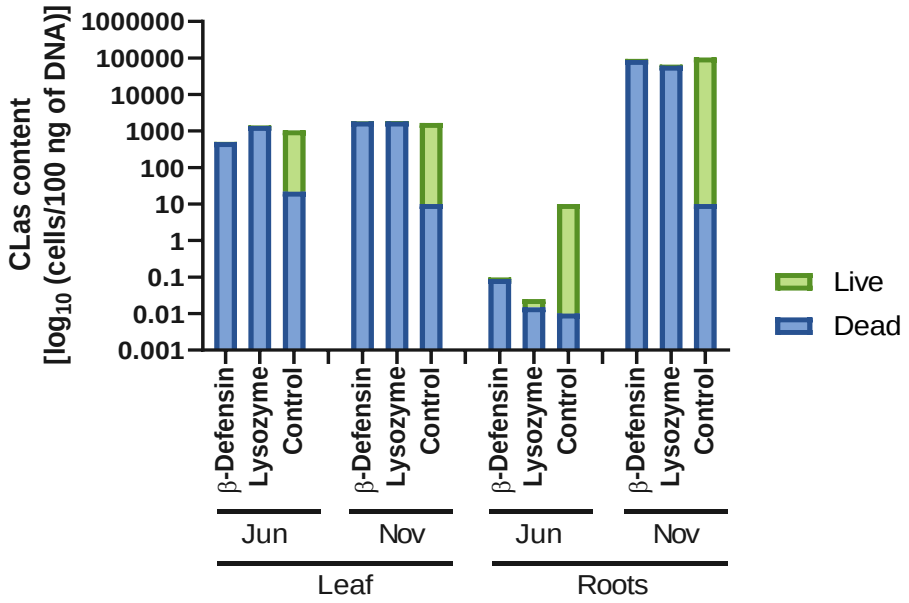


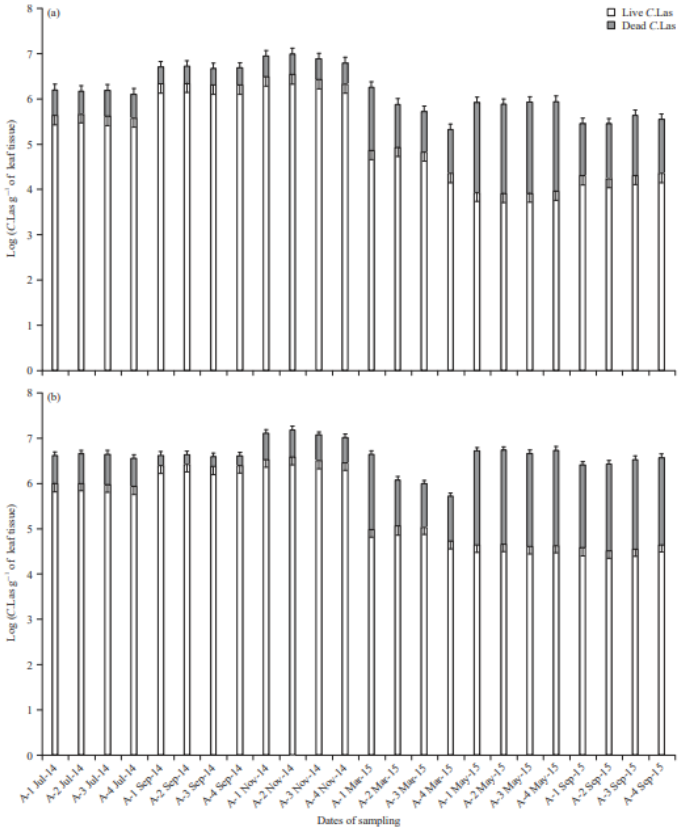
Figure 4. Characterization of the MSN/AMPs complexes. (**A**) Concentration of AMPs released from MSN5.4, (**B**) evaluation of antimicrobial activity by plaque diffusion (**, $p \leq 0.0028$; ****, $p < 0.0001$), (**C**) evaluation of antimicrobial activity in liquid medium (***, $p \leq 0.0001$) and (**D**) effect of pepsin on MSN/AMPs complexes (***, $p \leq 0.0005$; ****, $p < 0.0001$).

Marcelino-Pérez et al., 2021

AMPs mitigan HLB



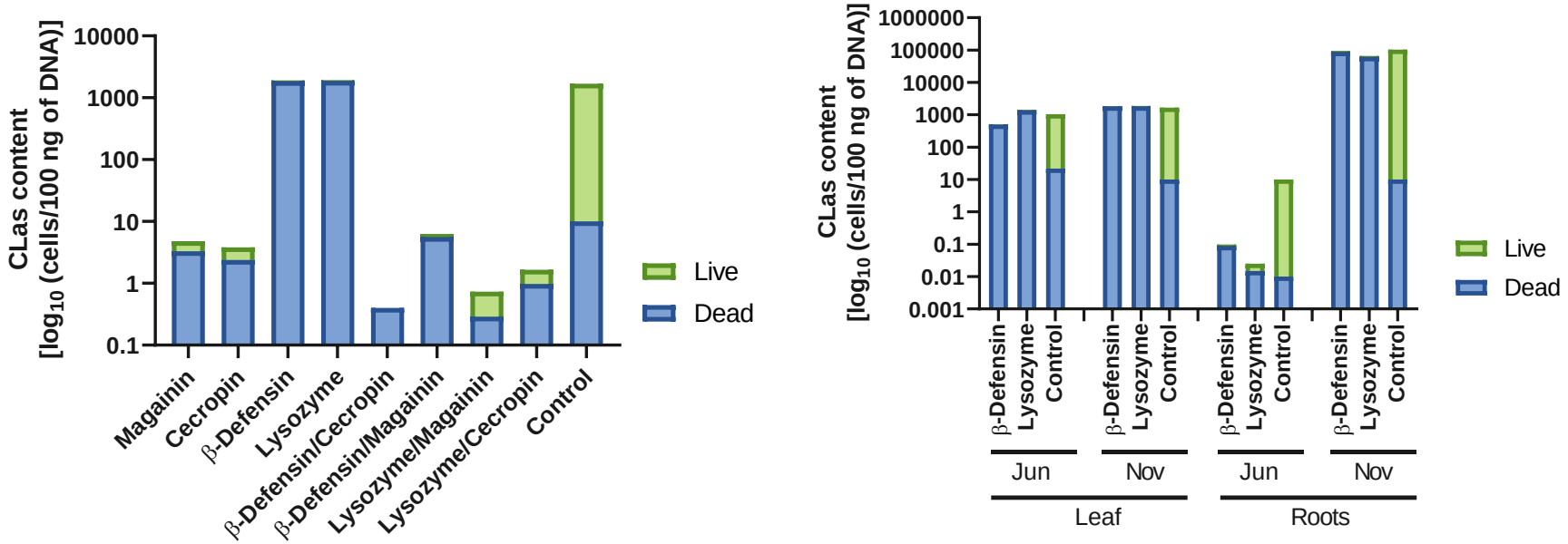




López-Buenfil et al., 2017; Guerra-Lupián et al., 2018; Calderón-Pérez et al., 2021

Otros Antimicrobianos mitigan HLB

A



B

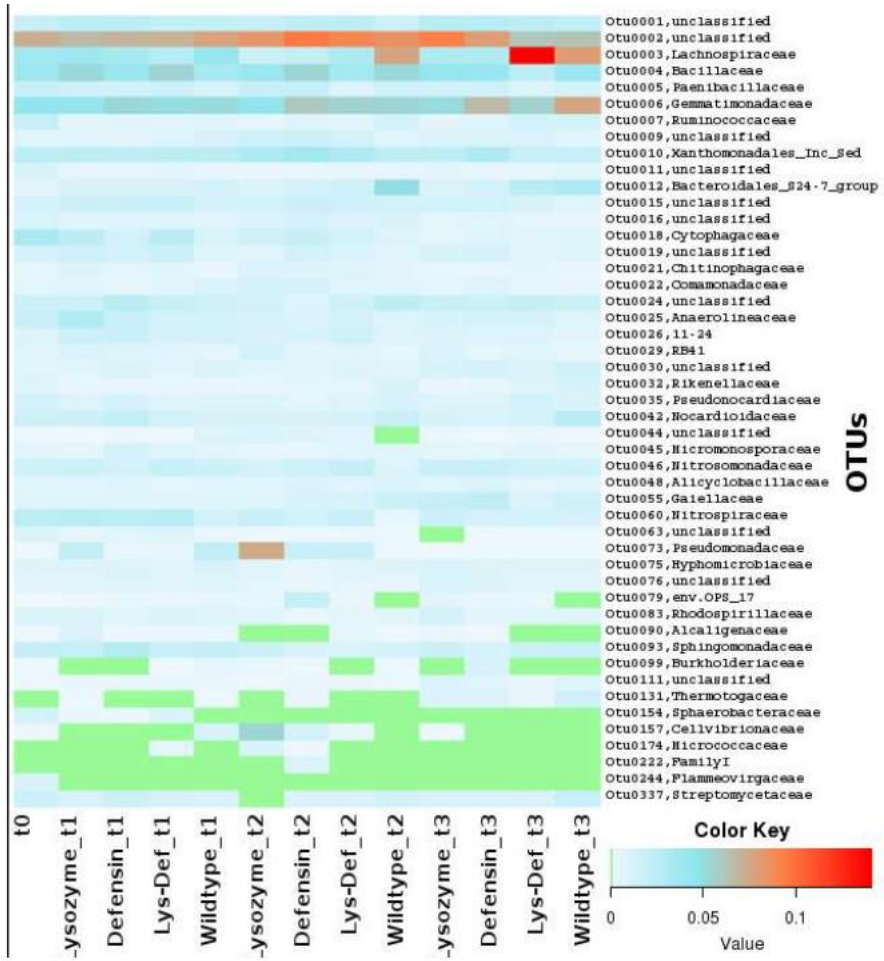
Calderón-Pérez et al., 2022.

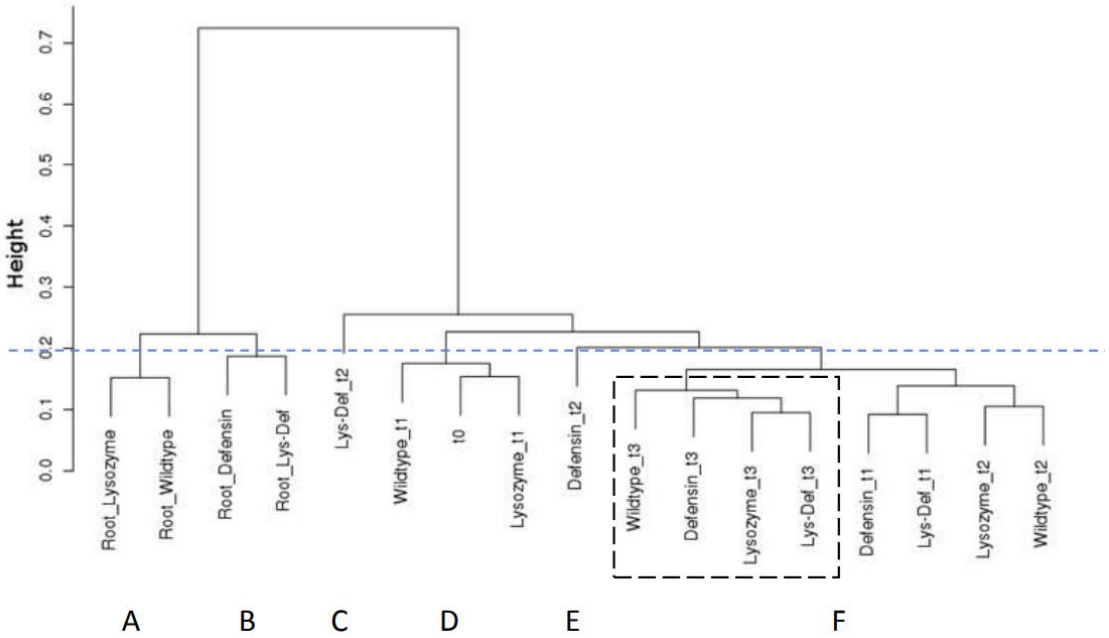
Expresión en limón persa

Zecua-Nájera, 2016, Ramírez-Ortega, 2017.

La diversidad bacteriana no se afecta en cítricos GM

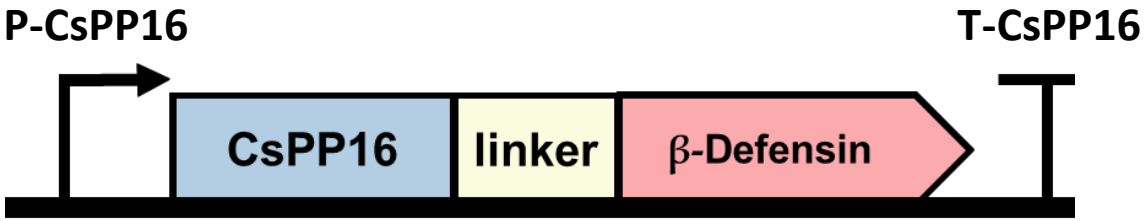
0, 3, 6 and 9 months





Edición de genes usando CRISPR-Cas9

- Traslocación del locus CsPP16



Cinvestav

Nuevas variedades de soya

Más proteína

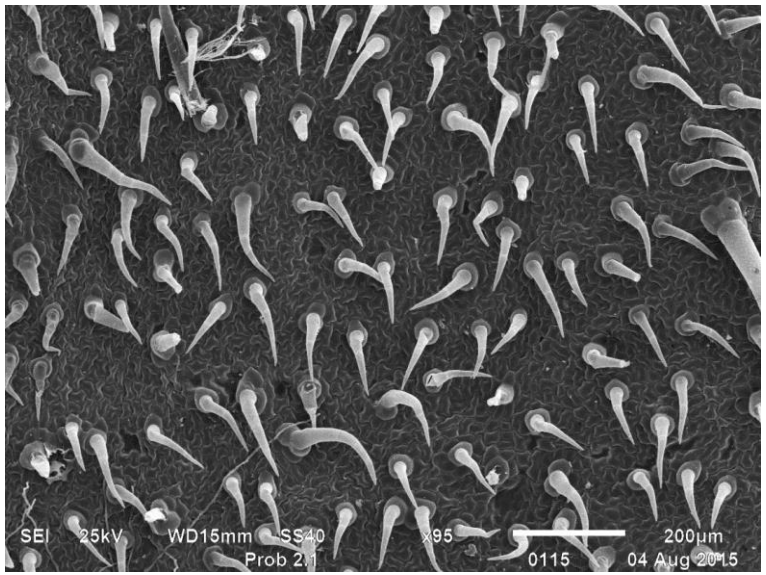
Más lípidos

Bradyrhizobium japonicum

Tomate y soya con menos tricomas para reducir infestación de mosquita blanca

Normal

glabrata



Cinvestav

Reducción de tricomas por edición de genomas

Galeano, Valenzuela & Ramírez-Pool et al., 2022.

ToBRFV

Cinvestav

SADER SECRETARÍA DE AGRICULTURA Y DESARROLLO RURAL · SENASICA

INTRODUCCIÓN

Tobamovirus

- *Tobacco mild green mosaic virus* (TMGMV)
- *Tobacco mosaic virus* (TMV)
- *Tomato brown rugose fruit virus* (ToBRFV)
- *Tomato mosaic virus* (ToMV)
- *Tomato mottle mosaic virus* (ToMMV)

DISTRIBUCIÓN

- ToMV, TMV, TMGMV: ampliamente distribuidos
- ToMMV: Brasil, China, Irán, México, USA

Alishir et al., 2011. New Disease Reports (2011) 23, 30.

Scholthof, 2000. www.apsnet.org

Chin y Miller, 2019. http://u.osu.edu

Alishir et al., 2016. New Disease Reports (2016) 33, 1.

Síntomas en planta por *Tobamovirus*

Moteado clorótico

Distorsión

Abultamiento

TMV: Moteado
Breman, 1989. Plant Pathology Circular No. 322

ToBRFV, maduración desigual

TMV Lesiones necróticas
Tsyplenkov A.E, 2009. http://www.agrostles.ru

www.sader.gob.mx
www.senasica.gob.mx

Cinvestav

SADER
SECRETARÍA DE AGRICULTURA Y DESARROLLO RURAL

SENASICA

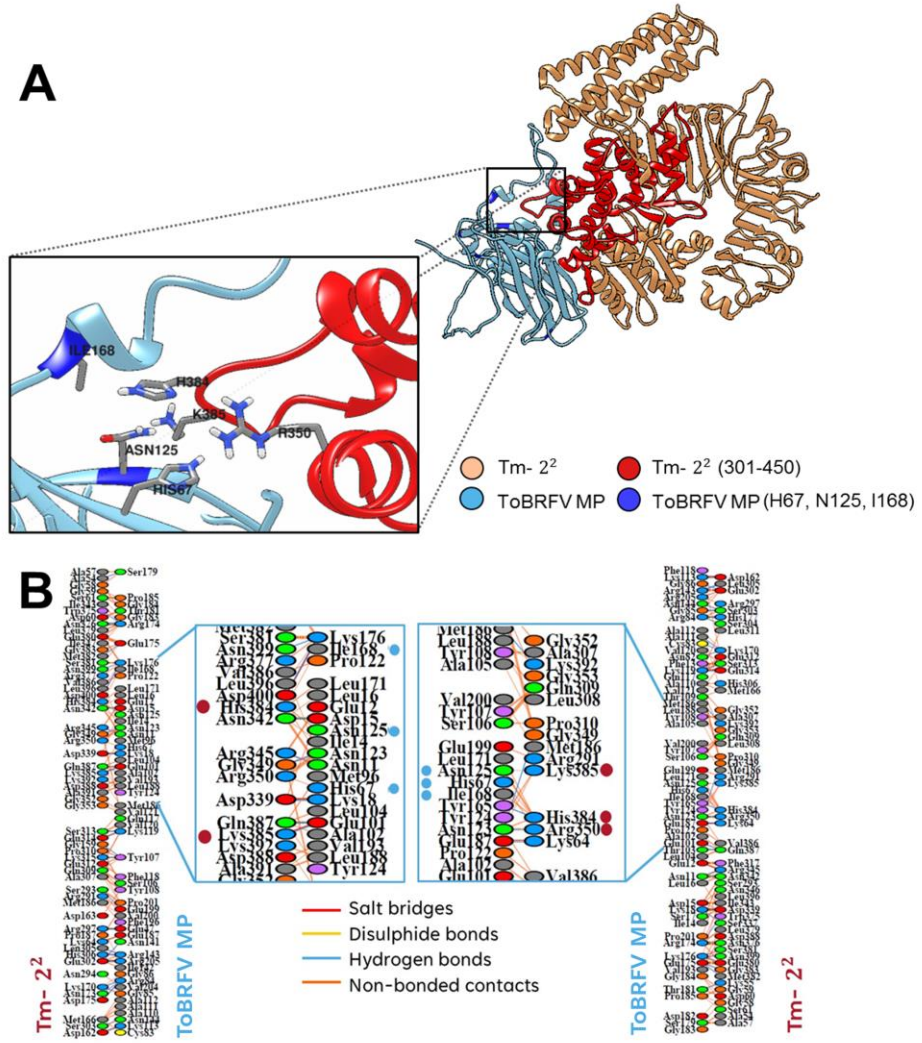
Síntomas de manchas amarillas y rayado de frutos de chile infectados con ToBRFV

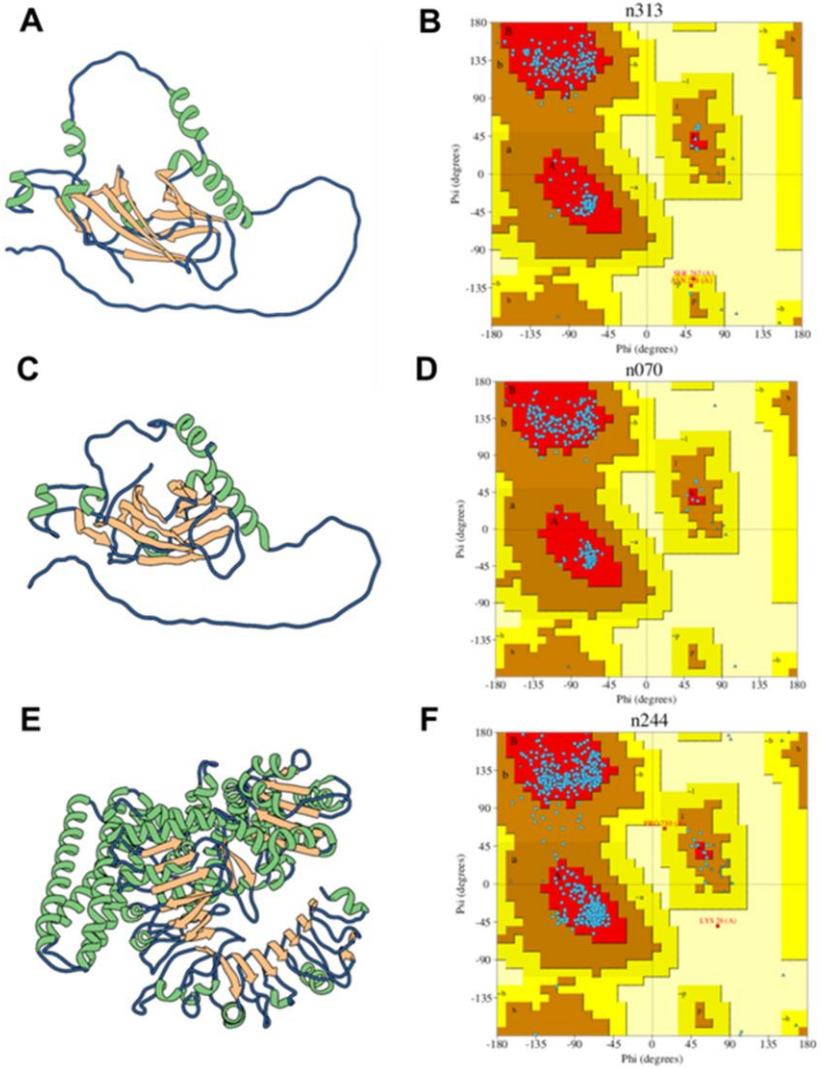
Síntomas de maduración irregular y manchas color marrón en frutos de chile infectados con ToBRFV

www.sader.gob.mx
www.senasica.gob.mx

Genotipos asíntomáticos en chile

Mutagénesis del receptor Tm-2^2

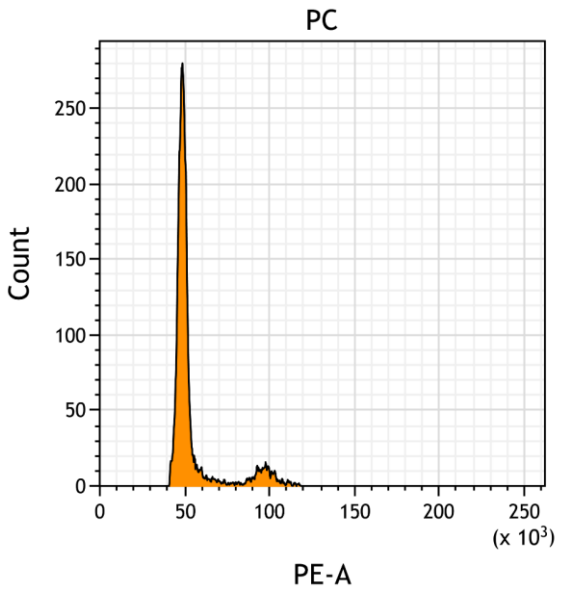




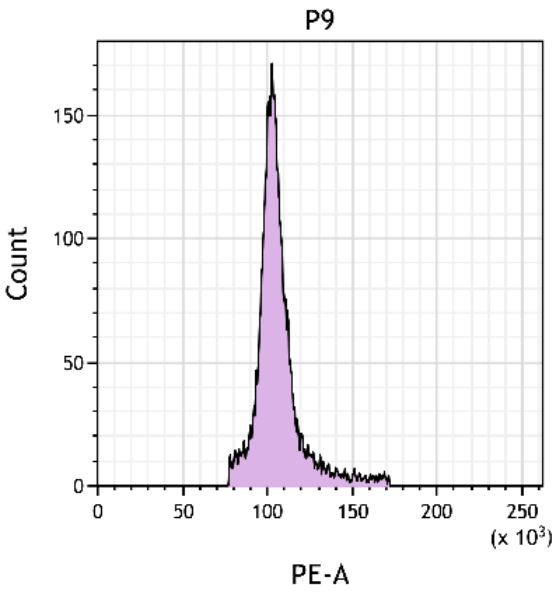
Rivera-Márquez et al., 2022.

Aumento de diversidad genética por poliploidía

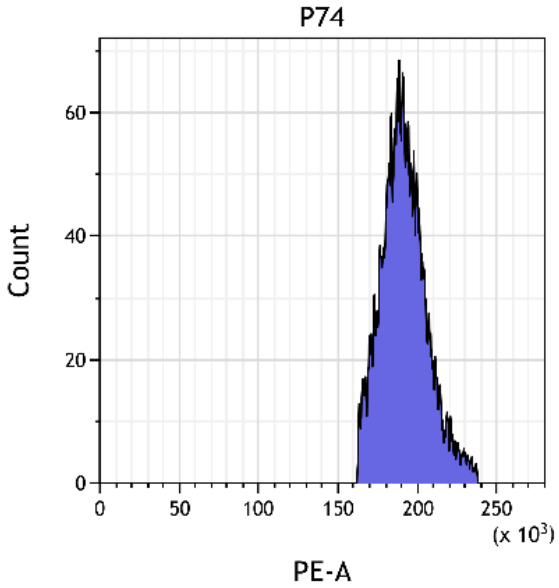
2n

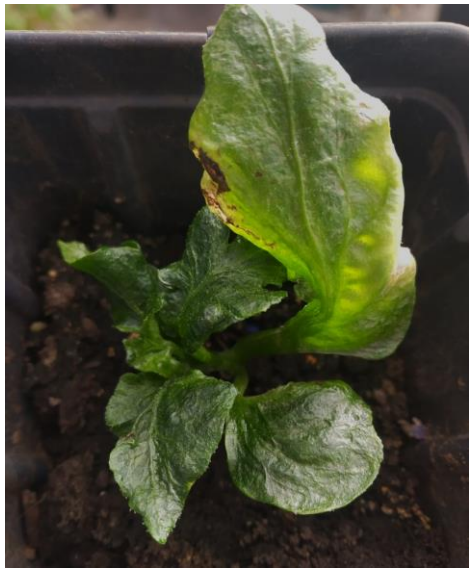


4n

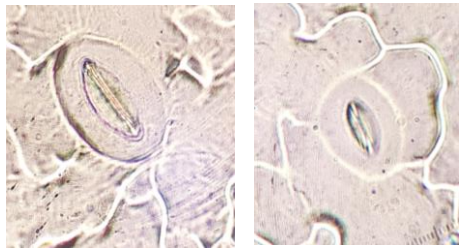


8n



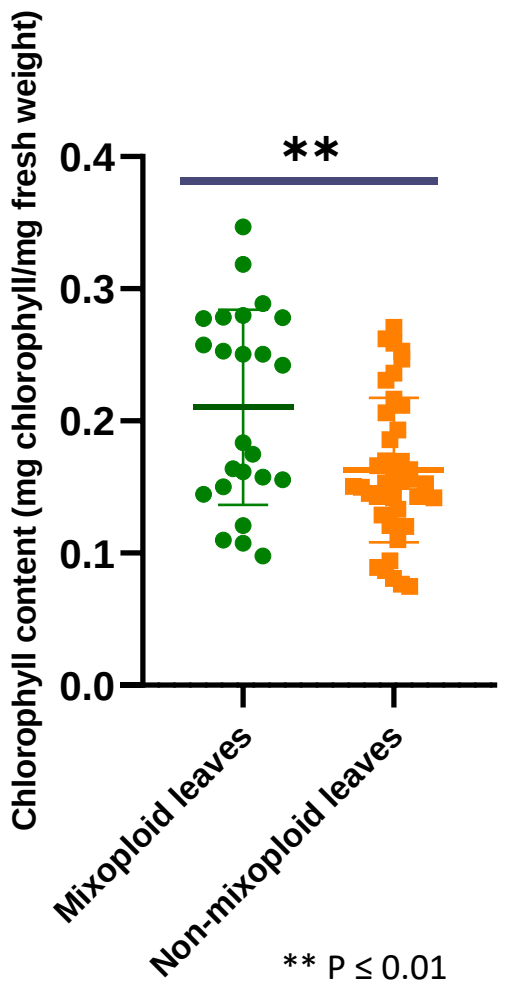


4n



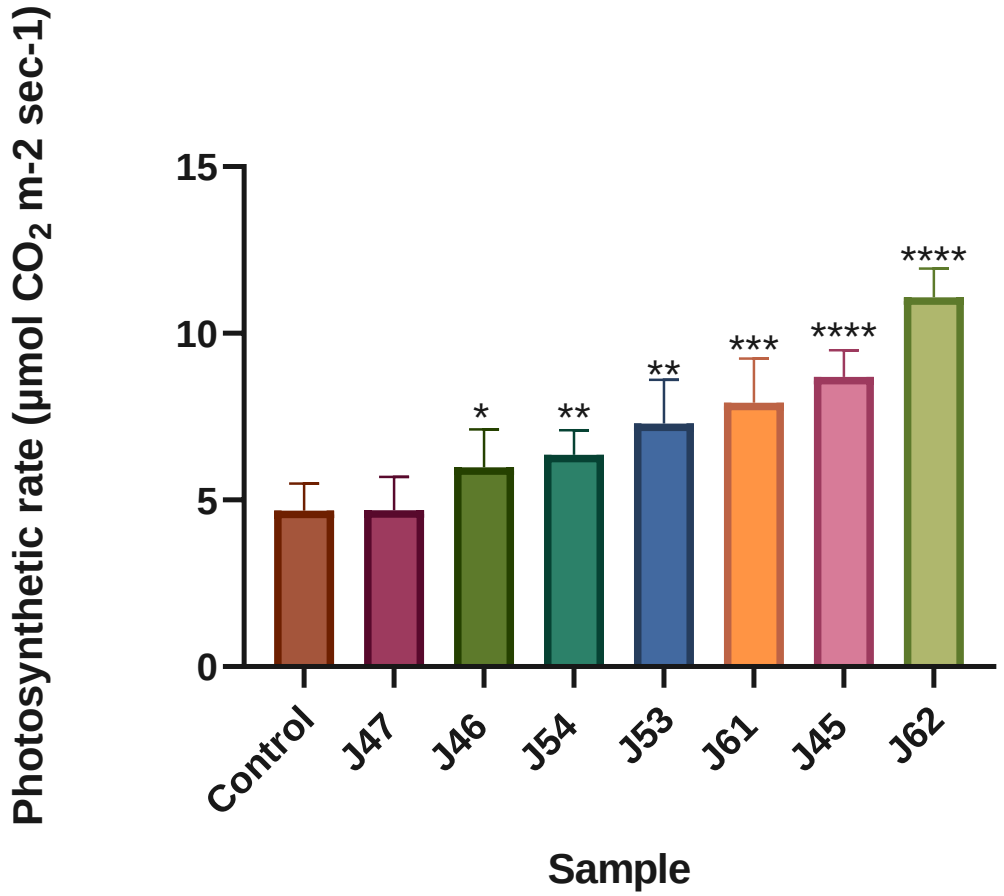
Tomates mixoploides muestran mejor fotosíntesis

Chlorophyll content



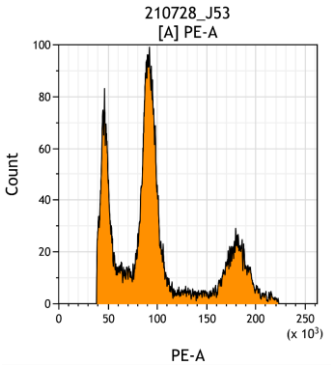
** P ≤ 0.01

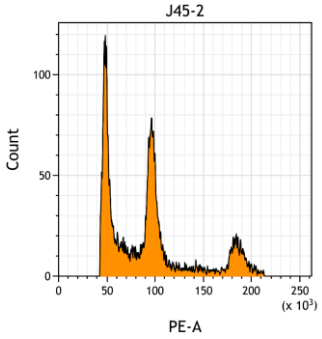
Photosynthetic rate

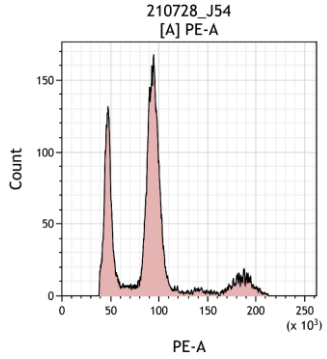


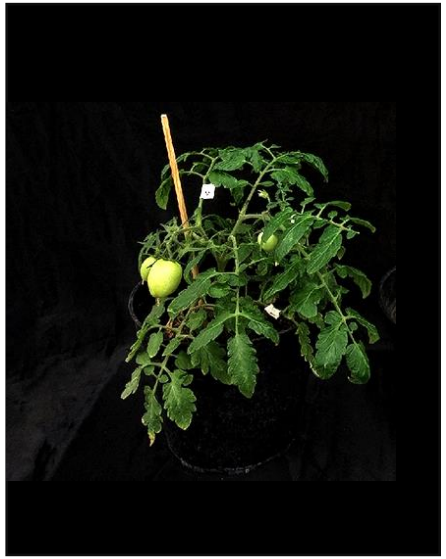
* P ≤ 0.05, ** P ≤ 0.01, *** P ≤0.001, **** P ≤ 0.0001

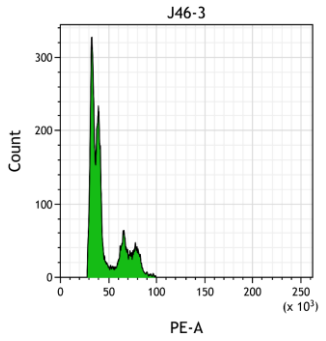
Plantas de tomate mixoploides

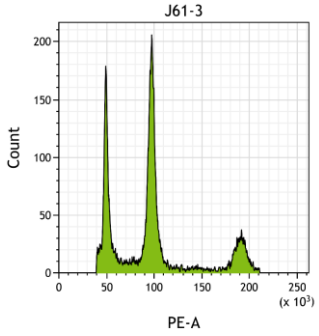


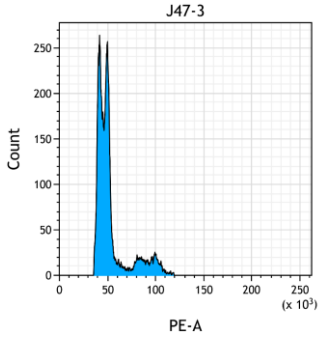






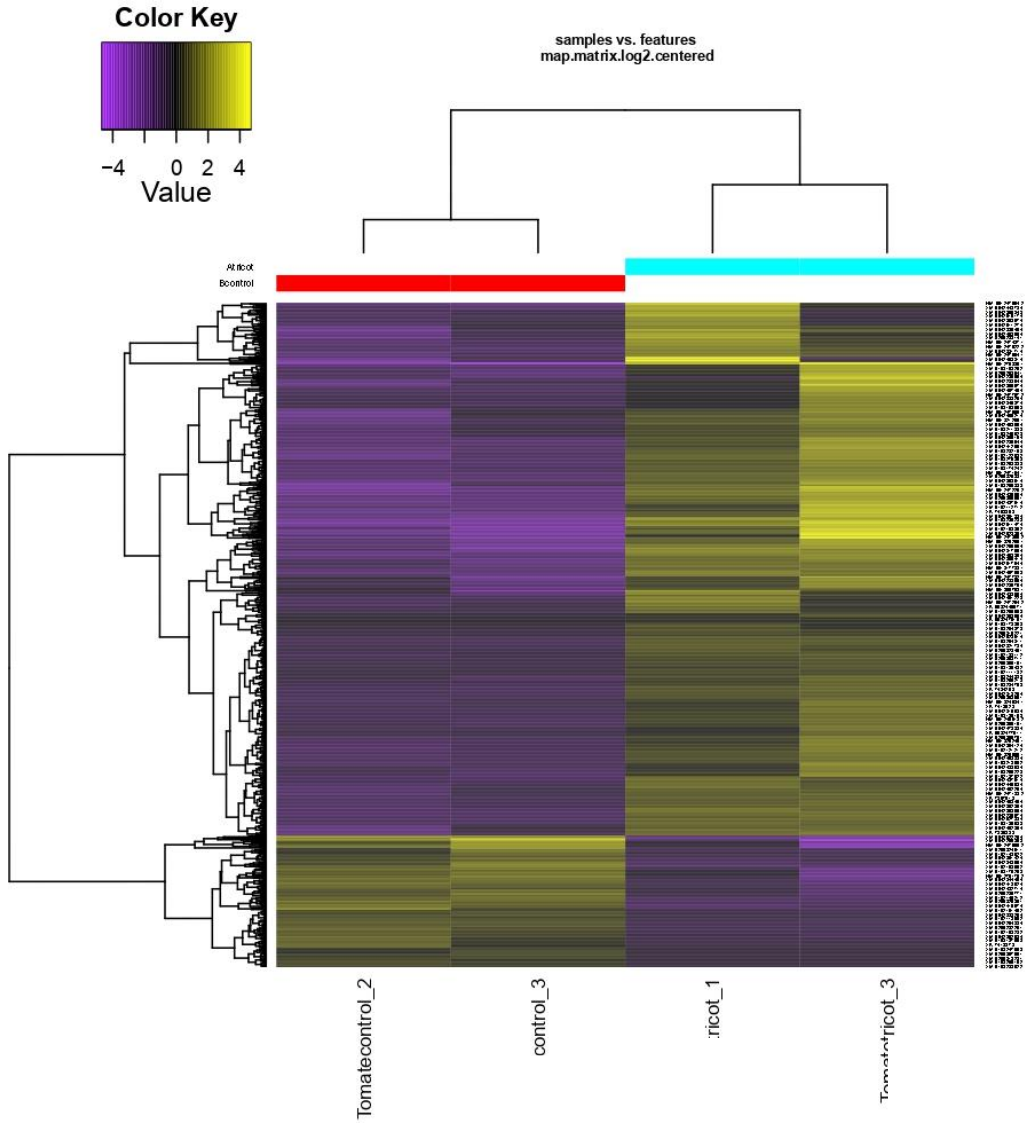






Germoplasma de tomate con tres cotiledones

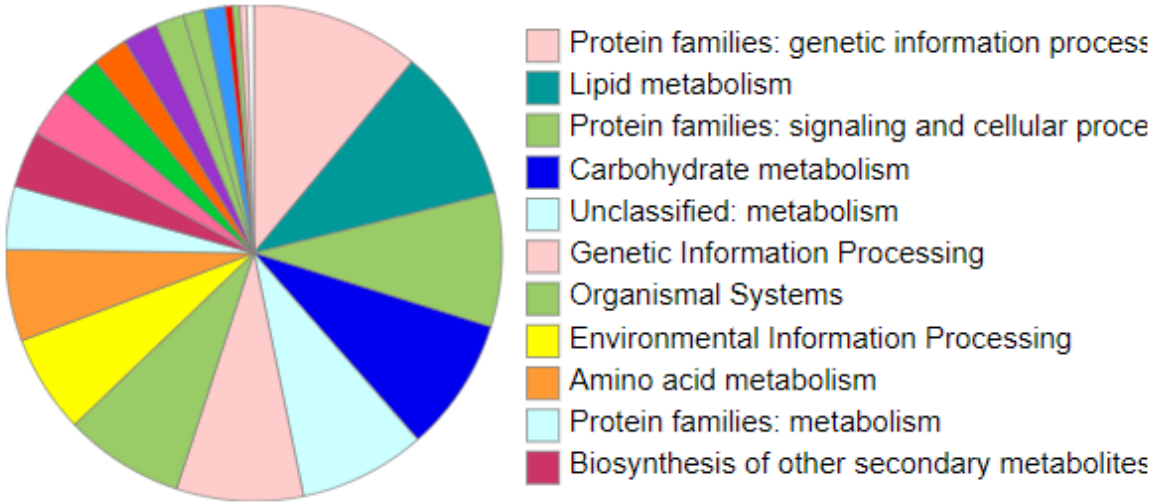
Análisis de transcriptoma de tomate 3-C



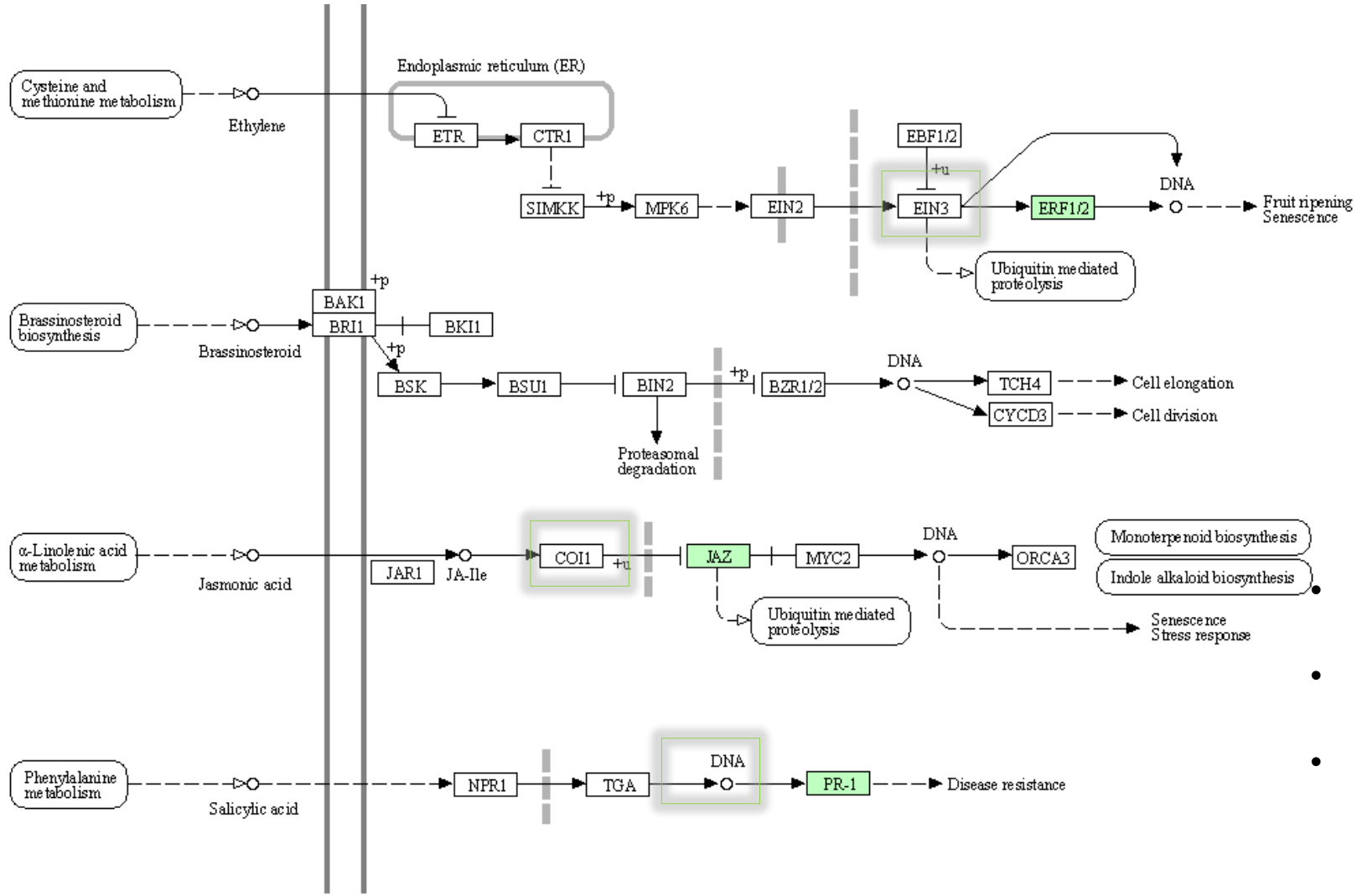
Heatmap of Control vs Tricot tomato plants F2

614 overexpressed transcripts and **152 repressed.**

Functional category

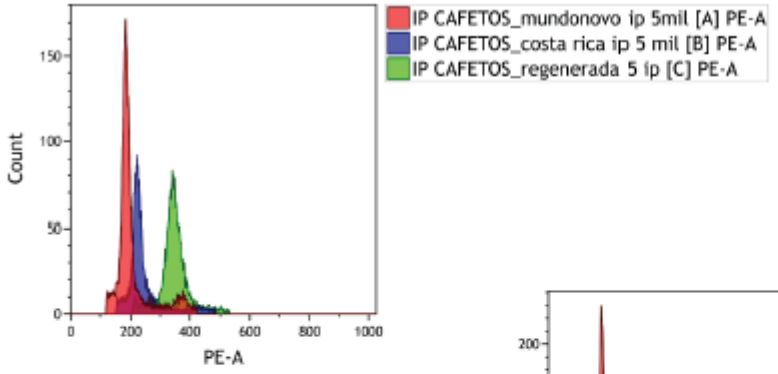


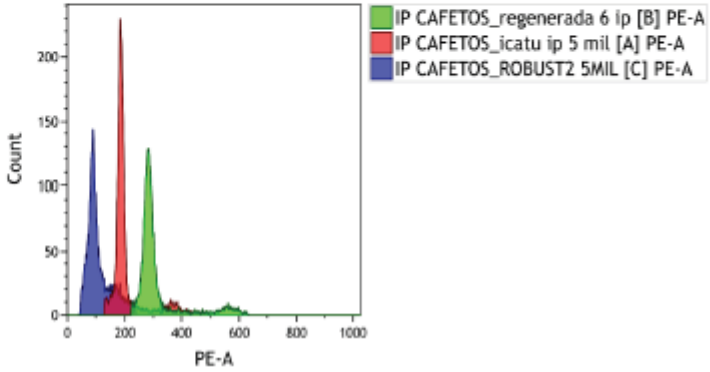
Sobreexpresión de transcritos de síntesis de hormonas y de respuesta de sistema inmune innato



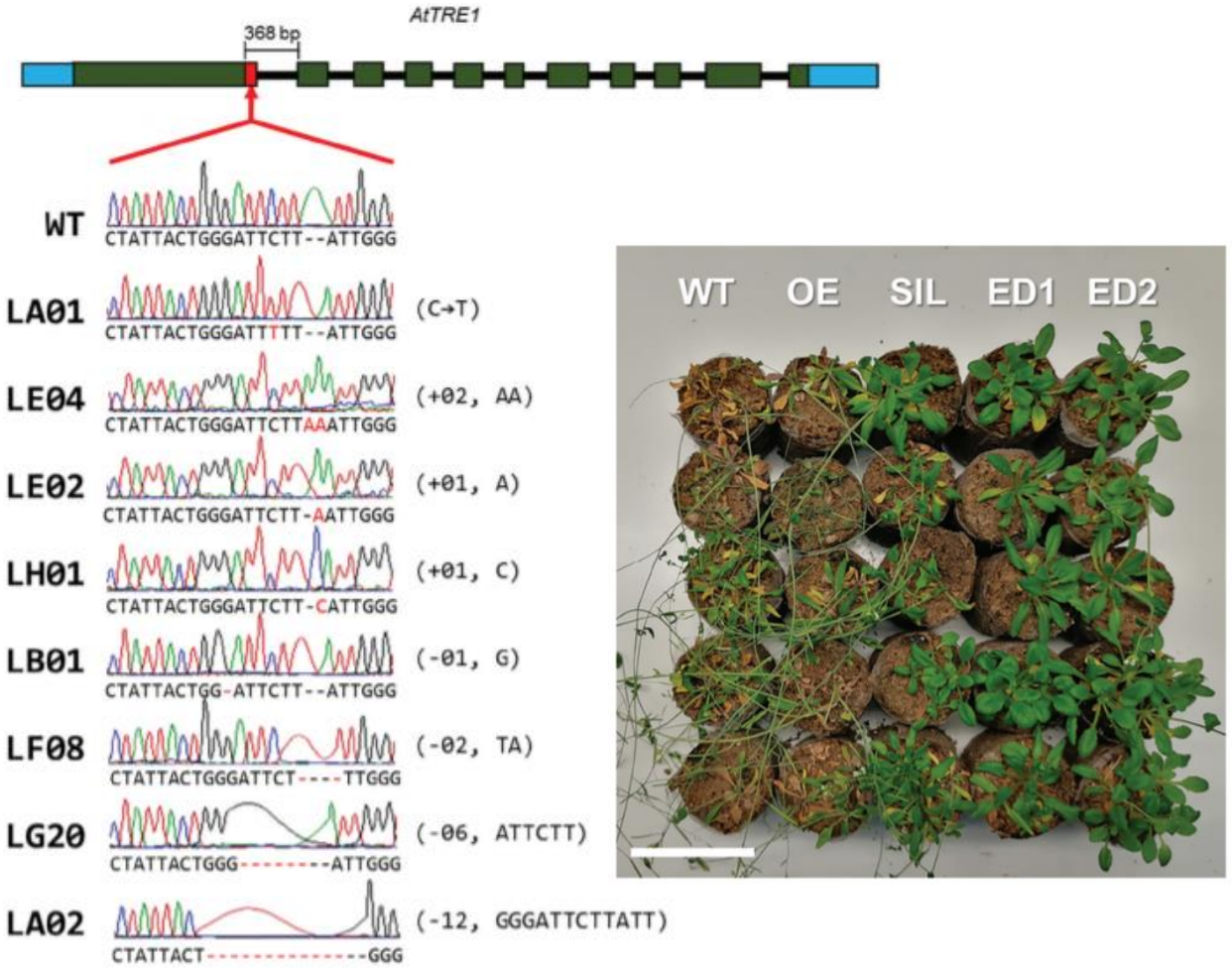
- **ERF1 ethylene-responsive transcription factor 1**
- **JAZ jasmonate ZIM domain-containing protein**
- **PR1 pathogenesis-related protein 1**

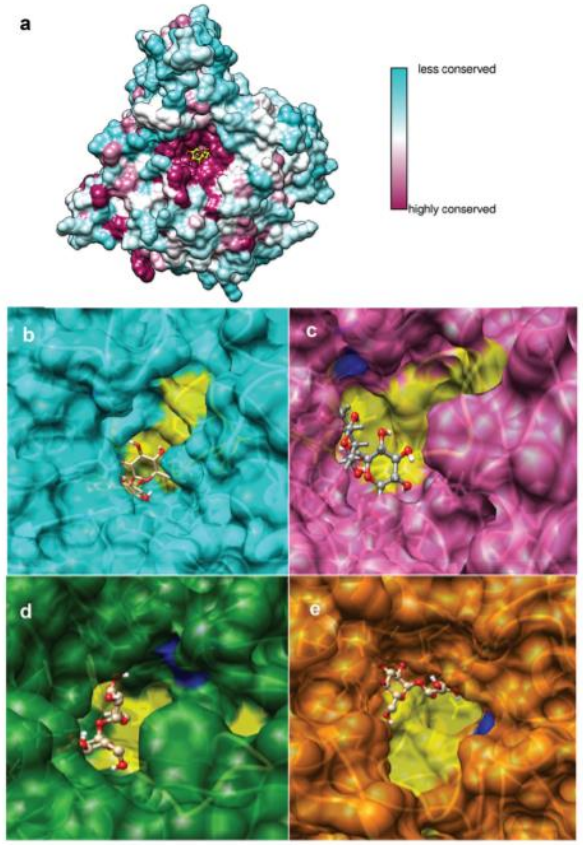
Nuevas variedades de café por cruza asexual





Edición de genes para tolerancia a sequía



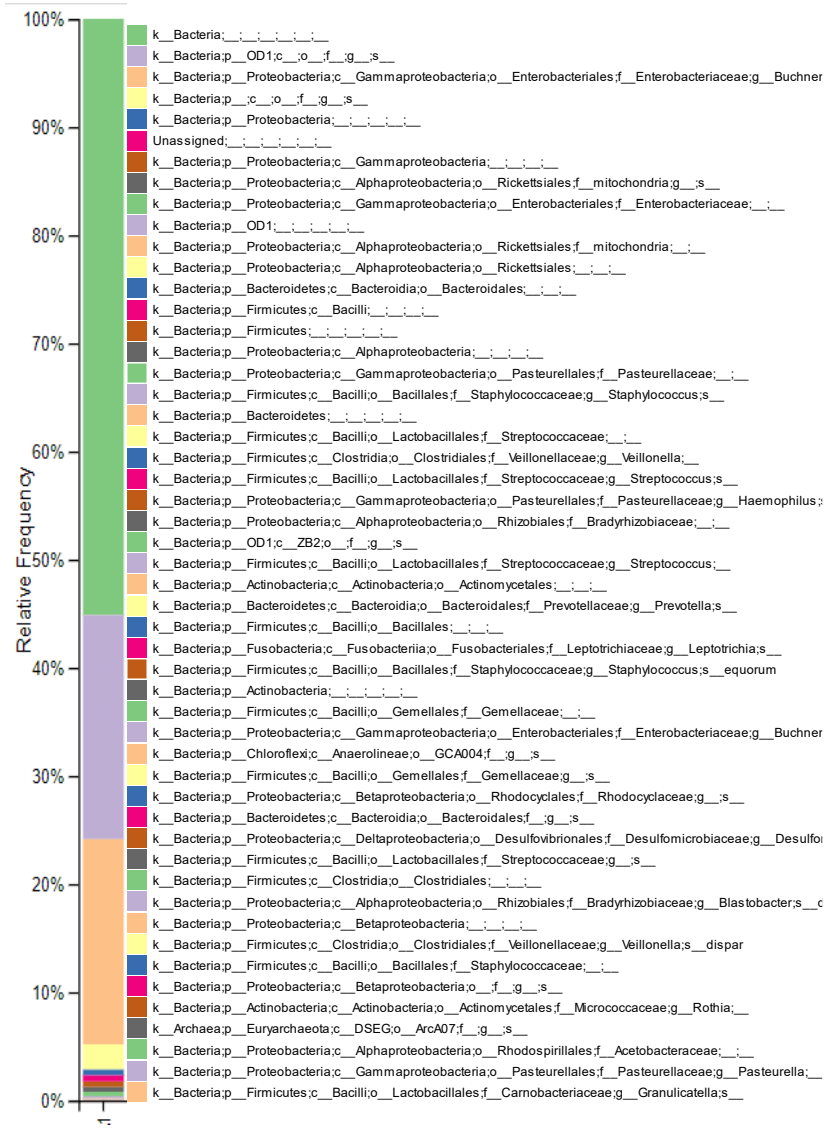


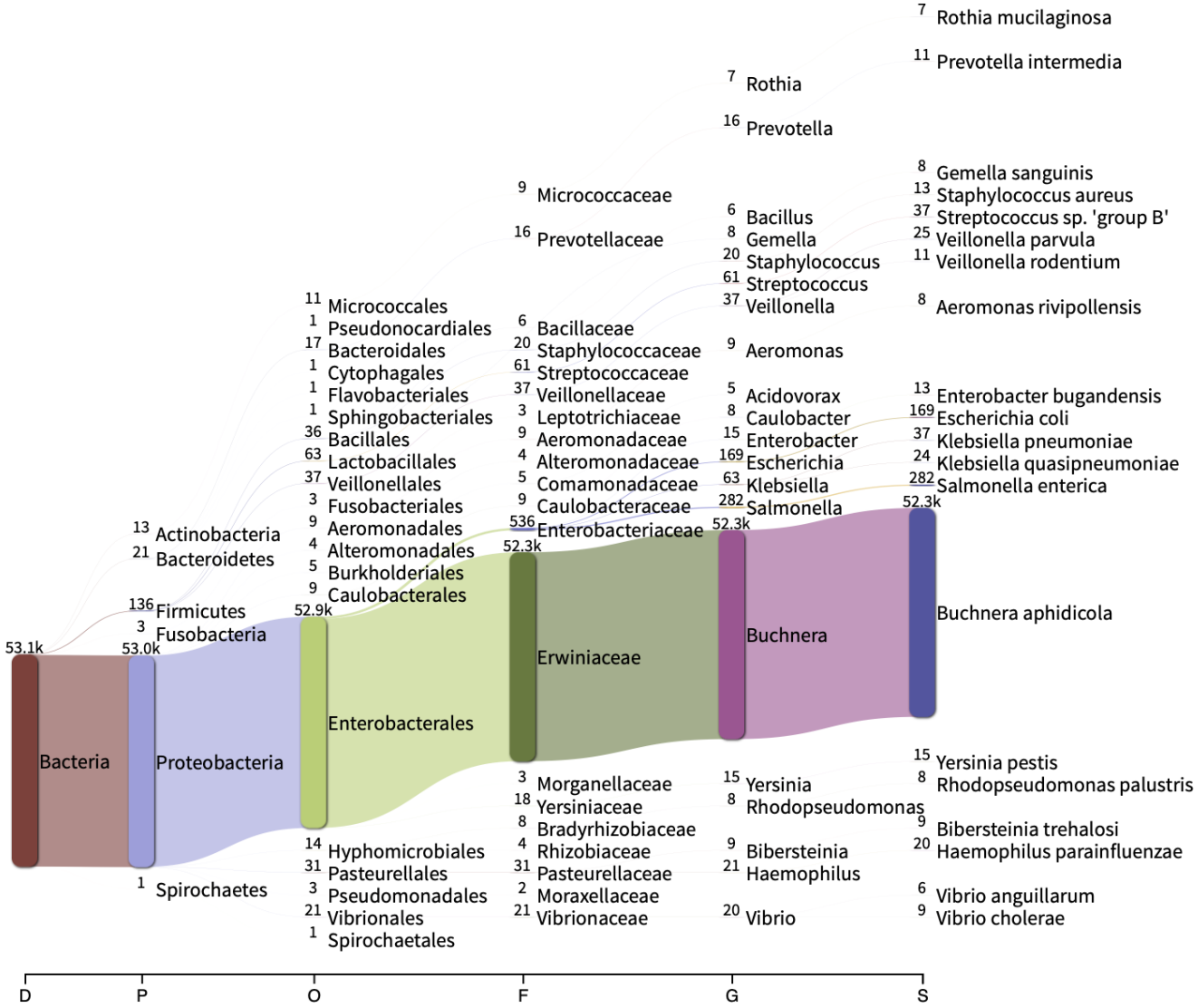
Núñez-Muñoz et al., 2021.

Microbioma de *Melanaphis saccharis (pulgón amarillo)*
Campus experimental INIFAP Celaya

Colaboración Dr. Bujanos

Microbioma de *Melanaphis saccharis*

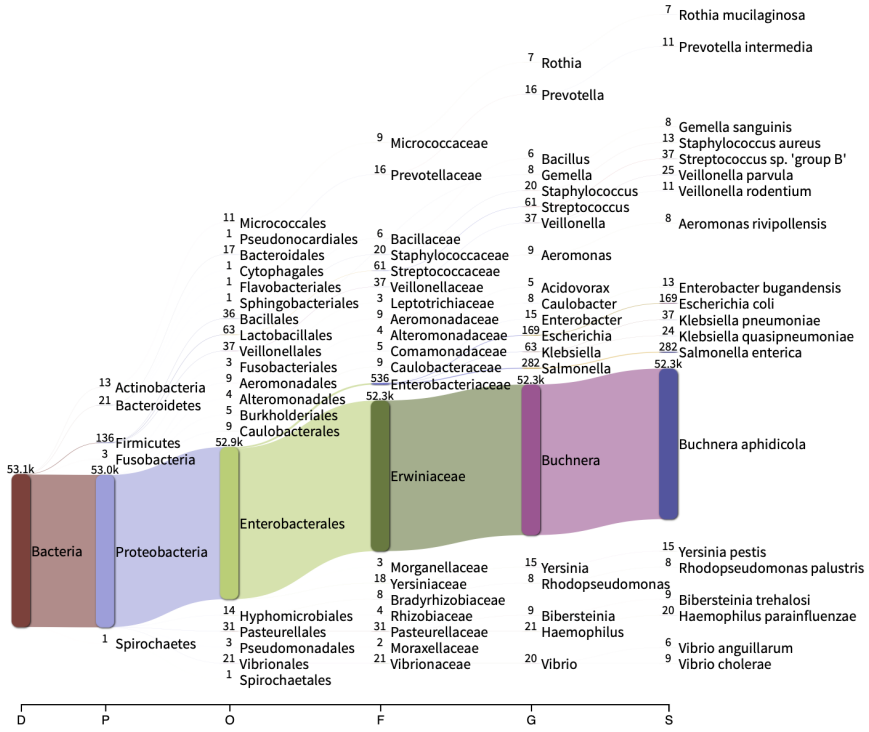




Xoconostle-Cázares et al., 2023.

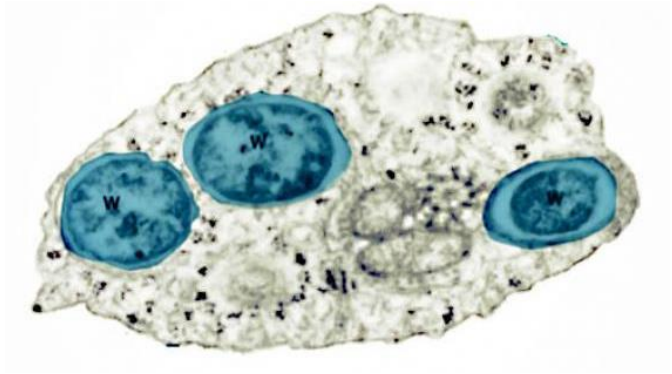
Estimación de diversidad de especies en *M. sacchari*

118,936 secuencias



Type	Index or measure	Value
Observed Richness	OTUs richness	110.000
	Singletons	0.5090
	Dobletons	0.1273
	Good's coverage	0.9989
Richness estimators	ACE	246.000
	Chao2 (S_{chao})	365.080
	Jacknife-1	270.998
	Jacknife-2	343.990
Evenness	Pielou J	0.0300
Dominance	Berger-Parker (d)	0.9860
	Simpson (D)	0.0279
Combined diversity	Shannon (H')	0.1198

Diversidad de *Wolbachia* en *M. saccharis*



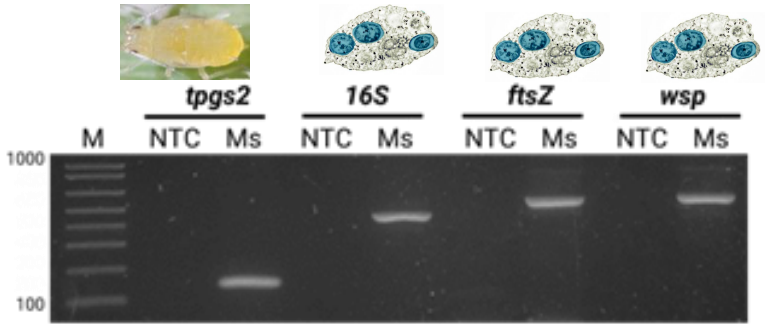


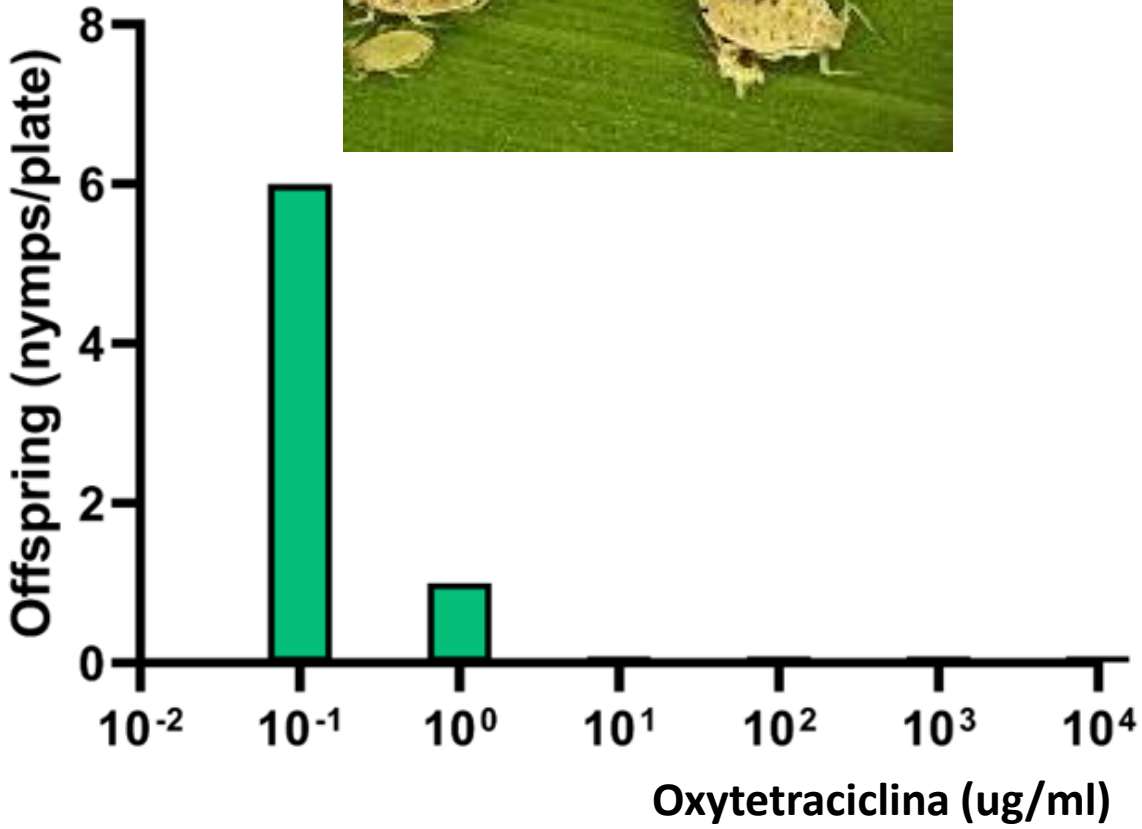
Figure 2. PCR detection of *Wolbachia* spp. in *M. sacchari* using specific primers against *M. sachhari* genome (*tpgs2*) and Wolbachia (*16S*, *ftsZ* and *wsp*).

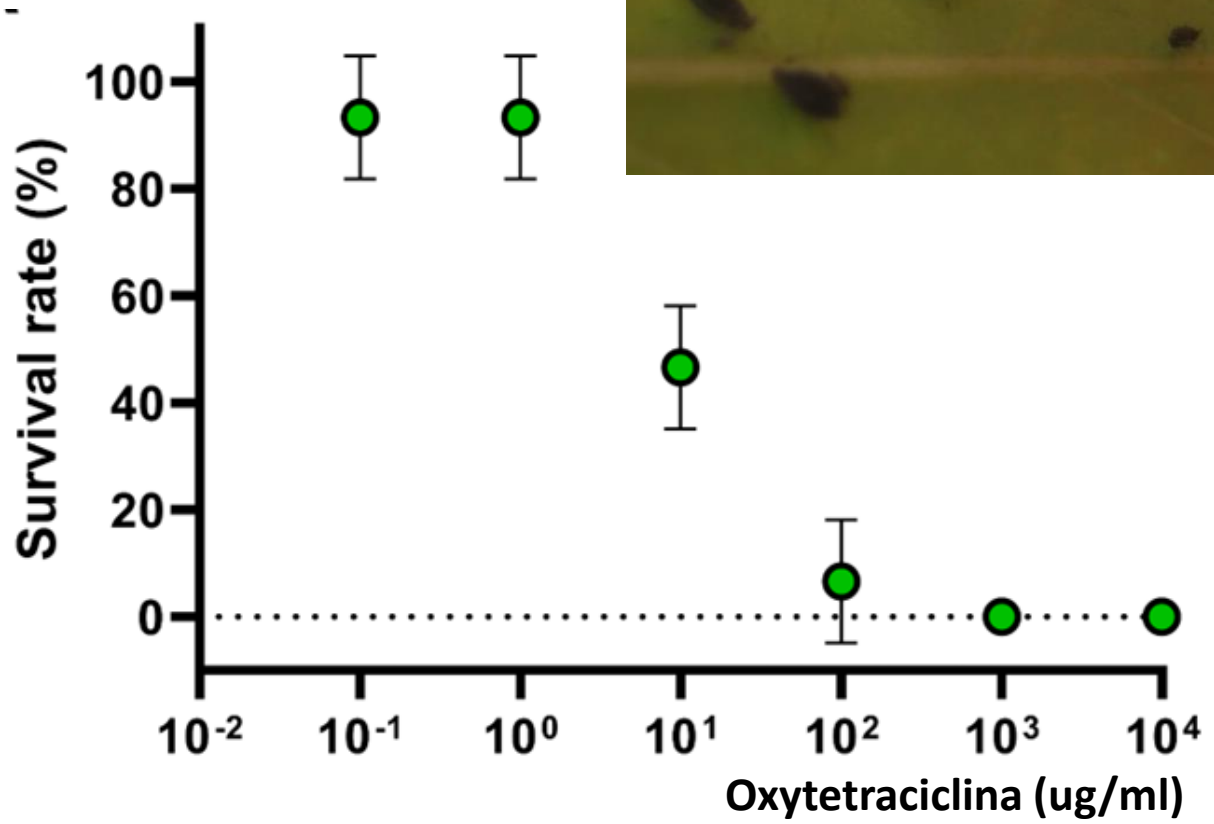
Host	Count
Homalodisca vitripennis	864
Formica fusca	768
Proasellus ibericus	402
Wolbachia, ambiguous taxa	243
Zorotypus caudelli	58
Wolbachia uncultured	48
Wolbachia sp. PL13	30
Radopholus similis	25
* *Rhinocyllus conicus*	19
Wolbachia pipientis	17
Mesaphorura yosii	15
* *Bangasternus orientalis*	12
Pentastiridius leporinu	1
Total	**2502**

Wolbachia induce la partenogénesis en insectos

¿Podríamos cambiar la microbiota del pulgón amarillo?

Efecto de antibióticos en hembras de *M. sacchari*





Xoconostle-Cázares et al., 2023.

Bacterias benéficas como bioinoculantes

- **Bacteria producing AMP**
  - **Aerobial fermentation**
  - **Formulation**

Uso of beneficial de *Bacillus* as plant growth and development promoter

Identificación de *Xylella fastidiosa en vides*

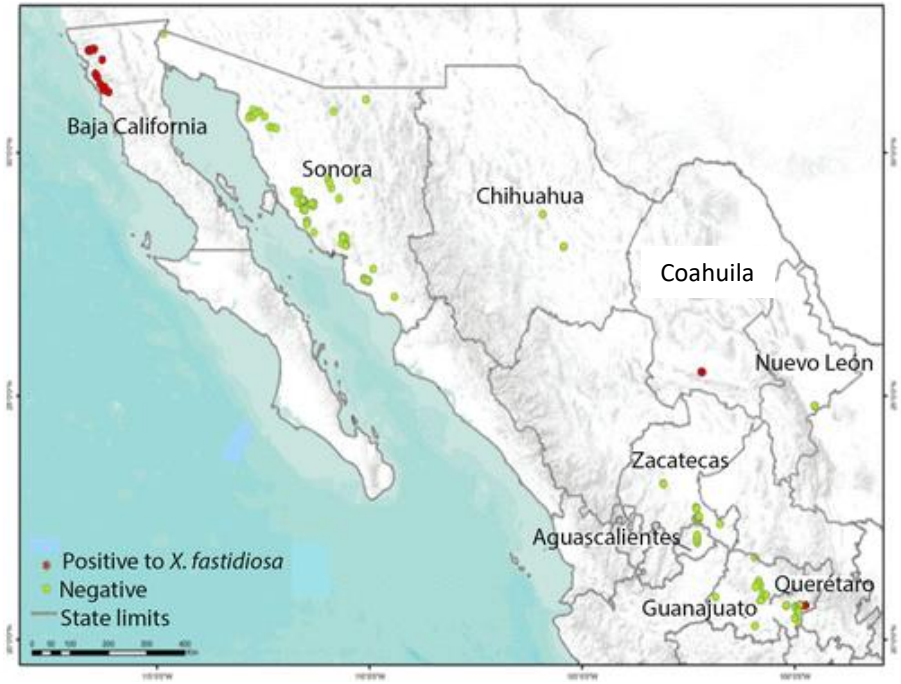


Table 2. Allelic profile of *Xylella fastidiosa* isolates from Mexican vineyards[a]

		MLST loci							
Sample	**State**	**ST**	***leu*A**	***pet*C**	***mal*F**	***cys*G**	***hol*C**	***nuo*L**	***glt*T**
Vid	Baja California	ST1	1	1	1	1	1	1	1
Vid	Coahuila	ST1	1	1	1	1	1	1	1
Vid	Querétaro	NI	1	1	9*	23*	25*	1	1
	…	NI	…	…	44C-T	21C-A	56 G-A	…	…
	…	NI	…	…	99T-G	53T-C	86 T-C	…	…
	…	NI	…	…	…	495T-C	131 T-C	…	…
	…	NI	…	…	…	…	134 T-C	…	…
	…	NI	…	…	…	…	185 T-C	…	…
	…	NI	…	…	…	…	218 G- A	…	…

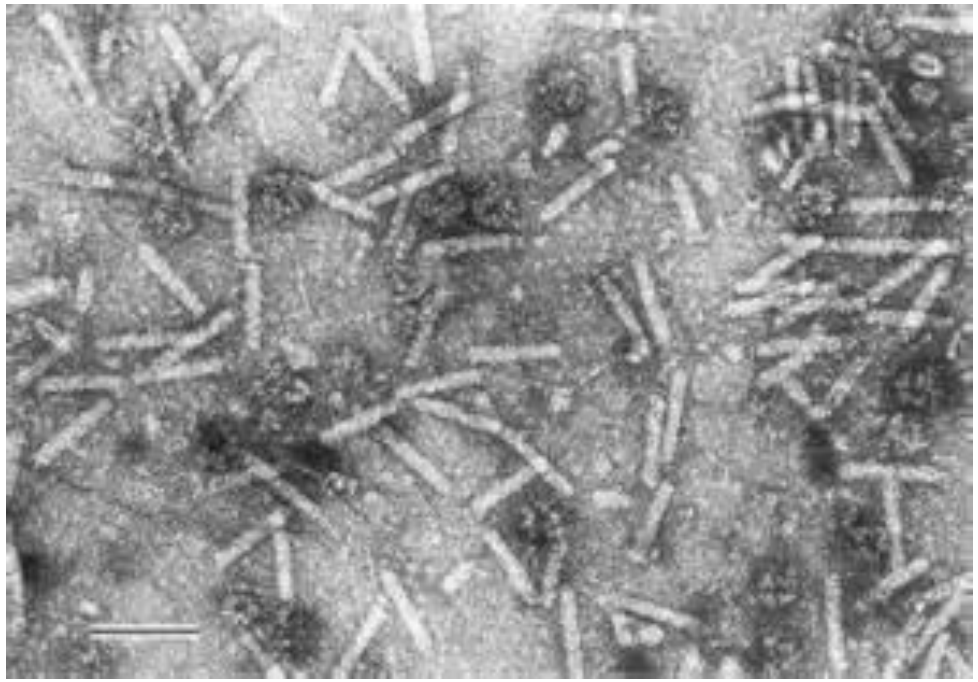
[a] MLST = multilocus sequence typing, ST = sequence type, NI = noninformative, and an asterisk (*) indicates substitutions.

Aguilar-Granados et al., 2021.

Identificación de fagos líticos (control biológico).

- *Xylella fastidiosa*
- *X. citri*
- *X. campestris*
- *C. michiganensis*
- *R. solanacearum*

- *Salmonella sp*
- *Listeria monocytogenes*

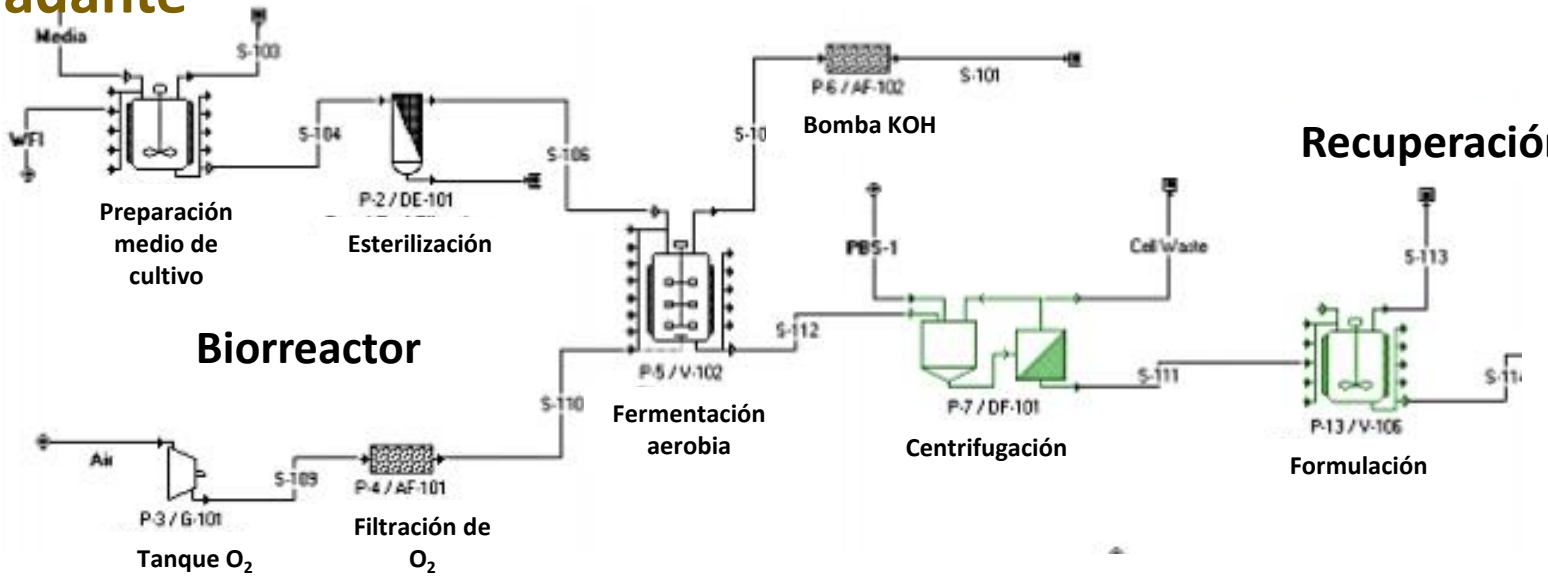


Aguilar-Granados et al., 2022.

Bioingeniería de la producción de fagos líticos

Operaciones unitarias a capacidad instalada de 10 L

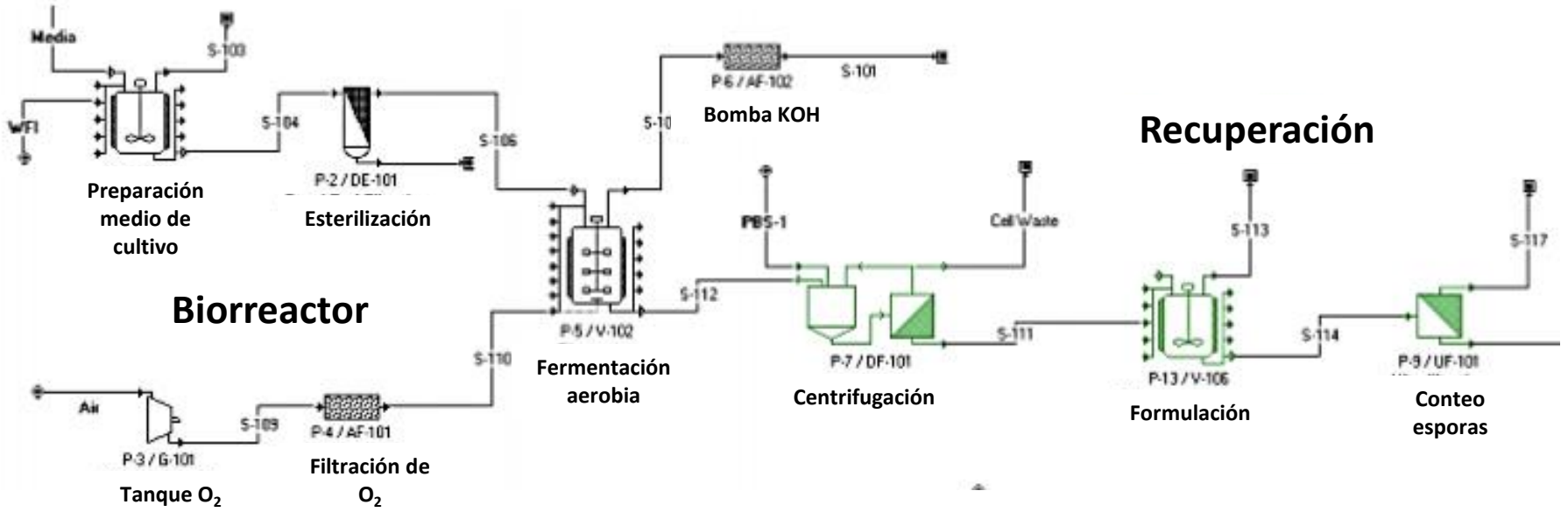
- **Bancos de bacterias y fagos**
- **Fermentación en biorreactor aeróbico**
  (lote alimentado, control pH, O_2, temperatura)
- **Centrifugación**
- **Ultrafiltración de sobrenadante**
- **Formulación**
- **Conteo de UFP**
- **Pruebas de estabilidad**



Bioingeniería de la producción de esporas de *Bacillus*

Operaciones unitarias a capacidad instalada de 10 L

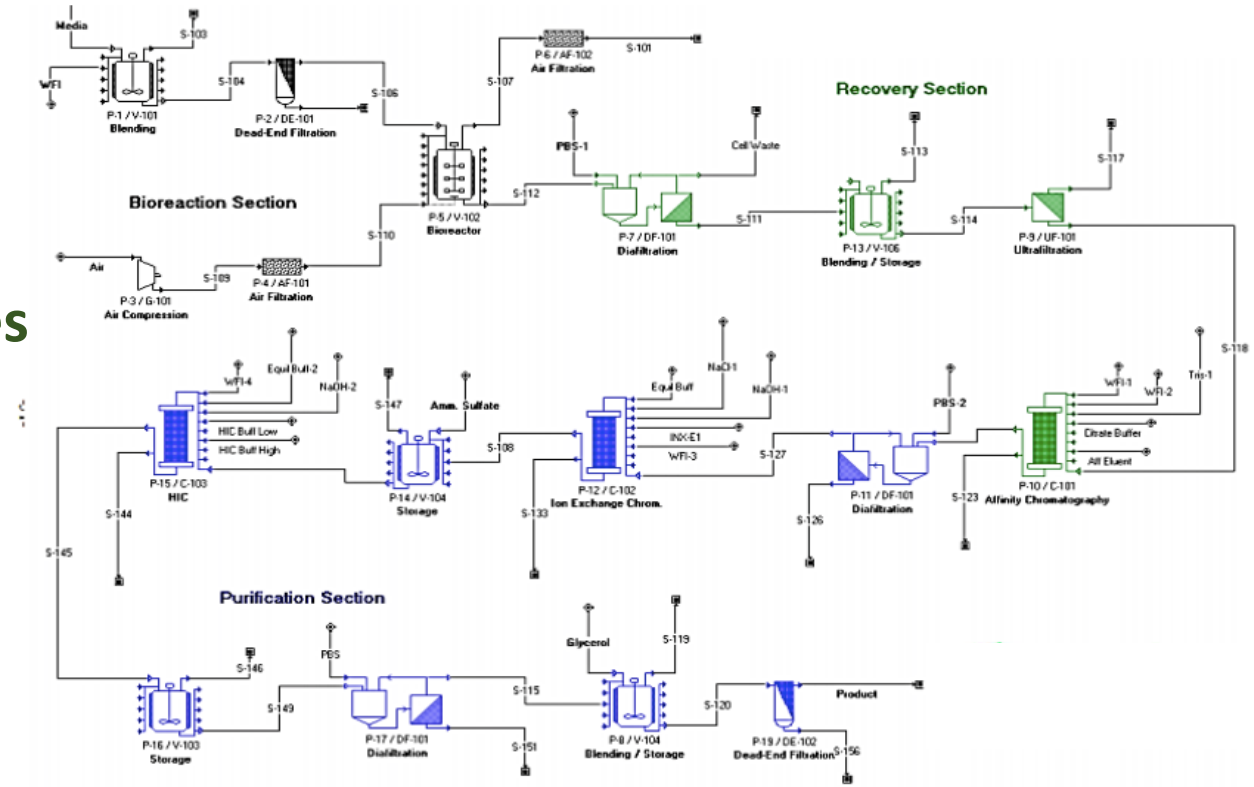
- **Banco celular**
- **Fermentación en biorreactor aeróbico**
  (lote alimentado, control pH, O_2, temperatura)
- **Centrifugación**
- **Secado**
- **Formulación**
- **Conteo de esporas**
- **Pruebas de estabilidad**



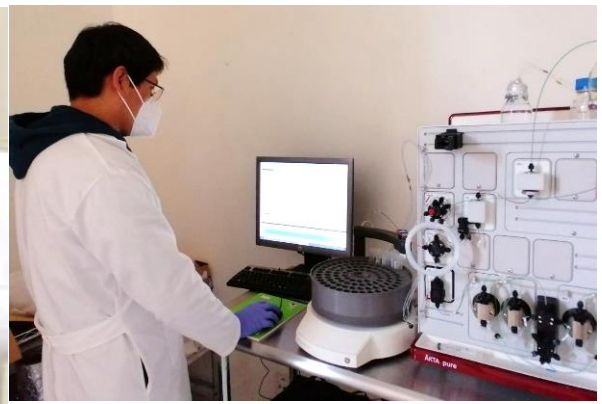
Bioingeniería de la producción de **lactonasa** para bloquear ***Quorum Sensing*** en bacterias

Operaciones unitarias a capacidad instalada de 10 L

Banco celular
Fermentación en biorreactor aeróbico (lote alimentado, control pH, O_2, temperatura)
Centrifugación
Lisis celular por sonicación
Purificación de cuerpos de inclusión
Solubilización de IB
Cromatografía de intercambio de iones
Replegamiento de proteínas
Esterilización por filtrado
Cuantificación de proteínas



Producción en cuarto limpio



Núñez-Muñoz et al., 2021.

Cinvestav

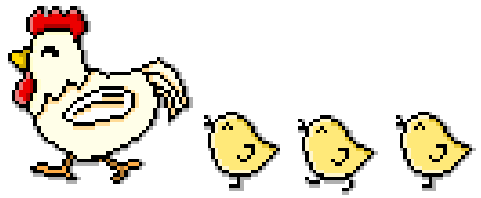
Vacunas recombinantes de uso pecuario

Genes sintéticos que codifican antígenos vacunales

Botulismo - Antigenic domains of C and D toxins of C*lostridium*

Influenza H7N3 - Hemaglutinin and neuraminidase ID in Mexico.

Adyuvantes nuevos - *Interleukins 2 and 4*



Grupo Interdisciplinario

CINVESTAV

Dr. Roberto Ruiz Medrano, Biotecnología
Dra. Beatriz Xoconostle Cázares, Biotecnología
Dra. Berenice Calderón Pérez
Dr. José Abrahán Ramírez Pool
Dra. América Padilla Viveros, Transdisciplinario
Dr. Marco Antonio Vega López, Infectómica
Dr. José Luis García Cordero, U Monterrey
Dr. Edgar Morales, Bioquímica
Dr. Salvador Gallardo, Física
Dr. Octavio Ramos Flores, UPEAL
Dr. Jorge Fernández Hernández, UPEAL
Aux. MC. Brenda Vargas Hernández
Aux. Leandro Nuñez Muñoz
Dra. Guadalupe Segura Covarrubias
Dr. Rogelio González González
Dr. Gabriel Marcelino Pérez
M.C. Diana Tapia Sidas
M.C. Ana Olivares
Dr. Daniela Roa, Bioquímica
Dr. Víctor Rosales, LANSE
Dr. Leopoldo Santos, Biomedicina
Dra. Leticia Cedillo, Biomedicina
Dr. Luis Brieba Castro

SILANES

Dr. Jorge González
M.C. Araceli Olguín
M.C. Marcela Farías

IMSS

Dra. Rosalía Lira Carmona
Dr. Eduardo Ferat.
Dr. Constantino López
Dr. Niels Wacher Hansen
Dr. César Raúl González Bonilla
Dr. Mauro Eduardo Berta Damaski

LEI

Dr. Cecilia Padierna Mota

VCGA Consultores

UC Davis, USA

Dr. Bill Lucas
Dr. Bob Gilbertson
Dr. María Rojas

Estudiantes graduados


Beatriz Xoconostle Cázares


Roberto Ruiz Medrano


Berenice Calderón Pérez


Angélica Concepción Martínez Navarro

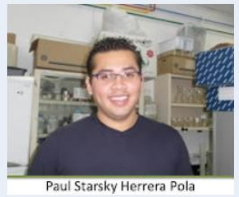

Jorge Luis Ruiz Salas


Diego Fernando Gutiérrez Galeano


Roberto Toscano Morales


Miguel Ángel Guerra Lupián


Santiago Valentín Gordillo


Paul Starsky Herrera Pola


Rebeca Zecua Nájera

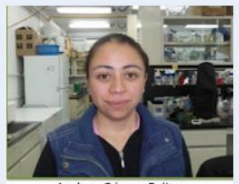

Emma Oliva Fuentes Ramírez


Kenny Alejandra Agreda Laguna


Elena María Peñaranda Lizarazo


Lilian Rocío Duplan Bermúdez


Jessica Citlalli Cedillo Jiménez

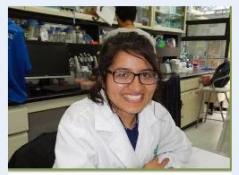

Andrea Gómez Feline

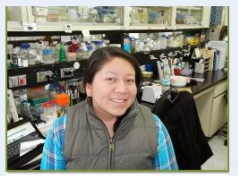

José Luis Cruz Jaramillo


Ariane Regina Razo Rodríguez


Lidia Guadarrama Valencia


Carlos Gabriel Espinoza Amador


Brenda Beatriz Xoconostle Morán

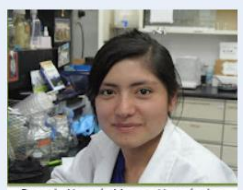

Laura Sofía Castillo Ortega


José Abrahán Ramírez Pool


Cristina Niño Navarro


José Andrés Galeana López


Silvia Beatriz Altamirano Morales


Brenda Yazmín Vargas Hernández


José Luis Sánchez Figueroa


Lourdes López Cruz

Financiamiento y Asesoría

Aliados

VCGA
Consultores

Roberto Ruiz Medrano
Beatriz Xoconostle Cázares

rmedrano@cinvestav.mx
beatriz_xoconostle@yahoo.com
bxoconos@cinvestav.mx

www.*biotecnologia.cinvestav.mx*
lab-biotecnologia.blogspot.com/
@XoconostleC