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Ecogenomic characterization of Begomovirus in Natural and Agricultural ecosystems to understand the origin of new diseases

JUAN JOSÉ MORALES AGUILAR

TESIS

PRESENTADA COMO REQUISITO PARCIAL PARA OBTENER EL GRADO DE

DOCTOR EN CIENCIAS EN BIOTECNOLOGÍA

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SECRETARÍA DE INVESTIGACIÓN Y POSGRADO

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1.- Se designa al aspirante el tema de tesis titulado:
Ecogenomic characterization of begomovirus in natural and agricultual ecosystems to understand the origin of new diseases

De manera general el tema abarcará los siguientes aspectos:
Characterize begomovirus diversity by ecogenomics analysis from natural ecosystems and agroecosystems in North states of Mexico
Characterize molecular and biologically begomovirus isolated from agroecosystems and their association to emerging diseases in *Solanaceus* crops
Determine the infective capacity of begomovirus isolated from *main* plant families from natural ecosystems and their potential to induce new diseases in *Solanaceus* crops

2.- Se designan como Directores de Tesis a los Profesores:
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ACTA DE REVISIÓN DE TESIS

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Ecogenomic characterization of Begomovirus in Natural an Agricultural ecosystems to understand the origin of new diseases

Presentada por el alumno:

Morales	Aguilar	Juan José
Apellido paterno	Apellido materno	Nombre(s)

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aspirante de:

DOCTOR EN CIENCIAS EN BIOTECNOLOGÍA

Después de intercambiar opiniones los miembros de la Comisión manifestaron ***APROBAR LA TESIS***, en virtud de que satisface los requisitos señalados por las disposiciones reglamentarias vigentes

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The present work was carried out in the Laboratory of Molecular Virology of the Department of Agricultural Biotechnology, of the Interdisciplinary Research Center for Regional Integral Development, Sinaloa Unit of the National Polytechnic Institute (CIIDIR-IPN), under the direction of Dr. Jesús Méndez Lozano and Dr. Andreas Voloudakis.

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Albert Einstein

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Resumen

La agricultura es una de las actividades socioeconómicas mas importantes en todo el mundo. La producción de cultivos se ve reducida en parte por el efecto de factores bióticos. Los virus son de especial consideración. Dentro de los virus que afectan a las plantas, el género *Begomovirus* (Familia *Geminiviridae*), posee mas de 320 especies reconocidasd por el (ICTV) y representan una amenaza constante para la producción de alimentos debido a que causan enfermedades afectado el rendimiento y calidad de los cultivos. Para mejorar y asegurar la producción alimentaria es necesario conocer la diversidad y distribución de virus con especial cuidado al género *Begomovirus*, debido a esto, en este trabajo se determino la presencia de begomovirus en plantas cultivadas y no cultivadas de los estados del norte del Pacifico Mexicano, para lo cual se collectaron 260 plantas de cultivos de tres familias principalmente y 422 plantas no cultivadas de las cuales se identificaron 132 especies y 34 familias. Para todas las plantas colectadas se les extrajo DNA y se determino la presencia de geminivirus mediante PCR. De las plantas cultivadas se obtuvieron 22 genomas de diferentes virus mediante el uso de RCA. De las plantas no cultivadas se juntaron las muestras de DNA pertenecientes a la misma especie y se amplifico mediante el uso de amplificación por circulo rodante (RCA) para su posterior procesamiento por secuenciación masiva (HTS) y analisis bioinformático. En plantas cultivadas se observo la presencia de al menos cinco virus associados a enfermedades de los cultivos de tomate y chile, que estan afectando las agroregiones: Comarca Lagunera en Coahuila, valle Poanas, Durango y Huatabampo, Sonora. Los analisis de HTS indican la presencia de al menos 52 virus relacionados a cultivos y 35 relacionados a begomovirus que infectan plantas no cultivadas, sugiriendo que las plantas no cultivadas son hospederos de una diversidad de begomovirus y que se encuentran presentes en diferentes regiones agro climaticas. Por ultimo se analizo el riesgo potencial de un begomovirus presente en plantas no cultivadas, se determinó que tienen la capacidad de infectar tomate, el cual es una planta de interes economico también se determino que no representan una amenaza para el cultivo del tomate por si mismo, sin embargo la posibilidad de estar presente en infecciones mixtas abre una puerta hacia fenomenos de recombinación, ruptura de tolerancia y sugieren que pueden ser origen de nuevas enfermedades.

Summary

Agriculture is one of the most important socio-economic activities in the world. Crop production is reduced by the effect of abiotic and biotic factors. Viruses are of special consideration, among the plant viruses, the genus *Begomovirus* (Family *Geminiviridae*), has more than 320 species recognized by the (ICTV) and represent a constant threat to food production because of the cause symptomatology of the disease that affects the yield and quality. To improve and ensure food production it is necessary to know the diversity and distribution of viruses with special care to the genus *Begomovirus*, due to this, in this work the diversity of begomovirus was determined in cultivated and non-cultivated plants of the northern states of the Mexican Pacific. To accomplish this 260 crop leaf plants were collected (including three plant-families) and 422 non-cultivated plants of which 132 species and 34 families were identified. All samples were georeferenced, photographed and preserved in ice. Total DNA was extracted, plant positive to geminiviruses was determined by PCR. Twenty-two full-length genomes of different viruses were obtained from a cultivated plant. From the non-cultivated plants, pools of DNA samples belonging to the same species were submitted to amplification by rolling circle amplification (RCA) and then processed by High through sequence (HTS), and bioinformatic analysis. The results of the cultivated plants indicated the presence of at least five viruses associated with diseases of tomato and pepper crops, in the agro-region known as La Comarca Lagunera in Coahuila, also in Poanas Valley, Durango and Huatabampo , Sonora. HTS analysis indicated the presence of at least Fifty-two crop-related viruses and thirty-five related to begomoviruses that infect non-cultivated plants, suggesting that non-cultivated plants are host to a variety of begomoviruses in different agro-climatic regions. Finally, the potential risk of a begomovirus present in non-cultivated plants was analyzed, showing that has the capacity to infect plants of economic importance such as tomato, however, due to the symptomless infection, suggest that, they are not a threat to the tomato crop by themselves but the possibility of being present in mixed infections opens a door towards recombination phenomena, rupture of tolerance and suggest that they can be the origin of new diseases.

Introduction

Viruses are ubiquitous and can affect plants. Plants viruses are economically important due to, the alteration of normal plant development behavior, the crop losses all worldwide in terms of quantity and/ or quality, these losses have been very devastating and are of great concern for the developing countries as Mexico. (Hull, 2014; Nicaise, 2014). Fast and precise identification is the main focus of attention in the field of virology, either in an ecological or phytopathological approach. Since this knowledge can allow the prevention of the dissemination of these viruses as well as the understanding of the roles they play in the habitat (Roossink, 2015; Ronssinsk and stobe).

Geminivirus (family Geminiviridae), are important plant viruses worldwide, they are circular single-stranded (ss) DNA viruses packed into icosahedral twinned-shaped particles, which cause severe diseases in major crop plants worldwide (Leke, Mignouna, Brown, & Kvarnheden, 2015; Varma & Malathi, 2003; Zerbini et al., 2017). The viruses that belong to this family are classified in nine genera (*Becurtovirus, Begomovirus, Capulavirus, Curtovirus, Eragovirus, Grablovirus, Mastrevirus, Topocuvirus, and Turncurtovirus*) according to their genome organization, the host range, and type of insect vector (Zerbini et al., 2017).

The genus Begomovirus

The Begomovirus the most diverse genus (>320 species), and comprise economically important viruses. They are transmitted by the polyphagous insect vector whitefly (*Bemisia tabaci*). They infect to diverse dicotyledonous plants worldwide (Hull, n.d.; Zerbini et al., 2017). The genomes of begomoviruses that are native to the New World (NW) usually are bipartite, consisting of two components that are designated DNA-A and DNA-B. In contrast, most of the known Old World (OW) begomoviruses have monopartite genomes consisting of single DNA molecules homologous to the DNA-A component of bipartite begomovirus. The DNA-A component encodes viral functions required for viral DNA-A and DNA-B replication, transcription and vector-assisted transmission, whereas DNA-B component encodes proteins required for cell-

to-cell and long-distance viral particles movement in host plants (Vincent N Fondong, 2013).

Begomovirus associated to crops diseases

Begomoviruses are important plant-infecting pathogens. Diseases complexes caused by begomoviruses are an emerging threat to vegetable productions worldwide(Blawid, Fontenele, Lacorte, & Ribeiro, 2013; Chang-Sidorchuk, González-Alvarez, Navas-Castillo, Fiallo-Olivé, & Martínez-Zubiaur, 2017; Domínguez-Durán et al., 2018; Leke et al., 2015, 2013; Macedo et al., 2018; Mohammed, El Siddig, El Hussein, Navas-Castillo, & Fiallo-Olivé, 2018a; Saeed & Samad, 2017; ZHAN, CAO, WANG, & ZHOU, 2018). Begomovirus disease has been observed and reported in Mexico since 1990, and for almost three decades of research, begomovirus has been associated to crops disease. There are about 17 begomovirus species associated with crops diseases in Mexico (Table 1), crops as tomato, pepper bean, pumpkin, soy, tobacco, watermelon, papaya, and okra. Some of these begomoviruses have been reported so far only in Mexico, e.g. *Pepper Huasteco yellow vein virus* (PHYVV), another first reported in Mexico follows by reports in other countries in America, e.g. *Pepper golden mosaic virus* (PepGMV), others begomoviruses first reported in other country and introduced to Mexico some time ago e.g. *Tomato yellow leaf curl virus* (TYLCV) or recently introduced e.g. *Watermelon chlorotic stunt virus* (WmCSV) (Ascencio-Ibáñez et al., 1999; Domínguez-Durán et al., 2018; J. Antonio Garzon-Tiznado, 1993).

Table 1. Relation of begomovirus associated to crop diseases in Mexico.

Virus	Crop	References
Chino del tomate virus	Tomato	(A. M. Idris, Lee, & Brown, 1999; Mauricio-castillo, Argüello-astorga, Bañuelos-hernández, & Ambríz-, 2014)
Pepper golden mosaic virus	Tomato	(Judith K. Brown & Poulos, 1990; R J Holguín-Peña, Vázquez-Juárez, &

		Rivera-Bustamante, 2004; Ramón Jaime Holguín-Peña, Vázquez Juárez, & Rivera-Bustamante, 2004)
	Pepper	(Jose A Garzon-Tiznado, Acosta-Garcia, Torres-Pacheco, Gonzalez-Chavira, Rivera-Bustamante, Maya-Hernandez, & Guevara-Gonzalez, 2002; Hernández-espinal et al., 2018; Rodelo-Urrego, Garcia-Arenal, Pagan, García-Arenal, & Pagán, 2015; Torres-Pacheco, Garzon-Tiznado, Herrera-Estrella, & Rivera-Bustamante, 1993b)
	Tobacco	(Paximadis et al., 1999)
	Soy bean	(Méndez-Lozano, Quintero-Zamora, et al., 2006)
Pepper Huasteco yellow vein virus	Tomato	(Bañuelos-Hernández, Mauricio-Castillo, Cardenas-Conejo, Guevara-González, & Arguello-Astorga, 2012; A. M. Idris et al., 1999)
	Pepper	(Jose A Garzon-Tiznado, Acosta-Garcia, Torres-Pacheco, Gonzalez-Chavira, Rivera-Bustamante, Maya-Hernandez, & Guevara-Gonzalez, 2002; Hernández-espinal et al., 2018; Melendrez-Bojorquez et al., 2016; Rodelo-Urrego, Garcia-Arenal, et al., 2015; Torres-Pacheco et al., 1993b)

	Pumpkin	(Jose A Garzon-Tiznado, Acosta-Garcia, Torres-Pacheco, Gonzalez-Chavira, Rivera-Bustamante, Maya-Hernandez, & Guevara-Gonzalez, 2002)
	Papaya	(Jose A Garzon-Tiznado, Acosta-Garcia, Torres-Pacheco, Gonzalez-Chavira, Rivera-Bustamante, Maya-Hernandez, & Guevara-Gonzalez, 2002)
	Bean	(Jose A Garzon-Tiznado, Acosta-Garcia, Torres-Pacheco, Gonzalez-Chavira, Rivera-Bustamante, Maya-Hernandez, & Guevara-Gonzalez, 2002)
Tomato yellow leaf curl virus	Tomato	(Ascencio-Ibáñez et al., 1999; Bañuelos-Hernández et al., 2012; J K Brown & Idris, 2006)
	Pepper	(Cardenas-Conejo et al., 2010; Hernández-espinal et al., 2018)
	Tomatillo	(Gamez-Jimenez, Romero-Romero, Santos-Cervantes, Leyva-Lopez, & Mendez-Lozano, 2009)
Tomato mottle virus	Tomato	(Garrido-Ramirez & Gilbertson, 1998)
Tomato leaf curl Sinaloa virus	Tomato	(a M. Idris & Brown, 1998)
Tomato severe leaf curl virus	Tomato	(Bañuelos-Hernández et al., 2012; J. A. Mauricio-Castillo et al., 2006a)

Tomato chino La Paz virus	Tomato	(Bañuelos-Hernández et al., 2012; R J Holguín-Peña, Vázquez-Juárez, & Rivera-Bustamante, 2005)
	Pepper	(Cardenas-Conejo et al., 2010)
Cucurbit leaf curl virus	Melon	(J K Brown et al., 2000)
Rhynchosia golden mosaic virus	Tobacco	(Ascencio-Ibanez, Arguello-Astorga, Mendez-Lozano, & Rivera-Bustamante, 2002)
	Soy bean	(Mendez-Lozano et al., 2006)
Okra yellow mottle Iguala virus	Okra	(De La Torre-Almaraz, Monsalvo-Reyes, Romero-Rodriguez, Argüello-Astorga, & Ambriz-Granados, 2006)
Watermelon chlorotic stunt virus	Watermelon	(Domínguez-Durán et al., 2018)
Tobaco apical stunt virus	Tobacco	(Paximadis et al., 1999)
Euphorbia mosaic virus	Pepper	(Gregorio-Jorge, Bernal-Alcocer, B??uelos-Hernndez, et al., 2010)
Bean golden yellow mosaic virus	Bean	(Garrido-Ramirez, Sudarshana, & Gilbertson, 2000)
Bean calico mosaic virus	Bean	(Bronw, 1999)
Cotton leaf crumple virus	Cotton	(a M. Idris & Brown, 2004)
Tomato golden mottle virus	Tomato	(J. A. Mauricio-Castillo, Argüello-Astorga, Ambriz-Granados, & Alpuche-Solís, 2007)

The non-cultivated plants also are infected by begomovirus

The real challenges of the begomoviral diseases are in non-cultivated hosts, many of these viruses are new species, some resulting from recombinations, others causing diseases in non-cultivated plants (Al-Aqeel, Iqbal, & Polston, 2018; Alabi, Villegas, Gregg, & Murray, 2016; Ferro et al., 2017; Fontenele et al., 2018; Murtaza,

Mubin, Nawaz-ul-rehman, & Amrao, 2018; Sohrab & Daur, 2018; Zhao, Zhong, Zhang, Ding, & Zhang, 2018). The ecological role that they are playing in these uncultivated plants is a big question and needs to do more research (Malmstrom, Melcher, & Bosque-Pérez, 2011; Marilyn J Roossinck, 2011; Stobbe & Roossinck, 2014), but what is certain, is that non-cultivated plants could be a source of viral inoculum to cultivated plants (Aguiar, Alves, Queiroz, Nascimento, & Lima, 2017; Basak, 2016; Bekele et al., 2018; Paz-Carrasco et al., 2014; Perry, McLane, Thompson, & Fuchs, 2018; Strydom & Pietersen, 2017; Tahir, Amin, Haider, Mansoor, & Briddon, 2015). In Mexico non-cultivated plants are host of some begomovirus (Table 2), nevertheless the information that has been acquired is important, it comprehends only a few plants species of some plant families considering to Mexico as the 4th place of megadiverse countries worldwide (Luna-Vega, Espinosa, Rivas, & Contreras-Medina, 2013), too much have to be done to known the diversity of begomovirus in non-cultivated plants in Mexico.

Table 2. Relation of begomovirus isolated from non-cultivated host in Mexico.

Virus	Host	References
Sida yellow mosaic Yucatan virus	*Sida acuta*	(Cecilia Hernández-Zepeda, Idris, Carnevali, Brown, & Moreno-Valenzuela, 2007)
Corchorus yellow vein Yucatan virus	*Corchorus siliquosus*	(Cecilia Hernández-Zepeda et al., 2007)
Desmoniun leaf distortion virus	*Desmonium glabrum*	(Cecilia Hernández-Zepeda, Arguello-Astorga, Germán, Idris, & Moreno-Valenzuela, 2009)
Rhynchosia yellow mosaic Yucatan virus	*Rhynchosia minima*	(C Hernández-Zepeda et al., 2010)
Sida mosaic Sinaloa virus	*Sida acuta*	(J. A. Mauricio-Castillo et al., 2014)

Euphorbia mosaic virus	*Euhporbia heterophylla*	(C. Hernández-Zepeda, Idris, Carnevali, Brown, & Moreno-Valenzuela, 2007)
Tomato golden mottle virus	*Solanum rostrarum*	(J. A. Mauricio-Castillo et al., 2007)
Pepper Huasteco yellow vein virus	*Alstroemeriae spp, Helianthus spp., Solanum rostrarum*	(Cervantes-Díaz et al., 2009; Jose A Garzon-Tiznado, Acosta-Garcia, Torres-Pacheco, Gonzalez-Chavira, Rivera-Bustamante, Maya-Hernandez, Guevara-Gonzalez, et al., 2002)

The Begomovirus have the potential to infect different plant families

The Begomoviruses have been found in a wide variety of plants including several families. Some examples of these are: some begomoviruses were first reported in a *Malvaceous* host but also encountered affecting plants of the family *Fabaceae*, *Solanaceae*, Collins reported that once the begomovirus is isolated can back-inoculated to his natural host (Collins et al., 2009). It had been studied virus first reported in *Euphorbiaceous* host then infecting *Solanaceae* plants and again once isolated could biolistic-inoculated another plant species of *Solanaceae* plant family (Gregorio-Jorge, Bernal-Alcocer, Bañuelos-Hernández, et al., 2010). A begomovirus isolated from *Euphorbiaceae* plant can infect *Fabaceae* plants and some *Solanaceae* plants and also got back to *Euphorbiaceae* plants (C. Hernández-Zepeda et al., 2007). In another study, Hernandez-Zepeda studied a begomovirus first reported in *Fabaceae* plants and could infect *Fabaceae* plants and *Solanaceae* plant *N. benthamiana* (C Hernández-Zepeda et al., 2010). It has been found that the monopartite begomovirus TYLCV has a large host range including species of the *Amaranthaceae, Chenopodiaceae, Compositae, Convolvulaceae, Cruciferae, Euphorbiaceae, Geraniaceae, Leguminosae, Malvaceae, Orobanchaceae, Plantaginaceae, Primulaceae, Solanaceae, Umbelliferae,* and *Urticaceae* plant families (Papayiannis,

Box, & Katis, 2011; Papayiannis et al., 2010). Bladwid isolated a new Begomovirus from *Corchorus hirtus* a plant belonging to *Malvaceae* plant family and biolistic-inoculated to another *Malvaceae* plant species and two Solanaceae plants (Blawid et al., 2013). Barreto characterized a begomovirus, first reported in Tomato a Solanaceae plant and that once isolated could biolistic infect some plants of the family *Asteraceae, Fabaceae, Euphorbiaceae and other Solanaceae* plants (Barreto, Hallwass, Aquino, & Inoue-Nagata, 2013). A virus present in *Solanaceae* plant family has been reported infecting other plants families like *Cucurbitaceae, Fabaceae, Malvaceae, Compositeae*, also another plant species of *Solanaceae* plant family (Sánchez-Campos et al., 2013) (figure 2). These results suggest that some begomoviruses have the ability to infect several plant species, even belonging to different plant families.

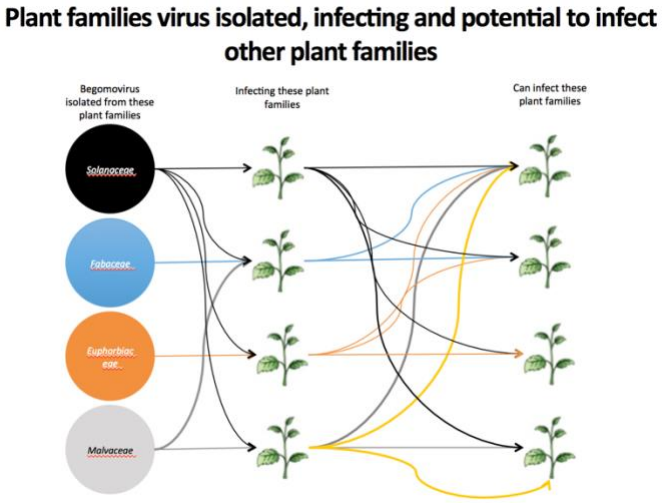

Figure 2. Select begomovirus and their potential to infect different plant species. The color of the lines show, the potential of some begomoviruses to infect different families of plants.

Metagenomics to discover virus

Traditional methods such as serology, polymerase chain reaction, have limited use in research to identify the infectious agents in which do not have any knowledge. In contrast, the state of the art in genomics technologies such as microarrays and next-generation sequencing (NGS) may be attractive tools for the detection of new pathogens (Chiu, 2013). Subsequent NGS studies in oceans, mines, soils, and lagoons

have helped to understand a little more about the importance of microbial communities for the evolution of life in different environments (Angly et al., 2006; Barba, Czosnek, & Hadidi, 2013; Coghill, 2013; Corinaldesi, 2015; Czotter et al., 2018; Edwards et al., 2006; Jo et al., 2017; Rusch et al., 2007; Sainju, Dris, & Singh, 2003; Sogin et al., 2006; Williamson et al., 2008; Winter, Garcia, Weinbauer, DuBow, & Herndl, 2014). With the arrival of new technologies as rolling circle amplification and Next-generation sequencing (NGS), discovering certain pathogens as begomovirus has increased. The use of these tools using circular DNA also called "circomics" has led us to the identification of new genomes, infecting plants (Dayaram et al., 2013; Jo et al., 2017; Patricia Soares Wyant et al., 2012). Also found a virus and its subviral satellite particles (Fei et al., 2011; Ng et al., 2012). Different approaches with NGS have been analyzed and used in order to obtain the plant viromes, including RNA-seq (using small RNA and messenger DNA, mRNA) (François, Filloux, Fernandez, Mylène, & Roumagnac, 2018; Jones, Baizan-Edge, MacFarlane, & Torrance, 2017; Pagán, 2018; Marilyn J Roossinck, 2012). All this technology has proved to be efficient to obtain begomovirus genomes from a sample with multiple viruses (A. Idris et al., 2014). The potential use of this technology can lead us to know more about the biodiversity of begomoviruses present in the region, also this could finally help elucidate programs, sustainable strategies and biotechnological development packages that allow us to respond to actual agricultural viral problematic (M. J. Roossinck, Martin, & Roumagnac, 2015; Marilyn J. Roossinck & García-Arenal, 2015).

Justification

Nowadays, the agricultural sector is particularly important for the economy, only the potato and tomato crops contribute to 50% of vegetable production worldwide. Agriculture in Mexico is one of the most important activities, both socially and economically for the foreign exchange earnings and employment generation. Also, is known that production, yield, and quality can be strongly affected by viruses. Within the Geminiviridae viral plant-family, the Begomovirus genus is the most abundant among them and are responsible for significant losses in various agricultural regions worldwide. Interestingly, many of the non-cultivated plants that live around the agroecosystems belong to the five or six families of plants of the main crops and probably these uncultivated plants can host viruses that are a potential risk to cause diseases in economically important crops. Evolution and discovery of mixed begomovirus infections make us think about the possibility of the emergence of new variants of viruses that may impact on commercially valuable plant host. The use of new technologies such as next-generation high throughput sequencing and bioinformatics, allow us to analyze a large number of plants populations, this will provide us, the opportunity to have a more throughout knowledge about the ecology and evolution of begomoviruses to anticipate possible diseases that may arise in the field leading us to a sustainable agriculture.

Hypothesis

The Begomovirus diversity isolated from non-cultivated plants in natural and agricultural ecosystems in Northern Mexico allows us to understand the origin of new viral diseases.

General objective

Characterization of begomovirus in natural and agricultural ecosystems to understand the origin of new viral diseases.

Specific objectives

- To analyze molecularly and biologically begomovirus isolated from agroecosystems and their association to emerging diseases in horticultural crops.

- To characterize begomovirus diversity by ecogenomics analysis from natural ecosystems from Northern States of Mexico.

- To determine the infective capacity of begomovirus isolated from main plant families from natural ecosystems and their potential to induce new diseases in *Solanaceus crops*.

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Chapter I.

Analyzing begomovirus isolated from agroecosystems and their association to emerging diseases in crops.

Pulished in:

Identification of *Tomato yellow leaf curl virus, Pepper Huasteco yellow vein virus* and *Pepper golden mosaic virus* associated with pepper diseases in Mexico Summited in: Morales-Aguilar JJ, Camacho-Beltrán E, Rodríguez-Negrete EA, Santos-Cervantes ME, Leyva-López NE, A, López-Luque CA, Jiménez-Díaz F, Voloudakis A, Méndez-Lozano J. 2018. Identification of *Tomato yellow leaf curl virus*, *Pepper Huasteco yellow vein virus* and *Pepper golden mosaic virus* associated with pepper diseases in Mexico. Can. J. Plant Pathol.

Identification of Tomato yellow leaf curl virus, Pepper Huasteco yellow vein virus and Pepper golden mosaic virus associated with pepper diseases in Mexico

Abstract

New diseases in pepper plantations were discovered in La Comarca Lagunera (CL) region in September 2014, the severity of which increased by October 2016. Pepper plants exhibited mild and severe yellow leaf mosaic, deformation, stunting and chlorotic leaves. In addition, whiteflies were observed on symptomatic plants, suggesting a possible begomovirus aetiology. In this study, naturally infected pepper plants were collected during three consecutive years to identify the potential begomovirus present in pepper in CL. PCR detection using degenerate and specific primers indicated that 47 out of 49 pepper plants were infected by begomoviruses mainly in mixed infection. The complete begomovirus genomes were isolated from a representative symptomatic pepper plant and two clones for each begomovirus were fully sequenced for the corresponding year of collection (2014 to 2016). Phylogenetic analysis of complete genomes of CL begomovirus pepper isolates indicated a close homology with Tomato yellow leaf curl virus (designated TYLCV-CL) displaying 99.9–100% identity with TYLCV Sinaloa isolate, and the bipartite Pepper huasteco yellow vein virus (designated PHYVV-CL) displaying 94.5% and 84.2% identity with first PHYVV isolate from Tamaulipas for DNA A and DNA B, respectively, and 97–98% identity with PHYVV Sinaloa isolate for DNA B. In 2016, Pepper golden mosaic virus (designated PepGMV-CL) was also found that consisted of DNA-A and DNA-B genome displaying 97% and 93.5% identity with PepGMV isolate Tamaulipas. To our knowledge, this is the first report of a pepper disease associated with TYLCV in double or triple infection either with PHYVV and/or PepGMV in Mexico.

Introduction

Pepper (*Capsicum annuum* L.) is an economically important crop in Mexico and makes up 1.93% of the country's worldwide exports (FAOSTAT, 2013). In 2015, the national pepper production was 2.7 million metric tons valued at US$1,252 million (SIAP, 2015). The Comarca Lagunera (CL) is a growing economic region in Northern

Mexico comprising counties of the two states, namely Durango and Coahuila. Since 2014, farmers and small producers have described the emergence of virus-like diseases affecting crop yield. In 2016, the increased severity of these pepper idsases and high populations of whitefly, as observed in the affected areas, has led to a yield reduction in pepper cultivation in CL.

Members of genus *Begomovirus* (Family *Geminiviridae*) are associated with different crop diseases, causing an enormous concern for global agriculture (Leke et al., 2015) especially under global warming that could alter the distribution of their insect vectors. In Mexico, a pepper disease named “rizado amarillo” was initially described as the coinfection of *pepper huasteco yellow vein virus* (PHYVV) and *Pepper golden mosaic virus* (PepGMV) (Garzon-Tiznado et al., 1993). Thereafter, both viruses were reported to infect pepper in several Mexican states such as Guanajuato, Jalisco, Oaxaca, Queretaro, San Luis Potosi, Sinaloa, Sonora, Tamaulipas and Yucatán, causing severe reductions in pepper yield production (Garzon-Tiznado, 1993; Torres-Pacheco et al., 1993; Garzón-Tiznado et al., 202; Méndez-Lozano et al., 2003; Rodelo-Urrego et al., 2015). An in crease in sweet pepper and tomato disease was reported in Sinaloa state in the last few years, which was found to be associated with a new isolate fo PHYVV (Melendrez-Bojorquez et al., 2016; Moreno-Félix et al., 2018).

Tomato yellow leaf curl virus (TYLCV), one of the most devastating begomoviruses affecting tomatoes worldwide, was first detected in Mexico in the Yucatán peninsula in 1999 (Ascencio-Ibáñez et al., 1999) and then reported in Sinaloa in tomato and tomatillo crops (Gámez-Jiménez, 2007; Gámez-Jimenez et al., 2009). Subsequently, it was found singly and in mixed infections with other begomoviruses, affecting mainly tomato plants in the states of Sonora and Tamaulipas (Hernández-Zepeda et al., 2007; Bañuelos-Hernández et al., 2012).

Infection of *Capsicum sp.* crops by TYLCV has been reported in several countries including Southern Spain (Reina et al., 1999), Dominican Republic (Salati et al., 2002), Cuba (Qiñones et al., 2002), Jamaica (Roye et al., 1999) and Mexico; mixed infection of TYLCV with a bipartite begomovirus *Tomato chino La Paz virus* (ToChLPV) were reported in Baja California Sur (Cardenas-Conejo et al., 2010). The objective of

this work was to detect and identify using molecular methods the begomoviruses associated in pepper in CL, Mexico.

Methods

Plant sampling

During 2014-2016, surveys for pepper diseases were carried out in six open fields in four couties of CL, according to the locations of pepper production during the year of survey (Fig. 1a). Forty-nine mature pepper plants were collected in Torreón, Coahuila (12 in September 2014), Tlahualilo, Durango (three in May 2015), Lerdo, Durango (eight in May 2015 and 17 in October 2016) and Francisco I. Madero, Coahuila (nine in October 2016).

DNA isolation and PCR detection

Total genomic DNA was purified form leaves using the CTAB method (Doyle & Doyle, 1990) PCR was employed on extracted dNA samples to determine the begomovirus present using degenerated primers (Mauricio-Castillo et al., 2007). In addition, PCR using a set of primers specific for TYLCV, which amplify a 180 bp fragment (Rodríguez-Negrete et al., 2014), a set for PepGMV which amplifies a 120 pb fragment (Carrillo-Tripp et al., 2007) and for PHYVV, which amplifies a 161 bp fragment (Supplementary table), were performed to determine mixed viral infections.

Cloning and sequencing of viral DNA

In order to obtain the putative full-length begomovirus monomeric component (~2.7 kb fragment), total DNAs from representative pepper samples collected in 2014, 2015 and 2016 were amplified by rolling circle amplification (RCA) with Φ-29 DNA polymerase (TempliPhi, Ge Healthcare, US) as described previously (Inoue-Nagata et al., 2004) or by a PCR strategy with overlapping primers for TYLCV and PHYVV DNA-A using high fidelity polymerase (iProof™ High-Fidelity DNA Polymerase, BIO-RAD®, US). RCA amplification products were digested with *BamH*I, *Sac*I, *Apa*I and *EcoR*I and

cloned either into *Bam*HI-digested pBluescript SK- vector (Agilent, US) or *Sac*I-, *Apa*I- and *Eco*RI-digested pGreen0029 vector (Hellens et al., 2000). The PCR amplified genomes of 2.7 kb were cloned into pGEM-T Easy Vector System® (Promega, USA) or NEB® PCR cloning kit (New England BioLabs, USA). Two independent clones of each viral component obtainded from samples of the corresponding year were fully sequenced using the primer walking strategy. The assemblies of the sequences were obtained using the SeqMan program (DNASTAR Inc., Madison, USA), and genome comparisons were performed employing Mega 7.0 (Kumar et al., 2016). One sequence of each genome component per year was submitted to the GenBank. Recombination analysis was performed in RDP4 with the default settings using all sevent methods: RDP, GENCONV, BooScan, MaxChi, SiScan, Chimera and 3Seq (Martin et al., 2015).

Results and discussions

Plant sampling and symptoms

In september 2014, a new viral diseas was reported by the growers in a single pepper farm at Torreón, Coahuila and subsequently the same symptomatology was observed in May 2015, where 30% of open field pepper plants showed mild yellow mosaic, deformation and chlorotic leaves, and symptomatic plants were randomly distributed (Fig. 1b). By October 2016, symptoms showed a dramatic increase in severity that included severe yellow mosaic, deformation and chlorotic leaves and stunting that reduced the quality and yield to an extent that rendered farmers not able to harvest (Fig. 1b, c and d). The suspected viral disease incidence increased up to 90% and a high population of whitefly was observed in the surveyed pepper fields (Fig. 1e). In order to determine the agent of the disease, a total of 49 symptomatic pepper leaf samples were collected in four counties of CL during 2014-2016, according to the pepper crop distribution in CL during the year of survey (Fig. 1a).

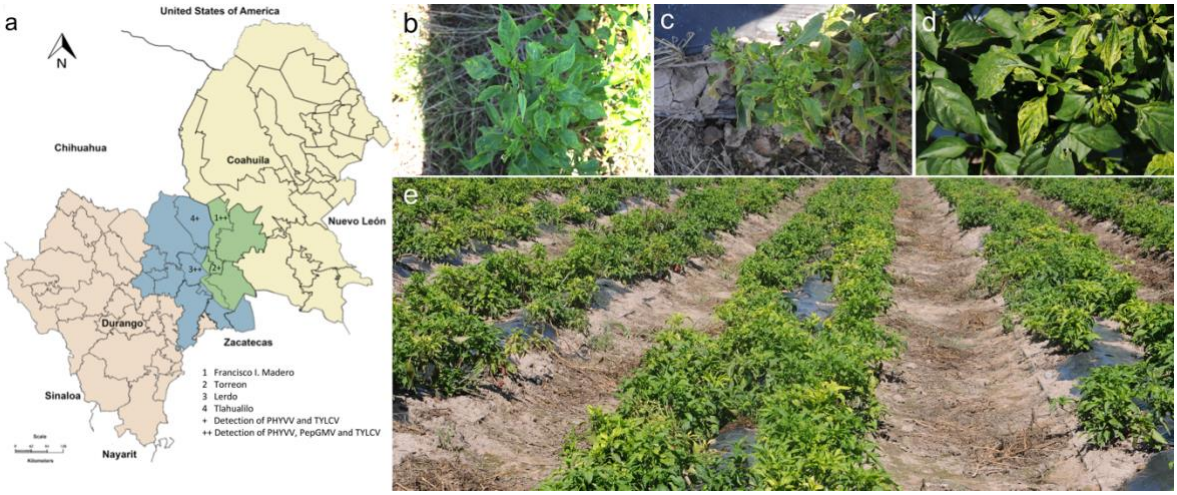

Fig. 1. Symptoms observed in pepper open fields in Comarca Lagunera. (a) Map of CL which includes surveyed counties of Durango state (blue) and Coahuila state (green) of CL. (b) A pepper plant showing yellow mosaic, deforming and chlorotic leaf. (c) A stunted pepper plant with severe leaf deformation and yellowing. (d) A pepper plant with chlorotic leaf and yellow mosaic. (e) General view of pepper open field surveyed in 2016 with a 90% disease incidence caused by begomoviruses when double or triple infection were detected.

Molecular detection and identification of begomoviruses in pepper samples

To investigate the presence of begomoviruses, DNA extracted from symptomatic plants were used as a template in PCR employing degerated primers. The analysis cofirmed the occurrence of begomoviruses in either single or mixed infections (data not shown). Complete begomovirus genomes were obtained using either RCA or PCR amplification from representative pepper samples collected in 2014, 2015, and 2016. Two selected clones were fully sequenced per year, which were 99 – 100% identical to each other. Sequence analyses of selected genomes of the corresponding year indicated that isolates LV157-2014, LV447-2015 and LV56-2016 showed the arrangement of genes typical of the Old World monopartite begomoviruses; the sequences were submitted to GenBank (KX440610, KX440606 and MF945598) and designated as TYLCV-CL. Sequence analysis revealed that TYLCV-CL isolates showed the highest identity of 99.7 – 100% to TYLCV Sinaloa isolate (KU836749). In

contrast, LV165/*Sac*I-2014 and LV42-2016 isolates had a genome organization of a begomovirus DNA-A; the sequences were submitted to GenBank (KY24179 and MG582068) and designated PHYVV-CL DNA A. Isolates LV163/*Bam*HI-2014, LV42/EcoRI-2016 had a genome organization of a begomovirus DNA-B; the sequences were submitted to GenBank (KX440614 and MG582069) and designated PHYVV DNA-B. Sequence analysis revealed that PHYVV-CL shared identity 94.5% for DNA-A and 84.2% for DNA-B to PHYVV isolate Tamaulipas DNA A and B (X70418 and X70419), respectively. Interestingly, PHYVV-CL showed 95% and 98% to PHYVV isolate Sinaloa for DNA A and DNA B (KP890827 and KP890828), respectively. Finally isolate LV46/ECORI-2016 had a genome organization of a begomovirus DNA-A, the sequence was submitted to GenBank (MF109819) and designated PepGMV-CL DNA-A; and LV46/ApaI-2016 isolate had a genome organization of a begomovirus DNA-B, the sequence was submitted to GenBank (MF109821) and designated PepGMV-CL DNA B- Genetic analysis revealed that PepGMV-CL shared 97% and 93.5% identity with PepGMV DNA-A (U57457) and DNA B (AF499442), respectively.

Genome analysis confirms TYLCV, PHYVV and PepGMV associated with pepper disease in CL.

Phylogenetic analysis of the complete genomes of TYLCV-CL, PHYVV-CL and PepGMV-CL with selected begomovirus sequences available in the GenBank showed the highest nucleotide identity with TYLCV, PHYVV and PepGMV as described above (Fig. 2a). The TYLCV-CL genome analysis indicates a nucleotide identity above 99% to the closest TYLCV reported in Sinaloa, Mexico, suggesting that this virus remains genetically stable in a new agro region. To date, it is not known to what extent TYLCV-CL contributes to the pepper infections since the *Capsicum* species have been reported as asymptomatic to single infection with TYLCV (Morilla et al., 2005; Polston et al., 2006; Kil et al., 2014); further study is needed to determine the role of TYLCV-CL in single or mixed infections with PHYVV-CL and/or PepGMV-CL in pepper hybrids cultivated in Mexico. Based on genome sequence analysis of PHYVV-CL DNA-A and DNA-B, PHYVV-CL DNA-B (isolates LV163/BamHI-2014 and LV46/EcoRI-2016) had low identity (82%) when compared with PHYVV DNA B Tamaulipas (Garzón-Tiznado,

1993); whereas identity to PHYVV DNA B Sinaloa isolate (Melendrez-Bojorquez et al., 2016) was 98% (Fig. 2b). It has been suggested that the DNA B component contributed significantly to symptom severity in cassava (Patil & Fauquet, 2015) and this may be the case for the previously described PHYVV-Sin and for PHYVV-CL DNA B obtained from CL reported in this work. A recombination analysis of PHYVV-CL DNA-B component was performed, but no recombination events were detected based on the parameters used in this study, suggesting that DNA B of PHYYV-CL isolate evolved by a different mechanism perhaps due to accu- mulation of point mutations.

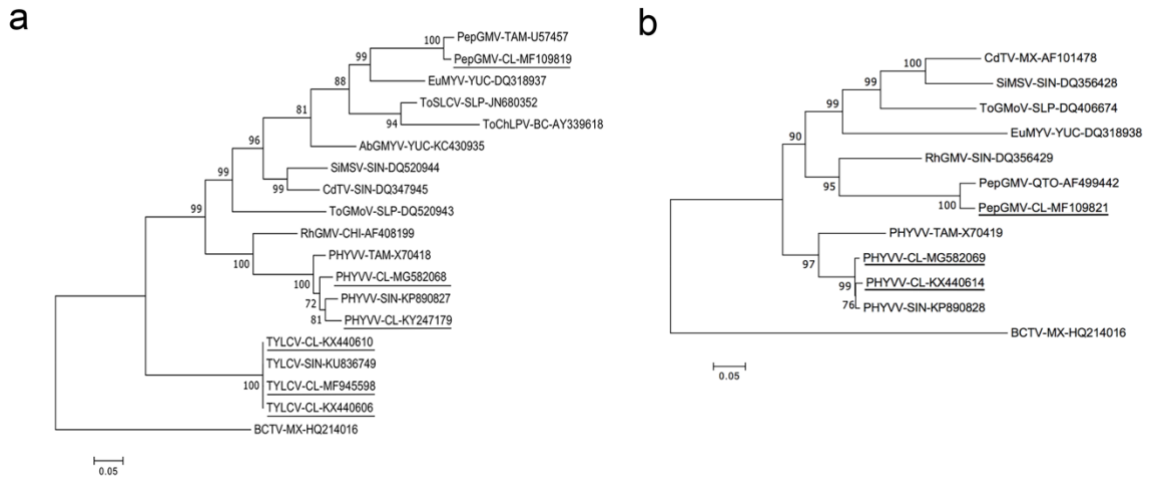

Fig. 2. Phylogenetic trees based on multiple sequence alignment of the complete components. (A) DNA-A. (B) DNA-B. Phylogenetic trees were constructed using maximum likelihood method. Numbers represent bootstrap percentages values out of 1000 replicates using MEGA 7. Nodes with clade credibility values of 65% are shown. *Beet curly top virus* (BCTV) was used as a root. Sequences of begomoviruses isolated in this work are underlined. Acronym of virus sequences used for this alignment were as follow: *Pepper golden mosaic virus* (PepGMV*), Pepper Huasteco yellow vein virus* (PHYVV), *Tomato yellow leaf curl virus* (TYLCV), *Rynchosia golden mosaic virus* (RhGMV), *Chino del tomate virus* (CdTV), *Sida mosaic Sinaloa virus* (SiMSV), *Tomato severe leaf curl virus* (ToSLCV), *Tomato chino La Paz virus* (ToChLPV), *Abutilon golden mosaic Yucatan virus* (AbGMYV), *Euphorbia mosaic virus* (EuMYV), *Tomato golden mottle virus* (ToGMV). The abbreviations after the acronyms represent the states of Mexico where the isolates were obtained from: SIN (Sinaloa), CHI (Chiapas),

YUC (Yucatan), SLP (San Luis Potosí), BC (Baja California), SON (Sonora), QTO (Querétaro), TAM (Tamaulipas), MX (No knowledge of the state of collection) and CL (La Comarca Lagunera).

Begomoviruses mixed infection associated with pepper diseases

In order to individually analyze naturally infected pepper plants from CL, samples from the 2014, 2015 and 2016 crops were evaluated for the presence of TYLCV-CL, PHYVV-CL and PepGMV-CL that were previously identified. PCR specific detection confirmed the presence of bego- moviruses in 47 out of 49 pepper plant samples with mixed infection being common (Fig. 3). In 2014, 10 out of 12 plants were positive for a single infection of TYLCV on 3 plants (Fig. 3a; lanes 2, 4 and 11), PHYVV on 3 plants (Fig. 3a; lanes 1, 3 and 9) or mixed infection with both viruses on 4 plants (Fig. 3a; lanes 7, 8, 10 and 12). In 2015, mixed infections with TYLCV and PHYVV were detected on 9 out of 11 plants (Fig. 3b; lanes 14–17 and 19–23), whereas single infections with TYLCV or PHYVV were detected only in one plant for each virus (Fig. 3b; lanes 13 and 18). In 2016, PepGMV was also detected in addition to TYLCV and PHYVV, and it is tempting to propose that PepGMV-CL could be a possible factor contributing to observed symptom severity. Interestingly, mixed infection was also common in the 26 plants tested either with three viruses (TYLCV, PHYVV and PepGMV) in 13 plants (Fig. 3c and d; lanes 26–34, 38, 40–41 and 49) or with two viruses (TYLCV and PepGMV) in the remaining 13 plants (Fig. 3c and d; lanes 24–25, 35–39, 42–48). The constant detec- tion of TYLCV suggested the prevalence and spread of this virus in CL. This is the first report of TYLCV in a new pepper-growing region in Mexico. It was postulated that once TYLCV is introduced in a new region, it will prevail as a source of emerging diseases (Rojas et al., 2005; Hoon et al., 2011; Lugo-Melchor et al., 2011; Yang et al., 2014). PHYVV and PepGMV have been well-documented affect- ing pepper crops in Mexico (Garzón-Tiznado, 1993; Méndez-Lozano et al., 2003; Holguín-Peña et al., 2004; Cardenas-Conejo et al., 2010; Ndunguru et al., 2015; Rodelo-Urrego et al., 2015; Melendrez-Bojorquez et al., 2016). In spite of this, CL region had no previous reports of the presence of TYLCV, PHYVV and PepGMV in pepper.

Mixed infections of begomoviruses are currently asso- ciated with a more severe disease in pepper; it is well-documented that in the case of PHYVV and PepGMV, a synergistic effect increases the disease severity in pepper (Méndez-Lozano et al., 2003). Mixed infections offer the opportunity to begomoviruses to evolve through recombination, resulting in novel pathogenic phenotypes similar to the recombination of TYLCV and TYLCSV documented in tomato (Monci et al., 2002; Lefeuvre & Moriones, 2015). Pepper diseases are of great concern to farmers in CL since the severity of the disease has increased in the last few years. The first step to viral disease management is the identification of the virus or viruses causing the disease. In this study, mole- cular identification was done in pepper samples, and our findings indicated the occurrence of TYLCV with either PHYVV and/or PepGMV in mixed infection on pepper in CL, a finding reported for the first time in Mexico. The potential of viruses to evolve under mixed infections is always a risk; a follow-up in pepper-begomovirus pathosystems to check whether novel recombinant begomoviruses will develop is intriguing from an evolutionary point of view.

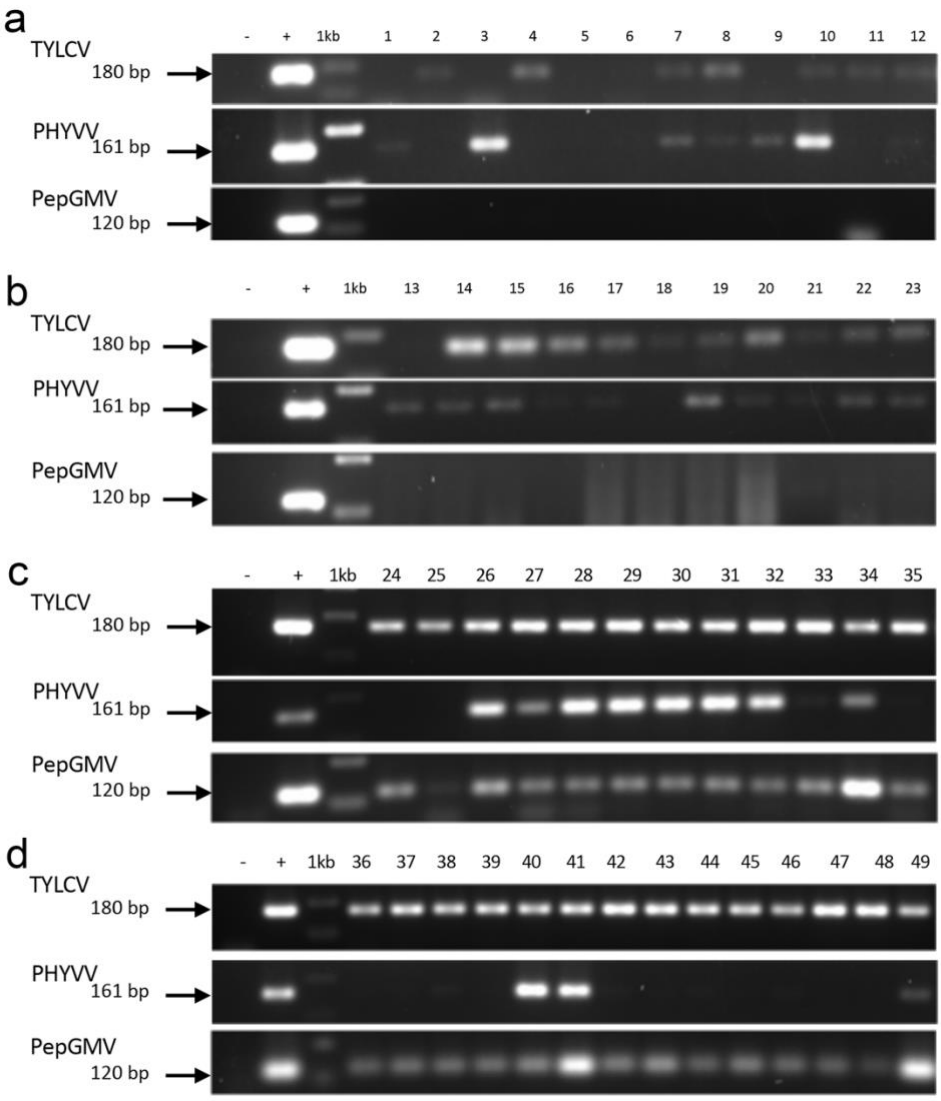

Fig. 3. Molecular detection by PCR using specific primers for the begomoviruses TYLCV, PHYVV and PepGMV. (A) Samples collected during 2014 in CL open fields at Torreón, Coahuila; Lines 1–12. (B) Samples collected during 2015 in CL open fields at Tlahualilo, Durango; Lines 13–23. (C and D) Samples collected during 2016 in CL open fields at Francisco I. Madero, Coahuila; Lines 24–49. (-) Negative control, (+) DNA of PHYVV as positive control, (1Kb) molecular maker (Invitrogen, USA).

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Chapter II.

High-throughput sequencing reveals differential begomovirus species diversity in non-cultivated plants in northern-pacific Mexico

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High-throughput sequencing reveals differential begomovirus species diversity in non-cultivated plants in northern-pacific Mexic. Edgar A. Rodríguez-Negrete[2, +], Juan J. Morales-Aguilar[1, +], Gustavo Domínguez-Duran[1], Gadiela Torres-Devora[1], Erika Camacho-Beltrán[1], Norma E. Leyva-López[1], Andreas E. Voloudakis[3], Eduardo R. Bejarano[4] and Jesús Méndez-Lozano[1, *]

High-throughput sequencing reveals differential begomovirus species diversity in non-cultivated plants in northern-pacific Mexico

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Abstract: Plant DNA viruses of the genus *Begomovirus* have been documented as the most genetically diverse in the family *Geminiviridae* and represent a serious threat for the global horticultural production, especially upon climate change. It is important to characterize the existing begomoviruses in nature since the viral genetic diversity in non-cultivated plants could lead to future disease epidemics in crops. In this study, high throughput sequencing (HTS) was employed to determine virus diversity on samples collected in a survey performed during 2005-2015 in seven states of northern-pacific Mexico, areas of diverse climatic conditions where different vegetable crops are intensively cultivated. In total 132 plant species, belonging to 34 families, were identified and sampled in those natural ecosystems

surrounding the cultivated areas. HTS analysis and subsequent *de novo* assembly revealed a list of geminivirus-releated signatures with 80 to 100% DNA homology with begomoviral sequences present in the genome databank. The analysis revealed 52 crop- and 35 non cultivated-infecting geminivirus-signatures that, interestingly, were present in different plant species. Such an analysis could deepen our knowledge in geminivirus diversity and help to predict emerging viruses in crops in different agro-climatic regions.

Keywords: Geminivirus; High-throughput sequencing; Non-cultivated plants; Viral biodiversity.

1. Introduction

Agroecosystems are used for the production of food, feed, fuel, fiber and other harvestable goods providing human support and health (Garbach, Milder, Montenegro, Karp, & DeClerck, 2014). Mexico has 196,437,500 ha, of which approximately 13% correspond to agricultural land. In 2016, 21.9 million ha were cultivated, with agricultural production of 26,032 million tons having a value of 26,760 million of dollars, which allowed the country to be ranked eleventh in world production of crops.

Plant diseases caused by begomovirus among other RNA viruses have been the main concern in Mexican horticulture through the years with important negative impact in crop production, of tomato, pepper, bean, pumpkin, melon, soybean, tomatillo, tobacco, watermelon, and cotton (Domínguez-Durán et al., 2018; Garrido-Ramirez & Gilbertson, 1998; R. J. Holguín-Peña, Arguello-Astorga, Brown, & Rivera-Bustamante, 2007; Melendrez-Bojorquez et al., 2016; Méndez-Lozano, Leyva-López, et al., 2006; Torres-Pacheco et al., 1993a). Begomoviruses (family *Geminiviridae*) are characterized by their geminate particles that encapsidate a circular single-stranded (ss) DNA genome (monopartite and bipartite) of about 2.8 kb in size. They are whitefly (*Bemisia tabaci*) transmitted, infecting a large number of plant species worldwide causing serious crop losses being the biggest global threat (Moffat, 1999). Important diseases caused by geminiviruses include maize streak disease (Harkins et al., 2009), cassava mosaic disease (BASAVAPRABHU L

Patil & Fauquet, 2009), cotton leaf curl disease (Briddon & Markham, 2000), and tomato leaf curl disease (Accotto, Navas-Castillo, Noris, Moriones, & Louro, 2000). In Mexico, the first report of tomato diseases caused by geminiviruses came from Sinaloa state in 1970 that later confirmed to be *Chino del tomato virus* (CdTV) (J. K. Brown, 1988). However, the first serious disease was reported in pepper crop, named “rizado amarillo” that was described as the coinfection of *Pepper huasteco yellow vein virus* (PHYVV) and *Pepper golden mosaic virus* (PepGMV) (J Antonio Garzon-Tiznado, 1993). Recently, the introduction of *Tomato yellow leaf curl virus* (TYLCV) in Sinaloa had a dramatic negative impact on tomato production. In different agro-climatic regions of Mexico begomovirus diseases are commonly caused by mixed infections with different begomoviruses, affecting tomato and pepper crops (Jose Antonio Garzon-Tiznado et al., 2002; Hernandez-Zepeda, Idris, Carnevali, Brown, & Moreno-Valenzuela, 2007; Melendrez-Bojorquez et al., 2016). Coinfections of non-cultivated plant- and crop-adapted begomoviruses have been reported in soybean, tobacco and pepper plants in Sinaloa, Chiapas and Jalisco states of Mexico (Ascencio-Ibáñez, Argüello-Astorga, Méndez-Lozano, & Rivera-Bustamante, 2007; Gregorio-Jorge, Argüello-Astorga, et al., 2010; Jorge Armando Mauricio-Castillo et al., 2014). Geminiviruses exhibit high mutations rates and large recombination frequency within and between species, both means for rapid adaptive evolution. Several reports indicated the emergence of recombinant species in geminiviruses (Fiallo-Olivé, Trenado, Louro, & Navas-Castillo, 2019; Cecilia Hernández-Zepeda, Varsani, & Brown, 2013; Lefeuvre & Moriones, 2015; Padidam, Sawyer, & Fauquet, 1999). For example *Tomato yellow leaf curl Malaga virus* (TYLMaV) is a recombinant of *Tomato yellow leaf curl Sardinia virus* (TYLCSV) and *Tomato yellow leaf curl virus* mild strain (TYLCV) that -unlike its “parental” genomes- has gained the ability to infect common bean and wild *Solanum nigrum* (Monci, Sánchez-Campos, Navas-Castillo, & Moriones, 2002). More importantly, TYLMaV accumulated to the same levels in susceptible and resistant tomato, indicating that the recombination event and subsequent selection led to the generation of a resistance-breaking isolate (Díaz-Pendón, Sánchez-Campos, Fortes, & Moriones, 2019).

It is proposed that global warming will influence the epidemiology of plant virus diseases mainly due to the alteration in the distribution of the insect vectors and in the host range (Anonymous, 2016; Aregbesola, Legg, Sigsgaard, Lund, & Rapisarda, 2018). New emerging diseases caused by geminiviruses appear more frequent lately that could be associated with climate change (Canto, Aranda, & Fereres, 2009). The viral quasispecies (non-identical but related genomes) present in a host plant could be generated by recombinant genomes providing an improved fitness potential to the viruses that may initiate infection in new host species or cause more severe disease symptoms in an established host by overcoming plant resistance. The generation of quasispecies depends on the host-virus interaction, the environmental conditions as well as the cultivation practices (Duffy & Holmes, 2007; Harkins et al., 2009; Lefeuvre et al., 2010; Monjane et al., 2011).

New mutant or recombinant viral genomes could arise in non-cultivated plants that upon transmission, successful infection of crops and their adaptation in the new host they could cause significant crop damage. Vector-transmission is an evolutionary barrier for plant viruses to expand their host range. Alterations in vector-transmission, most likely through mutations in the viral coat protein sequences, could increase the risk of emergence of a plant pathogen in a crop. Begomoviruses are transmitted by whitefly *Bemisia tabaci* but lately, aphid vectoring was confirmed for the *Capulovirus Alfalfa leaf curl virus* (ALCV) (Bernardo et al., 2013a; Varsani et al., 2017). Vector metagenomics could assist in exploring the diversity of geminiviruses present in their insect vectors and define the coat protein sequences that they are carried over. Although the coat protein has a structural role for the virus, the CP sequences diverge (Rybicki, 1994) due to a high mutation rate. In addition, it is possible that such CP mutations could establish new means of transmission such as seed transmission. Therefore, it is important to study the complexity and heterogeneity of the geminiviral quasipecies in the wild reservoir hosts and understand the factors that generate the genetic variation since such knowledge will be extremely useful to develop resistance strategies (e.g. RNAi-based approaches) and, as consequence, prevent crop losses.

High-throughput sequencing (HTS) has provided the means to detect known viruses as well as to identify novel viruses in plants (Barba, Czosnek, & Hadidi, 2014; Massart, Olmos, Jijakli, & Candresse, 2014; Pereira, Alfenas-Zerbini, Cascardo, Andrade, & Murilo Zerbini, 2012; Pooggin, 2018; Q. Wu, Ding, Zhang, & Zhu, 2015). As a result, sensitive and accurate diagnosis of viral infection has been achieved rendering this method extremely useful for quarantine purposes. The development of bioinformatics tools and the design of various pipelines have contributed significantly towards the deep analysis of the vast amount of the HTS data produced (Rampelli et al., 2016)

The current next generation sequence (NGS) technologies have a couple of drawbacks; firstly the contigs/singeltons need to be annotated *de novo* via short read assembly, a process that may create chimeras deriving from different genomes in a sample, and secondly the accurate differentiation of sequences; thus, confirmation is required by cloning followed by Sanger sequencing. The hope is that the latest single-molecule NGS technologies, where long reads are obtained, could address both the above-mentioned issues, especially when their sequence reading error rates drop significantly to the levels of the previous NGS technologies. The resolution of the metagenome of a sample could identify genetic variations of a viral population providing the necessary input to study viral genome evolution and determine which environmental factors affect the generation of new plant pathogens from benign viruses.

It is well accepted that non-cultivated (wild) host plants play a key role in generation of viral genetic variation maintaining sequence heterogeneity, without modifying the consensus sequences, useful for viral adaptation purposes in nature. Only recently, metagenomics studies on non-cultivated plant species have recently attracted the attention of plant researchers (Bernardo et al., 2017; Pooggin, 2018). Mexico is considered one of the most megadiverse countries worldwide (Llorente-bousquets & Ocegueda, 2008), and despite the fact that some non-cultivated plants species have been reported as geminivirus reservoir (Hernandez-Zepeda et al., 2007; J. A. Mauricio-Castillo et al., 2007), until to date the knowledge of geminivirus distribution in Mexican natural ecosystems is limited. To this extent we aimed at

determining the genetic diversity of begomoviruses in non-cultivated species that are present close to cultivated crops (agro-ecological interface) in northern-pacific Mexico. In the present study, upon rolling circle amplification (RCA), applied on obtained DNA samples, several begomoviral-signature and begomoviruses sequences were identified in the wild plant species during a ten-year survey. This supports the presence of a niche for begomovirus evolution neighboring important cultivation areas in northern-pacific Mexico.

2. Materials and Methods

2.1. Plant sample collection

A total of 422 non-cultivated plants (both symptomatic and asymptomatic) located between crops and wild vegetation zones (designated as agro-ecological interphase), in seven states in Northern-Pacific region of Mexico during the period 2005-2015 were collected, GPS documented, photographed, and identified to the species level. Thus, 132 species of plants belonging to 34 families were identified. The sampling regions were grouped as follows: 1) Baja California (BC), 2) Sonora (SO), 3) Sinaloa (SI), 4) Colima-Nayarit (CN), and 5) Coahuila-Durango (CD) states of Mexico. Samples were placed in ice and brought to the laboratory and stored at -80^{0}C until processed. The plants were collected in non-protected areas; additionally, an herbarium was established with most of the plant family's specimen.

2.2. DNA isolation, RCA and library construction

Total DNA was extracted from individual plants using the CTAB method (Doyle & Doyle, 1987), and the isolated DNA, upon estimation of its concentration spectrophotometrically, was used as template for PCR-based *Begomovirus* detection using degenerated universal primers (**Supplementary Table S3**). For each sampling region, total DNA from *Begomovirus* PCR-positive plants, belonging to the same plant species, was mixed in equimolar concentration. 100 ng of each DNA mixture was used for circular DNA-molecule enrichment by rolling circle amplification (RCA) using the illustra TempliPhi DNA Amplification Kit (GE Healthcare, USA), following the manufacturer´s instructions. Then, all the RCA

products per sampling region were pooled in equimolar concentrations, and cleaned using phenol:chloroform:isoamyl alcohol (25:24:1)/potassium acetate (5 M) and ethanol 100% precipitation (1/10 v/v, 1/2 v/v, respectively). DNA integrity was analyzed by agarose gel electrophoresis, and the cleaned RCA mixtures were used for NGS library construction that was sequenced by a commercial facility (LANGEBIO-Irapuato, MX) using Illumina Nextera XT paired end 2X150 bp protocol on a MiSeq 500. The same procedure was followed for each sampling region to obtain one library per region (in total five libraries).

2.3. Metagenomic analysis of Geminivirus-related signatures

Reads obtained from each library were trimmed employing the trimmomatic tool (Bolger, Lohse, & Usadel, 2014) with parameters (TRAILING: 30, HEADCROP:5) followed by quality check analysis by FASTQC (https://www.bioinformatics.babraham.ac.uk). Each library was filtered for human, bacteria, plant, and eukaryotic viruses reads using the ViromeScan pipeline (Rampelli et al., 2016) in order to obtain Geminivirus-related reads. All filtered libraries were subjected to *de novo* assembly using SPAdes (Kulikov et al., 2012), and both contigs (≥78 bp) and unassembled reads were compared against the GeneBank non-redundant database using BLASTn hosted in the Galaxy server (Altschul, Gish, Miller, Myers, & Lipman, 1990). Geminivirus-related signatures were sorted by contig length and analyzed manually. Contigs obtained in the present study are available in: https://www.dropbox.com/sh/ha6pkzls9217dhf/AAADNUa0TfYj3EZ8bb315cSga?dl=0.

2.4. Begomovirus full-length genome amplification, cloning, and sequence analysis

Full-length geminivirus genomes were obtained following a previously described protocol (Inoue-Nagata, Albuquerque, Rocha, & Nagata, 2004). In brief, total DNA (100 ng) from selected non-cultivated plants was used as template for viral circular DNA genomes enrichment by RCA as mentioned above. To obtain the viral monomeric full-length genomes, the RCA products were digested with selected

single-cutter restriction enzymes (BamHI, EcoRI, XbaI, or XhoI) depending on the virus under analysis. The expected linearized geminivirus full genomes (~2.7 kb) were recovered from 1% ultrapure agarose gels using PureLink Quick Gel Extraction Kit (Thermo Fisher Scientific, USA) according to the manufacturer´s instructions. The fragments were ligated into linearized pGreen 0029 plasmid (Hellens, Anne Edwards, Leyland, Bean, & Mullineaux, 2000) that was digested with the corresponding restriction enzymes. The resulting recombinant plasmids were transformed in *E. coli* DH5α, and positive clones were subjected to Sanger gene walking method sequencing. Genome assemblies were obtained using SeqMan (DNASTARInc, USA) and SnapGene (GLS Biotech LLC, USA) software. All pairwise comparisons were performed using the MUSCLE algorithm implemented in Mega 7 (Kumar, Stecher, & Tamura, 2016) and maximum likelihood phylogenetic tree(s) were constructed on both begomovirus components, with a 1000 bootstrap on both components to assess branch support. To analyze the nucleotide and amino acid identity, open reading frames (ORFs) were separated and individually compared with highest match homologous genome of each virus obtained from NCBI databank, using ClustalW algorithm implemented in Mega 7.

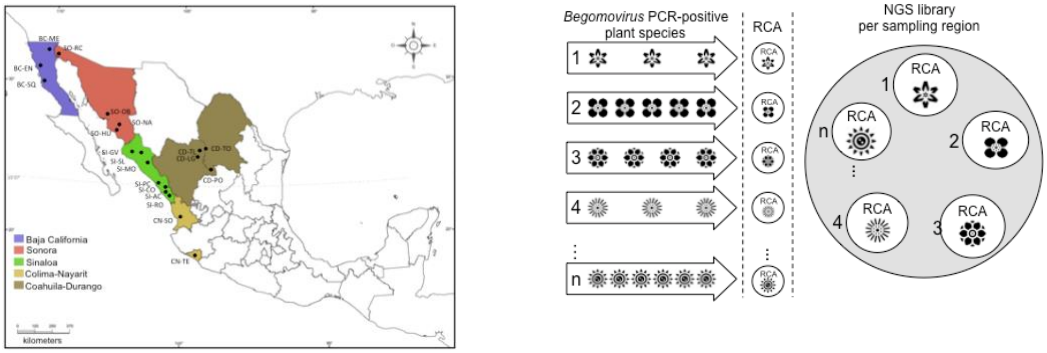
3. Results and Discussion

It is accepted that global warming will have an impact on global food security. In particular, crop yields are predicted to significantly decrease considering the 'worst' CO_2-emission scenario (A1FI) of the Intergovernmental Panel on Climate Change (Livermore, Fischer, Rosenzweig, Parry, & Iglesias, 2004). Plant pathogens will have varying responses to climate change and plant-pathogen warfare is expected to be altered (Velásquez, Castroverde, & He, 2018), imposing negative, neutral or positive effects on yields depending on the host-pathogen-environment interaction (the known 'disease triangle'). Disease pattern changes are anticipated due to alterations in host range of plant pathogens especially in rapidly evolving pathogens and disease severity will be influenced by increased CO_2, heavy rains, increased humidity, drought, and warmer winter temperatures (Luck et al., 2011). Studies towards understanding of the existing genetic diversity of plant viruses occurring in the agro-ecological interface, of the generation of new genomes with advantageous

features, of the relationship with their vectors will contribute significantly in human's preparation to adapt to climate change and sustain food production. In this study, high throughput sequencing (HTS) was employed to determine begomoviral diversity in plant samples collected in northern-pacific Mexico.

3.1. Non-cultivated plants from northern-pacific Mexico region as a reservoir of begomoviruses

Plant viruses have generally been studied as disease-causing infectious agents that have negative impact on their hosts (Marilyn J. Roossinck, 2012). Although the diversity of geminiviruses is wide, the non-cultivated plants may act as reservoir of known agriculturally important viruses, and these weed-hosted viral species, could potentially initiate the emergence of new diseases in cultivated plants in the future (Prajapat, Marwal, & Gaur, 2014)**.** To determine begomoviruses diversity in northern-pacific Mexico a survey was performed in the agro-ecological interface of crops during 2005-2015. Sampling areas were divided in five regions: Baja California, Sonora, Sinaloa, Colima-Nayarit, and Coahulia-Durango, and subdivided in three, four, seven, two, and four sampling points, respectively (**Figure 1. a**). A total of 422 non-cultivated plants (both symptomatic and asymptomatic), belonging to 34 families and 132 species were identified (**Supplementary Table S1 and Supplementary Figure S1**). Among the different families identified in each region, the most commonly distributed were the *Astaraceae*, *Solanaceae*, *Malvaceae*, and *Fabaceae*. It is noteworthy mentioning that those families were found to be the most predominant in natural ecosystems considering 200 plant families described in Mexico (Gutiérrez-García et al., 2017; Llorente-bousquets & Ocegueda, 2008). Using degenerated universal primers based on DNA A genome of genus *Begomovirus*, we were able to amplify the expected DNA fragment of 950-1100 bp in 252 out of the 422 (60%) tested individual plant specimens indicated that begomoviruses were present in 29 plant families collected from the five regions sampled (**Table 1**). These results suggested that non-cultivated plants represent a reservoir for begomoviruses that are widely distributed in northern-pacific Mexico.



(**a**) (**b**)

Figure 1. Northern-pacific Mexico sampling areas and HTS library construction. **(a)** Sampling areas were divided in five regions located in different biogeographic zones (according to the CONABIO classification): Baja California (BC), Sonora (SO), Sinaloa (SI), Colima-Nayarit (CN), and Coahulia-Durango (CD). BC-SQ: Baja California, San Quintin; BC-EN: Baja California, Ensenada; BC-ME: Baja California, Mexicali; SO-OB: Sonora, Obregon; SO-NA: Sonora, Navojoa; SO-HU: Sonora, Huatabampo; SO-RC: Sonora, Rio Colorado; SI-GV: Sinaloa, Guasave; SI-SL: Sinaloa, Sinaloa de Leyva; SI-MO: Sinaloa, Mocorito; SI-PC: Sinaloa, Playa Ceuta; SI-CO: Sinaloa, Concordia; SI-AC: Sinaloa, Agua Caliente; SI-RO: Sinaloa, El Rosario; CN-SO: Colima-Nayarit, Santa Maria del Oro; CN-TE: Colima-Nayarit, Tecoman; CD-TL: Coahuila-Durango, Tlahualilo; CD-LG: Coahuila-Durango, La Goma; CD-TO: Coahuila-Durango, Torreon; CD-PO: Coahuila-Durango, Poanas. Sampling areas are indicated in map by colored squares. **(b)** Diagram of sample processing to obtain HTS libraries. For each sampling area, total DNA from *Begomovirus* PCR-positive plants belonging to the same species was pooled in equimolar concentrations. The resulting DNA mix was used as template for RCA-mediated viral circular molecules enrichment. Finally, all resulting RCA products were pooled in equimolar concentrations and used for HTS library construction.

Table 1. Begomovirus detection in plants collected in the agro-ecological interphase from northern pacific regions of Mexico. Total DNA from each individual specimen was used as template for PCR-mediated begomovirus detection.

Plant Family[1]	Sampling region[2] Begomovirus PCR-positive/total plants collected				
	BC	**SO**	**SI**	**CN**	**CD**
Amaranthaceae		4/5	1/4	1/4	5/6
Apiaceae		1/1			
Asteraceae	4/10	8/16	8/22	3/4	0/15
Boraginaceae	0/1	4/5			
Brassicaceae	1/2	0/2	1/1		
Caesalpiniaceae			1/1		
Capparaceae			1/2		
Chenopodiaceae	4/4	6/10			
Convolvulaceae	0/2	2/2	3/4	1/4	
Cucurbitaceae	0/1		2/6	1/3	
Euphorbiaceae		2/3	5/15	0/2	
Fabaceae		1/2	49/72	0/1	
Hydrophyllaceae		1/1		0/1	
Malvaceae	5/6	14/18	21/33	9/13	9/9
Menispermaceae			1/1		
Nyctaginaceae			2/2	4/5	1/1
Onagraceae		1/2			
Papaveraceae		1/1			
Pedaliaceae			1/1		
Polygonaceae	1/2	3/5			
Portulacaceae			3/3	1/2	
Primulaceae	1/1				
Rhamnaceae		1/1	1/1		
Rubiaceae				2/2	
Sapindaceae			1/1		
Solanaceae	1/2	11/14	18/22	0/2	14/14
Sterculiaceae			1/2		
Verbenaceae			1/2	2/2	
Vitaceae			1/1	0/1	
Total positives	**17**	**60**	**122**	**24**	**29**

[1]Plant families PCR-negatives for begomovirus detection: *Apocynaceae*, *Asclepiadiaceae*, *Bignoniaceae*, *Commelinaceae* and *Malpiguiaceae*.

[2]Plant specimens belonging to 34 families were collected from five regions: Baja California (BC), Sonora (SO), Sinaloa (SI), Colima-Nayarit (CN), Coahuila-Durango (CD).

3.2. Metagenomics study reveals a number of geminviruses from non-cultivated plants

Following the pipeline described in the Materials and methods section, NGS resulted in 16 to 215 million reads for the five libraries. After human, bacteria, plant, and eukaryotic virus sequences depletion, an NCBI-GenBank database search was performed to identify the most closely related geminivirus sequences were identified,

obtaining between 6,000 and 4,6 millions of reads (**Table 2**). Subsequent annotation showed that more than 99% of the reads corresponded to genus *Begomovirus*, and the remaining 1% matches to the *Geminiviridae* genera *Curtovirus*, *Becurtovirus*, *Turncurtovirus*, *Topocuvirus* and *Mastrevirus*. No sequences with homology to the genera *Capulavirus*, *Eragrovirus*, and *Grablovirus* were identified in our study. The genus *Begomovirus* has been reported previously as the most widely distributed in Mexico (Ascencio-Ibáñez et al., 2007; Garrido-Ramirez & Gilbertson, 1998; C. Hernández-Zepeda et al., 2010; Hernandez-Zepeda et al., 2007; J. A. Mauricio-Castillo et al., 2006b; Méndez-Lozano, Leyva-López, et al., 2006; Torres-Pacheco et al., 1996); in addition, sporadic reports of other genera like *Curtovirus* and *Grablovirus* were described (Hernández-Martínez, Licea-Navarro, Pino-Villar, Carrillo-Tripp, & Gasperin-Bulbarela, 2018; Roberto Reveles Torres et al., 2012). Our data pointed out of the abundance and importance of the genus *Begomovirus* in Mexico; however, follow up studies of the other genera becomes imperative.

Table 2. NGS data summary of reads and contigs mapping to Geminivirus genomes.

Library name	Total reads	Total Geminivirus-related reads	Number of Geminivirus-related contigs	Smallest/largest Geminivirus-related contig
Baja California	16,056,866	6,156	92	78/2437[1]
Sonora	30,440,802	23,546	195	78/2293
Sinaloa	215,007,456	4,685,423	15,465	78/2723
Colima-Nayarit	33,159,620	2,475,219	8,368	78/2775
Coahuila-Durango	70,782,034	349,763	169	78/2858
Total	365,446,778	7,540,107	24,289	78/2858

[1] bp: Base pairs.

The *de novo* assembly of the geminivirus-related reads was carried out, resulting in 24,289 geminivirus-related contigs that ranged from 78 to 2,858 bp in length (**Table 2**). The generated contigs were used to search the NCBI-GenBank database in order to identify the most closely related geminivirus exemplars at the species level **(Supplementary Figure S2)**. **Table 3** shows the geminivirus-related

signatures ≥300 bp and ≥80% nucleotide homology against the best match regardless whether DNA A or B viral components were detected. Similar findings were described in a metagenomics analysis in whiteflies, in which only one component of a bipartite begomovirus was retrieved (Rosario et al., 2015). Additionally, the geminivirus-related signatures with 100-300 bp and/or <80% nucleotide homology against the best match in NCBI gene sequences are listed in **Supplementary Table S2.** It is important to note that short geminivirus-related signatures could hinder the correct classification of a begomovirus species or strain; nonetheless, profiling the phylogenetic composition of the viral communities is pivotal as a significant part of different plant-virus environment.

The analysis of the highest geminivirus-related signature sequence revealed a list of both bipartite and monopartite begomoviruses, including crop-adapted viruses in different plant families; with 14 bipartite genomes such as *Pepper husateco yellow vein virus* (PHYVV-signature) present in four regions *Pepper golden mosaic virus* (PepGMV-signature) present in four regions, *Pepper leafroll virus* (PepLRV-signature) present in one region, *Tomato chino la Paz virus* (ToChLPV-signature) present in two regions, *Tomato severe leaf curl virus* (ToSLCV-signature) present in two regions, *Tomato yellow spot virus* (ToYSV) present in three regions, *Potato yellow mosaic virus* (PYMV) present in one region, *Okra yellow mosaic mottle virus* (OYMMV-signature) present in four regions, *Cabbage leaf curl virus* (CabLCV-signature) present in two regions, *Bean calico mosaic virus* (BCaMV-signature) present in four regions, *Bean yellow mosaic Mexico virus* (BYMMV-signature) present in one region, *Vigna yellow mosaic virus* (ViYMV-signature) present in two regions, *Water melon chlorotic stunt virus* (WmCSV-signature) present in one region and *Squash leaf curl virus* (SLCV-signature) present in three region; and five with monopartite genomes, two belonging to the genus *Begomovirus*, namely *Chilli leaf curl virus* (ChiLCV-signature) present in one region, and *Tomato yellow leaf curl virus* (TYLCV-signature) present in four regions, *Sweet potato leaf curl virus* (SPLCV-signature) present in one region; additionally, one belonging to the genus *Curtovirus*, namely *Beet curly top virus* (BCTV-signature) and another belonging to genus *Topocovirus*, namely *Tomato pseudo-curly top virus* (TPCTV), both present in one

region (**Table 3**). Furthermore, nine non-cultivated plant-adapted included only begomovirus with bipartite genomes such as *Solanum mosaic Bolivia virus* (SoMBoV-signature), present in two regions, *Sida mosaic Sinaloa virus* (SiMSiV-signature) present in five regions, *Sida golden yellow spot virus* (SiGYSV-singnature) present in one region, *Malvastrum bright yellow mosaic virus* (MaBYMV-signature) present in three region, *Rhyncosia golden mosaic virus* (RhGMV-signature) present in five regions, and *Rhyncosia golden mosaic Sinaloa virus* (RhGMSV-signature) present in two regions, *Euphorbia mosaic virus* (EuMV-signature) present in three regions, *Euphorbia yellow mosaic virus* (EuYMV) present in one region and *Blechum leaf curl virus* (BlelCV-signature) present in one region, (**Table 3**). Interestingly, the presence of *Tomato pseudo-curly top virus* (TPCTV) in samples from Colima-Nayarit, is the first report of the genus *Topocovirus* in Mexico. The list of geminivirues are grouped as a potential of new viruses or strain of the best match virus, with molecular and biological validation being necessary.

The genus *Begomovirus* comprises the most common DNA viruses responsible for several plant-virus diseases in Mexico. Among them PHYVV, an endemic virus, and PepGMV have been documented as the most widespread and predominant in pepper crops (J. Antonio Garzon-Tiznado, 1993; Melendrez-Bojorquez et al., 2016; Rodelo-Urrego, García-Arenal, & Pagá, 2015; Torres-Pacheco et al., 1996). It is noteworthy that the introduction of the promiscuous TYLCV in Yucatán and later in Sinaloa states, with dramatic impact on crop yield, became the major concern in tomato crops in northern Mexico (Ascencio-Ibáñez et al., 1999). Interestingly, TYLCV did not exclude the “native” viruses and their co-infection with PHYVV or PepGMV caused severe disease in pepper crops (Morales-Aguilar et al., 2019). However, the identification of those viruses in non-cultivated plants as alternative host increases the opportunity to evolve through recombination events or other mechanisms; representing a latent possibility in nature. In fact, a new isolate of PHYVV described in pepper had significant sequence changes on DNA B genome, with a modified host range since this isolate was able to infect tomato plants causing severe symptoms (Melendrez-Bojorquez et al., 2016; Moreno-Félix et al., 2018). The other invasive virus which was introduced in Sonora state, apparently from Middle

East, was WmCSV (Domínguez-Durán et al., 2018) and it was detected in the present study with SLCV. Our results suggest that WmCSV and SLCV are present in non-cultivated plants collected in Coahuila-Durango region (**Table 3**), which implies that WmCSV virus has the potential to spread in Mexico and by adapting to new environments it has the potential to become an emerging disease in a new region. It is important to mention that those viruses could interact in mixed infection inducing more severe symptoms on crops as reported in Jordan (Abudy et al., 2010). The detection of ToChLPV, ToSLCV, OYMMV, BCaMV, and BYMMV viruses -that were reported previously in Mexico- indicate that they still occupy an ecological niche and could be a potential source of viral disease. The identification of SiMSiV and RhGMV in all regions sampled is intriguing. Perhaps both of them represent viruses well adapted to different hosts and environments with a potential risk to evolve in an emerging disease. SiMSiV was initially reported in Sinaloa state associated to *Sida rombifolia* (Jorge Armando Mauricio-Castillo et al., 2014); however, a negative impact on crops is not described as yet. On the other hand, RhGMV was previously reported causing disease in tobacco and soybean (Ascencio-Ibáñez et al., 2007; Méndez-Lozano, Leyva-López, et al., 2006) It is well known that non-cultivated plant-adapted virus normally do not induce disease symptoms in their host. Nonetheless, SiMSiV and RhGMV induce symptoms in the first reported host (*Sida rombifolia* and *Rhyncosia minima,* respectively) for both viruses with different wild species (reservoir) being suspected as the origin of the inoculum. Moreover, EuMV and EuYMV were reported previously in *Euporbia heterophylla* in Mexico and Brazil (Fernandes et al., 2011; Cecilia Hernández-Zepeda et al., 2007); whereas BlelCV, is a novel virus recently described in Chiapas state (Cantú-Iris et al., 2019). To the best of our knowledge the ChiLCV, PepLRV, ToYSV, PYMV, CabLCV, SPLCV, MaBYMV, and ViYMV geminivirus-related signatures have not been described and/or disease associated previously in Mexico and represents potential strains and/or novel viruses in which the biological role waits to be determined in the immediate future. It worths mentioning that viruses like ChilCV, PepLRV, and ToYSV are already associated to pepper, bean, and tomato diseases in Pakistan, Peru,

Ecuador and Brazil (Andrade et al., 2006; Martínez-Ayala et al., 2014; Shih et al., 2007).

3.3. Molecular validation of the predominant begomoviruses identified by HTS

The ecological role of begomoviruses identified by HTS studies in non-cultivated plants needs more efforts in order to understand the contribution of these plants for disease development (Malmstrom et al., 2011)(Marilyn J Roossinck, 2011)(Malmstrom et al., 2011; Marilyn J Roossinck, 2011; Stobbe & Roossinck, 2014). Non-cultivated plants could be a source of viral inoculum to cultivated plants (Aguiar et al., 2017; Basak, 2016; Bekele et al., 2018; Paz-Carrasco et al., 2014; Perry et al., 2018; Strydom & Pietersen, 2017; Tahir et al., 2015) and could contribute to viral evolution. Here, we described some non-cultivated plants at the agro-ecological interphase possessing geminivirus-signatures (**Table 3 and Supplementary Table S2**). The information acquired is an important progress towards elucidating the above-mentioned issues, but more work is needed for the validation of the described identities.

According to our survey carried out, plants belonging to the *Fabaceae, Malvaceae*, and *Solanaceae* families were the most widely distributed in all sampled areas. HTS analysis revealed that the TYLCV-signature, SiMSiV-signature, and RhGMV-signature, were detected in all 5 NGS libraries; whereas RhGMSV-signature was detected only in 2 out of 5 NGS libraries, suggesting that these begomovirus species are predominant. Initially, the presence of those viruses was confirmed by using viral species sequence-specific primers (**Supplementary Table S3**), in which an individual plant of the corresponding family was tested for virus presence. To characterize at the molecular level and confirm the biological nature of detected viruses, total DNA from *Nicotiana glauca* from Sinaloa (TYLCV PCR-positive); *Sida acuta* from Colima (SiMSiV PCR-positive), and two *Rhynchosia minima* both from Sinaloa (PCR-positive for RhGMV/RhGMSV), was used for RCA followed by viral full-length genome cloning. Sequence analysis of obtained clones is summarized in **Table 4 and 5.**

Table 3. Begomovirus signatures obtained by *de novo* assembly from the metagenomics study in plants collected in the agro-ecological interface from five regions in northern-pacific Mexico. Geminivirus-related reads for each NGS library were used for *de novo* assembly and generation of signatures.

Host adapted	Virus acronym[2]	Plant Family of first detection	Geminivirus-signatures of DNA-A/DNA-B[1] per region				
			Baja California	Sonora	Sinaloa	Colima-Nayarit	Coahuila-Durango
Crops	PHYVV	*Solanaceae*	ND[3]	98.5/96.5 LN848858.1/LN848912.1 1462/2124	99.6/100 LN848873.1/KP890828.1 251/594	100/99.6 X70418.1/ X70419.1 (583/1826)	96.9/98.5 LN848872.1/LN848922.1 955/1742
	PepGMV		ND/98.4 ND/AY928515.1 ND/524	ND	98.1/95.9 U57457.1/AY928515.1 1115/2388	88.2/84.3 AY905553.1/LN848829.1 136/147	99.4/95.9 LN848772.1/LN848841.1 1120/1562
	PepLRV		ND	88/ND KC769819.1/ND 458/ND	ND	ND	ND
	ChiLCV		ND	ND	81.2/NA[4] JN555601.1/NA 559/NA	ND	ND
	TYLCV		98.4/NA JQ354991.1/NA 131/NA	99.5/NA KU836749.1/NA 2540/NA	99.5/NA FJ012359.1/NA 1247/NA	99.7/NA EF523478.1/NA 1524/NA	99.4/NA FJ012358.1/NA 2048/NA
	ToChLPV		ND	89.1/NA AY339618.1/NA 120/NA	ND	81.9/NA HM459852.1/NA 337/NA	ND
	ToSLCV		ND	87.2/NA DQ347946.1/NA 359/NA	ND	99.5/NA KC479066.1/NA 411/NA	ND
	TPCTV		ND	ND	ND	82.3/NA X84735.1/NA 385/NA	ND
	ToYSV		ND	84.2/ND DQ336350.1/ND 470/ND	95.4/ND KJ742419.1/ND 155/ND	84.9/ND KX348173.1/ND 192/ND	ND

	PYMV		ND	ND	ND	78.8/ND FR851299.1/ND 321/ND	ND
	OYMMV	*Malvaceae*	ND/90.3 ND/GU972604.1 ND/2354	ND	93.6/96.4 GU990612.1/JX219471.1 174/226	98.9/94.9 GU990614.1/JX219471.1 1455/336	ND/94.9 ND/JX219471.1 ND/236
	CabLCV	*Brassicaceae*	ND	ND	97.4/ND AJ228570.1/ND 119/ND	84.2/82.8 MH359394.1/DQ178613.1 1645/157	ND
	BCaMV		ND	ND/95 ND/AF110190.1 ND/2576	97.2/96.7 AF110189.1/AF110190.1 2058/1296	92.3/88.9 AF110189.1/AF110190.1 353/135	97.9/82.9 AF110189.1/AF110190.1 1005/587
	BYMMV	*Fabaceae*	ND	85.3/ND FJ944023.1/ND 677/ND	ND	ND	ND
	ViYMV		ND	ND	86.6/86.7 KC430936.1/KC430937.1 758/369	89.6/86 KC430936.1/KC430937.1 242/115	ND
	WmCSV		ND	ND	ND	ND	100/100 KY124280.1/KY124281.1 239/1025
	SLCV	*Cucurbitaceae*	ND	94.2/ND KM595165.1/ND 104/ND	ND	80.6/83 KM595183.1/DQ285017.1 155/124	79.8/95.3 KM595165.1/M38182.1 188/1649
	SPLCV	*Convolvulaceae*	ND	ND	92.4/NA KX611145.1/NA 1818/NA	80/NA KJ013582.1/NA 261/NA	ND
	BCTV	*Amaranthaceae*	99.8/NA JX487184.1/NA 508/NA	ND	ND	ND	ND
Non-cultivated plants	SoMBoV	*Solanaceae*	ND	ND/84.7 ND/HM585436.1 ND/518	ND	ND/82.3 ND/HM585436.1 ND/655	ND
	SiMSiV	*Malvaceae*	96.3/ND DQ520944.1/ND 854/ND	95.8/96.7 DQ520944.1/DQ356428.1 2581/1582	96.9/90.2 DQ520944.1/DQ356428.1 1003/2085	95.6/87.7 DQ520944.1/DQ356428.1 1584/245	94.2/98.9 DQ520944.1/DQ356428.1 572/289
	SiGYSV		ND	84.6/ND	ND	ND	ND

			KX348185.1/ND 637/ND			
MaBYMV		96.9/ND KU058856.1/ND 1037/ND	ND	97.2/84.9 KU058865.1/KU058860.1 403/153	ND	94.8/94.5 KU058853.1/KU058859.1 1822/1282
RhGMV	*Fabaceae*	95/90 EU339939.1/EU339937.1 1049/536	88.9/ND EU021216.1/ND 253/ND	98.9/95.7 EU339939.1/EU339937.1 2086/675	92.2/85.4 EU339938.1/DQ356429.1 155/240	96.2/82.6 AF408199.1/EU339937.1 264/543
RhGMSV		ND	ND	93.4/96.2 DQ406672.1/DQ406673.1 1754/1794	91.2/89.2 DQ406672.1/DQ406673.1 727/353	ND
EuMV		ND	86.8/ND JN368145.1/ND 678/ND	ND	87.9/86.5 DQ318937.1/ DQ520942.1 158/104	ND/93.1 ND/ HQ185235.1 ND/249
EuYMV	*Euphorbiaceae*	ND	ND	ND	91.3/80.1 KY559516.1/KY559581.1 138/342	ND
BleICV	*Acanthaceae*	ND	ND	ND/79 ND/JX827488.1 ND/783	ND	ND

[1]Best match in %, accession numbers and contig length aligned is shown. Contig alignments of ≥300 bp in length were selected regardless whether one or both viral components (DNA A and B) were detected. Contigs alignments of <300 bp are also reported if at least one signature of ≥300 bp for the corresponding virus was detected.

[2]Virus acronyms: ***Monopartite Geminiviruses****: Beet curly top virus* (BCTV), *Chilli leaf curl virus* (ChiLCV), *Sweet potato leaf curl virus (SPLCV), Tomato pseudo-curly top virus* (TPCTV), *Tomato yellow leaf curl virus* (TYLCV); ***Bipartite Geminiviruses****: Bean calico mosaic virus* (BCaMV), *Blechum interveinal chlorosis virus* (BleICV), *Bean yellow mosaic Mexico virus* (BYMMV), *Cabbage leaf curl virus* (CabLCV), *Euphorbia mosaic virus* (EuMV), *Euphorbia yellow mosaic virus* (EuYMV), *Malvastrum bright yellow mosaic virus* (MaBYMV), *Okra yellow mosaic Mexico virus* (OYMMV), *Pepper golden mosaic virus* (PepGMV), *Pepper huasteco yellow vein virus* (PHYVV), *Pepper leafroll virus* (PepLRV), *Potato yellow mosaic virus* (PYMV), *Rhynchosia golden mosaic Sinaloa virus* (RhGMSV), *Rhynchosia golden mosaic virus* (RhGMV), *Sida golden yellow vein virus* (SiGYVV), *Sida*

mosaic Sinaloa virus (SiMSiV), *Squash leaf curl virus* (SLCV), *Solanum mosaic Bolivia virus* (SoMBoV), *Tomato chino la Paz virus* (ToChLPV), *Tomato severe leaf curl virus* (ToSLCV), *Tomato yellow spot virus* (ToYSV), *Vigna yellow mosaic virus* (ViYMV), *Watermelon chlorotic stunt virus* (WmCSV).

[3]ND: No detected.

[4]NA: Not applicable.

Table 4. Nucleotide and amino acid sequence identities (%) between DNA-A genome of Begomovirus isolates identified in the present study with best match sequences available in the database.

[1]

Clone code	Length (bp)	Accession No.	Virus acronym[1]	Reference genome	Complete genome	Virus gene[2]											
						CP		V2		Rep		TrAp		REn		C4	
					n[3]	n	a[4]	n	a	n	a	n	a	n	a	n	a
LV15-Ng-04	2781	MK643155	TYLCV	EF523478.1	99.9	99.6	100	99.7	99.1	99.9	100	99.8	100	99.5	98.5	100	100
LV15-Sa-03	2611	MK636866	SiMSiV	DQ520944.1	95.1	96.3	98.4	NA[5]	NA	94.9	95.8	96.5	93.7	95.6	93.2	94.8	98.4
LV17-Rm-02	2605	MK634355	RhGMV	EU339939.1	98.6	98.8	100	NA	NA	98.5	98.9	98.5	97.1	98.9	97.7	99.2	97.7
LV15-RM-02	2578	MK618662	RhGMSV	DQ406672.1	91.9	91	95.2	NA	NA	92.9	92.3	96.9	95.3	95.3	93.9	92	86.6

TYLCV: *Tomato yellow leaf curl virus*, SiMSiV: *Sida mosaic Sinaloa virus*, RhGMV: *Rhynchosia golden mosaic virus*, RhGMSV: *Rhynchosia golden mosaic Sinaloa virus*.

[2] CP (V1): Coat protein, V2: Precoat protein, Rep (C1): Replication associated protein, TrAP (C2): Transcriptional activator protein, REn (C3): Replication enhancer protein, C4: C4 protein.

[3] n: Nucleotide homologies in %.

[4] a: Aminoacidic homologies in %.

[5] NA: Not applicable.

Table 5. Nucleotide and amino acid sequence identities (%) between DNA-B genome of Begomovirus isolates identified in the present study and best match sequences available in the database.

Clon code	Length (bp)	Accession No.	Virus acronym[1]	Reference genome	Complete genome	MP[1]		NSP[2]	
					n[3]	n	a[4]	N	a
LV15-Sa-02	2583	MK643154	SiMSiV	DQ356428.1	91.3	92.7	95.6	90.3	92.2
LV17-Rm-06	2568	MK634539	RhGMV	DQ356429.1	91	94.8	99.3	91.7	91.6
LV15-Rm-08	2525	MK618663	RhGMSV	DQ406673.1	85.9	90.4	98.3	83.3	86.7

1 MP: Movement protein.

2 NSP: Nuclear shuttle protein.

3 n: Nucleotide homologies in %.

4 a: Aminoacidic homologies in %.

The clone LV15-Ng-04 (Accession number: MK643155) from *N. glauca* was 2781 nt in length and showed high nucleotide homology (99.9%) to TYLCV (Accession number: EF523478.1). Clones LV15-Sa-03 and LV15-Sa-02 (Accession numbers: MK636866, and MK643154), from *S. acuta*, were 2611 nt and 2583 nt in length and showed nucleotide homology of 95.1 and 91.3% with DNA-A and DNA-B of SiMSiV (Accession numbers: DQ520944.1, and DQ356428.1, respectively). Clones LV17-Rm-02 LV17-Rm-06 (Accession numbers: MK634355, and MK634539), from *R. minima*, were 2605 nt and 2568 nt in length and showed nucleotide homology of 98.6 and 91% with DNA-A and DNA-B of RhGMV (Accession numbers: EU339939.1, and DQ356429.1, respectively). Finally, clones LV15-Rm-02 LV15-Rm-08 (Accession numbers: MK618662, and MK618663), from *R. minima*, were 2578 nt and 2525 nt in length and showed nucleotide homology of 91.9 and 85.9% with DNA-A and DNA-B of RhGMSV (Accession numbers: DQ406672.1, and DQ406673.1, respectively). For all viral genomes obtained, the predicted stem-loop region containing the sequence TAATATTAC, found in the common region of family *Geminiviridae*, was identified. For bipartite begomoviruses, high nucleotide homology of the common region (CR), of 98, 91, and 87% (DNA-A versus DNA-B) was observed for SiMSiV, RhGMV, and RhGMSV, respectively. Furthermore, the array of regulatory elements (Iterons and TATA boxes) was conserved in all cases,

suggesting that corresponding DNA-A and DNB-B are cognates. According to the present taxonomic classification of ICTV (Judith K. Brown et al., 2015), for family *Geminiviradae*, the clones of TYLCV, SiMSiV, and RhGMV, obtained in this study, are classified as strains (≥94% DNA-A nucleotide homology), whereas the RhGMSV clones are classified as different isolates (≥91% DNA-A nucleotide homology).

Phylogenetic trees based on the nucleotides alignment with selected begomoviruses from the GenBank database, are shown in **Figure 2**. The results showed that TYLCV isolate LV15-Ng-04 from *N. glauca* clustered together with different TYLCV isolates from different regions of the world, and segregate more closely to Mexican and Asian isolates (Israel and China) (**Figure 2 A**). This data is in agreement with the TYLCV classification by geographic area, where Asian and American isolates are placed in Group I (Wan et al., 2014). SiMSiV is a *Malvaceae*-infecting virus, whereas RhGMV and RhGMSV are *Fabaceae*-infecting viruses. Phylogenetic analysis showed that SiMSiV (DNA A and B) isolated from *S. acute* is closely related to an isolate of SiMSiV from Sinaloa state (Figure 2 B and C). Similarly, isolates of RhGMV (DNA A and B) isolated from *R. minima* is clustered with isolates previously reported from soybean and weeds from Sinaloa state (Figure 2 B and C). Altogether, the HTS analysis strongly suggested the existence of possibly biologically active viruses in the agro-ecological interface with the potential of developing novel or emerging diseases to crops. Finally, infectious clones of TYLCV, SiMSinV, RhGMV and RhGMSV were also obtained; to be described elsewhere.

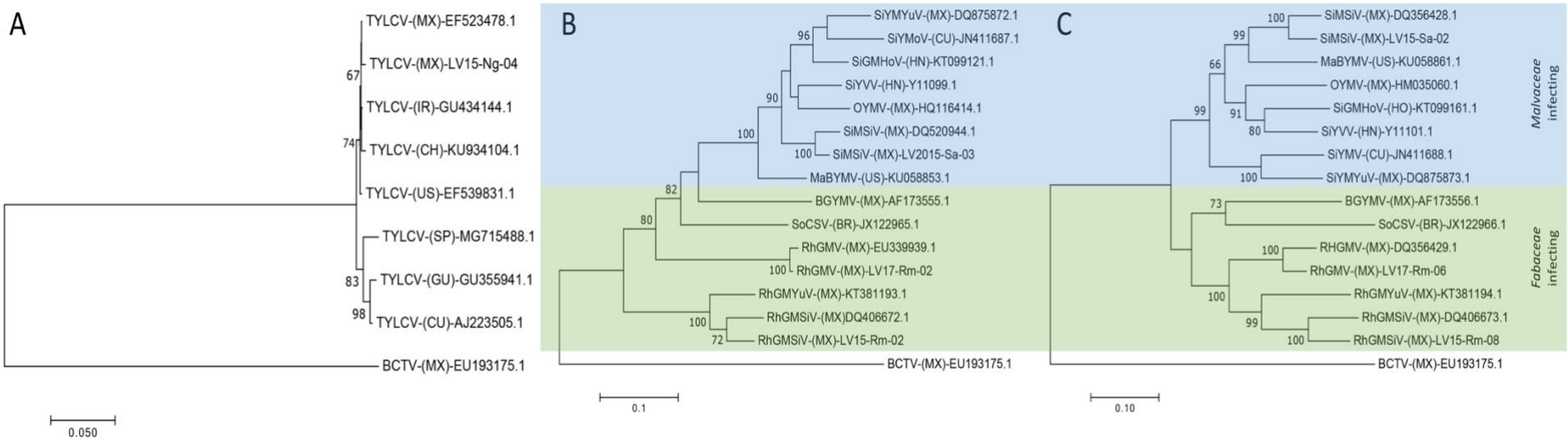


Figure 2. Phylogenetic trees based on multiple sequence alignment of complete monopartite (A) and bipartite begomovirus DNA-A (B), and DNA-B (C) with selected isolates obtained from NCBI. Trees were constructed by Maximum likelihood method with 1000 bootstrap replicates using MEGA7. Virus acronyms: *Bean golden yellow mosaic virus* (BGYMV), *Malvastrum bright yellow mosaic virus* (MaBYMV), *Okra yellow mosaic virus* (OYMV), *Rhynchosia golden mosaic Sinaloa virus* (RhGMSV), *Rhynchosia golden mosaic virus* (RhGMV), *Rhynchosia golden mosaic Yucatan virus* (RhGMYuV), *Sida golden mosaic Honduras virus* (SiGMHoV), *Sida mosaic Sinaloa virus* (SiMSiV), *Sida yellow mottle virus* (SiYMV), *Sida yellow mosaic Yucatan virus* (SiYMYuV), *Sida yellow vein virus* (SiYVV), *Soybean chlorotic spot virus* (SoCSV). Viral genomes Accession numbers are shown. Countries codes are as follow: Brazil (BR), Cuba (CU), Guatemala (GU), Ecuador (EC), Honduras (HN), Israel (IR), Mexico (MX), Puerto Rico (PR) and United States of America (US). As an out-group, *Beet curly top virus* sequence (BCTV) was used. *Malvaceae* and *Fabaceae* infecting virus are highlighted in blue and green, respectively.

3.4. Ecogenomic analyis of predominant begomoviruses

The metagenomic approach carried out in the present work, allowed us to identify the geminivirus diversity present in different plant families and geographical regions; however, it is crucial from an ecological point of view to determine the occurrence and dynamics of virus community in non-cultivated plants.

Begomovirus ecogenomic analyses were accomplished by sequence-specific PCR detection including the viruses PHYVV, TYLCV, SiMSiV, and RhGMV/RhGMSV as the widely distributed species in northern-pacific Mexico (**Table 3, Supplementary Table S3**). Thus, a total of 126 individual species sorted in the predominant plant families (*Fabaceae*, *Malvaceae*, and *Solanaceae*) were examined for the dynamic of the individual plant-virus infection in the five sampling regions (**Table 6, and Supplementary Figure 3**). The analysis revealed that TYLCV was detected in 89 plants, emerging as the predominant virus, followed by SiMSiV, RhGMV/RhGMSV, and PHYVV with 63, 62 and 46 detections, respectively. Moreover, the number of single infections were lower, namely the observed corresponded to TYLCV (13.54%) followed by SiMSiV and RhGMV/RhGMSV (3.12% and 1.04% respectively), suggesting that single infection is not usual (**Table 6, Figure 3)**. Interestingly, the dynamic of multiple (double, triple or quadruple) infections seem to be the rule in most of the plant species analyzed (**Figure 3**). Therefore, the most common viral infections detected in double was TYLCV-SiMSiV (14.58%); in triple were TYLCV-SiMSiV-RhGMV/RhGMSV (11.45%) and TYLCV-PHYVV-RhGMV/RhGMSV (10.4%); whereas, quadruple infections with TYLCV-PHYVV-SiMSiV-RhGMV/RhGMSV (31.25%) represented the highest viral complex founded in different plant species and in the five agroecological regions included in these study (**Table 6, and Figure 3**).

Table 6. Specific PCR detection of begomovirus in individual non-cultivated plants collected from different counties of the five northern pacific regions of Mexico included in this study.

Sampling area	Plant Family	Plant species	Collection year	Virus[1] specific PCR-positive samples				Negative samples	Total samples
				PHYVV	TYLCV	SiMSiV	RhGMV/RhGMSV		
BAJA CALIFORNIA									
Ensenada	*Malvaceae*	*Malva parviflora*	2015	ND[2]	1	ND	ND	0	1
	Solanaceae	*Nicotiana glauca*	2015	ND	1	1	1	0	1
San Quintin	*Malvaceae*	*Malva parviflora*	2015	ND	3	1	ND	1	4
SONORA									
Huatabampo	*Malvaceae*	*Abutilon palmeri*	2015	ND	1	1	1	1	2
		Abutilon trisulcatun	2015	ND	1	1	ND	0	1
		Anoda pedunculosa	2015	ND	ND	ND	ND	1	1
	Solanaceae	*Datura stramonium*	2015	1	1	1	1	0	1
		Nicotiana glauca	2015	ND	1	1	ND	1	2
		Nicotiana plumbanginifolia	2015	ND	1	1	ND	0	2
		Solanum. spp	2015	1	1	ND	1	0	1
		Solanum nigrescens	2015	ND	ND	1	ND	0	1
		Solanum. spp	2015	1	1	1	1	0	1
		Solanum verbascifolium	2015	1	1	ND	ND	0	1
Navojoa	*Fabaceae*	*Meliotus indica*	2015	ND	1	ND	1	0	1
	Malvaceae	*Malva parviflora*	2015	ND	2	3	1	2	5
		Malvella leprosa	2015	ND	1	1	ND	0	1
Obregón	*Malvaceae*	*Abutilon palmeri*	2015	ND	ND	ND	ND	1	1
		Sida rombifolia	2015	1	1	1	1	0	1
	Solanaceae	*Nicotiana glauca*	2015	1	1	1	1	0	1
		Nicotina plumbanginifolia	2015	1	1	1	1	0	1
Río Colorado	*Malvaceae*	*Malva parviflora*	2015	ND	1	1	ND	0	1
SINALOA									
Agua caliente	*Fabaceae*	*Rhynchosia minima*	2016	4	4	1	3	1	5
Concordia	*Malvaceae*	*Anoda pentaschista*	2012	ND	1	ND	ND	0	1
Guasave	*Fabaceae*	*Crotalaria juncea*	2016	1	3	2	3	0	3
		Lonchocarpus lanceolatus	2012	1	1	1	1	0	1
		Melilotus indicus	2015	ND	ND	ND	ND	1	1
			2016	1	1	ND	1	0	1
	Malvaceae	*Abutilon palmeri*	2012	ND	1	ND	ND	0	1
		Abutilon trisulcatun	2014	ND	1	ND	1	2	3
			2012	1	1	ND	1	0	1
		Herissantia crispa	2012	ND	1	ND	1	0	1
		Kosteletzkya depressa	2012	2	2	2	2	0	2
		Melochia piramydata	2014	4	4	4	4	0	4

	Solanaceae	*Datura reburra*	2012	ND	1	ND	ND	0	1
		Datura stramonium	2012	ND	ND	ND	ND	1	1
			2014	3	3	ND	3	1	4
		Nicotiana glauca	2012	ND	1	ND	1	1	2
		Solanum americanum	2012	ND	ND	ND	ND	1	1
		Solanum nigrescens	2012	ND	1	ND	1	0	1
Mocorito	*Malvaceae*	*Abutilon trisulcatun*	2012	1	1	ND	ND	1	2
		Sidastrum lodiegensis	2012	1	1	ND	1	0	1
	Solanaceae	*Datura discolor*	2012	ND	ND	ND	ND	2	2
		Solanum tridynamum	2012	ND	ND	ND	ND	1	1
Playa Ceuta	*Fabaceae*	*Rhynchosia minima*	2016	2	2	2	2	0	2
Rosario	*Fabaceae*	*Macroptilium atropurpureum*	2016	2	3	4	4	0	4
		Rhynchosia precatoria	2016	2	2	2	2	0	2
		Rhynchosia minima	2016	3	4	4	4	0	4
		Senna uniflora	2016	1	ND	1	1	0	1
			2014	1	1	1	1	0	1
	Malvaceae	*Abutilon trisulcatun*	2014	ND	1	ND	1	0	1
		Herissantia crispa	2014	1	1	1	1	0	1
		Melochia piramydata	2014	ND	ND	ND	ND	1	1
		Sida acuta	2014	2	2	2	2	0	2
	Solanaceae	*Physalis acutifolia*	2014	ND	ND	ND	ND	1	1
Sinaloa		*Datura inoxia*	2012	ND	1	ND	ND	0	1
		Solanum tridynamum	2012	ND	2	ND	ND	1	3
COLIMA/NAYARIT									
Tecomán	*Malvaceae*	*Herissantia crispa*	2014	1	5	5	5	0	5
		Malvastrum coromandelianum	2014	1	1	1	2	1	3
		Sida acuta	2014	ND	ND	+	ND		
COAHUILA/DURANGO									
La Goma	*Malvaceae*	*Sida acuta*	2015	ND	ND	ND	ND	1	1
		Sida rombifolia	2015	ND	2	1	ND	0	2
	Solanaceae	*Datura stramonium*	2015	ND	1	1	ND	0	1
Poanas		*Solanum elaeagnifolium*	2016	ND	ND	ND	ND	2	2
		Solanum rostrarum	2016	2	3	3	3	0	3
Tlahualilo	*Malvaceae*	*Sida rombifolia*	2015	ND	ND	1	1	2	3
	Solanaceae	*Solanum elaeagnifolium*	2015	1	1	1	ND	0	1
Torreón	*Malvaceae*	*Sphaeralcea angustifolia*	2015	ND	2	ND	ND	1	3
	Solanaceae	*Datura stramonium*	2015	ND	1	1	ND	0	1
		Nicotiana glauca	2015	1	2	1	ND	1	3
		Solanum elaeagnifolium	2015	ND	3	3	ND	0	3
Total				**46**	**89**	**63**	**62**	**30**	**126**

1 Virus acronyms: **PHYVV**, *Pepper huasteco yellow vein virus*; **TYLCV**, *Tomato yellow leaf virus*; **SiMSiV**, *Sida mosaic Sinaloa virus*; **RhGMV**, *Rhynchosia golden mosaic virus*; **RhGMSV**, *Rhynchosia golden mosaic Sinaloa virus*.

2 ND: No detected.

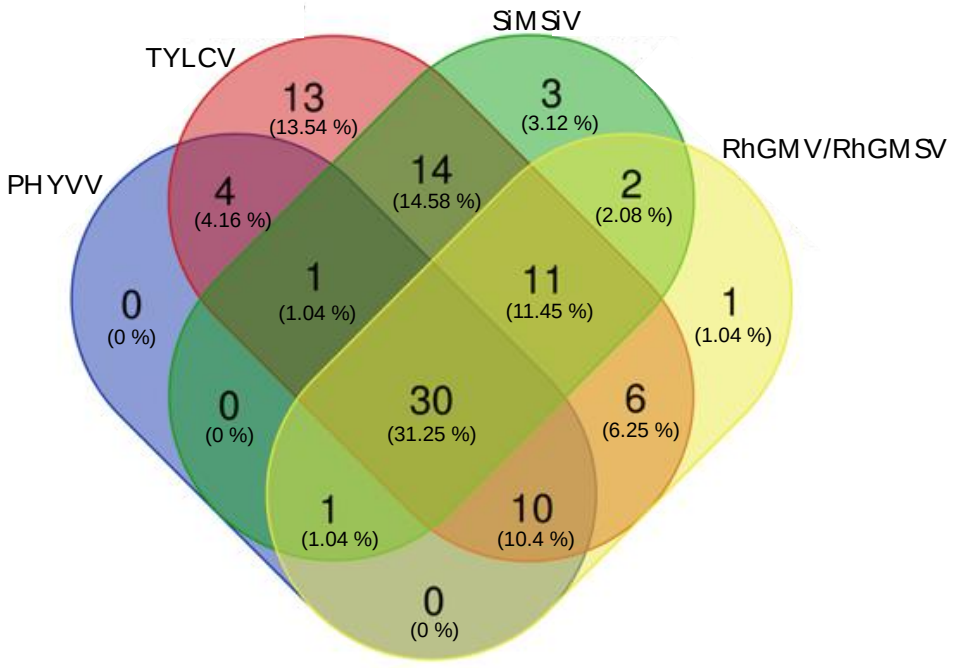


Figure 3. Venn diagram of predominant begomovirus specific PCR detection. A total of 126 individual species were examined for the presence of predominant begomovirus in the five sampling regions. Number of begomovirus-positive plants (96) showing single, double, triple or quadruple infections is reported. Values are

also reported in percentage in parenthesis. Virus acronyms: *Pepper huasteco yellow vein virus* (PHYVV), *Rhynchosia golden mosaic virus*, (RhGMV), *Rhynchosia golden mosaic Sinaloa virus* (RhGMSV), *Sida mosaic Sinaloa virus* (SiMSiV), *Tomato yellow leaf curl virus* (TYLCV).

TYLCV is the major viral concern for tomato production worldwide (Basak, 2016), recently identified as seed-transmitted virus (E.-J. Kil et al., 2016) and associated to crop diseases with other begomoviruses in both the old and new world (Barboza, Blanco-Meneses, Hallwass, Moriones, & Inoue-Nagata, 2013; J K Brown & Idris, 2006; Cardenas-Conejo et al., 2010; Dong, Luo, Ding, Zhang, & Yang, 2007; Gamez-Jimenez et al., 2009; Moriones & Navas-Castillo, 2008; Qinones, Fonseca, Martinez, & Accotto, 2002; Salati et al., 2002), also have been reported in many non-cultivated plant species worldwide including the families *Amaranthaceae, Asteraceae, Chenopodiaceae, Convolvulaceae, Euphorbiaceae, Fabaceae, Malvaceae*, and *Solanaceae* (Bedford et al., 1998; García-Andrés, Monci, Navas-Castillo, & Moriones, 2006; Jordá et al., 2007; Kashina, Mabagala, & Mpunami, 2003; E. J. Kil et al., 2014). In the present work, TYLCV was detected in new host species of the *Fabaceae* family (*Crotalaria juncea*, *Lonchocarpus lanceolatus*, *Macroptilium atropurpureum*, *Melilotus indicus*, *Rhychosia precatoria*, *Rhynchosia minima and Senna uniflora*), the *Malvaceae* family (*Abutilon trisulcatum*, *Anoda pentaschista*, *Herissantia crispa*, *kosteletzkua depressa*, *Malvastrum coromandelianum*, *Malvella leprosa*, *Melochia piramydata*, *Sida acuta*, *Sida rombifolia*, *Sidastrum lodiegensis and Sphaeralcea angustifolia*), and the *Solanaceae* family (*Datura discolor*, *Nicotiana plumbanginifolia*, *Nicotiana glauca*, *Solanum trydynamum*, *Solanum verbacifolium*). PHYVV is an endemic virus of Mexico, described as a major concern for pepper production (J. Antonio Garzon-Tiznado, 1993; Melendrez-Bojorquez et al., 2016; Morales-Aguilar et al., 2019; Rodelo-Urrego, García-Arenal, et al., 2015), additionally, it has been reported in several plant families (Cervantes-Díaz et al., 2009; Jose Antonio Garzon-Tiznado et al., 2002). Here, PHYVV was detected in new species hosts of the *Fabaceae* family (*Crotalaria juncea, Rhynchosia precatoria, Rhynchosia minima* and *Senna uniflora*), the *Malvaceae* family (*Abutilon trisulcatum*, *Herissantia crispa*, *Kosteletzkya*

depressa, *Malvastrum coromandelianum*, *Melochia piramydata*, *Sida acuta*, *Sida rombifolia*, *Sidastrum lodiegensis*), and the *Solanaceae* family (*Datura stramonium*, *Nicotiana glauca*, *Nicotiana plumbanginifolia*, *Solanum elaegnifolium*, *Solanum rostrarum* and *Solanum verbascifolium*). On the other hand, SiMSiV was reported infecting *Sida rombifolia* in Mexico; interestingly, this virus was detected also in several other malvaceous plants (*Abutilon palmeri, Anoda pentaschista, Herissantia crispa, Kosteletzkya depressa, Malva parviflora, Malvastrum coronomandelianum*) but also in the *Fabaceae* family (*Crotalaria juncea, Lonchocarpus lanceolatus, Macroptilium atropurpureum, Rhynchosia precatoria, Rhynchosia minima, Senna uniflora*) and the *Solanaceae* family (*Nicotiana plumbanginifolia, Datrua stramonium, Nicotiana glauca, Solanum elaeagnifolium, Solanum nigrescens and Solanum rostrarum*). Furthermore, RhGMV was first reported infecting in *Rhynchosia minima* in Honduras (Potter, Roca de Doyle, Nakhla, & Maxwell, 2000) and after infecting tobacco and soybean crops in Chiapas and Sinaloa states from Mexico, respectively (Ascencio-Ibáñez et al., 2007; Méndez-Lozano, Leyva-López, et al., 2006). The data showed that RhGMV species (RhGMV and/or RhGMSV) were able to infect not only *Rhynchosia minima* but also other fabaceous plants (*Crotalaria juncea*, *Macroptilium atropurpureum*, *Rhynchosia precatoria*, *Senna uniflora*, *Meliotus indica*, *Lochocarpus lanceolatus*, and *Meliotus indicus*), and other hosts belonging to the *Malvaceae* (*Abutilon palmeri*, *Abutilon trisulcatun*, *Herissantia crispa*, *Kosteletzkya depressa, Malva parviflora*, *Malvastrum coromandelianum*, *Melochia piramydata*, *Sida acuta*, *Sida rombifolia*, and *Sidastrum lodiegensis*), and the *Solanaceae* (*Datura stramonium, Nicotiana glauca*, *Nicotiana plumbanginifolia*, *Solanum nigrescens*, and *Solanum rostrarum*) families. This part of the study was oriented to individual plant samples, providing evidences for ecology of the viruses. The fact, of discovery different plants species harboring different viruses in multiple infection represent an important mixing vessel for geminivirus to evolve in different directions triggering by vector and environmental factors (Silva et al., 2012). Previous work substantially describes strains or new viruses in plants or whiteflies from different region and some imply the host plant and biological properties (Bernardo et al., 2013b; Fernandes et al., 2011; Cecilia Hernández-Zepeda et al.,

2007; Silva et al., 2012). Nonetheless, others were limited because the virus host was not determined (Rosario et al., 2016, 2015). The discovery of new viral sequences is not enough in terms of plant pathology (Marilyn J. Roossinck, 2012; Stobbe & Roossinck, 2014) and therefore, an in depth survey and biological determination is required that could enhanced our comprehension of the agroecological environmental impact on virus evolution.

Plant virus evolution is a complex process involving multiple ecological and genetic factors resulting in host-pathogen co-evolution. Studies on virus diversity have been documented in a wide number of non-cultivated plants, either described as different strains of existing virus or new viruses (Bernardo et al., 2013b; V. N. Fondong et al., 2000; Cecilia Hernández-Zepeda et al., 2007; Pita, Fondong, Sangaré, Kokora, & Fauquet, 2001; Ribeiro et al., 2003; Rosario et al., 2016, 2015; Silva et al., 2012). Noteworthy, however, is also documented that viral species are often co-infecting the same plants with the possibility of genetic interaction, given as a result an inter or intraspecific recombinant viruses resulting in more severe strains as the case of cassava mosaic diseases (CMD) (V. N. Fondong et al., 2000; Pita, Fondong, Sangaré, Otim-Nape, et al., 2001) and tomato yellow curl diseases (TYLCD) (Davino et al., 2009; Díaz-Pendón et al., 2019; Fiallo-Olivé et al., 2019; Monci et al., 2002). Plant disease emergence requires that a virus invades a new host from a reservoir resulting in a new infection dynamics and virus adaptation (Marilyn J. Roossinck & García-Arenal, 2015). In this sense, some geminiviruses acquired the ability to form a complex disease by assorting DNA A and B genomes like ACMV and EACMV in Uganda (Pita, Fondong, Sangaré, Otim-Nape, et al., 2001) or those were the DNA-A genome is similar to a bipartite begomovirues, but the DNA B is not yet described such ToChLPV and ToSLCV in Mexico (R. J. Holguín-Peña et al., 2007; J. A. Mauricio-Castillo et al., 2006b) or *Datura leaf curl virus* (DaLCV) in Sudan (Mohammed, El Siddig, El Hussein, Navas-Castillo, & Fiallo-Olivé, 2018b). In the present work, the geminivirus diversity was described in different regions and it is shown that TYLCV, SiMSiV, PHYVV, RhGMV and RhGMSV are the predominant viruses. In addition, a different multiviral complex was detected in several plant species taking into the consideration of high frequency of

mixed infections detected (**Figure 3**). However, a relevant issue is the ample host range size observed for these viruses and the genetic structural of the virome could be modified in an unpredictable manner. Towards this direction, work is in progress aiming at determining whether new viruses or strains constitute a potential risk for north-pacific Mexico agriculture.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1, **Figure S1**: Representation of non-cultivated plants belonging to predominant plant families collected in natural ecosystem in five regions of Northern-pacific Mexico, **Figure S2**: Generation of Geminivirus-related signatures workflow**, Figure S3**: PCR detection of five geminivirus species in three predominant plant families of non-cultivated plants from northern-pacific Mexico, **Table S1**: Non-cultivated plants collected from Northern-pacific regions of Mexico and determination of begomovirus host by PCR-test, **Table S2**: Geminivirus signatures obtained by *de novo* assembly from the metagenomic study, **Table S3**: List of PCR primers used in the present work.

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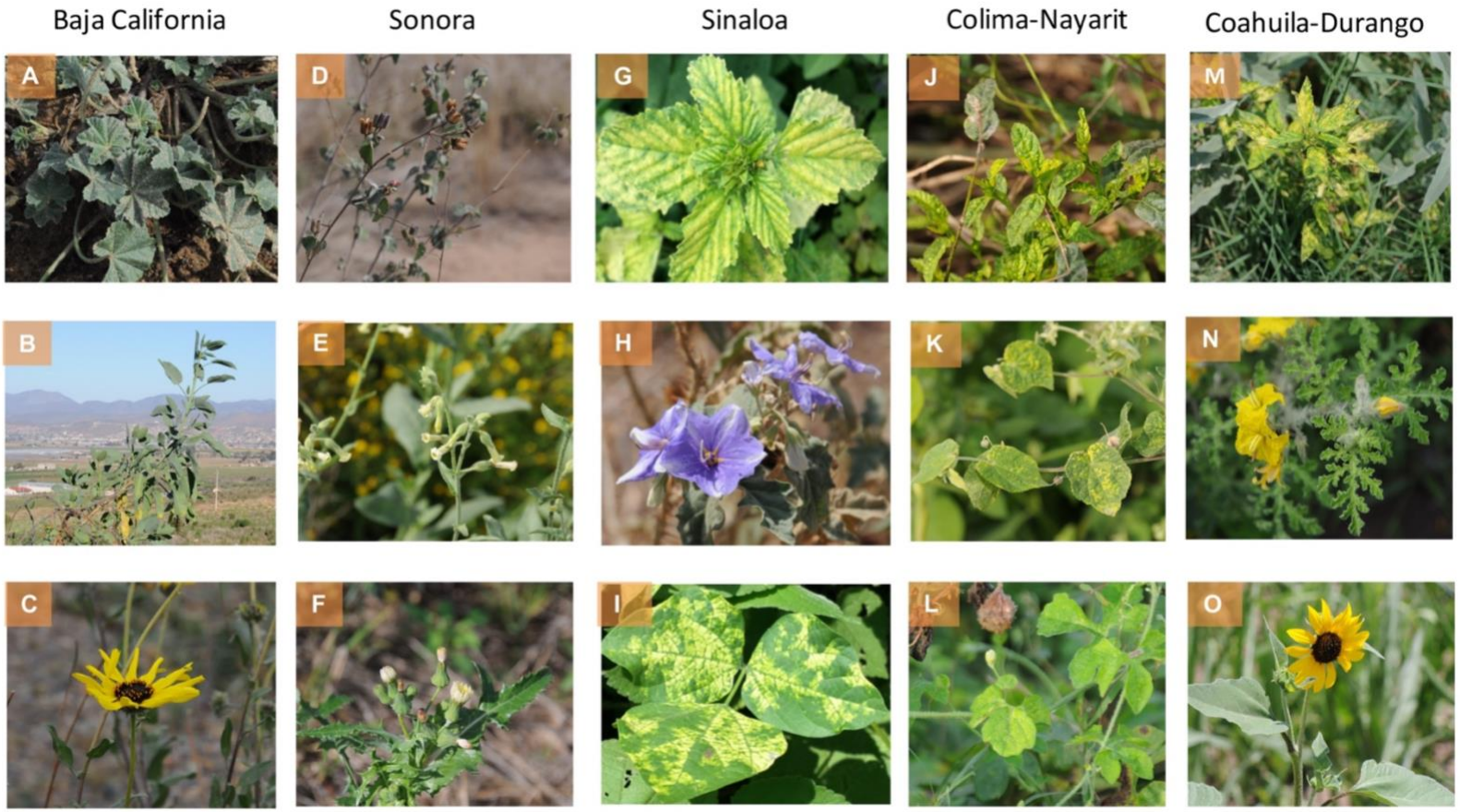
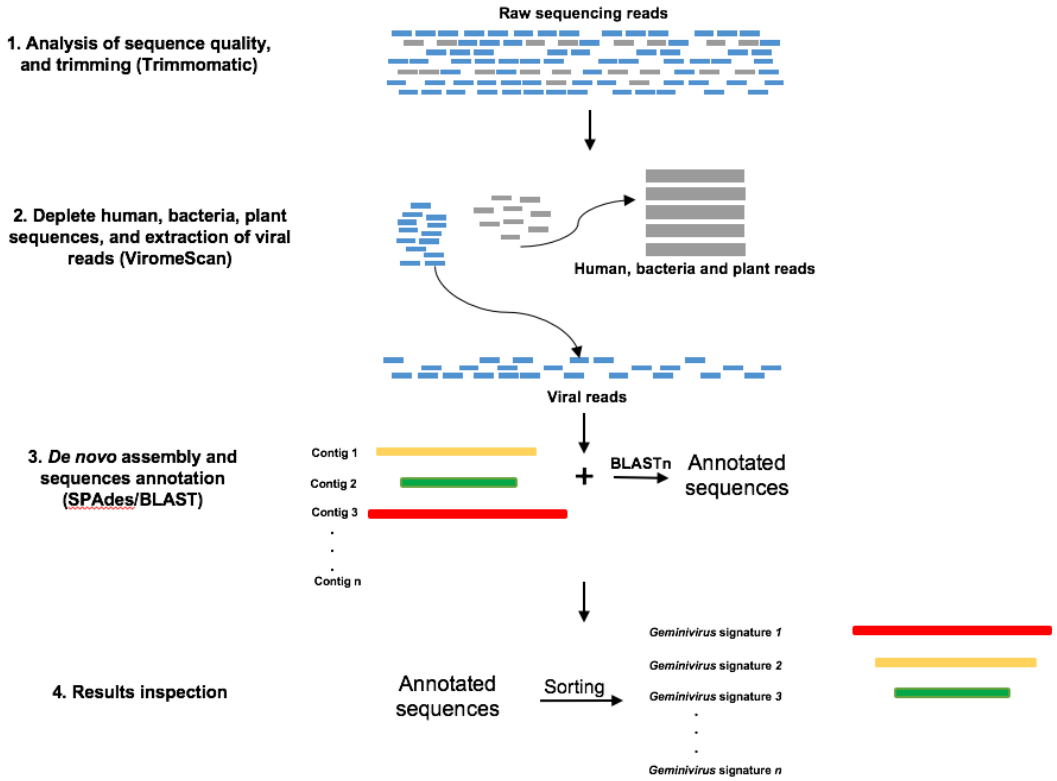
1.

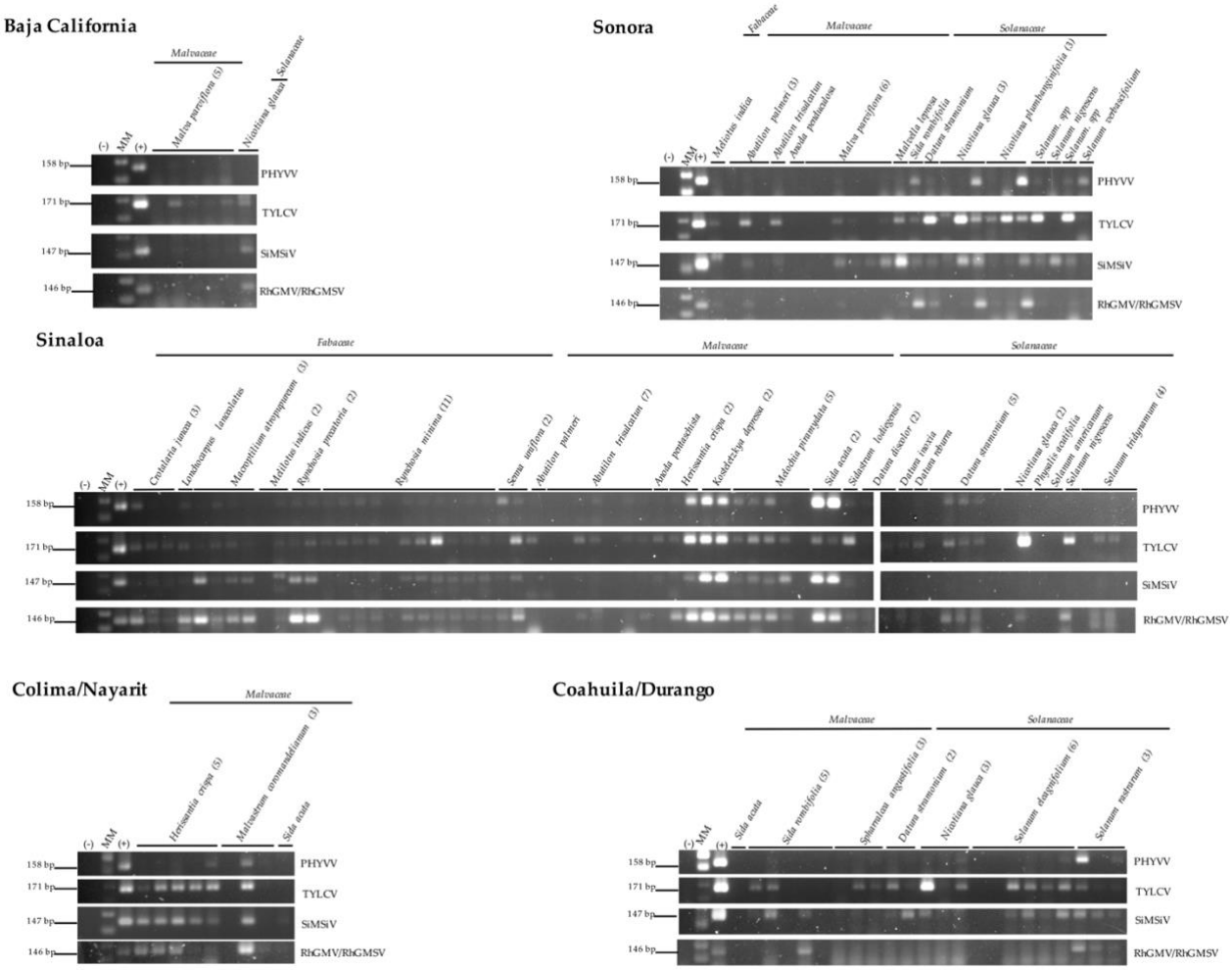
Supplemental Materials



Supplemental Figure 1. Representation of non-cultivated plants of the main plant families collected from Northern-pacific Mexico. ***Malvaceae*****:** A, *Malva parviflora.* D, *Abutilon trisulcatum.* G, Sida acuta. J, *Malvastrum coronomandelianum.* K, *Herissantia crispa.* M, *Sida rombifolia*. ***Solanaceae*****:** B, *Nicotiana glauca.* E, *Nicotiana plumbanginifolia.* H, *Solanum trydinamum*. N, *Solanum rostratum.* ***Asteraceae:*** C, *Helianthus niveus.* F, *Sonchus oleraceus.* O, *Helianthus annus*. ***Cucurbitacea:*** L, *Momardica charantia*. ***Fabaceae:*** I, *Rhynchosia minima*.



Supplemental Figure 2 Generation of Geminivirus-related signatures workflow. 1) Metagenomic reads obtained from each library were trimmed, 2) Each library was filtered (ViromeScan) for human, bacteria, and plant reads to obtain viral reads. **3**) All filtered libraries were *de novo* assembled using SPAdes, and contigs were compared against the GeneBank. non-redundant database using BLASTn for annotation. **4**) Geminivirus-related signatures were classified, and sorted by contig length.



Supplemental figure 3. PCR detection of five geminivirus species in three predominant plant families of no-cultivated plants from northern-pacific Mexico.

Supplemental Table 1. Non-cultivated plants collected from Northern pacific regions of Mexico and determination of begomovirus host by PCR-test

Sampled area	Collected year	Sample code	Scientific name	Family	Positive to begomovirus (PCR test with degenerate primers)*			Geolocalization
					M	B	M & B	
BC-EN	2015	15-255	*Encelia farinosa*	*Asteraceae*		1		31º 40'53.5" N, 116º37'02.7" W
BC-SQ	2014	14-132	*Encelia farinosa*	*Asteraceae*				30º26'0.596" N, 115º53'6.018" W
BC-SQ	2014	14-131	*Erigeron spp*	*Asteraceae*				30º26'0.596" N, 115º53'6.018" W
BC-EN	2015	15-253	*Gnaphalium americanum*	*Asteraceae*				31º 40'53.5" N, 116º37'02.7" W
BC-EN	2015	15-256	*Helianthus niveus*	*Asteraceae*			1	31º 40'53.5" N, 116º37'02.7" W
BC-EN	2015	15-249	*Helianthus niveus*	*Asteraceae*				31º 46'17.8" N, 116º33'12.1" W
BC-SQ	2015	15-246	*Helianthus niveus*	*Asteraceae*		1		30º34'31.8" N, 115º52'50.3"W
BC-SQ	2015	15-245	*Isocoma acradenia*	*Asteraceae*				30º34'31.8" N, 115º52'50.3"W
BC-SQ	2014	14-130	*Sonchus oleraceus*	*Asteraceae*		1		30º26'0.596" N, 115º53'6.018" W
BC-SQ	2015	15-239	*Sonchus oleraceus*	*Asteraceae*				30º30'57.1" N, 115º59'30.0"W
BC-SQ	2014	14-129	*Heliotropium curassavicum*	*Boraginaceae*				30º26'0.596" N, 115º53'6.018" W
BC-SQ	2015	15-243	*Brassica tournefortii*	*Brassicaceae*	1			30º34'31.8" N, 115º52'50.3"W
BC-EN	2015	15-248	*Raphanus raphanistrum*	*Brassicaceae*				31º 46'17.8" N, 116º33'12.1" W
BC-SQ	2015	15-240	*Chenopodium berlandieri*	*Chenopodiaceae*			1	30º30'57.1" N, 115º59'30.0"W
BC-SQ	2015	15-261	*Chenopodium berlandieri*	*Chenopodiaceae*	1			30º32'04.1" N, 115º57'13.5"W
BC-ME	2015	15-269	*Chenopodium spp*	*Chenopodiaceae*			1	32º24'42.5" N, 115º06'53.5" W
BC-SQ	2015	15-265	*Chenopodium spp*	*Chenopodiaceae*		1		30º32'04.1" N, 115º57'13.5"W
BC-ME	2015	15-271	*Convolvulus arvensis*	*Convolvulaceae*				32º19'53.2" N, 115º04'20.6" W
BC-SQ	2014	14-134	*Convolvulus arvensis*	*Convolvulaceae*				30º26'0.596" N, 115º53'6.018" W
BC-EN	2015	15-251	*Marah macrocarpa*	*Cucurbitaceae*				31º 40'53.5" N, 116º37'02.7" W

BC-EN	2015	15-257	*Malva parviflora*	*Malvaceae*			1	31º 40’53.5” N, 116º37’02.7” W
BC-SQ	2015	15-241	*Malva parviflora*	*Malvaceae*		1		30º30’57.1” N, 115º59’30.0”W
BC-SQ	2015	15-242	*Malva parviflora*	*Malvaceae*	1			30º30’57.1” N, 115º59’30.0”W
BC-SQ	2015	15-260	*Malva parviflora*	*Malvaceae*	1			30º32’04.1” N, 115º57’13.5”W
BC-SQ	2015	15-264	*Malva parviflora*	*Malvaceae*		1		30º32’04.1” N, 115º57’13.5”W
BC-SQ	2015	15-238	*Sphaeralcea ambigua*	*Malvaceae*				30º30’57.1” N, 115º59’30.0”W
BC-EN	2015	15-252	*Rumex crispo*	*Polygonaceae*				31º 40’53.5” N, 116º37’02.7” W
BC-ME	2015	15-270	*Rumex crispo*	*Polygonaceae*		1		32º20’06.8” N, 115º04’13.2” W
BC-SQ	2014	14-133	*Anagallis arvensis*	*Primulaceae*		1		30º26'0.596" N, 115º53'6.018" W
BC-SQ	2014	14-128	*Datura stramonium*	*Solanaceae*				30º26'0.596" N, 115º53'6.018" W
BC-EN	2015	15-254	*Nicotiana glauca*	*Solanaceae*		1		31º 40’53.5” N, 116º37’02.7” W
SO-HU	2015	15-288	*Amaranthus palmeri*	*Amaranthaceae*		1		26º 24’54.5”N, 109º01’15.1”W
SO-OB	2015	15-235	*Amaranthus palmeri*	*Amaranthaceae*		1		27º 36’22.5”N, 110º08’02.5”W
SO-NA	2015	15-362	*Amaranthus spinosus*	*Amaranthaceae*				26º96’00.39’’ N, 109º58 24.36’’ W
SO-HU	2015	15-389	*Amaranthus spp*	*Amaranthaceae*		1		26º54’97.04’’ N, 109º11’88.03’’ W
SO-HU	2015	15-280	*Amaranthus spp*	*Amaranthaceae*			1	26º 24’54.5”N, 109º01’15.1”W
SO-OB	2015	15-229	*Conium maculatum*	*Apiaceae*		1		27º 36’22.5”N, 110º08’02.5”W
SO-HU	2015	15-287	*Ambrosia ambrosoides*	*Asteraceae*		1		26º 24’54.5”N, 109º01’15.1”W
SO-OB	2015	15-224	*Ambrosia ambrosoides*	*Asteraceae*		1		27º33’33.5” N, 110º05’17.6” W
SO-HU	2015	15-286	*Ambrosia cordifolia*	*Asteraceae*				26º 24’54.5”N, 109º01’15.1”W
SO-HU	2015	15-293	*Artemisia absitium*	*Asteraceae*				26º 24’54.5”N, 109º01’15.1”W
SO-OB	2015	15-223	*Artemisia ludoviciana*	*Asteraceae*		1		27º33’33.5” N, 110º05’17.6” W
SO-OB	2015	15-214	*Gnaphalium spp*	*Asteraceae*		1		27º 08’09.9”N, 109º53’31.7”W
SO-OB	2015	15-237	*Helenium mexicanum*	*Asteraceae*		1		27º 36’22.5”N, 110º08’02.5”W
SO-NA	2015	15-335	*Helianthus annuus*	*Asteraceae*	1			26º 86’ 23.43’’N, 109º68’63.83 W
SO-NA	2015	15-339	*Helianthus spp*	*Asteraceae*				26º 86’ 23.43’’N, 109º68’63.83 W
SO-HU	2015	15-345	*Perityle microglossa*	*Asteraceae*				25º48'42.44" N, 108º13'9.302" W
SO-HU	2015	15-347	*Perityle spp*	*Asteraceae*	1			26º50'05.9"N, 109º31'51.8"

SO-HU	2015	15-350	*Perityle spp*	*Asteraceae*	1			26º50'05.9"N, 109º31'51.8"
SO-HU	2015	15-291	*Sonchus oleraceus*	*Asteraceae*				26º 24'54.5"N, 109º01'15.1"W
SO-NA	2015	15-338	*Sonchus oleraceus*	*Asteraceae*				26º 86' 23.43''N, 109º68'63.83 W
SO-NA	2015	15-340	*Sonchus oleraceus*	*Asteraceae*				26º 86' 23.43''N, 109º68'63.83 W
SO-NA	2015	15-360	*Sonchus oleraceus*	*Asteraceae*				26º96'00.39'' N, 109º58 24.36'' W
SO-HU	2015	15-283	*Handroanthus impetiginosus*	*Bignoniaceae*				26º 24'54.5"N, 109º01'15.1"W
SO-HU	2015	15-330	*Heliotropium curassavicum*	*Boraginaceae*		1		26º47'06.1"N, 109º40'52.8"W
SO-NA	2015	15-381	*Heliotropium curassavicum*	*Boraginaceae*				26º90'05.02''N, 109º52'28.42'' W
SO-NA	2015	15-383	*Heliotropium curassavicum*	*Boraginaceae*		1		26º90'05.02''N, 109º52'28.42'' W
SO-NA	2015	15-384	*Heliotropium curassavicum*	*Boraginaceae*		1		26º90'05.02''N, 109º52'28.42'' W
SO-OB	2015	15-215	*Heliotropium curassavicum*	*Boraginaceae*		1		27º 08'09.9"N, 109º53'31.7"W
SO-HU	2015	15-315	*Brassica juncea*	*Brassicaceae*				26º 24'54.5"N, 109º01'15.1"W
SO-RC	2015	15-275	*Diplotaxis muralis*	*Brassicaceae*				32º25'07.8" N, 114º49'34.7" W
SO-NA	2015	15-375	*Chenopodium album*	*Chenopodiaceae*	1			26º90'05.02''N, 109º52'28.42'' W
SO-NA	2015	15-379	*Chenopodium album*	*Chenopodiaceae*				26º90'05.02''N, 109º52'28.42'' W
SO-NA	2015	15-341	*Chenopodium album*	*Chenopodiaceae*	1			26º 86' 23.43''N, 109º68'63.83 W
SO-NA	2015	15-364	*Chenopodium album*	*Chenopodiaceae*	1			26º96'00.39''N, 109º58'24.36'' W
SO-NA	2015	15-336	*Chenopodium album*	*Chenopodiaceae*				26º 86' 23.43''N, 109º68'63.83 W
SO-OB	2015	15-217	*Chenopodium berlandieri*	*Chenopodiaceae*				27º 08'09.9"N, 109º53'31.7"W
SO-OB	2015	15-218	*Chenopodium berlandieri*	*Chenopodiaceae*		1		27º 08'09.9"N, 109º53'31.7"W
SO-RC	2015	15-273	*Chenopodium berlandieri*	*Chenopodiaceae*		1		32º20'06.3" N, 114º53'31.4" W
SO-HU	2015	15-307	*Chenopodium spp*	*Chenopodiaceae*		1		26º 24'54.5"N, 109º01'15.1"W
SO-HU	2015	15-316	*Chenopodium spp*	*Chenopodiaceae*				26º 24'54.5"N, 109º01'15.1"W
SO-HU	2015	15-318	*Convolvulus arvensis*	*Convolvulaceae*			1	26º 24'54.5"N, 109º01'15.1"W
SO-OB	2015	15-221	*Convolvulus arvensis*	*Convolvulaceae*		1		27º 08'09.9"N, 109º53'31.7"W
SO-HU	2015	15-348	*Cnidoscolus spp*	*Euphorbiaceae*				26º50'05.9"N, 109º31'51.8"
SO-HU	2015	15-349	*Cnidoscolus spp*	*Euphorbiaceae*	1			26º50'05.9"N, 109º31'51.8"
SO-OB	2015	15-220	*Ricinus communis*	*Euphorbiaceae*		1		27º 08'09.9"N, 109º53'31.7"W

SO-NA	2015	15-371	*Meliotus indica*	*Fabaceae*				26º99’98.46"N, 109º52’36.35" W
SO-NA	2015	15-365	*Meliotus indica*	*Fabaceae*	1			26º96’00.39’’N, 109º58’24.36’’ W
SO-OB	2015	15-236	*Nama jamaicensis*	*Hydrophyllaceae*		1		27º 36’22.5”N, 110º08’02.5”W
SO-HU	2015	15-278	*Abutilon palmeri*	*Malvaceae*		1		26º 24’54.5”N, 109º01’15.1”W
SO-HU	2015	15-290	*Abutilon palmeri*	*Malvaceae*		1		26º 24’54.5”N, 109º01’15.1”W
SO-OB	2015	15-230	*Abutilon palmeri*	*Malvaceae*		1		27º 36’22.5”N, 110º08’02.5”W
SO-HU	2015	15-277	*Abutilon trisulcatun*	*Malvaceae*			1	26º 24’54.5”N, 109º01’15.1”W
SO-HU	2015	15-285	*Anoda pedunculosa*	*Malvaceae*		1		26º 24’54.5”N, 109º01’15.1”W
SO-HU	2015	15-317	*Malva parviflora*	*Malvaceae*				26º 24’54.5”N, 109º01’15.1”W
SO-NA	2015	15-374	*Malva parviflora*	*Malvaceae*	1			26º90’05.02’’N, 109º52’28.42’’ W
SO-NA	2015	15-385	*Malva parviflora*	*Malvaceae*			1	26º90’05.02’’N, 109º52’28.42’’ W
SO-NA	2015	15-386	*Malva parviflora*	*Malvaceae*			1	26º90’05.02’’N, 109º52’28.42’’ W
SO-NA	2015	15-334	*Malva parviflora*	*Malvaceae*				26º 86’ 23.43’’N, 109º68’63.83 W
SO-NA	2015	15-337	*Malva parviflora*	*Malvaceae*	1			26º 86’ 23.43’’N, 109º68’63.83 W
SO-NA	2015	15-361	*Malva parviflora*	*Malvaceae*			1	26º96’00.39’’N, 109º58’24.36’’ W
SO-RC	2015	15-274	*Malva parviflora*	*Malvaceae*		1		32º25’07.8” N, 114º49’34.7” W
SO-NA	2015	15-373	*Malvella leprosa*	*Malvaceae*			1	26º90’05.02’’N, 109º52’28.42’’ W
SO-NA	2015	15-376	*Malvella leprosa*	*Malvaceae*				26º90’05.02’’N, 109º52’28.42’’ W
SO-NA	2015	15-378	*Malvella leprosa*	*Malvaceae*				26º90’05.02’’N, 109º52’28.42’’ W
SO-OB	2015	15-216	*Malvella leprosa*	*Malvaceae*				27º 08’09.9”N, 109º53’31.7”W
SO-OB	2015	15-227	*Sida rombifolia*	*Malvaceae*		1		27º 36’22.5”N, 110º08’02.5”W
SO-HU	2015	15-352	*Ludwigia octovalvis*	*Onagraceae*				26º50'05.9"N, 109º31'51.8"
SO-HU	2015	15-353	*Ludwigia octovalvis*	*Onagraceae*	1			26º50'05.9"N, 109º31'51.8"
SO-OB	2015	15-225	*Argemone mexicana*	*Papaveraceae*		1		27º 36’22.5”N, 110º08’02.5”W
SO-HU	2015	15-284	*Antigonon leptopus*	*Polygonaceae*			1	26º 24’54.5”N, 109º01’15.1”W
SO-NA	2015	15-377	*Rumex crispo*	*Polygonaceae*				26º90’05.02’’N, 109º52’28.42’’ W
SO-NA	2015	15-380	*Rumex crispo*	*Polygonaceae*	1			26º90’05.02’’N, 109º52’28.42’’ W
SO-NA	2015	15-382	*Rumex crispo*	*Polygonaceae*		1		26º90’05.02’’N, 109º52’28.42’’ W

SO-OB	2015	15-219	*Rumex crispo*	*Polygonaceae*				27º 08’09.9”N, 109º53’31.7”W
SO-HU	2015	15-282	*Gouania lupuloides*	*Rhamnaceae*		1		26º 24’54.5”N, 109º01’15.1”W
SO-HU	2015	15-329	*Datura stramonium*	*Solanaceae*		1		26º47’06.1”N, 109º40’52.8”W
SO-HU	2015	15-343	*Nicotiana glauca*	*Solanaceae*		1		26º50'05.9"N, 109º31'51.8"
SO-HU	2015	15-346	*Nicotiana glauca*	*Solanaceae*				26º50'05.9"N, 109º31'51.8"
SO-HU	2015	15-351	*Nicotiana glauca*	*Solanaceae*				26º50'05.9"N, 109º31'51.8"
SO-HU	2015	15-327	*Nicotiana glauca*	*Solanaceae*		1		26º47’06.1”N, 109º40’52.8”W
SO-OB	2015	15-213	*Nicotiana glauca*	*Solanaceae*			1	27º 08’09.9”N, 109º53’31.7”W
SO-HU	2015	15-344	*Nicotiana plumbanginifolia*	*Solanaceae*	1			26º50'05.9"N, 109º31'51.8"
SO-HU	2015	15-289	*Nicotina plumbanginifolia*	*Solanaceae*		1		26º 24’54.5”N, 109º01’15.1”W
SO-OB	2015	15-228	*Nicotina plumbanginifolia*	*Solanaceae*		1		27º 36’22.5”N, 110º08’02.5”W
SO-HU	2015	15-328	*Solanum lycopersicum*	*Solanaceae*		1		26º47’06.1”N, 109º40’52.8”W
SO-HU	2015	15-390	*Solanum nigrescens*	*Solanaceae*		1		26º 24’54.5”N, 109º01’15.1”W
SO-HU	2015	15-281	*Solanum pseudocapsicum*	*Solanaceae*				26º 24’54.5”N, 109º01’15.1”W
SO-HU	2015	15-331	*Solanum spp*	*Solanaceae*		1		26º47’06.1”N, 109º40’52.8”W
SO-HU	2015	15-342	*Solanum verbascifolium*	*Solanaceae*	1			26º50'05.9"N, 109º31'51.8"
SI-CO	2012	12-043	*Amaranthus palmeri*	*Amaranthaceae*				23º17'13.489"N, 106º4'51.193" W
SI-GV	2012	12-078	*Amaranthus palmeri*	*Amaranthaceae*				25º44'59.049" N, 108º39'46.189 W
SI-GV	2012	12-053	*Amaranthus palmeri*	*Amaranthaceae*	1			25º43'49.164" N, 108º24'27.845" W
SI-SL	2012	12-111	*Amaranthus palmeri*	*Amaranthaceae*				25º49'29.732"N, 108º14'4.196" W
SI-SL	2012	12-100	*Stemmadenia palmeri*	Apocynaceae				25º48'42.44" N, 108º13'9.302" W
SI-CO	2012	12-029	*Sarcostema spp*	*Asclepiadiaceae*				23º17'13.489"N, 106º4'51.193" W
SI-SL	2012	12-109	*Artemisia ludoviciana*	*Asteraceae*				25º48'42.44" N, 108º13'9.302" W
SI-SL	2012	12-108	*Franseria ambrosioides*	*Asteraceae*				25º48'42.44" N, 108º13'9.302" W
SI-GV	2012	12-065	*Helianthus annuus*	*Asteraceae*				25º43'49.164" N, 108º24'27.845" W
SI-GV	2014	14-127	*Parthenium hysterophorus*	*Asteraceae*				20º30'39.585" N, 108º30'39.252" W
SI-GV	2014	14-130-Sin	*Parthenium hysterophorus*	*Asteraceae*				20º30'39.585" N, 108º30'39.252" W
SI-GV	2014	14-128-Sin	*Parthenium hysterophorus*	*Asteraceae*				20º30'39.585" N, 108º30'39.252" W

SI-GV	2014	14-129-Sin	*Parthenium hysterophorus*	*Asteraceae*				20º30'39.585" N, 108º30'39.252" W
SI-GV	2012	12-049	*Parthenium hysterophorus*	*Asteraceae*				25º43'49.164" N, 108º24'27.845" W
SI-GV	2012	12-050	*Parthenium hysterophorus*	*Asteraceae*		1		25º43'49.164" N, 108º24'27.845" W
SI-GV	2012	12-052	*Parthenium hysterophorus*	*Asteraceae*				25º43'49.164" N, 108º24'27.845" W
SI-GV	2012	12-067	*Parthenium hysterophorus*	*Asteraceae*	1			25º43'49.164" N, 108º24'27.845" W
SI-GV	2012	12-068	*Parthenium hysterophorus*	*Asteraceae*				25º43'49.164" N, 108º24'27.845" W
SI-GV	2012	12-070	*Parthenium hysterophorus*	*Asteraceae*	1			25º43'49.164" N, 108º24'27.845" W
SI-GV	2012	12-072	*Parthenium hysterophorus*	*Asteraceae*		1		25º43'49.164" N, 108º24'27.845" W
SI-MO	2012	12-024	*Parthenium hysterophorus*	*Asteraceae*				25º28'55.277" N, 107º54'14.071" W
SI-RO	2012	14-137-Sin	*Parthenium hysterophorus*	*Asteraceae*			1	23º0'8.665"N, 105º51'26.246" W
SI-RO	2012	15-151-Sin	*Parthenium hysterophorus*	*Asteraceae*				23º0'8.665"N, 105º51'26.246" W
SI-GV	2012	12-076	*Porophyllum punctatum*	*Asteraceae*		1		25º43'49.164" N, 108º24'27.845" W
SI-GV	2012	12-063	*Sonchus oleraceus*	*Asteraceae*	1			25º43'49.164" N, 108º24'27.845" W
SI-GV	2012	12-074	*Sonchus oleraceus*	*Asteraceae*				25º43'49.164" N, 108º24'27.845" W
SI-SL	2012	12-112	*Sonchus oleraceus*	*Asteraceae*				25º48'42.44" N, 108º13'9.302" W
SI-GV	2012	12-060	*Xanthium strumarium*	*Asteraceae*	1			25º43'49.164" N, 108º24'27.845" W
SI-MO	2012	12-015	*Amphilophium paniculatum*	*Bignoniaceae*				25º28'55.277" N, 107º54'14.071" W
SI-GV	2015	15-276	*Diplotaxis muralis*	*Brassicaceae*		1		32º25'07.8" N, 114º49'34.7" W
SI-SL	2012	12-101	*Caesalpinia platyloba*	Caesalpiniaceae		1		25º48'42.44" N, 108º13'9.302" W
SI-CO	2012	12-036	*Polinesia dodecandra*	*Capparaceae*	1			23º17'13.489"N, 106º4'51.193" W
SI-SL	2012	12-107	*Polinesia dodecandra*	*Capparaceae*				25º49'29.732"N, 108º14'4.196" W
SI-GV	2012	12-059	*Ipomoea purpurea*	*Convolvulaceae*				25º43'49.164" N, 108º24'27.845" W
SI-CO	2012	12-042	Ipomoea spp	*Convolvulaceae*		1		23º17'13.489"N, 106º4'51.193" W
SI-GV	2012	12-084	Ipomoea spp	*Convolvulaceae*			1	25º44'59.049" N, 108º39'46.189 W
SI-GV	2012	12-086	Ipomoea spp	*Convolvulaceae*	1			25º44'59.049" N, 108º39'46.189 W
SI-SL	2012	12-103	*Citrullus lanatus*	*Cucurbitaceae*				25º48'42.44" N, 108º13'9.302" W
SI-GV	2012	12-087	*Cucumis anguria*	*Cucurbitaceae*				25º44'59.049" N, 108º39'46.189 W
SI-GV	2012	12-055	Cucumis dipsaceus	*Cucurbitaceae*	1			25º43'49.164" N, 108º24'27.845" W

SI-GV	2012	12-061	*Momardica charantia*	*Cucurbitaceae*	1			25º43'49.164" N, 108º24'27.845" W
SI-MO	2012	12-003	*Momardica charantia*	*Cucurbitaceae*				25º28'55.277" N, 107º54'14.071" W
SI-MO	2012	12-019	*Momardica charantia*	*Cucurbitaceae*				25º28'55.277" N, 107º54'14.071" W
SI-CO	2012	12-041	*Acalypha polystachya*	*Euphorbiaceae*				23º17'13.489"N, 106º4'51.193" W
SI-GV	2012	12-064	*Acalypha polystachya*	*Euphorbiaceae*	1			25º43'49.164" N, 108º24'27.845" W
SI-MO	2012	12-009	*Acalypha polystachya*	*Euphorbiaceae*				25º28'55.277" N, 107º54'14.071" W
SI-MO	2012	12-027	*Acalypha polystachya*	*Euphorbiaceae*				25º28'55.277" N, 107º54'14.071" W
SI-MO	2012	12-001	*Croton spp*	*Euphorbiaceae*				25º28'55.277" N, 107º54'14.071" W
SI-MO	2012	12-002	*Euphorbia heterophylla*	*Euphorbiaceae*				25º28'55.277" N, 107º54'14.071" W
SI-RO	2014	14-138-Sin	*Euphorbia heterophylla*	*Euphorbiaceae*			1	23º0'8.665"N, 105º51'26.246" W
SI-RO	2014	14-139-Sin	*Euphorbia heterophylla*	*Euphorbiaceae*		1		23º0'8.665"N, 105º51'26.246" W
SI-RO	2014	14-140-Sin	*Euphorbia heterophylla*	*Euphorbiaceae*		1		23º0'8.665"N, 105º51'26.246" W
SI-RO	2015	15-152-Sin	*Euphorbia heterophylla*	*Euphorbiaceae*				23º0'8.665"N, 105º51'26.246" W
SI-RO	2015	15-153-Sin	*Euphorbia heterophylla*	*Euphorbiaceae*				23º0'8.665"N, 105º51'26.246" W
SI-MO	2012	12-021	*Manihot spp*	*Euphorbiaceae*		1		25º28'55.277" N, 107º54'14.071" W
SI-GV	2014	14-136-Sin	*Ricinus communis*	*Euphorbiaceae*				20º30'39.585" N, 108º30'39.252" W
SI-GV	2012	12-058	*Ricinus communis*	*Euphorbiaceae*				25º43'49.164" N, 108º24'27.845" W
SI-SL	2012	12-110	*Ricinus communis*	*Euphorbiaceae*				25º48'42.44" N, 108º13'9.302" W
SI-GV	2016	19	*Crotalaria juncea*	*Fabaceae*	1			25º43'01.45" N, 108º19'42.45 W
SI-GV	2016	20	*Crotalaria juncea*	*Fabaceae*	1			25º43'01.45" N, 108º19'42.45 W
SI-GV	2016	21	*Crotalaria juncea*	*Fabaceae*	1			25º43'01.45" N, 108º19'42.45 W
SI-GV	2012	12-069	*Lonchocarpus lanceolatus*	*Fabaceae*		1		25º43'01.45" N, 108º19'42.45 W
SI-ES	2016	32	*Macroptilium atropurpureum*	*Fabaceae*				23º00'08.66 N, 105º51'26.23 W
SI-ES	2016	33	*Macroptilium atropurpureum*	*Fabaceae*				23º00'08.66 N, 105º51'26.23 W
SI-ES	2016	34	*Macroptilium atropurpureum*	*Fabaceae*				23º00'08.66 N, 105º51'26.23 W
SI-RO	2016	19	*Macroptilium atropurpureum*	*Fabaceae*		1		23º00'08.66 N, 105º51'26.23 W
SI-RO	2016	25	*Macroptilium atropurpureum*	*Fabaceae*		1		23º00'08.66 N, 105º51'26.23 W
SI-RO	2016	26	*Macroptilium atropurpureum*	*Fabaceae*		1		23º00'08.66 N, 105º51'26.23 W

SI-RO	2015	15-155	*Macroptilium atropurpureum*	*Fabaceae*		23º00'08.66 N, 105º51'26.23 W
SI-RO	2016	17	*Macroptilium atropurpureum*	*Fabaceae*	1	23º00'08.66 N, 105º51'26.23 W
SI-GV	2015	15-495	*Melilotus indicus*	*Fabaceae*	1	23º00'08.66 N, 105º51'26.23 W
SI-GV	2016	22	*Melilotus indicus*	*Fabaceae*	1	23º00'08.66 N, 105º51'26.23 W
SI-AB	2016	**43**	*Rhynchosia Minima*	*Fabaceae*		22º34'27.113" N, 108º32'30.951" W
SI-AB	2016	44	*Rhynchosia Minima*	*Fabaceae*		22º34'27.113" N, 108º32'30.951" W
SI-AB	2016	45	*Rhynchosia Minima*	*Fabaceae*		22º34'27.113" N, 108º32'30.951" W
SI-AB	2016	46	*Rhynchosia Minima*	*Fabaceae*		22º34'27.113" N, 108º32'30.951" W
SI-AB	2016	47	*Rhynchosia Minima*	*Fabaceae*		22º34'27.113" N, 108º32'30.951" W
SI-AC	2016	1	*Rhynchosia minima*	*Fabaceae*		23º09'39.09" N, 106º05'26.09" W
SI-GV	2012	12-082	*Rhynchosia minima*	*Fabaceae*		25º44'59.049" N, 108º39'46.188" W
SI-GV	2016	48	*Rhynchosia Minima*	*Fabaceae*		25º32'31.16" N, 108º32'46.154" W
SI-GV	2016	49	*Rhynchosia Minima*	*Fabaceae*		25º32'31.16" N, 108º32'46.154" W
SI-GV	2016	50	*Rhynchosia Minima*	*Fabaceae*		25º32'31.16" N, 108º32'46.154" W
SI-GV	2016	51	*Rhynchosia Minima*	*Fabaceae*		25º32'31.16" N, 108º32'46.154" W
SI-GV	2016	52	*Rhynchosia Minima*	*Fabaceae*		25º32'31.16" N, 108º32'46.154" W
SI-GV	2014	14-120	*Rhynchosia Minima*	*Fabaceae*		25º32'31.16" N, 108º32'46.154" W
SI-GV	2014	14-119	*Rhynchosia minima*	*Fabaceae*		25º32'31.16" N, 108º32'46.154" W
SI-GV	2014	14-121	*Rhynchosia minima*	*Fabaceae*		25º32'31.16" N, 108º32'46.154" W
SI-GV	2014	14-122	*Rhynchosia minima*	*Fabaceae*		25º32'31.16" N, 108º32'46.154" W
SI-PC	2016	35	*Rhynchosia Minima*	*Fabaceae*		23º50'37.66" N, 107º00'33.67" W
SI-PC	2016	36	*Rhynchosia Minima*	*Fabaceae*		23º50'37.66" N, 107º00'33.67" W
SI-PC	2016	37	*Rhynchosia Minima*	*Fabaceae*		23º50'37.66" N, 107º00'33.67" W
SI-PC	2016	38	*Rhynchosia Minima*	*Fabaceae*		23º50'37.66" N, 107º00'33.67" W
SI-PC	2016	39	*Rhynchosia Minima*	*Fabaceae*		23º50'37.66" N, 107º00'33.67" W
SI-PC	2016	40	*Rhynchosia Minima*	*Fabaceae*		23º50'37.66" N, 107º00'33.67" W
SI-RO	2016	22	*Rhynchosia Minima*	*Fabaceae*		23º0'8.665"N, 105º51'26.246" W
SI-RO	2014	14-142	*Rhynchosia Minima*	*Fabaceae*		23º00'08.66 N, 105º51'26.24 W

SI-RO	2014	14-143	*Rhynchosia minima*	*Fabaceae*				23º00'08.66 N, 105º51'26.24 W
SI-AC	2016	7	*Rhynchosia precatoria*	*Fabaceae*				23º09'39.09" N, 106º05'26.09" W
SI-AC	2016	8	*Rhynchosia precatoria*	*Fabaceae*				23º09'39.09" N, 106º05'26.09" W
SI-AC	2016	9	*Rhynchosia precatoria*	*Fabaceae*				23º09'39.09" N, 106º05'26.09" W
SI-AC	2016	10	*Rhynchosia precatoria*	*Fabaceae*				23º09'39.09" N, 106º05'26.09" W
SI-AC	2016	11	*Rhynchosia precatoria*	*Fabaceae*				23º09'39.09" N, 106º05'26.09" W
SI-AC	2016	12	*Rhynchosia precatoria*	*Fabaceae*				23º09'39.09" N, 106º05'26.09" W
SI-AC	2016	13	*Rhynchosia precatoria*	*Fabaceae*				23º09'39.09" N, 106º05'26.09" W
SI-ES	2016	27	*Rhynchosia precatoria*	*Fabaceae*				22º58'7.266"N, 105º54'34.657"W
SI-ES	2016	28	*Rhynchosia precatoria*	*Fabaceae*				22º58'7.266"N, 105º54'34.657"W
SI-ES	2016	29	*Rhynchosia precatoria*	*Fabaceae*				22º58'7.266"N, 105º54'34.657"W
SI-ES	2016	30	*Rhynchosia precatoria*	*Fabaceae*				22º58'7.266"N, 105º54'34.657"W
SI-ES	2016	31	*Rhynchosia precatoria*	*Fabaceae*				22º58'7.266"N, 105º54'34.657"W
SI-RO	2016	20	*Rhynchosia precatoria*	*Fabaceae*				23º00'08.66 N, 105º51'26.24 W
SI-RO	2016	21	*Rhynchosia precatoria*	*Fabaceae*				23º00'08.66 N, 105º51'26.24 W
SI-RO	2016	23	*Rhynchosia precatoria*	*Fabaceae*		1		23º00'08.66 N, 105º51'26.24 W
SI-RO	2016	24	*Rhynchosia precatoria*	*Fabaceae*		1		23º00'08.66 N, 105º51'26.24 W
SI-RO	2014	14-141	*Rhynchosia precatoria*	*Fabaceae*				23º00'08.66 N, 105º51'26.24 W
SI-RO	2016	16	*Rhynchosia precatoria*	*Fabaceae*				23º00'08.66 N, 105º51'26.24 W
SI-AC	2016	2	*Rhynchosia minima*	*Fabaceae*	1			23º09'39.09" N, 106º05'26.09" W
SI-AC	2016	3	*Rhynchosia minima*	*Fabaceae*	1			23º09'39.09" N, 106º05'26.09" W
SI-AC	2016	4	*Rhynchosia minima*	*Fabaceae*	1			23º09'39.09" N, 106º05'26.09" W
SI-AC	2016	5	*Rhynchosia minima*	*Fabaceae*	1			23º09'39.09" N, 106º05'26.09" W
SI-AC	2016	6	*Rhynchosia minima*	*Fabaceae*	1			23º09'39.09" N, 106º05'26.09" W
SI-PC	2016	41	*Rhynchosia minima*	*Fabaceae*	1			23º50'37.66" N, 107º00'33.67" W
SI-PC	2016	42	*Rhynchosia minima*	*Fabaceae*	1			23º50'37.66" N, 107º00'33.67" W
SI-RO	2016	15	*Rhynchosia minima*	*Fabaceae*			1	23º00'08.66 N, 105º51'26.24 W
SI-RO	2016	16	*Rhynchosia minima*	*Fabaceae*		1		23º00'08.66 N, 105º51'26.24 W

SI-RO	2016	17	*Rhynchosia minima*	*Fabaceae*		1		23º00'08.66 N, 105º51'26.24 W
SI-RO	2016	18	*Rhynchosia minima*	*Fabaceae*			1	23º00'08.66 N, 105º51'26.24 W
SI-RO	2016	14	*Senna uniflora*	*Fabaceae*	1			23º00'08.66 N, 105º51'26.24 W
SI-RO	2014	14-144	*Senna uniflora*	*Fabaceae*		1		23º00'08.66 N, 105º51'26.24 W
SI-RO	2015	15-157	*Senna uniflora*	*Fabaceae*				23º00'08.66 N, 105º51'26.24 W
SI-SL	2012	12-098	*Senna uniflora*	*Fabaceae*				25º48'42.44" N, 108º13'9.302" W
SI-SL	2012	12-099	*Mascagnia macroptera*	*Malpiguiaceae*				25º48'42.44" N, 108º13'9.302" W
SI-SL	2012	12-095	*Mascagnia macroptera*	*Malpiguiaceae*		1		25º48'42.44" N, 108º13'9.302" W
SI-GV	2012	12-044	*Abutilon palmeri*	*Malvaceae*		1		25º43'49.164" N, 108º24'27.845" W
SI-GV	2012	12-085	*Abutilon palmeri*	*Malvaceae*				25º43'49.164" N, 108º24'27.845" W
SI-SL	2012	12-094	*Abutilon palmeri*	*Malvaceae*				25º48'42.44" N, 108º13'9.302" W
SI-GV	2014	14-131-Sin	*Abutilon trisulcatun*	*Malvaceae*			1	20º30'39.585" N, 108º30'39.252" W
SI-GV	2014	14-132-Sin	*Abutilon trisulcatun*	*Malvaceae*				20º30'39.585" N, 108º30'39.252" W
SI-GV	2014	14-133-Sin	*Abutilon trisulcatun*	*Malvaceae*			1	20º30'39.585" N, 108º30'39.252" W
SI-GV	2014	14-134-Sin	*Abutilon trisulcatun*	*Malvaceae*		1		20º30'39.585" N, 108º30'39.252" W
SI-GV	2012	12-075	*Abutilon trisulcatun*	*Malvaceae*		1		25º43'49.164" N, 108º24'27.845" W
SI-MO	2012	12-004	*Abutilon trisulcatun*	*Malvaceae*	1			25º28'55.277" N, 107º54'14.071" W
SI-MO	2012	12-011	*Abutilon trisulcatun*	*Malvaceae*			1	25º28'55.277" N, 107º54'14.071" W
SI-MO	2012	12-023	*Abutilon trisulcatun*	*Malvaceae*				25º28'55.277" N, 107º54'14.071" W
SI-MO	2012	12-025	*Abutilon trisulcatun*	*Malvaceae*				25º28'55.277" N, 107º54'14.071" W
SI-MO	2012	12-028	*Abutilon trisulcatun*	*Malvaceae*				25º28'55.277" N, 107º54'14.071" W
SI-RO	2014	14-145-Sin	*Abutilon trisulcatun*	*Malvaceae*			1	23º0'8.665"N, 105º51'26.246" W
SI-RO	2015	15-156-Sin	*Abutilon trisulcatun*	*Malvaceae*				23º0'8.665"N, 105º51'26.246" W
SI-CO	2012	12-034	*Anoda pentaschista*	*Malvaceae*	1			23º17'13.489"N, 106º4'51.193" W
SI-GV	2012	12-071	*Herisantia crispa*	*Malvaceae*	1			25º43'49.164" N, 108º24'27.845" W
SI-RO	2014	14-149-Sin	*Herisantia crispa*	*Malvaceae*		1		23º0'8.665"N, 105º51'26.246" W
SI-GV	2012	12-090	*Kosteletzkya depressa*	*Malvaceae*		1		25º43'49.164" N, 108º24'27.845" W
SI-GV	2012	12-091	*Kosteletzkya depressa*	*Malvaceae*		1		25º43'49.164" N, 108º24'27.845" W

SI-GV	2014	14-115	*Melochia piramydata*	*Malvaceae*			1	20º30'39.585" N, 108º30'39.252" W
SI-GV	2014	14-116	*Melochia piramydata*	*Malvaceae*			1	20º30'39.585" N, 108º30'39.252" W
SI-GV	2014	14-117	*Melochia piramydata*	*Malvaceae*			1	20º30'39.585" N, 108º30'39.252" W
SI-GV	2014	14-118	*Melochia piramydata*	*Malvaceae*			1	20º30'39.585" N, 108º30'39.252" W
SI-RO	2014	14-148-Sin	*Melochia piramydata*	*Malvaceae*		1		23º0'8.665"N, 105º51'26.246" W
SI-GV	2014	14-113	*Sida acuta*	*Malvaceae*				20º30'39.585" N, 108º30'39.252" W
SI-GV	2014	14-114	*Sida acuta*	*Malvaceae*				20º30'39.585" N, 108º30'39.252" W
SI-RO	2014	14-146-Sin	*Sida acuta*	*Malvaceae*		1		23º0'8.665"N, 105º51'26.246" W
SI-RO	2014	14-147-Sin	*Sida acuta*	*Malvaceae*		1		23º0'8.665"N, 105º51'26.246" W
SI-RO	2015	15-154-Sin	*Sida acuta*	*Malvaceae*				23º0'8.665"N, 105º51'26.246" W
SI-MO	2012	12-005	*Sidastrum lodiegensis*	*Malvaceae*	1			25º28'55.277" N, 107º54'14.071" W
SI-MO	2012	12-007	*Sidastrum lodiegensis*	*Malvaceae*				25º28'55.277" N, 107º54'14.071" W
SI-MO	2012	12-026	*Sidastrum lodiegensis*	*Malvaceae*				25º28'55.277" N, 107º54'14.071" W
SI-GV	2012	12-048	*Coccolus diversifolius*	*Menispermaceae*		1		25º43'49.164" N, 108º24'27.845" W
SI-MO	2012	12-022	*Boerhavia spp*	*Nyctaginaceae*	1			25º28'55.277" N, 107º54'14.071" W
SI-GV	2012	12-046	*Salpianthus macrodonthus*	*Nyctaginaceae*		1		25º43'49.164" N, 108º24'27.845" W
SI-GV	2012	12-088	*Ludwigia erecta*	*Onagraceae*				25º44'59.049" N, 108º39'46.189 W
SI-GV	2012	12-083	*Ludwigia octovalvis*	*Onagraceae*				25º44'59.049" N, 108º39'46.189 W
SI-CO	2012	12-038	*Matynia annua*	*Pedaliaceae*		1		23º17'13.489"N, 106º4'51.193" W
SI-GV	2012	12-079	Portulaca oleraceae	*Portulaceae*		1		25º44'59.049" N, 108º39'46.189 W
SI-GV	2012	12-080	Portulaca oleraceae	*Portulaceae*		1		25º48'42.44" N, 108º13'9.302" W
SI-GV	2012	12-081	Portulaca oleraceae	*Portulaceae*		1		25º48'42.44" N, 108º13'9.302" W
SI-SL	2012	12-093	*Karwinskia humboldtiana*	Rhamnaceae		1		25º48'42.44" N, 108º13'9.302" W
SI-CO	2012	12-035	*Borreria laevis*	*Rubiaceae*				23º17'13.489"N, 106º4'51.193" W
SI-GV	2012	12-045	*Paullinia fuscescens*	*Sapindaceae*		1		25º43'49.164" N, 108º24'27.845" W
SI-MO	2012	12-008	*Datura discolor*	*Solanaceae*	1			25º28'55.277" N, 107º54'14.071" W
SI-MO	2012	12-016	*Datura discolor*	*Solanaceae*			1	25º28'55.277" N, 107º54'14.071" W
SI-SL	2012	12-106	*Datura inoxia*	*Solanaceae*			1	25º48'42.44" N, 108º13'9.302" W

SI-GV	2012	12-051	*Datura reburra*	*Solanaceae*	1			25º43'49.164" N, 108º24'27.845" W
SI-GV	2012	12-092	*Datura stramonium*	*Solanaceae*		1		25º44'59.049" N, 108º39'46.189 W
SI-GV	2014	14-123	*Datura stramonium*	*Solanaceae*			1	20º30'39.585" N, 108º30'39.252" W
SI-GV	2014	14-124	*Datura stramonium*	*Solanaceae*		1		20º30'39.585" N, 108º30'39.252" W
SI-GV	2014	14-125	*Datura stramonium*	*Solanaceae*		1		20º30'39.585" N, 108º30'39.252" W
SI-GV	2014	14-126	*Datura stramonium*	*Solanaceae*		1		20º30'39.585" N, 108º30'39.252" W
SI-GV	2012	12-089	*Nicotiana glauca*	*Solanaceae*		1		25º44'59.049" N, 108º39'46.189 W
SI-GV	2012	12-056	*Nicotiana glauca*	*Solanaceae*	1			25º43'49.164" N, 108º24'27.845" W
SI-RO	2014	14-150-Sin	*Physalis acutifolia*	*Solanaceae*		1		23º0'8.665"N, 105º51'26.246" W
SI-MO	2012	12-018	*Physalis Angulata*	*Solanaceae*				25º28'55.277" N, 107º54'14.071" W
SI-GV	2012	12-066	Solanum americanum	*Solanaceae*	1			25º43'49.164" N, 108º24'27.845" W
SI-CO	2012	12-039	*Solanum nigrescens*	*Solanaceae*				23º17'13.489"N, 106º4'51.193" W
SI-GV	2012	12-054	*Solanum nigrescens*	*Solanaceae*	1			25º43'49.164" N, 108º24'27.845" W
SI-MO	2012	12-012	*Solanum nigrescens*	*Solanaceae*				25º28'55.277" N, 107º54'14.071" W
SI-MO	2012	12-006	Solanum tridynamum	*Solanaceae*	1			25º28'55.277" N, 107º54'14.071" W
SI-MO	2012	12-014	Solanum tridynamum	*Solanaceae*				25º28'55.277" N, 107º54'14.071" W
SI-SL	2012	12-096	Solanum tridynamum	*Solanaceae*	1			25º48'42.44" N, 108º13'9.302" W
SI-SL	2012	12-102	Solanum tridynamum	*Solanaceae*	1			25º48'42.44" N, 108º13'9.302" W
SI-SL	2012	12-105	Solanum tridynamum	*Solanaceae*		1		25º48'42.44" N, 108º13'9.302" W
SI-CO	2012	12-030	*Melochia piramydata*	*Sterculiaceae*				23º17'13.489"N, 106º4'51.193" W
SI-CO	2012	12-031	*Waltheria americana*	*Sterculiaceae*			1	23º17'13.489"N, 106º4'51.193" W
SI-GV	2012	12-057	*Verbenaceae spp*	*Verbenaceae*	1			25º43'49.164" N, 108º24'27.845" W
SI-GV	2012	12-073	*Verbenaceae spp*	*Verbenaceae*				25º43'49.164" N, 108º24'27.845" W
SI-MO	2012	12-013	*Vitaceae spp*	*Vitaceae*		1		25º28'55.277" N, 107º54'14.071" W
CN-SM	2014	14-176	*Amaranthus hybridus*	*Amaranthaceae*			1	21º20'35.76" N, 104º40'15.124" W
CN-SM	2014	14-140	*Amaranthus retroflexus*	*Amaranthaceae*				21º20'35.76" N, 104º40'15.124" W
CN-TE	2014	14-145	*Amaranthus spinosus*	*Amaranthaceae*				18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-181	*Amaranthus spinosus*	*Amaranthaceae*				18º50'53.07" N, 103º49'54.892" W

CN-SM	2014	14-170	*Cosmos sulphureus*	*Asteraceae*	1			21º20'35.76" N, 104º40'15.124" W
CN-SM	2014	14-174	*Cosmos sulphureus*	*Asteraceae*			1	21º20'35.76" N, 104º40'15.124" W
CN-TE	2014	14-199	*Erigeron longipes*	*Asteraceae*				18º50'53.07" N, 103º49'54.892" W
CN-SM	2014	14-178	*Melampodium rosei*	*Asteraceae*			1	21º20'35.76" N, 104º40'15.124" W
CN-TE	2014	14-152	*Commelina diffusa*	*Commelinaceae*				18º50'53.07" N, 103º49'54.892" W
CN-SM	2014	14-141	*Ipomoea hederaceae*	*Convolvulaceae*				21º20'35.76" N, 104º40'15.124" W
CN-SM	2014	14-171	Ipomoea spp.	*Convolvulaceae*				21º20'35.76" N, 104º40'15.124" W
CN-SM	2014	14-175	Ipomoea spp.	*Convolvulaceae*			1	21º20'35.76" N, 104º40'15.124" W
CN-TE	2014	14-202	*Merremia quinquefolia*	*Convolvulaceae*				18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-147	*Momardica charantia*	*Cucurbitaceae*				18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-179	*Momardica charantia*	*Cucurbitaceae*				18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-195	*Momardica charantia*	*Cucurbitaceae*			1	18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-143	*Acalypha seetosa*	*Euphorbiaceae*				18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-150	*Euphorbia hypericifolia*	*Euphorbiaceae*				18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-149	*Rhynchosia spp*	*Fabaceae*				18º50'53.07" N, 103º49'54.892" W
CN-SM	2014	14-136	*Nama hispida*	*Hydrophyllaceae*				21º20'35.76" N, 104º40'15.124" W
CN-TE	2014	14-166	*Herisantia crispa*	*Malvaceae*				18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-167	*Herisantia crispa*	*Malvaceae*		1		18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-168	*Herisantia crispa*	*Malvaceae*				18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-169	*Herisantia crispa*	*Malvaceae*		1		18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-182	*Herisantia crispa*	*Malvaceae*			1	18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-183	*Herisantia crispa*	*Malvaceae*			1	18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-184	*Herisantia crispa*	*Malvaceae*			1	18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-194	*Herisantia crispa*	*Malvaceae*				18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-192	*Malvastrum coromandelianum*	*Malvaceae*			1	18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-196	*Malvastrum coromandelianum*	*Malvaceae*			1	18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-197	*Malvastrum coromandelianum*	*Malvaceae*			1	18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-153	*Sida acuta*	*Malvaceae*		1		18º50'53.07" N, 103º49'54.892" W

CN-SM	2014	14-172	*Sida Collina*	*Malvaceae*				21º20'35.76" N, 104º40'15.124" W
CN-TE	2014	14-186	*Boerhavia coccinea*	*Nyctaginaceae*				18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-187	*Boerhavia coccinea*	*Nyctaginaceae*			1	18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-188	*Boerhavia coccinea*	*Nyctaginaceae*			1	18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-189	*Boerhavia coccinea*	*Nyctaginaceae*			1	18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-193	*Boerhavia coccinea*	*Nyctaginaceae*			1	18º50'53.07" N, 103º49'54.892" W
CN-SM	2014	14-137	Portulaca oleraceae	*Portulaceae*				21º20'35.76" N, 104º40'15.124" W
CN-TE	2014	14-148	Portulaca oleraceae	*Portulaceae*		1		18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-185	*Galium mexicanum*	*Rubiaceae*			1	18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-198	*Richardia scabia*	*Rubiaceae*			1	18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-144	*Solanum nigrescens*	*Solanaceae*				18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-180	*Solanum nigrescens*	*Solanaceae*				18º50'53.07" N, 103º49'54.892" W
CN-TE	2014	14-146	*Priva iappulaceae*	*Verbenaceae*		1		18º50'53.07" N, 103º49'54.892" W
CN-SM	2014	14-173	*Verbenaceae spp*	*Verbenaceae*			1	21º20'35.76" N, 104º40'15.124" W
CN-TE	2014	14-151	*Cissus sicyoides*	*Vitaceae*				18º50'53.07" N, 103º49'54.892" W
CD-GO	2015	15-432	*Amaranthus spp*	*Amaranthaceae*		1		25º29'28.04" N, 103º41'12.80 W
CD-GO	2015	15-435	*Amaranthus spp*	*Amaranthaceae*	1			25º29'28.04" N, 103º41'12.80 W
CD-PO	2016	2016-12	*Amaranthus spp*	*Amaranthaceae*		1		24º00'37.45" N, 104º07'31.73 W
CD-PO	2016	2016-13	*Amaranthus spp*	*Amaranthaceae*		1		24º00'37.45" N, 104º07'31.73 W
CD-PO	2016	2016-9	*Amaranthus spp*	*Amaranthaceae*		1		24º00'37.45" N, 104º07'31.73 W
CD-TO	2015	15-416	*Amaranthus spp*	*Amaranthaceae*				26º04'52.61" N, 102º55'30.01" W
CD-GO	2015	15-441	*Artemisia absitium*	*Asteraceae*				25º29'28.04" N, 103º41'12.80 W
CD-UJED	2015	15-418	*Artemisia absitium*	*Asteraceae*			1	25º47'14.25" N, 103º21'10.37" W
CD-TO	2015	15-413	*Bahia absinthifolia*	*Asteraceae*			1	25º32'46.79" N, 103º16'55.40" W
CD-TO	2015	15-414	*Bahia absinthifolia*	*Asteraceae*			1	25º32'46.79" N, 103º16'55.40" W
CD-GO	2015	15-445	*Helianthus spp*	*Asteraceae*				25º29'28.04" N, 103º41'12.80 W
CD-GO	2015	15-446	*Helianthus spp*	*Asteraceae*	1			25º29'28.04" N, 103º41'12.80 W
CD-TO	2015	15-401	*Helianthus spp*	*Asteraceae*			1	25º33'21.45" N, 103º22'17.73" W

CD-UJED	2015	15-425	*Sonchus oleraceus*	*Asteraceae*				25º47'14.25" N, 103º21'10.37" W
CD-UJED	2015	15-426	*Sonchus oleraceus*	*Asteraceae*				25º47'14.25" N, 103º21'10.37" W
CD-UJED	2015	15-427	*Sonchus oleraceus*	*Asteraceae*				25º47'14.25" N, 103º21'10.37" W
CD-UJED	2015	15-419	*Sonchus oleraceus*	*Asteraceae*				25º47'14.25" N, 103º21'10.37" W
CD-UJED	2015	15-420	*Sonchus oleraceus*	*Asteraceae*			1	25º47'14.25" N, 103º21'10.37" W
CD-TO	2015	15-410	*Xanthium echinatum*	*Asteraceae*				25º32'46.79" N, 103º16'55.40" W
CD-TO	2015	15-411	*Xanthium echinatum*	*Asteraceae*	1			25º32'46.79" N, 103º16'55.40" W
CD-TO	2015	15-412	*Xanthium echinatum*	*Asteraceae*	1			25º32'46.79" N, 103º16'55.40" W
CD-GO	2015	15-452	*Sida acuta*	*Malvaceae*		1		25º29'28.04" N, 103º41'12.80 W
CD-GO	2015	15-439	*Sida rombifolia*	*Malvaceae*		1		25º29'28.04" N, 103º41'12.80 W
CD-GO	2015	15-440	*Sida rombifolia*	*Malvaceae*		1		25º29'28.04" N, 103º41'12.80 W
CD-UJED	2015	15-422	*Sida rombifolia*	*Malvaceae*		1		25º47'14.25" N, 103º21'10.37" W
CD-UJED	2015	15-423	*Sida rombifolia*	*Malvaceae*		1		25º47'14.25" N, 103º21'10.37" W
CD-UJED	2015	15-424	*Sida rombifolia*	*Malvaceae*			1	25º47'14.25" N, 103º21'10.37" W
CD-TO	2015	15-408	*Sphaeralcea angustifolia*	*Malvaceae*		1		25º32'46.79" N, 103º16'55.40" W
CD-TO	2015	15-409	*Sphaeralcea angustifolia*	*Malvaceae*		1		25º32'46.79" N, 103º16'55.40" W
CD-TO	2015	15-417	*Sphaeralcea angustifolia*	*Malvaceae*		1		26º04'52.61" N, 102º55'30.01" W
CD-GO	2015	15-447	*Boerhavia coccinea*	*Nyctaginaceae*			1	25º29'28.04" N, 103º41'12.80 W
CD-PO	2016	2016-47	*Portulaca oleraceae*	*Portulacaceae*				24º00'37.45" N, 104º07'31.73 W
CD-PO	2016	2016-29	*Portulaca oleraceae*	*Portulacaceae*				24º00'37.45" N, 104º07'31.73 W
CD-PO	2016	2016-30	*Portulaca oleraceae*	*Portulacaceae*				24º00'37.45" N, 104º07'31.73 W
CD-PO	2016	2016-31	*Portulaca oleraceae*	*Portulacaceae*				24º00'37.45" N, 104º07'31.73 W
CD-PO	2016	2016-32	*Portulaca oleraceae*	*Portulacaceae*				24º00'37.45" N, 104º07'31.73 W
CD-PO	2016	2016-33	*Portulaca oleraceae*	*Portulacaceae*				24º00'37.45" N, 104º07'31.73 W
CD-GO	2015	15-442	*Datura stramonium*	*Solanaceae*			1	25º29'28.04" N, 103º41'12.80 W
CD-TO	2015	15-402	*Datura stramonium*	*Solanaceae*	1			25º33'21.45" N, 103º22'17.73" W
CD-TO	2015	15-403	*Nicotiana glauca*	*Solanaceae*			1	25º32'46.79" N, 103º16'55.40" W
CD-TO	2015	15-404	*Nicotiana glauca*	*Solanaceae*			1	25º32'46.79" N, 103º16'55.40" W

CD-TO	2015	15-415	*Nicotiana glauca*	*Solanaceae*			1	26º04'52.61" N, 102º55'30.01" W
CD-PO	2016	2016-17	*Solanum elaeagnifolium*	*Solanaceae*		1		24º00'37.45" N, 104º07'31.73 W
CD-PO	2016	2016-18	*Solanum elaeagnifolium*	*Solanaceae*		1		24º00'37.45" N, 104º07'31.73 W
CD-TO	2015	15-405	*Solanum elaeagnifolium*	*Solanaceae*	1			25º32'46.79" N, 103º16'55.40" W
CD-TO	2015	15-406	*Solanum elaeagnifolium*	*Solanaceae*			1	25º32'46.79" N, 103º16'55.40" W
CD-TO	2015	15-407	*Solanum elaeagnifolium*	*Solanaceae*			1	25º32'46.79" N, 103º16'55.40" W
CD-UJED	2015	15-421	*Solanum elaeagnifolium*	*Solanaceae*			1	25º47'14.25" N, 103º21'10.37" W
CD-PO	2016	2016-1	*Solanum rostrarum*	*Solanaceae*		1		24º00'37.45" N, 104º07'31.73 W
CD-PO	2016	2016-15	*Solanum rostrarum*	*Solanaceae*		1		24º00'37.45" N, 104º07'31.73 W
CD-PO	2016	2016-16	*Solanum rostrarum*	*Solanaceae*		1		24º00'37.45" N, 104º07'31.73 W

* PCR-Base amplification length related to monopartite (M), bipartite (B) or mixed infection where both amplification were present (M&B).

Supplemental Table 2. Begomovirus metagenomic signatures obtained by de novo assembly. **Geminivirus-related** reads for each NGS library were used for *de novo* assembly and generation of Begomovirus signatures. Contigs of 200-500pb in length, and/or <90% nucleotide identity against best match reference sequence were selected.

Host adapted	Virus acronym[1]	Family of first detection	DNA-A/DNA-B Best match% (Accession number) per region		
			Sonora	Sinaloa	Colima-Nayarit
Crops	ToYSV	*Solanaceae*	84.2/ND[2] (DQ336350.1/ND)	95.5/ND (KJ742419.1/ND)	84.9/ND KX348173.1/ND)
	ToSLCV	*Solanaceae*	87.1/ ND (DQ347946.1/ND)	-	99.5/ND (KC479066.1/ND)
	ToChLPV	*Solanaceae*	86/- (AY339619.1/ND)	-	81.9/ND (HM459852.1/ND)
	CdTV	*Solanaceae*	-	ND/92.9 (ND/EU339940.1)	86.5/ND (DQ885456.1/ND)
	TPCTV	*Solanaceae*	-	-	82.3/NA (X84735.1/NA)
	CLCrV	*Malvaceae*	ND/98 (ND/AY742221.1)	ND/87.2 (ND/AF480941.1)	-
	ViYMV	*Fabaceae*	-	86.6/ND (KC430936.1/ND)	89.7/ND (KC430936.1/ND)
Non cultivated plants	EuMV	*Euphorbiaceae*	86.8/ND (JN368145.1/ND)	-	87.9/- (DQ318937.1/ND)
	EuYMV	*Euphorbiaceae*	ND/80.1 (ND/KY559581.1)	-	ND/81.7 (ND/ KY559604.1)
	CaGMV	*Fabaceae*	89/ND (AF439402.1/ND)	94.7/ND (AF439402.1/ND)	90.5/ND (AF439402.1/ND)
	BlelCV	*Acanthaceae*	-	ND/79 (ND/JX827488.1)	-

[1] Virus acronyms: *Blechum leaf curl virus* (BlelCV), *Calopogonium golden mosaic virus* (CaGMV), *Chino del tomate virus* (CdTV), *Cotton leaf crumple virus* (CLCrV), *Euphorbia mosaic virus* (EuMV), *Euphorbia yellow mosaic virus* (EuYMV), *Tomato chino La Paz virus* (ToChLPV), *Tomato pseudo-curly top virus* (TPCTV), *Tomato severe leaf curl virus* (ToSLCV), *Tomato yellow spot virus* (ToYSV), *Vigna yellow mosaic virus* (ViYMV).

[2] ND: No detected; [3] NA: Not Applicable.

Supplemental Table 3. List of PCR primers used in the present work.

Virus	Name	Primer sequence 5' to 3'	Amplification lenght (pb)	Reference
Begomovirus	DGR-SAR	GAGTCTAGATGCTGACCTCCTCTAGCWGATCTGC	950 pb - 1150 pb	Mauricio-Castillo et al., 2007
	CP-70-BamHI	CACGGATCCGATTGRACCTTACANGGNCCTTCACA		
TYLCV	qPCR-TYLCV-F	GAAGGCTGAACTTCGACAGC	171	Rodriguez-Negrete et al., 2014
	qPCR-TYLCV-R	GGACTTTACATGGGCCTTCAC		
PHYVV	qPCR-PHYVV-F	GGCGATACCGTAGAATGGGGAGAA	158	Morales-Aguilar et al., 2019
	qPCR-PHYVV-R	TGAAGGAAGAAATGCTGGGGTTGT		
RhGMV	qPCR-RhGMV-R	GCACTCGAGCAAATACAGCG	177	This work
	qPCR-RhGMV-F	ATGTGCTGACCTGGTTGAGG		
RhGMSV	qPCR-RhGMSV-F	CTTGGTACCCCTATGGATTTTGGC	146	This work
	qPCR-RhGMSV-F	CGTTGCTGGCATACTGTCCACC		
SiMSiV	qPCR-SiMSV-F	TTGGCAAGATATGGATGGATGA	147	This work
	qPCR-SiMSV-R	CAGTGCTGGGCTCGTTGTCG		

Chapter III.

Malvastrum bright yellow mosaic virus associated to new host *Sida rombifolia* with potential to infect tomato.

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Abstract

Malvastrum bright yellow mosaic virus (MaBYMV) is a bipartite begomovirus that causes severe diseases to *Malvaceous* plants, particularly *Malvastrum coronomandelianum*. *Sida rombifolia* plants showing begomovirus-associated symptoms and virus vector whitefly infestation were collected near tomato crops in Durango, Mexico. Using rolling circle amplification (RCA) and sequencing, the full-length genome of an isolate of MaBYMV was obtained. Agrobacterium-mediated virus inoculation to *Solanaceous* (*Nicotiana benthamiana and Solanum Lycopersicum*) shows that can be infected by MaBYMV. To the best of our knowledge, this is the first report of MaBYMV capable of infect Solanaceous host.

Keywords: Begomovirus, Sida rombifolia, Tomato, Mexico

Geminivirus (family Geminiviridae) are circular single-stranded (ss) DNA viruses packed into icosahedral twinned-shaped particles that cause severe diseases in major crop plants worldwide. The geminivirus are classified into nine genera (*Becurtovirus, Begomovirus, Capulavirus, Curtovirus, Eragovirus, Grablovirus, Mastrevirus, Topocuvirus,* and *Turncurtovirus*) on the basis of genome organization, the host range and type of insect vector (Zerbini et al., 2017). The Begomovirus the most diverse genus (>320 species), and comprise economically important viruses, also are transmitted by the polyphagous insect vector whitefly (*Bemisia tabaci*). The genomes of begomoviruses that are native to the New World (NW) usually are bipartite, consisting of two components that are designated DNA-A and DNA-B. In contrast, most of the known Old World (OW) begomoviruses have monopartite genomes consisting of

single DNA molecules homologous to the DNA-A component of bipartite begomovirus. The DNA-A component encodes viral functions required for viral DNA-A and DNA-B replication, transcription and vector-assisted transmission, whereas DNA-B component encodes proteins required for cell-to-cell and long distance viral particles movement in host plants (Vincent N Fondong, 2013). In Mexico the begomovirus is associated to diseases of the cultivars: tomato, pepper, bean, soy, tobacco, watermelon, okra (Ascencio-Ibanez et al., 2002; Bronw, 1999; Domínguez-Durán et al., 2018; Melendrez-Bojorquez et al., 2016; Méndez-Lozano, Quintero-Zamora, et al., 2006). Furthermore, weeds may act as an alternative host for some agriculturally significant begomovirus (Basak, 2016; Liu, Xie, & Zhou, 2009; Polston, Cohen, Sherwood, Ben-Joseph, & Lapidot, 2006). Weeds-infecting begomovirus has been found to infect crops (Ascencio-Ibanez et al., 2002; Tahir et al., 2015). *Malvaceous* weeds are also frequently infected by begomovirus, which cause a wide range of symptoms including mosaics, yellow veins and curling leaf (Alabi et al., 2016; Fiallo-Olivé, Martínez-Zubiaur, Moriones, & Navas-Castillo, 2010; Fiallo-Olivé, Zerbini, & Navas-Castillo, 2015; Graham, Martin, & Roye, 2010; a M. Idris, Hiebert, Bird, & Brown, 2003; J. A. Mauricio-Castillo et al., 2014). *S. rombifolia* is a ubiquitous non-cultivated plant belonging to *Malvaceae* plant family distributed in Mexico (Naturalista, 2011), and host of begomovirus (Fiallo-Olivé, Navas-Castillo, Moriones, & Martínez-Zubiaur, 2012; Rodríguez-Pardina et al., 2006; Patrícia Soares Wyant, Gotthardt, Schäfer, Krenz, & Jeske, 2011). The aim of this study was to characterize the begomovirus present in non-cultivated malvaceous plant *S. rombifolia* and its potential to infect economically important crop as tomato. *Malvaceous* plant *S. rombifolia* collected Summer 2015 exhibiting typical geminivirus symptoms (yellow and green mosaics and leaf

deformation) also, asymptomatic plants were observed near tomato fields (Fig. 1A and B), although the insect vector (*B. tabaci*) was present, suggesting a viral etiology. Total DNA was extracted from two symptomatic and one asymptomatic plant using a CTAB-Based method (Doyle, 1991). Then putative full-length begomovirus genome components were amplified by rolling circle amplification (RCA) with ϕ-29 DNA polymerase (TempliPhi, Ge Healthcare) as previously described (Inoue-Nagata et al., 2004). The result concatamers were digested with two different enzymes (*Sac*I and *Eco*RI). The *Eco*RI and yield one fragment of ~2.7 Kpb and *Sac*I yielded 3 fragments of 2.7, 1.9 and .8 kpb putatively corresponding to a full-length monomeric component (2.7 kpb) and split component of (1.8 and 0.8 Kpb). Both 2.7 kpb fragments were cloned into *Eco*RI and *Sac*I digested pGreen vector (Hellens et al., 2000), transformed in E. coli DH5α and one colony of each fragment of sample Sr-15-423 was fully sequenced using the primer walking strategy by designed specific primers. The assembly and comparison of the sequences were obtained using the SeqMan Pro and MegAling programs [DNASTAR Inc., Madison, Wi, USA], and it shows the presence of two putative viral full-length genomes of 2619 pb and 2593 pb corresponding to DNA-A and DNA-B, respectively. Phylogenetic analysis based on the alignment of the complete nucleotide sequences available in the GenBank database resulted in high nucleotide sequence identity with MaBYMV (DNA-A 93 % and DNA-B 92%) with accession number (KU058854 and KU058857respectively), based in the present demarcation criteria this result indicates that it is another isolated of MaBYMV (Judith K. Brown et al., 2015), Supplemental figure 1. The isolates of MaBYMV-[MX] (DNA-A and DNA-B were deposited in GenBank in process respectively), hereafter MaBYMV-

[Mx]. Additionally, a phylogenetic tree based on multiple sequence alignment of the complete DNA-A and DNA-B sequences with another begomovirus that infect malvaceous plants showed that MaBYMV-[MX] DNA-A and DNA-B cluster with MaBYMV-[US] (Fig. 1).

Fig. 1. *Sida rombifolia* symptomatic (A) and asymptomatic (B).

To evaluate the infectivity of MaBYMV-[MX], viral infectious clones were obtained according to the protocol previously described (C.-Y. Wu et al., 2008). The RCA-derived product from sample Sr-15-423 was partially digested with two units of *Eco*RI for DNA-A and *Sac*I for DNA-B within 20 min, and the digestion product corresponding

to viral dimer tandem was excised and purified from 1% agarose gel. Viral dimers were cloned into *Eco*RI- and *Sac*I-digested pGreen 0029 binary vector, transformed in E. coli, and dimeric constructs were corroborated by *Pvu*I and *Hind*III for DNA-A and DNA-B respectively. Agrobacterium strains harboring viral infectious clone were obtained by electro- transformation of GV3101 A. tumefaciens strain. Viral agroinoculation assays were performed according to the methodology previously described (Cañizares et al., 2015). The potential to induce infection in Solanaceous (*S. lycopersicum* and *N. benthamiana* plants) was investigated by agroinoculation. Plants at 4-5 leaf stage were inoculated with A. tumefaciens strain harboring pGreen infectious clone. As control *N. benthamiana* and *S. lycopersicum* plants were inoculated with A. tumefaciens strain harboring pGreen empty vector. Inoculated plants were maintained in the greenhouse and viral infection was evaluated at 36 days post inoculation (dpi) and corroborated by viral PCR detection using MaBYMV-[MX] specific primers. Inoculated plants of *N. benthamiana* displayed mild symptoms at 36 dpi (Fig. 2C) and (4/10) were positive (Fig. 3A); whereas plants of *S. lycopersicum* show not symptoms (Fig. 2D) but (10/10) were positive (Fig. 3B).

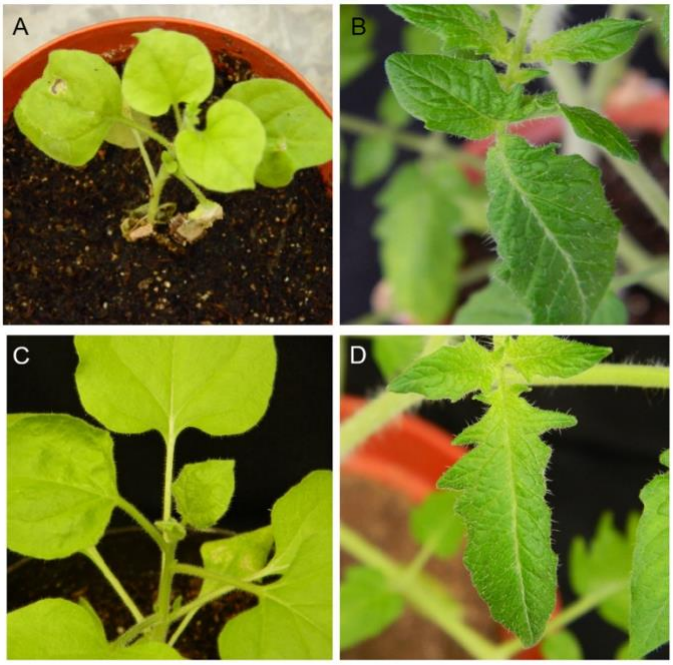

Fig. 2. Infectivity assay using BCaMV-[MX] agroinfectious clone. *N. benthamiana*, *S. lycopersicum*, plants were inoculated with *A. tumefaciens* strains harboring pGreen empty vector (A-B) or viral infectious clone (C-D). All images obtained at 36 dpi.

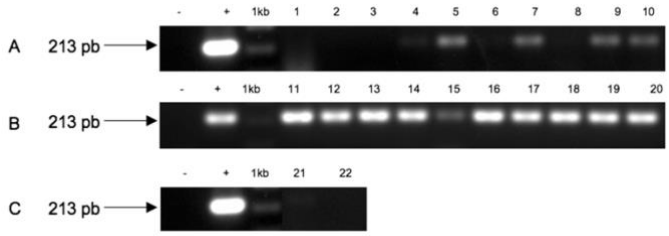

Fig. 3. PCR test of infectivity of MaBYMV-[MX] viral infectious clone in *N. benthamiana* (A) and *S. lycopersicum* (B), and *A. tumefaciens* strains harboring pGreen empty vector (C), at 36 dpi.

Weed has been reported as virus reservoir for crops especially those harboring economically important begomovirus as TYLCV, PHYVV, PepGMV (Barreto et al., 2013; Jose A Garzon-Tiznado, Acosta-Garcia, Torres-Pacheco, Gonzalez-Chavira,

Rivera-Bustamante, Maya-Hernandez, Guevara-Gonzalez, et al., 2002; Smith, Seijo, Vallad, Peres, & Druffel, 2015). Begomovirus naturally infected weeds have been poorly studied, however, only a few reports have shown the potential of these begomoviruses to infect crops (Ascencio-Ibanez et al., 2002; Paz-Carrasco et al., 2014; Tahir et al., 2015). Members of the malvaceae plant family currently has been associated as host of begomovirus in North and South America (Barreto et al., 2013; Castillo-Urquiza et al., 2008; Fiallo-Olivé et al., 2015; J. A. Mauricio-Castillo et al., 2014; Passos et al., 2017). Little is known about the potential to infect crops. In this work, we describe the detection and obtainment of infective clones of MaBYMV, from a new host *R. minima*. Analysis of infectivity showed mild symptomatology in the experimental host *N. benthamiana* (Fig2. C) and none in *S. lycopersicum* (Fig. 2D), some other begomoviruses has presented strong symptomatology in one host and mild or none symptomatology in other (Basak, 2016; Cardenas-Conejo et al., 2010; Polston et al., 2006). The decrease in symptomatology could increase vertical transmission in some viruses (Pagán, Montes, Milgroom, & García-Arenal, 2014) couple with this, and the recent analysis of begomovirus seed transmission (E.-J. Kil et al., 2016, 2017) open a great area of concern and study weeds as potential viral inoculum for crop protection. Considering some weeds as *S. rombifolia* well distributed and present annually (Vibrans, 2012), also weeds can be host of begomovirus economically important, and considering that mixed infection could increase disease severity (Méndez-Lozano et al., 2003) even recombination (Graham et al., 2010; C Hernández-Zepeda et al., 2010; Stewart et al., 2014). Is the great concern to study the begomovirus present in weeds and considered of great risk those that can infect economically important crops.

Investigation with MaBYMV in mixed infections and the potential to cause new diseases in crops are in progress.

Conflict of interest

The authors hat they have not conflict of interest.

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Supplemental Figure 1: Phylogenetic relationship or MaBYMV-[MX] DNA-A and DNA-B

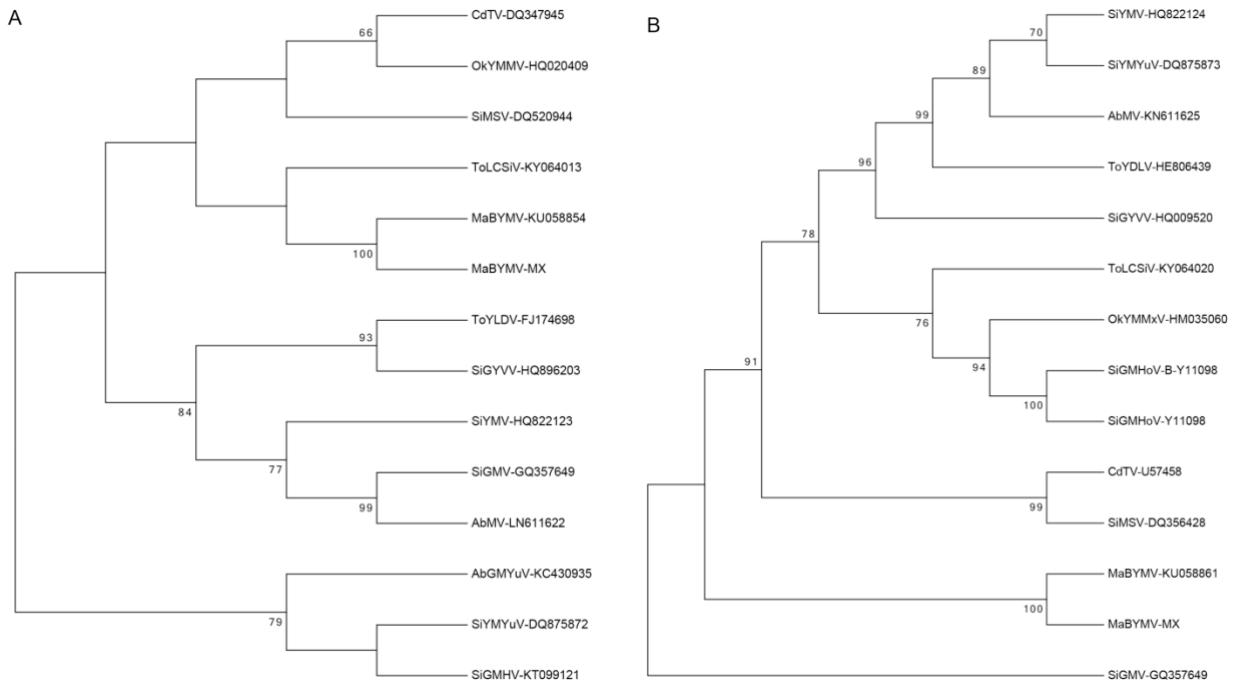

Fig. 1. Phylogenetic relationship of MaBYMV-MX DNA-A (A) and DNA-B (B). Tree generated using the maximum-likehood algorithm implemented in MEGA6 program. Only the consensus ML tree is presented. Numbers at branch internodes represented bootstrap values (1,000). Virus abbreviations: MaBYMV, *Malvastrum bright yellow mosaic virus*; SiMSV, *Sida mosaic Sinaloa virus*; CdTV, *Chino del tomate virus*; OkYMMV, *Okra yellow mosaic Mexico virus*; ToYLDV, *Tomato yellow leaf distortion virus*; AbMV, *Abutilon mosaic virus*; SiYMYuV, *Sida yellow mosaic Yucatan virus*; SiGYVV, *Sida golden yellow vein virus*; ToLCSiV, *Tomato leaf curl Sinaloa Virus*; SiGMV, *Sida golden mottle virus*. Virus sequences accession numbers are shown in the figure followed by virus acronym. MaBYMV-MX.

Integrative discussion.

Geminiviral infections are of great concern to agriculture worldwide, some of these viruses are related to Geminivirus emerging diseases constantly such as the case of Casava mosaic virus (CaMV), *Watermelon chlorotic stunt virus* (WmCSV) in the old world, *Peper golden mosaic virus* (PepGMV) in the new world and *Tomato yellow leaf curl virus* (TYLCV) distributed worldwide. There are many factors that can favor the emergence of new diseases, the introduction of viruses to new ecosystems (Domínguez-Durán et al., 2018), the introduction of new plant materials to endemic areas (Melendrez-Bojorquez et al., 2016), appearance of more aggressive virus variants (Melendrez-Bojorquez, 2016), virus recombination (Alabi et al., 2016), expansion of cultivation areas, decrease in diversity, increase in vector populations (Roossinck, 2011, Roossinck et al., 2015). Of course, the re-emergence of endemic viruses from one season to another causing problem is not considered a new disease as such but it is also a key point to put in consideration. Knowing the Begomoviruses that are currently in the main agricultural areas allows us to know the diseases that crop-producers are currently dealing with. In this paperwork we described the molecular characterization of two virus complexes (pepper and tomato complexes), those viral-complexes were located in an area of Mexico known as the Comarca Lagunera, where begomoviral diseases are not reported.

In pepper complex, TYLCV, Pepper Huasteco yellow vein virus (PHYVV), and Pepper golden mosaic virus (PepGMV) were characterized, affecting pepper crops, all viruses show homologies above 91% with other variants of the same species reported in Mexico, which indicates that they are new variants but belong to the same species. In our work we found toTYLCV interacting with PHYVV during two years of cultivation, It was until the third season that we detected a third member (PepGMV). The infection of PHYVV and PepGMV in pepper has already been previously characterized (Méndez-Lozano et al., 2003) and it is common to find them in pepper in single or mixed infection (Melendrez-Bojorquez et al., 2016; Rodelo-Urrego et al. al., 2015). There are few reports of PHYVV, PepGMV with TYLCV in tomato in San Luis Potosi and Sinaloa, Mexico (Bañuelos-Hernández et al., 2012; Hernandez-Espinal et al., 2018), however,

the role that TYLCV could be playing in this interaction it is unknown and will require more research.

In the tomato complex, TYLCV, *Tomato chino La Paz virus* (ToCHLPV), *Tomato severe leaf curl virus* (ToSLCV), and *Sida mosaic Sinaloa virus* (SiMSV) associated with tomato diseases were characterized. All these viruses have already been characterized in Mexico, however never in a complex like this. TYLCV, a virus known as the most devastating tomato crop, was detected for the first time in, Yucatan, Mexico in 1999 (Ascencio-Ibañez et al., 1999,), then the second introgression to Mexico in Sonora, then it was detected again in Sinaloa, Mexico affecting tomatillo (Gaméz-Jiménez et al., 1999), has been found in coinfection with ToChLPV Baja California (Cardenas-Conejo et al., 2010) and also with ToSLCV in San Luis Potosí (Bañuelos-Hernández et al., 2012), ToChLPV is a virus endemic to Mexico, of which it has been poorly characterized. Variants of ToSLCV have been reported in Mexico and in other Latin American countries such as Nicaragua, Guatemala (Rojas et al., 2000, Rosario et al., 2015). SiMSV has been found associated with the *Sida acuta* disease in Sinaloa (Mauricio-Castillo et al., 2014).

In both complexes (pepper and tomato) the presence of TYLCV indicates that this virus has a plasticity to move to several hosts, in the case of TYLCV in pepper (*Capsicum annum*), it is believed that it can be its end host (Morilla et al., 2005, Polston et al., 2006, Kil et al., 2014), in the case of TYLCV in tomato (*Solanum lycopersicum*), TYLCV is one of the begomoviruses that has caused the most damage to this crop in the world (Moriones & Navas-Castillo 2000; Mabvakure et al., 2016), not only could be infecting different alternate hosts, but also can be transmitted by seed in tomato and pepper (Kil et al., 2016; Kil et al., 2017), this suggests that it can remain in alternate hosts and seeds and be associated with emerging diseases in each growing season. It is also of great importance to consider not only TYLCV but also the whole Begomovirus complex that, being in mixed infections, recombination’s can be carried out to help, the evolution of these viruses (Silva et al., 2014).

Thanks to Next-generation sequencing technology, we are able to generate large amounts of sequencing data (Grada & Weinbrecht, 2013). This tool has the potential to determine the viral population of an environmental sample (Metagenomic) up to a single plant in a set of several samples (Ecogenomic) (Roossinck et al., 2012, Roossinck et al., 2015) without the need for antibodies or prior knowledge of the viral sequences. This massive amount of data along with the help of bioinformatic tools at hand (reviewed in Jones et al., 2017), is being exploited by plant virology, this has resulted in an increase in the discovery of new viruses, changing the taxonomy and generating families, genera, species, variants and quasispecies (Reviewed in Prabha et al., 2013; Jo et al., 2017; Jo et al., 2018; Wu et al., 2018, King et al., 2018; Hadidi et al., 2016). The ability to obtain a more comprehensive picture of infections in a plant known as virome (Czotter et al., 2018; Jo et al., 2018) that could help in the diagnosis of plant diseases (Czotter et al., 2018; Claverie et al., 2018).

In this paperwork we were able to identify several begomoviruses, some of them just accepted officially within the Begomovirus taxonomy (King et al., 2018), as is the case of *Malvastrum bright yellow mosaic virus* (Alabi et al., 2018; Kin et al. al., 2018), others already known, of great importance and already reported in Mexico as is the case of TYLCV, where in Mexico, TYLCV has been reported continuously across the country since the first outbreak in 1999 (Ascencio-Ibañez et al. , 1999), others that were not known to be in Mexico as is the case of *Tomato chlorotic mottle Guyane virus* (ToCMGuV), reportedly affecting tomato in French Guiana (Lett et al., 2015) and others poorly characterized as is the case of *Calopogonium Golden mosaic virus* (CaGMV) (Diaz et al., 2002). All these results reinforce the idea of the great Begomovirus diversity that exists in non-cultivated plants in Northern Mexico, although it is known that increasing diversity decreases the risk of disease (Rodelo Urrego et al., 2015). It is known that weeds serve as a source of inoculum and emergence of diseases in crops of agricultural interest, as well as being meeting points for several viruses and in some evolutionary processes such as recombination, these hot spots can serve as a cradle for the appearance of new variants that could be a potential risk for crops (Rocha et al., 2013, Barreto et al., 2013, Paz-Carrasco et al., 2013, Aguiar et al., 2018, Ranabaht et

al., 2018), with that in though the role of Begomovirus in non-cultivated host must be analyzed one by one to go deeper in the understanding.

In our paperwork, it was possible to determine a new host of *Malvastrum bright yellow mosaic virus* - MaBYMV of which, was no report so far in Mexico and had only been reported in the USA (Alabi et al., 2016), MaBYMV has been reported as causing diseases in *Malvastrum spp*, however, we found it, infecting *Sida rombifolia* (family *Malvaceae*). *Sida rombifolia* is widely distributed in the Mexican territory (Vibrans, 2016), Analysis of infectivity showed mild symptomatology in the experimental host *N. benthamiana* (Chapter III. Fig. 2. C) and none in *S. lycopersicum* (Chapter III. Fig. 2D), infectivity assays of other begomoviruses have presented strong symptomatology in one host and mild or none symptomatology in other (Basak, 2016; Cardenas-Conejo et al., 2010; Polston et al., 2006), this suggests that the symptomatology is host-virus dependent, also the decrease in symptomatology could increase vertical transmission in some viruses (Pagán et al., 2014) couple with this, and the recent analysis of begomovirus seed transmission (E.-J. Kil et al., 2016, 2017) open a great area of concern and study weeds as potential viral inoculum for crop protection. *Sida rombifolia* is well distributed and present annually (Vibrans, 2012), also weeds can be host of begomovirus economically important as *Tomato yellow leaf curl virus* (Chapter II. Supplemental figure 3), and considering that mixed infection could increase disease severity (Méndez-Lozano et al., 2003) even recombination (Graham et al., 2010; C Hernández-Zepeda et al., 2010; Stewart et al., 2014) it is of the best interest to fully understand and characterize the *Begomovirus* diversity and distribution in non-cultivated areas as much as the cultivated ones.

Integrative conclusions

Tomato yellow leaf curl virus, *Pepper Huasteco yellow vein virus* and *Pepper golden mosaic virus* are associated to pepper diseases at La Comarca Lagunera, in the North of Mexico

Tomato yellow leaf curl virus, *Tomato chino La Paz virus*, *Tomato severe leaf curl virus* and *Sida mosaic Sinaloa* virus are associated to tomato diseases at La Comarca Lagunera, in the North of Mexico.

The Next-generation sequencing is a useful tool to determine the Begomovirus population in non-cultivated plants and our approach can help to determine the main *Begomovirus* located in one selected area.

There is diversity of Begomovirus and are distributed in North States of Mexico, this results with the concern that begomovirus mixed infection in host plants provide a perfect scenario for recombination leading to the evolution of potentially agriculturally relevant *Begomovirus,* plants belong to Malvaceae, Solanaceae, and Fabaceae must be in constant surveillance in case of emerging viral diseases.

Sida mosaic Sinaloa virus and *Malvastrum bright yellow mosaic virus* has the potential to infect tomato and *N. benthamiana* plants and tomato plants develop symptomless infection, this findings suggest that can go unnoticed in Tomato plants which is of great concern for the potential that Begomovirus has to recombine or interact with others begomovirus despite the fact that the *Begomovirus* TYLCV has proven to be seed-transmissible.

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Appendix 1. Additional results

In preparation to be sumitted to Crop protection as a Short communication

***Tomato yellow leaf curl virus* with new world Begomovirus associated with Tomato diseases in Northen, Mexico.**

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Abstract

Tomato (*Solanum lycopersicum*) is an economically important crop worldwide. *Tomato yellow leaf curl virus* (TYLCV) is the most devastating Begomovirus affecting tomato crops worldwide. In norther, Mexico recurrent virus-related symptomatology as yellow mosaic, severe curly leaf and stunted plants has been observed in different tomato hybrids, in different annual crop season. *Tomato yellow leaf curl virus* (TYLCV), *Tomato*

chino La Paz virus (ToChLPV), *Tomato severe leaf curl virus* (ToSLCV), and *Sida mosaic Sinaloa virus* (SiMSinV). TYLCV was found widely distributed in the area every year monitored, both in tomato and in non-cultivated plants. These results suggest that despite TYLCV-tolerant tomato crops, TYLCV subsists and associated with other Begomoviruses, together can become a potential risk. Non-cultivated plants can act as natural reservoirs of begomoviruses and as sources of inoculum for tomato plants, favoring the occurrence of epidemics. To our knowledge, first report of TYLCV with ToChLPV, ToSLCV, and SiMSinV associated to tomato diseases. In addition, this is first report of SiMSinV in mixed infection.

1. Introduction

Mexico is one of the leading tomato producers in the world. In 2018, national tomato production was 3.5 million tons with a total value of approximately US $1.6 billion (SIAP, 2018). Tomato crops are seriously affected by several viral diseases.

The genus Begomovirus (family Geminiviridae) are important plant pathogens consisting of circular, single-stranded DNA (ssDNA) genomes ranging from 2.6 to 2.8 kb packed into 1 or 2 icosahedral twine-shaped particles. The genomes of begomoviruses that are native to the New World usually are bipartite (DNA-A and DNA-B), in contrast, most of the known Old World begomoviruses have monopartite genomes consisting of single DNA molecules homologous to the DNA-A component of bipartite Begomovirus (Roshan, Kulshreshtha, & Hallan, 2017). *Tomato yellow leaf curt virus* (TYLCV) is one of the most devastating viral diseases of tomato worldwide resulting in enormous economic losses (can be up to 80%) to the growers. TYLCV is monopartite, encodes the replication-associated protein (Rep), the coat protein (CP),

proteins C4 and V2 associated with pathogenicity and virus-host interactions, as well as the replication enhancer protein (REn) and the transcription activator protein (TrAP) that participate in replication and gene expression (Wartig L. et al., 1997). TYLCV is transmitted plant-to-plant by insect vector whitefly (*Bemisia tabaci*) in a persistent circulative manner (Ghanim & Medina, 2007; Rosen et al., 2015). It has a broad geographical distribution and continues to spread to new regions (Mabvakure et al., 2016). Symptoms observed in infected tomato plants vary widely depending on the time of disease onset, environmental conditions, and tomato cultivar. In addition to stunting and flower abortion, along with a size reduction of leaflets and yellowing of young leaves is observed. Reduction in size can also occur in fruits of affected plants without obvious symptoms, leading to significant reductions in yield. Plants infected at early growth stages will be severely stunted, abort blooms and not bear fruit (Moriones & Navas-Castillo, 2010). In addition, TYLCV has wide host range and can infect other plant species including cultivated and non-cultivated plant species belonging to different families, Amaranthaceae, Chenopodiaceae, Compositae, Convolvulaceae, Cruciferae, Euphorbiaceae, Geraniaceae, Leguminosae, Malvaceae, Orobanchaceae, Plantaginaceae, Primulaceae, Solanaceae, Umbelliferae, Urticaceae and Cucurbitacea (Khan, Tiwari, Khan, Ji, & Chun, 2013). The efficiency in adapting TYLCV and that may be present along with native viruses makes it a potential risk anywhere in the world. In this work, we took on the task of monitoring the natural occurrence and extension of Begomoviruses in different years.

2. Materials and methods

Surveys of tomato plants were conducted during the growing seasons of 2007, 2012, 2015 and 2016 in north Mexico over an area called “La Comarca Lagunera” (**Figure 1A**). Among the 54 tomato plants collected, 9.3%, 33%, 5.6% and 52% were from years 2007, 2012, 2015 and 2016 respectively. Only plants that showed symptoms were collected (**Figure 1B**). In addition, in 2016, six non-cultivated plants were collected. The presence of Begomoviruses was assessed by PCR using the degenerate primers Rep-DGRSAR (GAGTCTAGATGCTGACCTCCTCTAGCWGATCTGCCGTC) and CP70-BamHI (CACGGATCCGATTGRACCTTACANGGNCCTTCACAACC), the PCR products obtained are 1100 bp in length for monopartite, and 950 bp for bipartite (Mauricio-Castillo et al., 2007). PCR products were cloned into pGem-T easy vector and sequenced.

To obtain full-length Begomovirus component, total DNAs from representative tomato samples collected were amplified by rolling circle amplification (RCA) with Φ-29 DNA polymerase (TempliPhi, Ge Healthcare, USA) as described previously (Inoue-Nagata et al., 2004). RCA amplification products were digested with BamHI, SacI and XbaI, then monomeric products were cloned into pGreen0029 vector. Two independent clones for each viral component obtained from samples of the corresponding year were obtained and fully sequenced using the primer walking strategy. The assemblies of the sequences were obtained using SeqMan program (DNASTAR Inc., Madison, USA), and genome comparisons were performed employing Mega 7.0.

To understand, natural occurrence and extension of these Begomoviruses throughout different years, was performance PCR-test with specific primers for TYLCV, ToChLPV, ToSLCV, and SiMSinV. Specific primers were used for TYLCV (qPCR-TYLCV-F: 5’-

GAAGGCTGAACTTCGACAGC-3' and qPCR-TYLCV-R: 5'-GGACTTTACATGGGCCTTCAC-3') (Rodríguez-Negrete et al., 2014), ToChLPV (ToChLPV-F: 5'- GTTTGCTGACCTCCTCTAGC-3' and ToChLPV-R: 5'-GCCTCGAGGAACATCGGC-3'), ToSLCV (YMAC-F-5'-CGTGAATTCTTATTGTAYATGGCRTGTACDCATGC-3' and ToSLCV-Rev 5'-GANTCGAGHACGGGBAAGAC-3') and SiMSinV (Cp-SiMSV-F: 5'-TTGGCAAGATATGGATGGATGA-3' and Cp-SiMSV-R 5'-CAGTGCTGGGCTCGTTGTCG-3').

3. Results and discussion

PCR-test with degenerate primers, showed the presence of Begomovirus in 45 of 54 samples. The analysis of complete nucleotide sequences (DNA-A) showed the presence of *Tomato yellow leaf curl virus* (TYLCV), *Tomato chino La Paz virus* (ToChLPV), *Tomato severe leaf curl virus* (ToSLCV), and *Sida mosaic Sinaloa virus* (SiMSinV) (GenBank Accession Nos. KX427166, MH678590, MH678589, and KX440613, respectively), showed 99%, 98%, 92%, and 94% nucleotide sequence identity with previously reported GenBank sequences (Accession Nos. EF523478, DQ347949, JN680352, and DQ520944, respectively). The natural occurrence and extension of these Begomoviruses throughout different years his analysis showed the presence of TYLCV in 52 of 54 samples (96.3%). Among the 54 plants tested, 50 (92.6%) were found to have mixed viral infections. Combination of viruses found in mixed infection was: TYLCV- ToChLPV (1.9%), TYLCV- ToSLCV (1.9%), TYLCV- SiMSinV (18.5%), TYLCV- ToChLPV- ToSLCV (3.5%), TYLCV- ToChLPV- SiMSinV (9.3%), TYLCV- ToSLCV- SiMSinV (13%), and TYLCV- ToChLPV- ToSLCV- SiMSinV

(42.6%) (**Table 1 and Figure 2**). The infections with the four viruses was only detected in 2016. In addition, six non-cultivated plants were taken at random, all of which tested positive for the presence of TYLCV. The results show that TYLCV has adapted and distributed very well over time. The ToChLPV and ToSLCV virus were also present in non-cultivated plants. There are several reports that indicate that TYLCV is found in mixed infection with native Begomoviruses. In 2010, the presence of TYLCV and ToChLPV in pepper plants in Baja California Peninsula was reported (Cardenas-Conejo et al., 2010). In addition, in 2018 TYLCV was found to be double or triple infected with the native PepGMV and PHYVV viruses, and this interaction was associated with diseases of pepper plants in Mexico (Morales-Aguilar et al., 2019). Non-cultivated plants can act as natural reservoirs of begomoviruses and as sources of inoculum for tomato plants, favoring the occurrence of epidemics. In addition, *Tomato chino La Paz virus* (ToChLPV) and *Tomato severe leaf curl virus* (ToSLCV) are interesting because both have been found exclusively in mixed infections with other Begomoviruses, and their genomic component B has not been identified (Mauricio-Castillo et al., 2007; Mauricio-Castillo et al., 2007b; Rojas et al., 2005). We confirmed this in our analysis, ToChLPV and ToSLCV were found in samples together with TYLCV or/and SIMSinV (**Figure 2**). These observations suggest that these viruses can possibly use other Begomoviruses (such as, TYLCV) for their movement and carry out their infectious cycle. In conclusion, this is first report of TYLCV with native viruses ToChLPV, ToSLCV, and SiMSinV associated to tomato diseases.

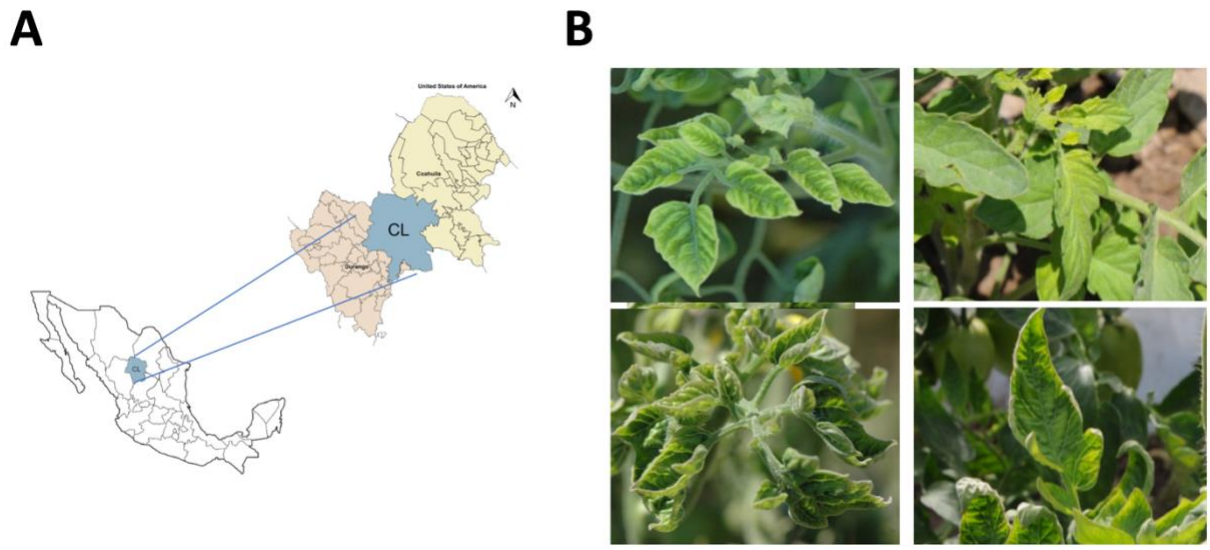

Figure 1. Symptoms observed in tomato plants in La Comarca Lagunera (CL), Mexico. (A) Geographic localization of CL in Mexico. (B) Several tomato plants showing chlorotic leaf, leaf curl, and yellowing.

Table 1. Relation of plants with specific begomovirus detection

	Virus specific PCR-positive samples				
Collection year	#	**TYLCV**	**ToChLPV**	**ToSLCV**	**SiMSinV**
2007	1	+	-	+	+
2007	2	+	+	-	-
2007	3	+	+	-	+
2007	4	+	-	-	-
2007	5	+	+	-	+
2012	6	+	-	-	+
2012	7	+	+	-	+
2012	8	+	-	-	+
2012	9	+	-	-	+
2012	10	+	-	-	+
2012	11	+	-	-	+
2012	12	+	+	+	-
2012	13	-	-	-	-
2012	14	+	-	-	+
2012	15	-	-	-	-
2012	16	+	-	-	+
2012	17	+	-	+	-
2012	18	+	+	-	+
2012	19	+	-	-	+
2012	20	+	+	+	-
2012	21	+	-	+	+
2012	22	+	-	-	+

2012	23	+	-	-	-
2015	24	+	-	-	+
2015	25	+	-	-	-
2015	26	+	+	-	+
2016	27	+	-	+	+
2016	28	+	-	+	+
2016	29	+	+	+	+
2016	30	+	+	+	+
2016	31	+	+	+	+
2016	32	+	-	+	+
2016	33	+	+	+	+
2016	34	+	+	+	+
2016	35	+	+	+	+
2016	36	+	+	+	+
2016	37	+	+	+	+
2016	38	+	+	+	+
2016	39	+	-	+	+
2016	40	+	+	+	+
2016	41	+	+	+	+
2016	42	+	+	+	+
2016	43	+	+	+	+
2016	44	+	+	+	+
2016	45	+	-	+	+
2016	46	+	+	+	+
2016	47	+	+	+	+
2016	48	+	+	+	+
2016	49	+	+	+	+
2016	50	+	+	+	+
2016	51	+	+	+	+
2016	52	+	+	+	+
2016	53	+	+	+	+
2016	54	+	+	+	+

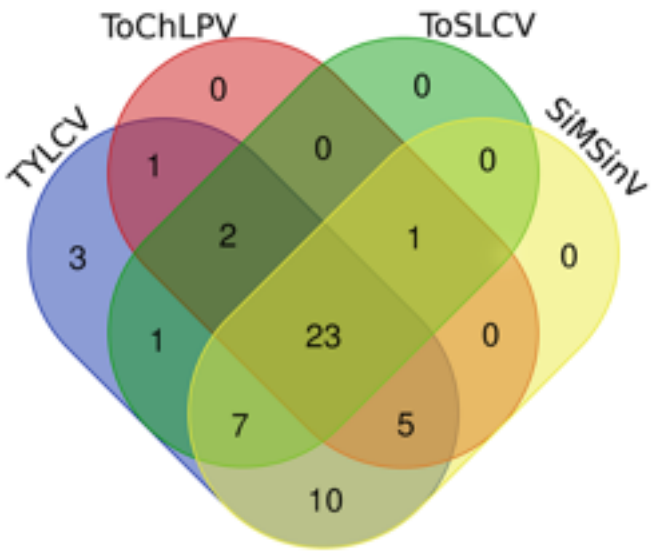

Figure 2. Veen diagram of begomovirus specific detection.

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Identification of *Tomato yellow leaf curl virus, Pepper huasteco yellow vein virus* and *Pepper golden mosaic virus* associated with pepper diseases in northern Mexico

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