Article

First incidence of yellow vein virus of huasteco pepper in soybean in Mexico

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Abstract

In the state of Sinaloa, soybeans are one of the few crop alternatives during the summer; therefore, its reactivation is being reconsidered. The whitefly (*Bemisia tabaci* Genn, Biotype 'B') was one of the main causes of the reduction of soybean growing areas in Mexico as a result of direct damage to flower production and plant yield, as well as of the transmission of geminiviruses. During the summer of 2013-2014, a total of 11 soybean varieties were evaluated to determine the incidence of viral diseases in the Valley of Culiacán and Valle del Fuerte, Sinaloa, Mexico. Symptomatology, amplification of DNA by polymerase chain reaction (PCR) and sequence analysis of amplified fragments confirmed the incidence of yellow vein virus of huasteco pepper (PHYVV) in soybean grown in the west of Sinaloa, Mexico. Sequence analysis showed 100% of nucleotide and amino acid identities with respect to the PHYVV1GMES isolate from Mexico (GenBank accession number KT022087). This is the first report on the emergence of PHYVV in soybean in Mexico.

Keywords: begomovirus, enzymatic sequence, yellow vein virus of huasteco pepper.

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Introduction

Soy (*Glycine max* L.) Merr. also, known as 'golden bean' or 'soy bean' consists of more than 40% protein and 20% oil and for this reason has been recognized as a potential supplemental source of edible oil and nutritional food throughout the world. Mexico imports practically all the soybean that it consumes, from the year 2000 to 2008, the average annual production of soybean was only 119 924 t, while the imports were 3 905 596 t (http: //siap.sagarpa.gob.mx./ventana.php?idLiga =1592&type=0). In the state of Sinaloa, soybeans are one of the few crop alternatives during the summer (Martinez *et al*., 1998); therefore, its reactivation is being reconsidered. The whitefly (*Bemisia tabaci* Genn, Biotype 'B') was one of the main causes of the reduction of soybean growing areas in Mexico, as a result of direct damage to flower production and plant yield, as well as of geminiviruses transmission (Cortéz *et al*., 2005).

Geminiviruses are a diverse and widely distributed group of plant pathogens that cause great economic losses throughout the world. Its genome consists of one or two molecules of circular single-stranded DNA with a size of 2.5 to 3 Kb (Lazarowitz, 1992), known as DNA-A and DNA-B, which encodes all the functions of viruses required for DNA replication, control of gene expression, insect transmission and movement in the plant (Hanley *et al*., 1999). The single-stranded circular DNA of the genus *Begomovirus*, family *Geminiviridae*, are among the most important plant viruses (Brown and Bird, 1992, Moriones and Navas, 2000). This group of viruses is known as one of the economic factors that limit crops in many parts of the world, especially in tropical and subtropical regions (Zerbini *et al*., 2005). Begomoviruses only infect species of dicotyledonous plants and are transmitted exclusively by the whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae) in a non-circulating way (King *et al*., 2012; Briddon, 2015).

These viruses are considered viruses of emerging plants, due to their increasing incidence and the severity of the diseases they cause (Polston and Anderson, 1997). It has also been reported that these viruses infect new hosts and have spread to more geographic locations (Mansoor *et al*., 2003; Varma and Malathi, 2003). Recombination and mutation may play an important role in the establishment and emergence of new begomovirus strains/species (Mansoor *et al*., 2006). Although only a small number of monopartite begomovirus have been discovered, it is known that all their essential functions are encoded by a single component in the DNA-A of the bipartite genome of begomovirus (Navot *et al*., 1991; Rojas *et al*., 2001).

Currently, the International Committee of Virus Taxonomy (ICTV) recognizes that four genera (mastrevirus, topocuvirus, curtovirus and begomovirus) belong to the geminiviridae family (Fauquet *et al*., 2008). There are several reasons why the genomes of begomovirus are the most diversified group, including: high global distribution, whitefly transmission, presence of a mono or bipartite genome and the ability to infect dicotyledonous plants. Components A and B of the bipartite begomoviruses share a common region, ranging from 160 to 230 base pairs (bp), which contain the cis elements necessary for viral replication (Argüello *et al*., 1994). Component A produces the proteins involved in the replication and encapsidation of the viral genome (Gutiérrez *et al*., 2004), while component B encodes two proteins that promote the movement of the virus through the plant (Gafni and Epel, 2002).

Currently, ICTV has noticed more than two hundred confirmed and tentative species of the genus Begomovirus (ICTV, King *et al*., 2012). These viruses are transmitted by insects of the homoptera (whiteflies and leafhoppers) to a variety of mono and dicotyledonous plants, producing symptoms such as: mosaics, specks, streaks, leaf deformation, dwarfism, yellowing and chlorosis. From an economic point of view, this transforms the Begomovirus group into one of the most important groups of plant pathogens, as a result of its high incidence, distribution and severity of symptoms.

In Mexico, this type of phytopathogen has caused great confusion about the etiology of different diseases, which were initially called 'filling', when their presence was detected in tomato crops (*Solanum lycopersicum* L.) in the state of Sinaloa (Gonzalez and Cervantes, 1973). In addition to this, infection by the virus has been called 'tigers plant' in Puebla (Garzón and Galindo, 1985) due to the symptoms of yellowing caused in pepper crops (*Capsicum* spp.).

Finally, in the pepper crops in the south of Tamaulipas, it is known as the 'curly yellow' disease (Garzón *et al*., 1993). It has been determined that these diseases are of geminiviral etiology and can be caused by the Huasteco pepper virus (PHV) (Brown *et al*., 1989; Brown and Bird, 1992; Garzon *et al*., 1993; Torres *et al*., 1996; Garzón *et al*., 2002). Currently, it is believed that the PHV is restricted to species of the Solanaceae family, especially tomato and pepper crops. Recently, it was detected and identified that the huasteco pepper yellow vein virus (PHYVV) affects tomato (Lugo *et al*., 2011) and pepper in Culiacan, Sinaloa (Retes *et al*., 2016; Melendrez *et al*., 2016).

Materials and methods

Surveys and sample collection

During the summer of 2013-2014, a total of 11 varieties of soybeans were evaluated for the incidence of viral diseases in experimental fields operated by the National Institute of Agricultural and Livestock Forestry Research (INIFAP), in the Valley of Culiacán and the Valle del Fuerte, Sinaloa, Mexico. The incidence of the disease in these areas varies from 25.2 to 39.8%. The collected leaves were immediately placed in plastic bags and transported to the laboratory and kept at 4 °C. For long-term storage, the leaf samples were dried using silica gel or a lyophilizer and stored at -20 °C. Of these, three varieties (Cajeme, Nainari and Esperanza) showed distortion of the leaf (Figure 1).

Extraction of DNA

The total DNA was extracted from 0.05 g of dry foliar tissue using the previously described method (Velarde *et al*., 2015). The tissue was macerated in a sterile porcelain mortar and pestle and finally cooled to -70 °C. Three mL of extraction buffer was added containing: 30 mM sodium chloride (NaCl), 30 mM ethylenedinitrile tetracytic acid (EDTA) (pH 8.5) and 250 mM tris base (pH 8.5). The maceration product was placed in sterile 1.5 mL Eppendorf tubes. Subsequently, a volume of 100 µL of 10% ammonium cetyltrimethylbromide (CTAB) was added and incubated at 95 °C for 10 min.

Figure 1. Damage and symptoms of yellow leaves in soybean plants.

Then 250 µl of 5 M NaCl was added and incubated at -20 °C for 5 min and then centrifuged. The aqueous solution was added with a volume of cold absolute chloroform (v/v) and stirred with a vortex machine for a few seconds, and subsequently centrifuged at 10 000 rpm for 5 min, after this time, by pipetting, the aqueous solution was transferred to a new tube, adding a volume of cold absolute isopropanol (v/v) and homogenized manually.

Subsequently, the samples were stored in a refrigeration unit at -20 °C for one hour to allow the precipitation of the DNA. After this time, the samples in tubes were centrifuged at 13 000 rpm for 10 min to obtain the DNA pellet, for which it was waited two hours for the DNA to dehydrate and finally the DNA obtained from the different isolates were resuspended in 50 µL of nuclease-free water (Promega) and stored at 4 ºC for its preservation.

Polymerase chain reaction (PCR)

The DNA extracted from the tissues was analyzed by PCR using the degenerate oligonucleotides AV494 (direct; 5'- GCC(C/T)AT(G/A)TA(T/C)AG(A/G)AAGCC(A/C)AG-3') and AC1048 (reverse) 5-'GG(A/G)TT(A/G/T)GA(G/A)GCATG(T/A/C)GTACATG-3' (Wyatt and Brown, 1996), corresponding to the 3'and 5' ends of the capsid protein (PC) gene, respectively The cyclic profile consisted of 10 min of fusion at 95 °C, followed by 30 cycles of fusion, hybridization and polymerization of DNA for 1 min at 95 °C, 1 min at 55 °C and 1 min at 72 °C, respectively and a final extension of 10 min at 72 °C A thermal cycler was used (NyxTechnik Amplitronyx Series 6 A6 (ATC401 The final reaction mixture (15 µL) contained 100 nanograms of DNA, an equimolar mixture of dATP, dCTP, dGTP and dTTP, magnesium chloride (25 mM), PCR buffer (1X), DNA Taq polymerase (5U) (Promega® PCR Master Mix, catalog No. M7502) and 40 picomoles of each oligonucleotide (Sigma®).

The amplified products were analyzed in 1% agarose gels, stained with a Gel Red solution (Biotium catalog No. 41003), for which 15 µL of the amplified DNA were deposited in each well of the gel, adding 3 µL of the blue dye of bromophenol 6X (Sambrook and Rusell, 2001) and as reference the molecular weight markers 1Kb HyperLadder and HyperLadder 1kb Plus (Bioline). The run buffer and preparation of the agarose gels was 1X NaOH-borate (Brody and Scott, 2004). Run time was 30 min at 250 V (volts) and to visualize the DNA, the gel was exposed to a UV light transilluminator. The images were captured by a camera and scanned with the Launch Doc-ItLS (UVP) program. The sequence of oligonucleotides for all geminivirus strains and PCR conditions are given in Table 1.

Table 1. Alignment temperature conditions and sequences of degenerate primers AV494/AC1048 for the analysis of begomovirus PCR.

Initiator	Sequence (5' → 3')	Alignment °C/virus cycles	
F: AV494	GCC(C/T)AT(G/A)TA(T/C)AG(A/G)AAGCC(A/C)AG		
R: AC1048	GG(A/G)TT(A/G/T)GA(G/A)GCATG(T/A/C)GTACATG	55/30	all geminivirus

F= direct initiator; R= reverse initiator.

Enzymatic sequencing

Three DNA fragments of Geminivirus amplified by PCR on agarose gel based on silica columns (EZ-10 Spin Column BS354 DNA Gel Extraction Kit, Bio Basic Inc.), were purified, samples were sent for enzymatic sequencing using the method based on ddNTPs (Sanger *et al*., 1977) to the National Laboratory of Genomics for Biodiversity (LANGEBIO) of the Center for Research and Advanced Studies of the National Polytechnic Institute (CINVESTAV-IPN), Irapuato, Guanajuato, Mexico. A 3730 XL DNA sequencer (Applied Biosystems, Foster City, CA) and a Big Dye Terminator 3.1 kit (Applied Biosystems, Foster City, CA) were used for this purpose. The search for DNA sequence similarity was made using the BLAST program, comparing the values that identify the homology with the information found in the databases of the National Biotechnology Information Center (NCBI) (http: //www.ncbi.nlm nih.gov/BLAST/).

Phylogenetic analysis

The nucleotide sequences of the CP amplicon represent the region of 668 bp coordinated in the PHYVV, were compared with the golden mosaic virus Rhynchosia (RhGMV) and the Chinese tomato virus (CdTV), using the Neighbor-Joining phylogenetic dendrogram method (Saitou and Nei, 1987), based on the alignment of partial nucleotide sequences. Evolutionary distances were calculated using the Tajima-Nei method (Tajima and Nei, 1984). Evolutionary analyzes were performed on MEGA7 (Kumar *et al*., 2016).

Results and discussion

Identification of geminivirus

The genetic analysis showed amplicons of 668 bp of the CP gene obtained with a PCR of three randomly generated geminivirus isolates (Figure 2). Once purified, the amplified product was sent (Figure 3) for sequencing and subsequent search of databases using the BLASTn software: the resulting sequence of 668 bp had a 100% identity match with the sequence of a corresponding gene of the virus of the yellow vein of huasteco pepper 'HE967672' in the deposit of the Bank of Genes (GenBank) carried out by Rodelo *et al*. (2013). The sequence obtained was deposited in the GenBank of the National Biotechnology Information Center (NCBI), with the accession number KT022087. PHYVV was isolated from the variety Esperanza de soya.

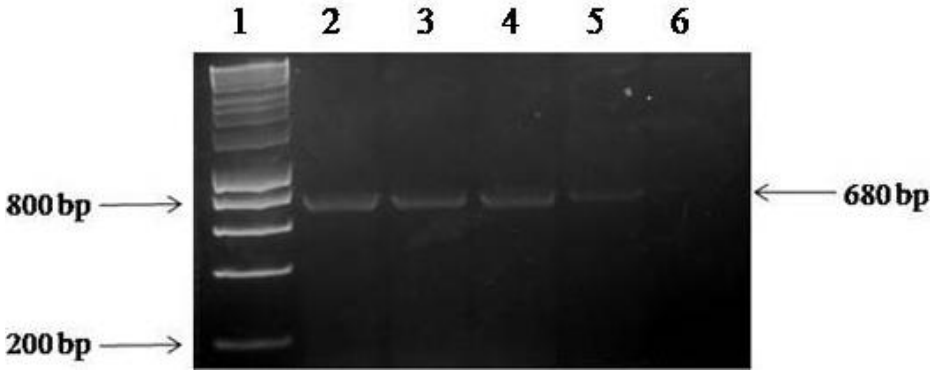

Figure 2. Fragments amplified by PCR from DNA extracted from different soybean plants with symptoms and without symptoms of begomovirus infection, using primers AV494 and AC1048. Lane 1= hyperLadder 1Kb (Bioline); Lane 2= DNA isolated from begomovirus in symptomatic soy plants (positive control); lanes 3-5= DNA isolated from geminivirus in symptomatic soy plants; lane 6= negative control (without DNA).

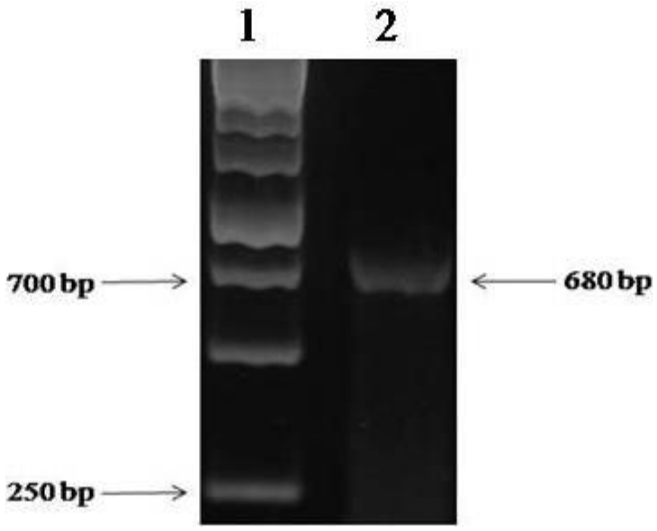

Figure. 3. Purification by PCR of begomoviruses using silica columns. Lane 1= hyperLadder 1kb Plus (Bioline), lane 2= geminivirus purification DNA in symptomatic soybean plants.

Phylogenetic analysis

The phylogenetic relationships of the Begomovirus group were investigated with the Neighbor-Joining method, using the CP gene sequences of RhGMV and CdTV. This phylogenetic analysis is shown in Figure 4. The sequence analysis of KT022087 (PHYVV 1GMES) was similar to the begomovirus genes and was clearly differentiated from the other Begomovirus strains found in pepper and several hosts (Figure 4). Multiple sequence alignments using the CLUSTAL W program showed that the 1GMES nucleotide sequence was 100% identical to the Mexican isolates of the virus. Within this region, PHYVV1GMES isolated from soybeans in Sinaloa exhibited 100% identity with PHYVV isolates in pepper in different states of Mexico. Soy is a temporary refuge for a virus that affects horticultural species that have great economic importance for Sinaloa. Therefore, it is necessary to use resistant varieties (such as Guayparime S-10), as well as to comply with an adequate program for the management of insect vectors. This document is the first report on the appearance of PHYVV in soybean in Mexico.

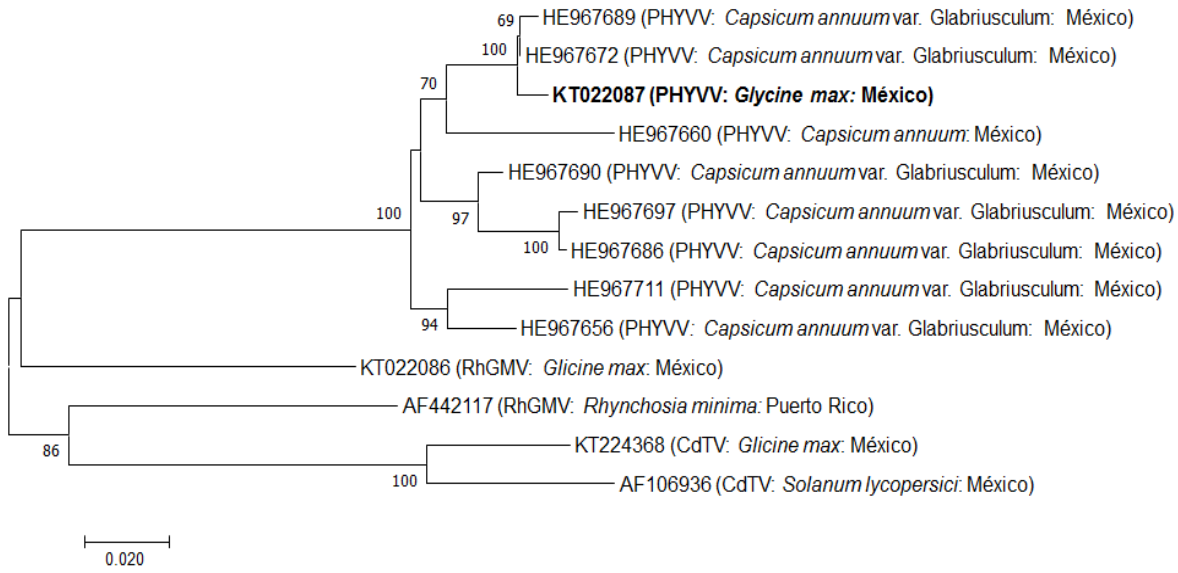

Figure 4. Phylogenetic dendrogram generated by the Neighbor-Joining method based on the partial alignment of nucleotide sequences of the capsid protein gene of PHYVV, with which the PHYVV, RhGMV and CdTV were selected and isolated. The values in the nodes represent the percentage of boot cores (1 000 replicas), only >50 values are shown.

Conclusions

Based on the analysis of the nucleotide sequence of the fragments amplified by means of the Polymerase Chain Reaction, the yellow vein virus of huasteco pepper was identified for the first time, infecting soybean plants in the state of Sinaloa, Mexico. This new knowledge regarding the PHYVV could provide information on its ability to infect plants of the Solanaceae family.

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