



Genomic characterization and transmission efficiency by its vector *Bemisia tabaci* of a novel recombinant strain of potato yellow mosaic virus

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Abstract

Potato yellow mosaic virus (PYMV) is one of the most important begomoviruses infecting tomatoes in the Caribbean basin. Viruses of this group are transmitted by whiteflies of the *Bemisia tabaci* cryptic species complex. Within this complex, the whitefly referred to as Middle East-Asia Minor 1 (MEAM1) is globally spread and recognised as a highly efficient begomovirus vector. This study reports the whole genome of a PYMV isolate infecting tomatoes in Táchira state, Venezuela. The DNA-A of this isolate shared the highest nucleotide identity (93.5%) with a PYMV isolate from *Solanum americanum*. The PYMV isolate described here revealed a putative recombination event involving PYMV and tomato chlorotic leaf distortion virus. Additionally, the PYMV transmission by the whitefly MEAM1 was evaluated. At 60 days post inoculation, the transmission efficiency was 83.6, 88.2 and 100% when using one, five and 20 adults per plants, respectively.

Keywords Begomovirus · Geminiviridae · *Solanum lycopersicum* · whitefly

The genus *Begomovirus* (family *Geminiviridae*) represents the largest group of plant viruses (Brown et al. 2015). They are transmitted by whiteflies of the *Bemisia tabaci* cryptic species complex (Rojas et al. 2018). The begomoviruses have a mono- or bipartite genome consisting of circular single-stranded DNA (Zerbini et al. 2017). Potato yellow mosaic virus (PYMV) is a bipartite begomovirus (i.e.: a genome possessing a DNA-A and DNA-B components) widespread in the Caribbean basin threatening tomato production in the region (Geraud-Pouey et al. 2016). In addition to potato and tomato, PYMV can infect *Capsicum annuum*, *Nicotiana benthamiana*, *N. tabacum*, *Datura stramonium*, *Petunia hybrida* and *Solanum americanum*

(Urbino et al. 2004; Romay et al. 2016). In Venezuela, PYMV is the most frequent begomovirus found in tomato-growing regions and causing severe yield losses (Nava et al. 2006; Geraud-Pouey et al. 2016). In 2007, a tomato plant showing mosaic symptoms and leaf deformation (Fig. 1a) was collected in Táchira state (sample 2007-07-30-02). In this plant, a PCR-based detection confirmed a begomovirus infection and the partial sequencing of the DNA-A showed 95% of nucleotide identity with a begomovirus species tentatively named tomato Venezuela virus (ToVEV) (Chirinos et al. 2012), which is closely related to PYMV. However, the full-length sequence of the DNA-A component is required to define the boundaries among begomovirus species (Brown et al. 2015). Hence, the whole genome sequencing of this begomovirus isolate was performed in this study to establish its correct identification at the species level. In addition, the transmission efficiency of this isolate by its vector, *B. tabaci*, was also evaluated.

Total DNA extraction from leaf tissue according to Gilbertson et al. (1991) was used to amplify the whole genome of the begomovirus infecting sample 2007-07-30-02 (hereafter referred as Ta935 isolate) by rolling circle amplification (RCA) using the TempliPhi kit (GE Healthcare, Munich, Germany). RCA products were digested with enzymes *EcoRI*, *HindIII*, *SspI*, and *XbaI*. The restriction pattern

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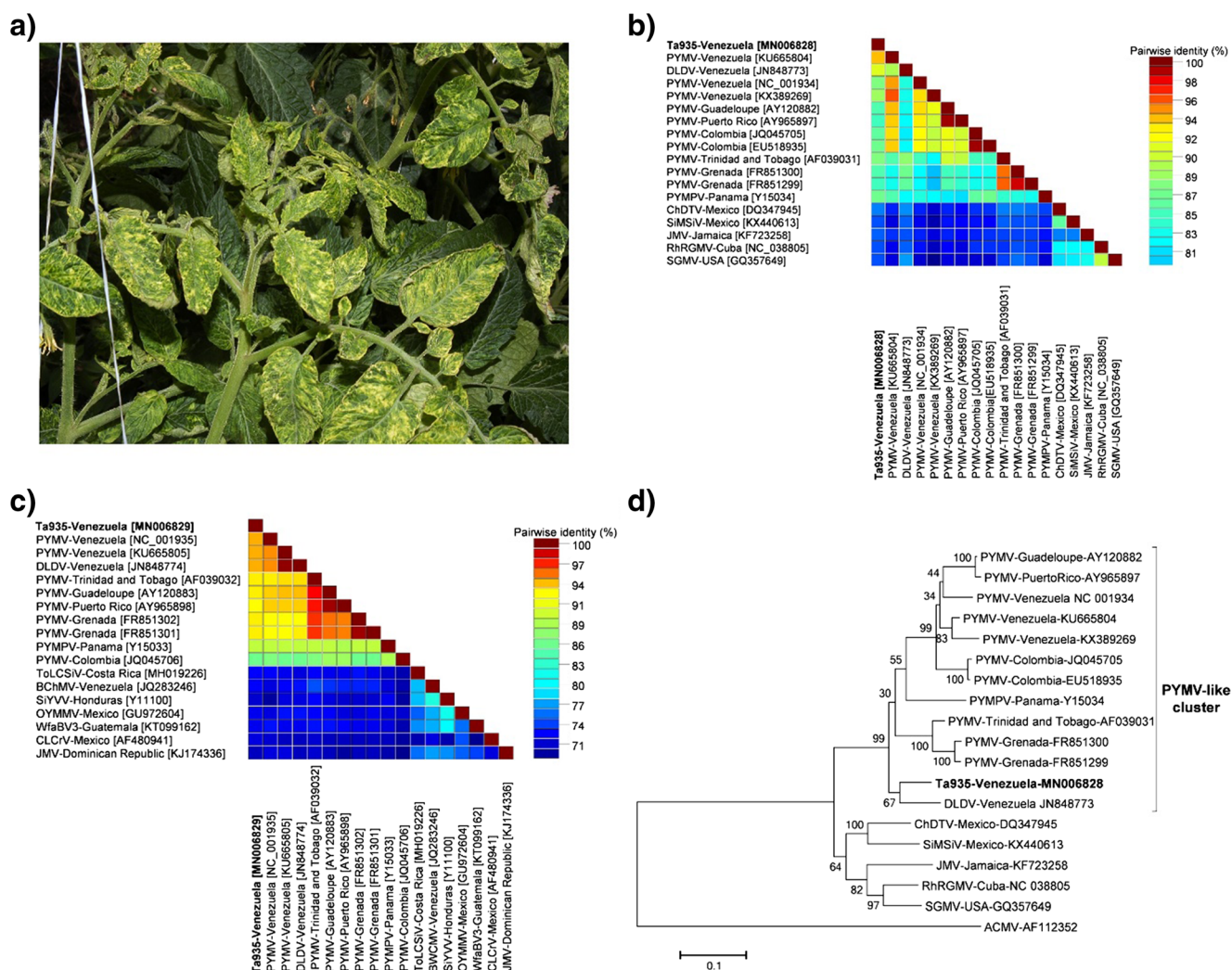


Fig. 1 Symptoms and genetic relationships of the new PYMV strain (Ta935 isolate) infecting tomato. **A)** Symptoms associated with Ta935 isolate of PYMV in tomato plants. **B)** Pairwise sequence identity analysis of DNA-A component of Ta935 isolate with related begomovirus sequences. **C)** Pairwise sequence identity analysis of DNA-B component of Ta935 isolate with related begomovirus sequences. **D)** Phylogenetic tree based on whole DNA-A sequences from Ta935 isolate and related begomoviruses. Phylogenetic tree was based on maximum likelihood method using GTR + G as nucleotide substitution model. Bootstrap values (500 iterations) are indicated for each node. African cassava mosaic virus (ACMV) sequences was used

of RCA products showed two different DNA components of ca. 2.6 kb. The RCA products digested with *Ssp*I were inserted into pBluescript II SK+ (Stratagene, La Jolla, USA) to transform *Escherichia coli* DH10B cells. Five clones were selected for sequencing and they corresponded to DNA-A and DNA-B components of a single begomovirus. The full-length of DNA-A was 2590 nucleotides (nt) (GenBank No. MN006828), while the full-length of DNA-B was 2538 nt (GenBank No. MN006829). Both components shared a common region of 161 nt with 94.4% identity, indicating that they are a cognate pair of the same viral isolate referred here as

as outgroup. Ta935 isolate is highlighted in bold. Countries and GenBank accession numbers were added to begomovirus isolates of bean chlorotic mosaic virus (BChMV), chilo del tomate virus (ChDTV), cotton leaf crumple virus (CLCrV), Datura leaf distortion virus (DLDV), Jatropha mosaic virus (JMV), okra yellow mosaic Mexico virus (OYMMV), potato yellow mosaic virus (PYMV), potato yellow mosaic Panama virus (PYMPV), Rhynchosia rugose golden mosaic virus (RhRGMV), Sida golden mosaic virus (SGMV), Sida mosaic Sinaloa virus (SiMSiV), Sida yellow vein virus (SiYVV), Sida yellow mottle virus (SiYMoV), tomato leaf curl Sinaloa virus (ToLCSiV) and whitefly-associated begomovirus V3 (WfABV3).

Ta935 isolate. The DNA components were compared with related begomovirus sequences available in GenBank. Sequence alignments were performed using MUSCLE (Edgard 2004) and pairwise sequence comparisons were conducted using SDT v.1.0 software (Muhire et al. 2014). The Fig. 1b showed that the DNA-A of isolate Ta935 shared the highest identity (93.5%) with a PYMV isolate (GenBank No KU665804) infecting *S. americanum* in Táchira state, Venezuela (Romay et al. 2016). Meanwhile, the DNA-B of this isolate shared the highest identity (91%) with the reference isolate of PYMV (GenBank No NC_001935) (Fig. 1c),

which was reported infecting potato in Venezuela (Roberts et al. 1986).

According to the demarcation threshold for begomovirus species proposed by the International Committee on Taxonomy of Viruses (ICTV), begomoviruses sharing DNA-A nucleotide identities equal or higher than 91% are considered to belong to the same species (Brown et al. 2015). Therefore, the isolate Ta935 should be considered as an isolate of PYMV. Moreover, the cut-off for begomovirus strain demarcation, suggested by the ICTV, is 94%. Thus, we propose isolate Ta935 as a new strain of PYMV (PYMV-[VE:Tachira-935:07]). It is noteworthy to mention that Ta935 isolate shared 92% of nucleotide identity with *Datura leaf distortion virus* (DLDV) (Fig. 1b). Although this percent identity is higher than 91% (cut-off for begomovirus species), the isolate Ta935 belongs to the species PYMV since it shares the highest percentage of pairwise identity with a PYMV isolate, in accordance to ICTV criteria (Brown et al. 2015). The nucleotide sequences of DNA-A from Ta935 isolate and related begomoviruses were aligned to estimate the optimal nucleotide substitution model and build a phylogenetic tree based on the maximum likelihood method using MEGA X (Kumar et al. 2018). The phylogenetic tree showed the isolate Ta935 within the PYMV-like cluster, in which two species, potato yellow mosaic Panama virus (PYMPV) and DLDV, were also included (Fig. 1d). Until now, only one full-length DNA-A of PYMPV and DLDV, respectively, are available in GenBank database. Although these begomoviruses are recognised as different species (Zerbini et al. 2017), the sequencing of more genomes from both begomoviruses might be needed to clarify whether they are different species or outlier isolates of PYMV. Furthermore, genetic recombination analyses were performed using RDP4 software (Martin et al. 2015). A recombination event was detected between nucleotides 133 and 1777, with a p -value < 0.05 , by the seven methods implemented in RDP4 software, suggesting a recombinant nature of this isolate. Among the recombination methods used in RDP4, the 3Seq method displayed the lowest p -value ($p = 6.19 \times 10^{-52}$). Interestingly, the recombination breakpoints were found spanning the genome region including the coat protein gene and the 3' end part of the AC1 gene that encodes the replication-associated protein (Rep). This genome region is not considered as a frequent recombination hot-spot in the genome of begomoviruses as compared with the 5' end of the Rep gene and the adjacent intergenic region (Lefevre et al. 2007). In fact, the putative recombination nature of Ta935 isolate is likely explained by the discrepancy between the pairwise sequence comparison (Fig. 1b) and the phylogenetic analysis (Fig. 1d). Tomato chlorotic leaf distortion virus (ToCLDV) (GenBank No JN241632) and an isolate of PYMV (GenBank No KX389269) were referred to as the potential major and minor parents, respectively. ToCLDV is a begomovirus reported infecting tomato and *Capsicum*

chinense in Zulia state, Venezuela (Zambrano et al. 2011). In a previous survey on Venezuelan tomato fields, this virus was found with a relative low prevalence (Geraud-Pouey et al. 2016). The isolate of PYMV considered as one of the putative parents of isolate Ta935 was found infecting the weed *S. americanum* in a tomato field in Lara state, Venezuela (Romay et al. 2016). In this country, tomato transplants are often produced in Lara state and then distributed to tomato growers throughout the major tomato-growing regions (Nava et al. 2006; Chirinos et al. 2014; Geraud-Pouey et al. 2016). This situation has contributed to disseminate begomoviral diseases including those caused by mixed infections, which lead to the generation of new recombinant isolates.

Additionally, in this work whitefly-mediated transmission assays of Ta935 were carried out to estimate the transmission efficiency of this isolate by its vector. Within the *B. tabaci* species complex, the most prevalent in Venezuela is the whitefly referred to as Middle East-Asia Minor 1 (hereon MEAM1) (Romay et al. 2011). Hence, a colony of non-viruliferous whitefly, previously identified as MEAM1 (Geraud-Pouey et al. 2009; Romay et al. 2010) was used for such assays. The transmission experiments were carried out according to Güerere et al. (2012). Individuals of a non-viruliferous whitefly colony raised on cotton (*Gossypium hirsutum*) were used to transmit Ta935 isolate to tomato seedlings, which were subsequently used as viral sources for transmission experiments. The PYMV infection on tomato plants was confirmed by PCR and sequencing of a ca. 1300 nt fragment of DNA-A using the universal primers for begomoviruses, PAL1v1978 and PAR1c715 (Rojas et al. 1993). Non-viruliferous whitefly adults were given a virus acquisition access period onto PYMV-infected tomato plants within an insect-proof cage for 48 h. Then, ten tomato plants were exposed to one, five and 20 adults of the whitefly MEAM1, respectively, and kept in separated insect-proof cages for two months for symptom expression. Six replicates were included in this study. The inoculated plants were evaluated at 30, 45 and 60 days post-inoculation (dpi) to record the onset of viral symptoms. Further, the begomovirus infection was confirmed by sequencing of PCR products using primers PAL1v1978 and PAR1c715. At 30, 45 and 60 dpi the percent of symptomatic plants were not significantly different when using one or five adults for virus inoculation and the transmission rate was around 80% at 60 dpi (Table 1). When inoculating PYMV with 20 adults per plant, the percent of symptomatic plants was significantly higher than inoculations mediated by one or five adults (Table 1).

Our results showed that the isolate Ta935 of PYMV can be transmitted with a high efficiency ($> 80\%$) by only one individual of the whitefly MEAM1, although the symptom appearance was delayed as compared to plants inoculated with 20 adults, in which 96.7% of plants displayed viral symptoms

Table 1 Percentages of tomato plants displaying PYMV symptoms after different days post inoculation (dpi) mediated by *B. tabaci*

Number of adults per plant	PYMV transmission rates (%) (<i>n</i> = 60 plants) ^a		
	30 dpi	45 dpi	60 dpi
1	13.3 ± 5.4b	59.8 ± 1.6b	83.6 ± 1.6a
5	18.3 ± 6.8b	64.2 ± 0.7b	88.2 ± 1.9a
20	75.0 ± 5.5a	96.7 ± 0.0a	100.0 ± 0.0a

^a Values with different letter indicate a significant difference between transmission rates observed when using 1, 5 and 20 adults within the indicated day post-inoculation (dpi). Values are means ± standard error. Means comparisons were performed with Tukey's test ($P < 0.05$)

at 45 dpi. Similar transmission rates were obtained by Cohen et al. (1983) after inoculations of the begomovirus squash leaf curl virus (SLCV) with one *B. tabaci* adult per plant and more than 15 adults were needed to reach a 100% transmission of SCLV. Overall, this study strengthens the need to produce virus- and vector-free transplants aiming to reduce the impact of viruliferous whitefly populations in the early stage of plant material. Such strategy along with others like removal of virus-infected plants during the growing season, selection plant dates and field locations represent efficient measures to control geminiviruses within an integrated pest management approach (Rojas et al. 2018).

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Compliance with ethical standards

Conflict of interest. The authors declare no conflict of interest.

Ethical approval. This article does not contain any studies with human participants or animals performed by any of the authors.

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