

**Characterization and genetic diversity of
Potato yellow mosaic virus from the Caribbean***

Brief Report

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Received August 5, 2002; accepted July 30, 2003

Published online November 4, 2003 © Springer-Verlag 2003

Summary. The begomovirus *Potato yellow mosaic virus* (PYMV) is responsible of significant yield losses in tomato in Guadeloupe. Four field isolates from Guadeloupe were analyzed in term of their host range using three inoculation methods (mechanical, grafting and insect vector), sequences analysis of PCR fragments and phylogenetic analysis of an infectious clone, PYMV-[GP]. *Capsicum annuum*, *Datura stramonium*, *Nicotiana benthamiana*, *N. tabacum* ‘Xanthi NC’, *Petunia hybrida*, and *Solanum tuberosum* were found to be hosts. All isolates from Guadeloupe, Martinique, Puerto Rico and the Dominican Republic were closely related to PYMV-[GP]. Sequence identity between PYMV-[GP] and PYMV-Ve from Venezuela and PYMTV from Trinidad and Tobago clearly confirmed that it is a new strain of PYMV.

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Since 1993, *Potato yellow mosaic virus* (PYMV) infects tomato in Guadeloupe [12]. Symptoms include mild to severe curling, a yellow mosaic on the leaves, and stunting. Infection also results in a general decrease of plant growth and yield losses. PYMV has become one of the most widespread bipartite begomoviruses of

*The sequences reported in this paper have been deposited into the GenBank under the accession numbers AF528501 to AF528503, AF528505, AY126611 to AY126614, AY120882 and AY120883.

tomato in the Caribbean. It was first identified in an isolate collected from potato in 1986 from Venezuela (PYMV-VE) [3, 13] and later from tomato in 1994 (PYMV-[Tom]) [8]. In the Caribbean, isolates of PYMV have been identified from tomato at Trinidad (*Potato yellow mosaic Trinidad virus*, PYMTV-TT) [16], Puerto Rico [12], Martinique [12], as well as Guadeloupe (PYMV-GP) [12]. The sequence of PYMTV-TT appears to be a recombinant virus derived from PYMV-VE and a virus similar to *Sida golden mosaic virus* from Honduras (SiGMHV) [16]. It has been proposed that *Tomato leaf curl virus* (ToLCV) from Panama is a strain of PYMV (*Potato yellow mosaic Panama virus*, PYMPV) based on sequence comparisons of this virus with PYMV-VE and PYMV-TT [4, 16]. More recently, PYMV has been proposed to be a synonym of *Tomato yellow mosaic virus* (ToYMV), a virus first recognized in Venezuela in the 1970's and recently partially sequenced [10]. Currently the taxonomy of PYMV strains is based primarily on genomic sequence and very few is known about biological characteristics.

Four isolates of PYMV from Guadeloupe were collected in different tomato producing areas in 1998: isolate GT, BT and MG were collected from symptomatic plants at Petit-Canal (Grande-Terre), Vieux-Habitants (Basse-Terre), and Marie-Galante Island respectively. A fourth isolate, IA, was obtained by placing whiteflies, collected from symptomatic tomato plants at Petit-Canal (Grande-Terre), onto a young *Lycopersicon esculentum* Mill. cv. 'Caraibo' plant. Isolate, IA was obtained by placing whiteflies, collected from symptomatic tomato plants onto a young *L. esculentum* cv. 'Caraibo' plant. Isolates GT, BT, MG and IA were maintained in an insect proof green house by stem grafting onto 'Caraibo' plant. Infectious clones of A and B component of PYMV (PYMV-[GP]) were obtained from a sample of a symptomatic tomato plant collected in 1995 at Baie Mahault (Basse-Terre). Isolate MQ, was collected in 1995 in Martinique from symptomatic tomato from Le Lorrain. The PYMV isolate from the Dominican Republic was obtained by PCR amplification from whiteflies collected in an abandoned tomato field near Santiago in 1995. Leaf samples from Martinique and Puerto-Rico were dried over calcium chloride for transport and storage.

The four isolates of PYMV from Guadeloupe (GT, BT, IA and MG) were tested for their ability to infect a range of plant species by grafting, whitefly transmission and mechanical inoculation. Test plants were: *L. esculentum* ('Caraibo'), *Nicotiana tabacum* L. 'Xanthi NC', *N. benthamiana* Domin., *Petunia hybrida* Hort. Vilm.-Andr., *Datura stramonium* L., *Capsicum annum* L. ('Narval' and 'Domingo'), and *Solanum tuberosum* L., progeny from crosses of 'Roseval' × 'Alemaria', 'Agata' × 'Penelope', and 'Stella' × 'Colmo'.

For whitefly transmission, adults, identified as *Bemisia tabaci* B biotype [15], were reared on virus free tomato plants. Groups of 50 whitefly adults were used to transmit virus from *L. esculentum* cv. 'Caraibo to all species cited above except *D. stramonium*. After a 48 h acquisition period on infected tomato plants and a 48 h inoculation period on test plants at the two-leaf stage, test plants were treated with imidacloprid to kill immatures and adults, then placed in greenhouses. Mechanical inoculation experiments were conducted by inoculating 12 to 15 day-old *L. esculentum*, *P. hybrida*, *D. stramonium*, *N. tabacum* and *N. benthamiana*

plants using 0.1M potassium phosphate buffer pH 8.0 containing 1% magnesium tri-silicate [7]. Graft transmission was accomplished by grafting 4 wk-old test plants of *L. esculentum*, *N. tabacum*, *N. benthamiana*, *S. tuberosum*, *P. hybrida* and *C. annuum* onto an infected stem (from *L. esculentum*, *N. benthamiana* or *C. annuum*). Five to fifteen plants were inoculated by each method. Successful transmission was determined 3 weeks after inoculation by visual assessment of symptoms and by ELISA (TYLCV Kit, Adgen Agrifood Diagnostic, Auchincruive, Ayr, Scotland, UK).

Total DNA was extracted from infected tomato plants using DNeasy Plant Mini Kit (Qiagen S.A. Courtaboeuf, France). Viral DNA was amplified using three different pairs of degenerate primers: MP16/MP82 [16] direct the amplification of a ~0.4 kbp fragment of the DNA-A of begomoviruses from the conserved nonanucleotide sequence to the 5'-region of the *CP* gene; PAL1v1978/PAR1c496 [14] amplify the 5' region of the *Rep* gene, the intergenic region and the 5'-region of the *CP* gene (~1.1 kbp) and PBL1v2040/PCRC154 [14] amplify a ~0.60 kbp region of the DNA-B component which includes the 5'-region of the movement protein (*MP*) gene, and the common region (CR). The PCR reactions were conducted as described in [14] and [16]. One to 3 PCR products of 0.4 kbp obtained from each of the four isolates were directly sequenced or cloned into pBluescript SK (+) as recommended by the manufacturer (Stratagene Cloning System, La Jolla, CA) and sequenced by Genome Express S.A. (Paris, France). The 1.1 kbp and the 0.6 kbp PCR fragments obtained from the PYMV isolate from the Dominican Republic and from Puerto Rico were cloned and sequenced as described before.

Sequences of the 0.4 kbp amplicon obtained from the 4 isolates from Guadeloupe were compared to each other and with the homologous sequence of PYMV-[GP] (GenBank accession numbers AY120882 and AY120883 for A and B components respectively). Sequences from PYMV-[GP] were then compared with 1.1 kbp and 0.6 kbp sequences obtained from the isolate from the Dominican Republic (PYMV-[DR], AY126611, A-comp; AY126614, B-component), Martinique (PYMV-[MQ], AY126610, A-component; AY126612, B-component), Puerto Rico (PYMV-[PR], AY126613, B-component), and with sequences from other viruses listed in Table 1 (Partial sequence of ToYMV is an unpublished sequence). Sequence comparisons were done using ClustalW. A BLASTN analysis was made using PYMV-[GP] sequences. Eight out of nine viruses with the highest bit scores were aligned and compared or translated, using Gap, part of the GenBank software package. Phylograms were generated using full-length viral sequences identified in the BLASTN analysis (see above). They were estimated using PAUP*'s heuristic method with maximum parsimony. Bootstrap replications (500) were performed using neighbor joining and tree bisectioning-reconnecting for heuristic method branch swapping.

The four viral isolates from Guadeloupe were transmitted by grafting, mechanical inoculation, and the whitefly vector to *L. esculentum* cv. 'Caraibo' and to *Nicotiana* species. Grafting and whitefly transmission resulted in 100% infection of tomato inoculated plants, however mechanical inoculation resulted in infection

Table 1. Sources and sequence accession numbers of viruses isolates used in the sequence alignments or comparisons in this paper

Virus	Source (country)	Accession number	Reference
PYMTV-TT	Tomato (Trinidad and Tobago)	AF039031, AF039032	[16]
PYMV-VE	Potato (Venezuela)	D00940, D00941	[3]
PYMPV	Tomato (Panama)	Y15034, Y15033	[4]
*PYMV-[Tom]	Tomato (Venezuela)	AF026553	[8]
*VeToV	Tomato (Venezuela)	AF026464	[8]
ToMoV	Tomato (Florida)	L14460, L14461	[1]
ChdTV-[IC]		AF101476, AF101478	[9]
ChdTV-[H8]		AF226664	[2]
ChdTV-[B52]		AF226666	[2]
SiGMHV	Honduras	Y11097, Y11098	[5]
AbMV		X15983, X15954	[6]

*Partial sequences

rates of only 25 to 50%. All isolates produced symptoms identical to those observed in the field: a yellow foliar mosaic, leaf curling, leaf distortion, reduction in leaf size and stunting of plants. Foliar symptoms appeared on newly developed leaves within 8 days after graft transmission or whitefly transmission, and 10 days after mechanical inoculation.

Symptoms on leaves were yellow spots and leaf distortion on inoculated and newly emerging leaves for *D. stramonium*, a bright yellow mosaic and distortion for *S. tuberosum*, a mild chlorosis and leaf curling leaves of *P. hybrida*, a mild yellow mosaic and distortion for *C. annuum* and a mild chlorosis and curling for the two *Nicotiana* species. Under our conditions, inoculation of *P. hybrida* was achieved only after grafting using infected *N. benthamiana* as scions. Successful inoculation of *C. annuum* with PYMV was achieved through whitefly transmission or graft transmission using infected pepper scions (7/10), but not with infected tomato scions (0/15).

Infectious full-length genomic clones of A and B component DNA of PYMV-[GP] were obtained from the same tissue. The two sequences showed a complete sequence identity in 154 nucleotide corresponding to the common region.

The 0.4 kbp fragment (intergenic region and 5' region of CP) obtained from PYMV isolates from Guadeloupe (GT, BT, MG and IA, Accession numbers AY528501 to AY528503 and AY528505) were at least 99.4% identical in nucleotide sequence to each other and 99.7 to 100% identical to equivalent region of PYMV-[GP]. Partial A and B components sequences obtained from each of the isolates from the Dominican Republic, Martinique, and Puerto Rico showed a complete sequence identity on 135 nucleotides suggesting that the two sequences belonged to the same virus. Sequence comparison based on these sequences showed that PYMV-[GP] was most closely related to PYMV isolates from the Dominican Republic, Martinique, and Puerto Rico (Table 2) with at least 99%

Table 2. Comparison of nucleic acid sequence of *Potato yellow mosaic virus* from Guadeloupe (PYMV-[GP]) with sequences of other isolates of PYMV and related viruses using partial sequences

Isolate	A component ~1100 nt	B component ~593 nt	CR ~154 nt
PYMV [DR]	99.6	98.2	100.0
PYMV [MQ]	99.6	98.4	100.0
PYMV [PR]	99.0	99.5	99.4
PYMV-[Tom]	93.7	N/A	95.6
ToYMV	93.1	N/A	96.3
PYMV-VE	91.7	73.8	95.5
PYMTV-TT	82.0	89.6	64.4
VeTo virus	80.5	N/A	74.0
PYMPV	74.2	82.6	75.2

nt = nucleotide; CR = Common region (non-coding)
(1.1 kbp from the A component and a 0.6 kbp from the B component)

and 98% identity on A and B component respectively. PYMV-[GP] shared less sequence identity in decreasing order with PYMV-[Tom], ToYMV, PYMV-VE and PYMTV-TT, VeTo virus and PYMPV.

Full length nucleic acid sequences alignment revealed that the A component of PYMV-[GP] had greater homology with that of PYMV-VE, however the B component had greater homology with that of PYMTV-TT (Fig. 1). Comparison of the predicted amino acid sequences of individual protein and nucleic acid sequence of the CR indicated that PYMV-[GP] shared greater homology with PYMV-VE in the CR, Rep, AC4, and CP and with PYMTV-TT in transcriptional activator protein (TrAP), replication enhancer (REn), movement protein (MP), and nuclear shuttle protein (NSP) (Table 3). After PYMTV-TT and PYMV-VE, PYMV-[GP] shared the greatest homology with PYMPV and less homology with ToMoV, SGMHV, ChdTV strains, and AbMV.

PYMV isolates from Guadeloupe are transmitted by insect vector, mechanical inoculation and grafting. The host range of these isolates was similar to that of PYMTV-TT and PYMV-VE in those hosts that were naturally infected or experimentally inoculated with these strains [4, 11, 13, 16, 17]. *D. stramonium* was found to be an experimental host which has not been reported for PYMV before, although it was reported as an host of ToYMV [17].

Comparison of partial sequences of A and B component including amino terminal of the *Rep*, the common region of both A and B component and the first 125 nucleotides of the *CP* revealed that the PYMV isolates from Guadeloupe, Martinique, Dominican Republic and Puerto Rico form a closely related group that have a greater sequence homology among themselves than with other PYMV isolates or other begomovirus species. This is consistent with the conclusion that no or little polymorphism was found in these region of the genome for PYMV

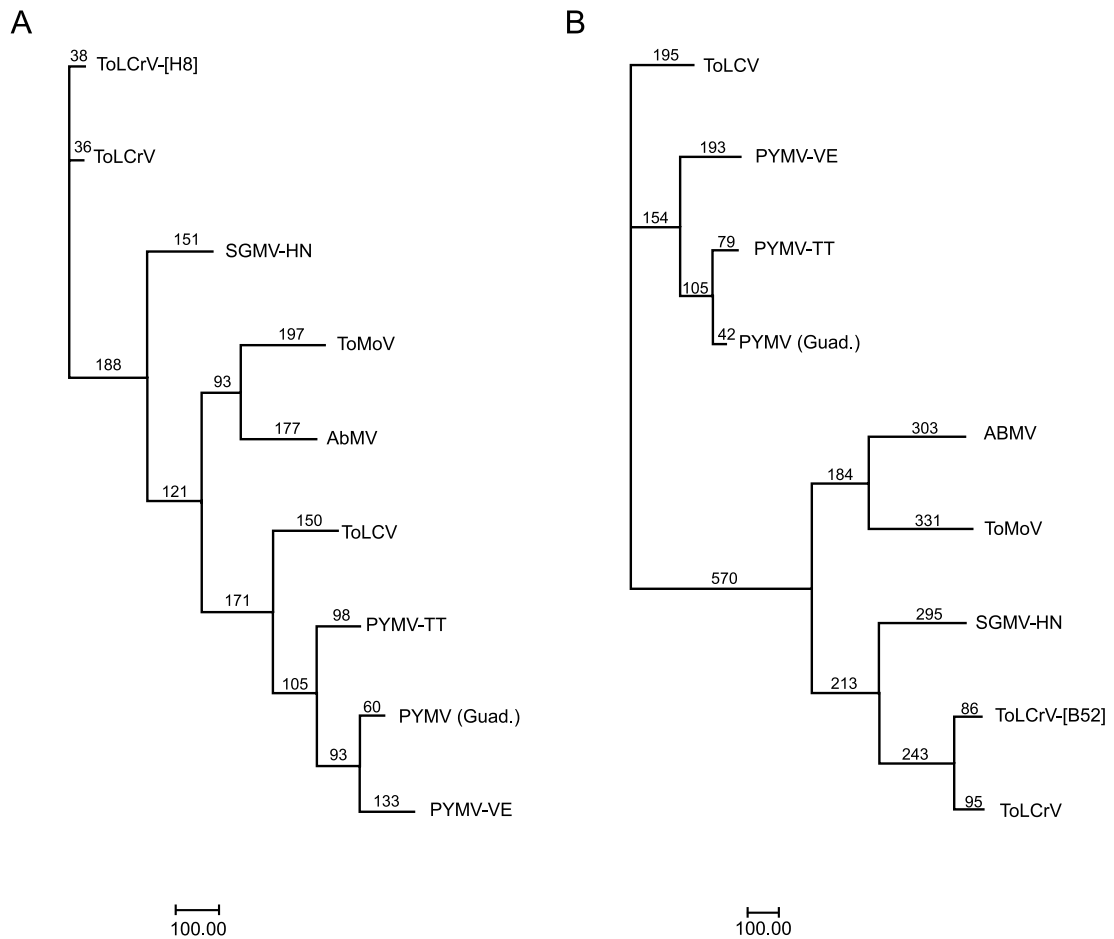


Fig. 1. Phylograms were generated using PAUP*s heuristic method with maximum parsimony. Bootstrap replications (500) were performed using neighbor joining and tree bisectioning-reconnecting for heuristic method branch swapping. Abbreviations are as described in Materials and methods. **A** Phylogram of full-length A component sequences; **B** Phylogram of full-length B component sequences

isolates amplified from 34 samples from Guadeloupe, Martinique, and Puerto Rico [12]. PYMV-[GP] could be an emergent indigenous virus or a recent introduction to these Caribbean islands widely spaced and the sequence of PYMV-[GP] could be representative of these isolates. According to the DNA-A nucleotide sequence identity (92.8%) with the type strain of PYMV (PYMV-Ve), we can state that PYMV-[GP] is a newly identified strain of PYMV.

The comparison with a partial sequence from ToYMV from Venezuela suggests that PYMV-[GP] is as closely related to ToYMV as it is to PYMV-VE. This is consistent with the proposition that PYMV could be considered a synonym of ToYMV.

Table 3. Comparison of the nucleotide sequence of common region and the amino acid sequence similarity of an infectious clone of *Potato yellow mosaic virus* from Guadeloupe (PYMV-[GP]) with other PYMV isolates and related viruses

Isolate	CR	Rep	TrAP	REn	AC4	CP	MP	NSP
PYMV-VE	91.7	94.75	96.90	92.98	84.88	99.20	92.25	97.62
PYMV-TT	82	87.57	98.45	96.99	66.28	98.80	96.90	99.32
ToLCV	74.2	86.19	92.25	93.23	58.14	99.20	86.82	97.28
ToMoV	73.5	83.15	86.05	89.47	61.63	95.24	72.87	91.16
SGMV-HN	72.6	83.98	83.72	83.46	67.44	95.24	74.81	90.82
ChdTV [IC]	72.7	84.25	84.50	87.22	65.12	97.62	74.71	89.80
ChdTV-[H8]	73.4	83.98	84.50	89.47	60.47	97.62		
ChdTV-[B52]							73.26	89.46
AbMV	70.4	82.04	83.72	85.71	58.14	94.05	73.26	90.14

CR = common region, Rep = replication protein, TrAP = transcriptional activator, Ren = replication enhancer, CP = coat protein, MP = movement protein, NSP = nuclear shuttle protein

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