

Detection of *Plantago asiatica* mosaic virus in lily hybrid plants (*Lilium* spp.) in Costa Rica grown from imported bulbs

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Abstract *Plantago asiatica* mosaic virus (PIAMV) was detected in Costa Rica infecting lily plants showing foliar chlorotic and necrotic streaking. Virus identity was established by TEM, sequencing and ELISA. Pathogenicity was confirmed on *Nicotiana benthamiana* by mechanical inoculation, and PIAMV was detected by ELISA and RT-PCR.

Keywords *Potexvirus* · ELISA · RT-PCR · PIAMV · International trade of infected plants · Emerging viral disease

Plantago asiatica mosaic virus (PIAMV) (genus *Potexvirus*; family *Alphaflexiviridae*; order *Tymovirales*) infects lily plants (*Lilium* spp.) among other hosts (Komatsu et al. 2017). PIAMV was initially described from *Plantago asiatica* in Russia (Kostin and Volkov 1976, in: Solovyev et al. 1994). Currently, PIAMV is distributed in several countries in Asia, Europe and the Americas, including Chile (Vidal et al. 2016), China (Xu et al. 2017), Hungary (Pájtli et al. 2015), Italy (Parrella et al. 2015), Japan (Ozeki et al. 2006), Korea (Lim et al. 2016), and the USA (Hammond et al. 2015), and causing up to 80% losses in The Netherlands (Anonymous 2010). In addition, a virus infecting heavenly bamboo (*Nandina domestica*) in California, named Nandina mosaic virus

(Zettler et al. 1980), is now recognized as a strain of PIAMV (Hughes et al. 2005).

Reports suggest that PIAMV is disseminated internationally by trading of infected bulbs (Hammond et al. 2015; Pájtli et al. 2015; Parrella et al. 2015). Moreover, PIAMV is mechanically transmitted (by sap) and no arthropod vector is known. The virus naturally infects hosts belonging to diverse plant families including Plantaginaceae (plantago), Liliaceae (lily), Primulaceae (primrose), Violaceae (violet), Scrophulariaceae (Chinese foxglove), and Berberidaceae (nandina) (Kostin and Volkov 1976, in: Solovyev et al. 1994; Komatsu et al. 2008, 2017; Kwon et al. 2017); and experimental hosts from additional plant families (Kostin and Volkov 1976; J. Hammond, unpublished).

During a survey to detect viruses belonging to the genus *Orthospovirus* (family *Tospoviridae*; order *Bunyavirales*) (Adams et al. 2017) in Costa Rica, two Asiatic lily hybrid plants were collected at a greenhouse in Cartago Province on June 2013 (samples T188 and T189). The symptoms included brown necrotic streaking and irregular leaf chlorosis (Fig. 1a, b). RNA was extracted (RNeasy Plant Mini Kit, Qiagen, Germany) and cDNA was obtained by retrotranscription (Maxima H Minus First Strand cDNA Synthesis Kit, Thermo Scientific, Lithuania) primed with random hexamers. cDNAs were tested by PCR (DreamTaq PCR Master Mix, Thermo Scientific, Lithuania) with five different primer pairs for orthospovirus detection (Eiras et al. 2001; Uga and Tsuda 2005). An amplicon under 500 bp was obtained for both plants, T188 and T189, using primer pair IYSV-837 / Tos-R15 (Uga and Tsuda 2005) and sequenced (Macrogen, Korea) in both directions. The resulting sequences (T188, KY807780; T189, KY807781) had top BLAST hits in GenBank (National Center for Biotechnology Information, USA) to isolates of PIAMV. The unexpected amplification of PIAMV sequence using primers designed to detect

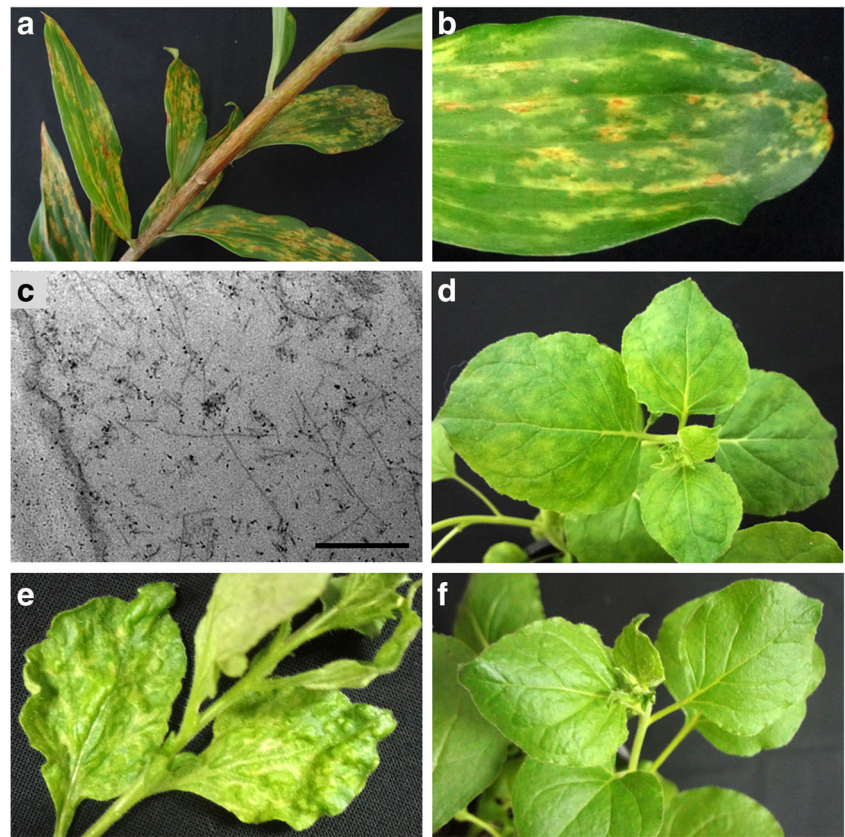
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Fig. 1 Detection and pathogenicity of *Plantago asiatica* mosaic virus (PIAMV) in Costa Rica. Symptoms of PIAMV observed on a *Lilium* sp. plant (T188) (a, b). Transmission electron microscopy (TEM) image from sample T188 leaf tissue with several flexuous rods in the cytoplasm of a cell (c); scale bar represent 500 nm. Symptoms observed on *Nicotiana benthamiana* after mechanical inoculation with sample T189, 22 days after inoculation (dai) (d), with sample T188, 36 dai (e), and buffer-inoculated plant, 22 dai (f)



tosspoviruses was investigated by aligning the sequences of primers IYSV-837 and Tos-R15 against the PIAMV genome. All alignments identified included multiple mismatches,

particularly with primer Tos-R15, which has five two-fold degenerate positions near the 3' end, and potential to prime on both strands of the PIAMV genome. The initial detection of

Table 1 *Plantago asiatica* mosaic virus (PIAMV) detection^a in lily samples in Costa Rica

Sample	DAS-ELISA ^b	Amplification by RT-PCR ^c (GenBank accession numbers for obtained sequences)	
		Potex 4 / Potex 5 (<i>Potexvirus</i>)	CP-F2 / CP-R3 (PIAMV)
Greenhouse lily, T188	+	+ (KY807782)	+ (KY807776)
Greenhouse lily, T189	+	+ (KY807784)	+ (KY807777)
<i>N. benthamiana</i> inoculated with T188	+	+ (KY807783)	+ ^f
<i>N. benthamiana</i> inoculated with T189	+	+ (KY807785)	+ (KY807778)
Supermarket lily, L1 ^d	Nt	–	–
Supermarket lily, L2 ^d	Nt	–	–
Supermarket lily, L3 ^d	Nt	+ (KY807786)	+ (KY807779)
Supermarket lily, L4 ^d	Nt	–	–
Potexvirus positive control (<i>CymMV</i>) ^e	Nt	+ (KY807788)	–

^a Positive result (+), negative result (–), or not tested (Nt)

^b Double antibody sandwich ELISA for the detection of *Nandina* mosaic virus, an isolate of the virus PIAMV

^c Primer pairs used herein for the detection of virus isolates belonging to: *i.* the viral genus *Potexvirus*, Potex4/Potex5 (Miglino et al. 2006), and *ii.* the species *Plantago asiatica* mosaic virus, CP-F2/CP-R3 (Hammond et al. 2015)

^d Asymptomatic plants

^e Cymbidium mosaic virus (*CymMV*) infecting *Phaius tankervilleae* (Moreira et al. 1998)

^f No final contig. Sequencing reaction with forward primer resulted in PIAMV sequence; however, the reaction with reverse primer failed

PIAMV using ‘tospovirus-specific’ primers was verified by testing with primer pairs for general potexvirus detection (Potex 4 / Potex 5, Miglino et al. 2006) and for specific detection of PIAMV (PIAMV CP-F2 / CP-R3, Hammond et al. 2015). As negative controls, four symptomless lily plants (two pots, 2 plants per pot) were purchased at a supermarket in San José Province. RNA was extracted from each plant (L1 to L4) and processed as

previously described. As a positive control for potexvirus detection, RNA was extracted from a *Phaius tankervilleae* plant infected with Cymbidium mosaic virus (CymMV) (genus *Potexvirus*) (Moreira et al. 1998). The two greenhouse lily plants (T188 and T189) and one of the supermarket (symptomless) plants (L3) yielded positive results with both generic potexvirus, and PIAMV-specific, primer pairs (Table 1).

Table 2 Pairwise nucleotide comparisons^a (estimates of evolutionary divergence between sequences) for 24 *Plantago asiatica* mosaic virus isolates and one isolate each of tulip virus x and Cymbidium mosaic virus

Part A. Virus isolates		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	KY807782_PIAMV_(T188)_CRI		0.003	0.003	0.004	0.016	0.004	0.016	0.014	0.014	0.014	0.015	0.014	0.014	0.013
2	KY807784_PIAMV_(T189)_CRI	0.007		0.002	0.004	0.016	0.004	0.016	0.014	0.014	0.014	0.015	0.014	0.014	0.013
3	KY807786_PIAMV_(L3)_CRI	0.007	0.003		0.004	0.016	0.004	0.016	0.014	0.014	0.014	0.015	0.014	0.015	0.013
4	KF471012_PIAMV_(Sorbonne)_NED	0.012	0.012	0.012		0.016	0.003	0.016	0.014	0.014	0.014	0.014	0.014	0.014	0.013
5	NC003849_PIAMV_RUS	0.169	0.168	0.168	0.174		0.016	0.015	0.016	0.016	0.016	0.016	0.016	0.016	0.016
6	LN794199_PIAMV_(Concador)_HUN	0.010	0.010	0.010	0.005	0.169		0.016	0.014	0.014	0.014	0.014	0.014	0.014	0.013
7	AY800279_NaMV_(PLH1)_USA	0.183	0.178	0.178	0.183	0.164	0.183		0.016	0.016	0.016	0.016	0.016	0.016	0.016
8	AB360790_PIAMV_(Li1)_JPN	0.141	0.141	0.141	0.142	0.176	0.139	0.183		0.000	0.000	0.003	0.002	0.003	0.011
9	AB360791_PIAMV_(Li2)_JPN	0.141	0.141	0.141	0.142	0.176	0.139	0.183	0.000		0.000	0.003	0.002	0.003	0.011
10	AB360792_PIAMV_(Li3)_JPN	0.141	0.141	0.141	0.142	0.176	0.139	0.183	0.000	0.000		0.003	0.002	0.003	0.011
11	AB360793_PIAMV_(Li4)_JPN	0.142	0.142	0.142	0.144	0.178	0.141	0.184	0.005	0.005	0.005		0.002	0.003	0.011
12	AB360794_PIAMV_(Li5)_JPN	0.139	0.139	0.139	0.141	0.174	0.137	0.181	0.002	0.002	0.002	0.003		0.003	0.011
13	AB360795_PIAMV_(Li6)_JPN	0.142	0.142	0.142	0.144	0.174	0.141	0.183	0.007	0.007	0.007	0.008	0.005		0.011
14	AB360796_PIAMV_(Pr)_JPN	0.127	0.126	0.129	0.127	0.179	0.124	0.198	0.079	0.079	0.079	0.080	0.077	0.082	
15	LC155796_PIAMV_(Vi)_JPN	0.204	0.206	0.206	0.203	0.206	0.199	0.214	0.194	0.194	0.194	0.196	0.193	0.193	0.211
16	LC155795_PIAMV_(NJ)_JPN	0.198	0.194	0.194	0.199	0.181	0.199	0.184	0.198	0.198	0.198	0.199	0.196	0.198	0.219
17	KX245539_PIAMV_(CAAS01)_CHN	0.010	0.010	0.010	0.005	0.171	0.003	0.179	0.139	0.139	0.139	0.141	0.137	0.141	0.124
18	KU159093_PIAMV_(Ko-YS)_KOR	0.012	0.012	0.012	0.007	0.174	0.005	0.184	0.141	0.141	0.141	0.142	0.139	0.142	0.126
19	KU159089_PIAMV_(Ko-JJ_2-1)_KOR	0.008	0.008	0.008	0.003	0.171	0.002	0.181	0.141	0.141	0.141	0.142	0.139	0.142	0.126
20	KU159091_PIAMV_(Ko-JJ_2-2)_KOR	0.008	0.008	0.008	0.003	0.171	0.002	0.181	0.141	0.141	0.141	0.142	0.139	0.142	0.126
21	KU159090_PIAMV_(Ko-JJ_2-4)_KOR	0.008	0.008	0.008	0.003	0.171	0.002	0.181	0.141	0.141	0.141	0.142	0.139	0.142	0.126
22	KU159092_PIAMV_(Ko-JJ_3-1)_KOR	0.010	0.010	0.010	0.005	0.171	0.003	0.183	0.139	0.139	0.139	0.141	0.137	0.141	0.124
23	KU697313_PIAMV_(Gunwi)_KOR	0.162	0.156	0.159	0.164	0.127	0.161	0.191	0.173	0.173	0.173	0.174	0.171	0.173	0.176
24	KT717325_PIAMV_(kr)_KOR	0.010	0.010	0.010	0.005	0.173	0.003	0.183	0.141	0.141	0.141	0.142	0.139	0.142	0.126
25	AB066288_TVX_JPN	0.228	0.228	0.228	0.231	0.238	0.229	0.261	0.245	0.245	0.245	0.246	0.243	0.245	0.255
26	NC001812_CymMV_SGP	0.400	0.400	0.397	0.402	0.382	0.399	0.387	0.410	0.410	0.410	0.412	0.409	0.407	0.399

Part B. Virus isolates		15	16	17	18	19	20	21	22	23	24	25	26
1	KY807782_PIAMV_(T188)_CRI	0.016	0.016	0.004	0.004	0.004	0.004	0.004	0.004	0.016	0.004	0.017	0.020
2	KY807784_PIAMV_(T189)_CRI	0.016	0.016	0.004	0.004	0.004	0.004	0.004	0.004	0.015	0.004	0.017	0.020
3	KY807786_PIAMV_(L3)_CRI	0.016	0.016	0.004	0.004	0.004	0.004	0.004	0.004	0.016	0.004	0.017	0.020
4	KF471012_PIAMV_(Sorbonne)_NED	0.016	0.016	0.003	0.003	0.002	0.002	0.002	0.003	0.016	0.003	0.017	0.020
5	NC003849_PIAMV_RUS	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.018	0.019
6	LN794199_PIAMV_(Concador)_HUN	0.016	0.016	0.002	0.003	0.002	0.002	0.002	0.002	0.016	0.002	0.017	0.020
7	AY800279_NaMV_(PLH1)_USA	0.017	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.018	0.019
8	AB360790_PIAMV_(Li1)_JPN	0.016	0.017	0.014	0.014	0.014	0.014	0.014	0.014	0.016	0.014	0.018	0.020
9	AB360791_PIAMV_(Li2)_JPN	0.016	0.017	0.014	0.014	0.014	0.014	0.014	0.014	0.016	0.014	0.018	0.020
10	AB360792_PIAMV_(Li3)_JPN	0.016	0.017	0.014	0.014	0.014	0.014	0.014	0.014	0.016	0.014	0.018	0.020
11	AB360793_PIAMV_(Li4)_JPN	0.016	0.017	0.014	0.015	0.014	0.014	0.014	0.014	0.016	0.014	0.018	0.020
12	AB360794_PIAMV_(Li5)_JPN	0.016	0.017	0.014	0.014	0.014	0.014	0.014	0.014	0.016	0.014	0.018	0.020
13	AB360795_PIAMV_(Li6)_JPN	0.016	0.017	0.014	0.014	0.014	0.014	0.014	0.014	0.016	0.014	0.018	0.020
14	AB360796_PIAMV_(Pr)_JPN	0.017	0.017	0.013	0.013	0.013	0.013	0.013	0.013	0.016	0.013	0.018	0.020
15	LC155796_PIAMV_(Vi)_JPN		0.017	0.016	0.016	0.016	0.016	0.016	0.016	0.017	0.016	0.018	0.020
16	LC155795_PIAMV_(NJ)_JPN	0.221		0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.016	0.018	0.020
17	KX245539_PIAMV_(CAAS01)_CHN	0.199	0.198		0.003	0.002	0.002	0.002	0.002	0.016	0.002	0.017	0.020
18	KU159093_PIAMV_(Ko-YS)_KOR	0.201	0.198	0.005		0.002	0.002	0.002	0.003	0.016	0.003	0.017	0.020
19	KU159089_PIAMV_(Ko-JJ_2-1)_KOR	0.201	0.198	0.002	0.003		0.000	0.000	0.002	0.016	0.002	0.017	0.020
20	KU159091_PIAMV_(Ko-JJ_2-2)_KOR	0.201	0.198	0.002	0.003	0.000		0.000	0.002	0.016	0.002	0.017	0.020
21	KU159090_PIAMV_(Ko-JJ_2-4)_KOR	0.201	0.198	0.002	0.003	0.000	0.000		0.002	0.016	0.002	0.017	0.020
22	KU159092_PIAMV_(Ko-JJ_3-1)_KOR	0.203	0.199	0.003	0.005	0.002	0.002	0.002		0.016	0.002	0.017	0.020
23	KU697313_PIAMV_(Gunwi)_KOR	0.213	0.181	0.162	0.162	0.162	0.162	0.162	0.162		0.016	0.017	0.020
24	KT717325_PIAMV_(kr)_KOR	0.203	0.198	0.003	0.005	0.002	0.002	0.002	0.003	0.162		0.017	0.020
25	AB066288_TVX_JPN	0.253	0.253	0.231	0.231	0.229	0.229	0.229	0.229	0.221	0.231		0.020
26	NC001812_CymMV_SGP	0.379	0.410	0.399	0.402	0.400	0.400	0.400	0.402	0.394	0.399	0.425	

^a p-distance values shown below the diagonal (black font) calculated in Mega7 (Kumar et al. 2016) with 5000 permutations and the rate variation among sites was modeled with a gamma distribution (shape parameter = 1). Blue font values above the diagonal are standard error estimates

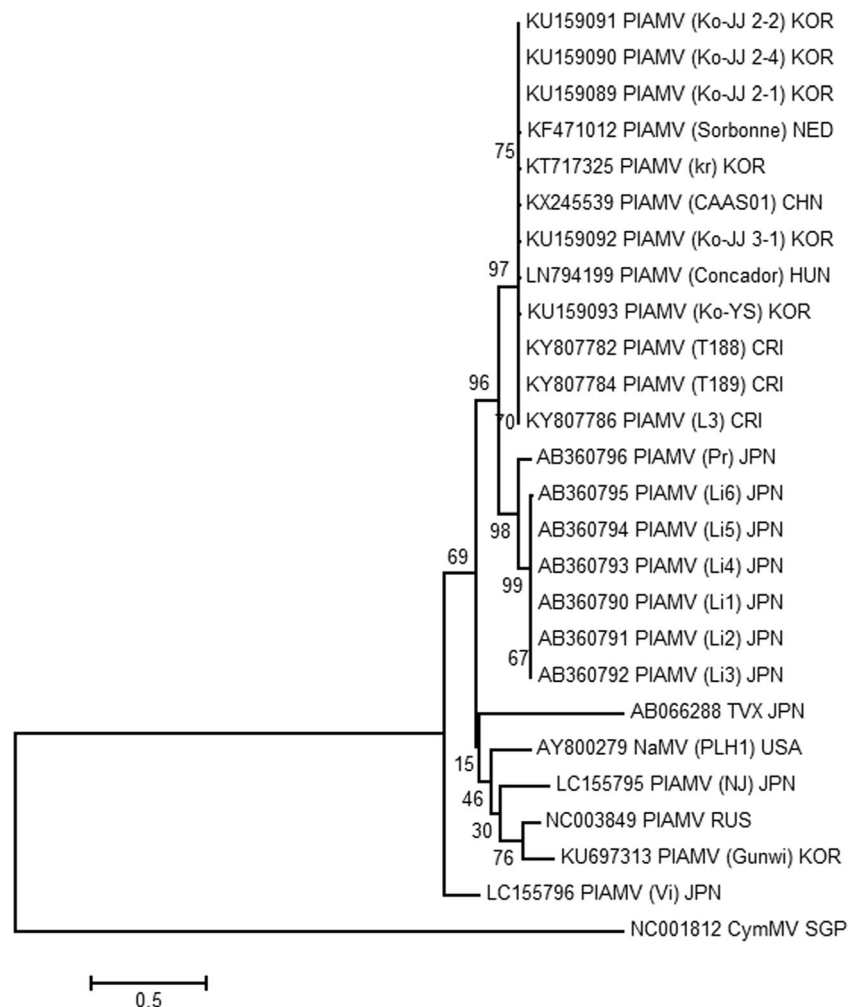
Small leaf segments (1 × 2 mm) were harvested around chlorotic and necrotic lesions on leaves of one greenhouse lily sample (T188) and were processed for transmission electron microscopy (TEM) as previously described (Montero-Astúa et al. 2017). We detected individual flexuous-rod shaped particles scattered in the cytoplasm (Fig. 1c) similar to those associated with potexviruses (Purcifull et al. 1966). Particle average length was measured from micrographs of tissue sections using ImageJ software (Schneider et al. 2012), selecting only those particles that were clearly visible and not in aggregates. Average particle length was estimated at 516 nm with a 95% confidence interval 499–533 nm ($n = 60$), consistent with PIAMV particle size (490 to 530 nm, Kostin and Volkov 1976, in: Solovyev et al. 1994).

The virus was mechanically inoculated to *Nicotiana benthamiana* plants and symptoms were observed. Frozen leaf tissue (stored at -35°C) from each greenhouse lily sample (T188 and T189) was ground in ice-cold 100 mM Na_2SO_3 using chilled mortars and pestles. The extracts were rub-inoculated with carborundum and cotton swabs on two-week old *N. benthamiana* plants (four plants per sample). No

symptoms were visible at 15 days after inoculation (dai). A mild mottle was observed at 22 dai (Fig. 1d) and young leaf corrugation and severe chlorotic patterns were observed 36 dai (Fig. 1e) on three and four plants inoculated with T188 and T189 extracts, respectively. One mock inoculated plant (buffer) presented no symptoms (Fig. 1f).

Leaf tissue from the two greenhouse lily samples (T188 and T189) and from the corresponding mechanically inoculated *N. benthamiana* plants tested positive by DAS-ELISA with commercial antibodies against the Nandina mosaic strain of PIAMV (Agdia Inc., Indiana, USA). Moreover, RT-PCR and sequencing (as described previously) from inoculated *N. benthamiana* tissue yielded positive results and sequences corresponding to PIAMV (Table 1). Virus partial sequences obtained from inoculated *N. benthamiana* plants (GenBank accession numbers: KY807783 and KY807785) had the same SNPs as the corresponding source virus sequences (KY807782 and KY807784). Therefore, we confirmed the identity of the virus and its transmission and pathogenicity to *N. benthamiana*. Additionally, we obtained for the first time sequence confirmation (KY807788) for CymMV from Costa

Fig. 2 Phylogenetic tree constructed with Plantago asiatica mosaic virus (PIAMV) sequences, each corresponding to two concatenated fragments of the virus genome, including a partial sequence of the RdRp ORF and a partial sequence of the coat protein ORF adding to a final alignment of 689 nucleotide positions with gaps. Each sequence code indicates GenBank accession number, virus species, (isolate, when available) and geographical origin (three letter country code). Sequences were aligned with MUSCLE algorithm in MEGA7 and phylogeny inferred with the maximum likelihood method using a Hasegawa-Kishino-Yano model (HKY) with G + I variation rate across sites and 5000 bootstrap replicates. Scale bar represents 0.05 substitutions per site



Rica (potexvirus positive control in *P. tankervilleae*, Moreira et al. 1998). The nucleotide sequences obtained for PIAMV and CymMV are available in GenBank under accession numbers KY807776-KY807788.

The partial sequences obtained with primer pairs Potex 4 / Potex 5 (Miglino et al. 2006) and CP-F2 / CP-R3 (Hammond et al. 2015) for samples T188, T189 and L3 were aligned with corresponding sequences from available PIAMV genomes from GenBank (Table 2) using MUSCLE (Edgar 2004) algorithm in MEGA7 (Kumar et al. 2016). The species *Tulip virus X* (Yamaji et al. 2001) and *Cymbidium mosaic virus* (Wong et al. 1997) were included as outgroups. The two sequence alignments: (i) a fragment of the polymerase ORF (3350–3616, nucleotide positions from Russian reference genome sequence NC003849) and (ii) a fragment of the coat protein ORF (5664–6059) were concatenated with FaBox (Villesen 2007). Phylogenetic analysis was done using a maximum likelihood method with a Hasegawa-Kishino-Yano model (HKY) and a gamma-shaped rate variation across sites with a proportion of invariable sites (G + I) and 5000 permutations. A Japanese isolate of tulip virus X (TVX) grouped closely with isolates of PIAMV as previously reported (Yamaji et al. 2001). Three putative PIAMV groups were indicated by the dendrogram (Fig. 2). Two clades are supported by a 96% bootstrap value: one group includes the three lily isolates from Costa Rica, plus isolates from China, Hungary, Korea and The Netherlands; a second clade includes Japanese isolates from lily and one from primrose (Pr, *Primula sieboldii*). A third loose group includes isolates from *N. domestica* (USA and Japan) and from *P. asiatica* (Russia and Korea). Two Japanese isolates, one from *N. domestica* (NJ) and another from *Viola grypoceras* (Vi) were separated from other Japanese isolates in accordance with a previous report (Komatsu et al. 2017). Komatsu et al. (2008) indicated that the species *Plantago asiatica mosaic virus* shows high biological and genomic variability. Pairwise nucleotide comparisons (calculated with MEGA7, Table 2) for the concatenate sequence dataset of 24 PIAMV isolates used herein has a mode of 0.141. The highest distance (0.221 ± 0.017) was found between two Japanese PIAMV isolates; one from viola (LC155795) and another from nandina (LC155795). Costa Rican isolates were very similar to one another (0.003 ± 0.002 , or 0.007 ± 0.003), and showed the highest distance (0.204 ± 0.016 , or 0.206 ± 0.016) to the Japanese PIAMV isolate from viola (LC155795), with the lowest distance values (0.008 ± 0.004) to three Korean lily isolates (KU159089, KU159090, and KU159091).

To our knowledge, this is the first report of PIAMV in Costa Rica. We hypothesize the virus was introduced with imported lily bulbs as has been indicated for other countries (Hammond et al. 2015; Pájtli et al. 2015; Parrella et al. 2015; Vidal et al. 2016; Xu et al. 2017). Symptomless infection of lily was noted in Chile (Vidal et al. 2016) and China (Xu et al.

2017). We also detected a symptomless infection in a commercialized lily plant increasing the risk for unobserved movement and transmission of the disease. These results suggested the need for enhanced virus testing schemes for bulbs and plants to avoid virus dissemination worldwide and emergence of novel diseases due to new virus-host encounters.

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References

- Adams MJ, Lefkowitz EJ, King AMQ, Harrach B, Harrison RL, Knowles NJ, Kropinski AM, Krupovic M, Kuhn JH, Mushegian AR, Nibert M, Sabanadzovic S, Sanfaçon H, Siddell SG, Simmonds P, Varsani A, Zerbini FM, Gorbalenya AE, Davison AJ (2017) Changes to taxonomy and the international code of virus classification and nomenclature ratified by the international committee on taxonomy of viruses (2017). Arch Virol 162:2505–2538. <https://doi.org/10.1007/s00705-017-3358-5>
- Anonymous (2010) *Plantago asiatica mosaic virus* on *Lilium* spp.: pest report – The Netherlands. Plant protection Service of the Netherlands. https://145.12.37.103/txmpub/files/?p_file_id=2001424. Accessed 30 Oct 2017
- Edgar RC (2004) MUSCLE: a multiple sequence alignment method with reduced time and space complexity. BMC Bioinform 5:113. <https://doi.org/10.1186/1471-2105-5-113>
- Eiras M, Resende RO, Missiaggia AA, de Ávila AC (2001) RT-PCR and dot blot hybridization methods for a universal detection of tospoviruses. Fitopatol Bras 26:170–175. <https://doi.org/10.1590/S0100-41582001000200009>
- Hammond J, Bampi D, Reinsel MD (2015) First report of *Plantago asiatica mosaic virus* in imported Asiatic and oriental lilies (*Lilium* hybrids) in the United States. Plant Dis 99:292. <https://doi.org/10.1094/PDIS-08-14-0792-PDN>
- Hughes PL, Harper F, Zimmerman MT, Scott SW (2005) Nandina mosaic virus is an isolate of *Plantago asiatica mosaic virus*. Eur J Plant Pathol 113:309–313. <https://doi.org/10.1007/s10658-005-0624-2>
- Komatsu K, Yamaji Y, Ozeki J, Hashimoto M, Kagiwada S, Takahashi S, Namba S (2008) Nucleotide sequence analysis of seven Japanese isolates of *Plantago asiatica mosaic virus* (PIAMV): a unique potexvirus with significantly high genomic and biological variability within the species. Arch Virol 153:193–198. <https://doi.org/10.1007/s00705-007-1078-y>
- Komatsu K, Yamashita K, Sugawara K, Verbeek M, Fujita N, Hanada K, Uehara-Ichiki T, Fuji S (2017) Complete genome sequence of two highly divergent Japanese isolates of *Plantago asiatica mosaic virus*. Arch Virol 162:581–584. <https://doi.org/10.1007/s00705-016-3110-6>
- Kostin VD, Volkov YG (1976) Some properties of the virus affecting *Plantago asiatica* L. Virusnye Bolezni Rastenij Dalnego Vostoka 25:205–210. (in Russian)
- Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. <https://doi.org/10.1093/molbev/msw054>
- Kwon SJ, Cho IS, Yoon JY, Jeong BN (2017) Identification and disease incidence of viruses infecting Chinese foxglove in Korea. Abstract P8–30, Asian Conference on Plant Pathology, Jeju, Korea

- Lim S, Igori D, Zhao F, Do YS, Cho IS, Choi GS, Moon JS (2016) Molecular detection and characterization of a divergent isolate of *Plantago Asiatica mosaic virus* in *Plantago asiatica*. *Virus Dis* 27: 307–310. <https://doi.org/10.1007/s13337-016-0329-5>
- Miglino R, Jodłowska A, van Schadewijk AR (2006) Detection and identification of a novel Potexvirus infecting allium by paramagnetic beads ssRNA isolation and one tube RT-PCR assay with a new Potexvirus genus primer set. *Acta Hort* (722):285–292. 10.17660/ActaHortic.2006.722.35
- Montero-Astúa M, Dejuk-Protti N, Vásquez E, Garita L, Moreira L (2017) First report of *Iris yellow spot virus* in Costa Rica. *Australas Plant Dis Notes* 12:18. <https://doi.org/10.1007/s13314-017-0243-8>
- Moreira L, Villalobos W, Hsu HT, Rodríguez-Cerezo E, Rivera C (1998) First report of *Cymbidium mosaic potexvirus* (CymMV) infecting the terrestrial orchid *Phaius tankervilleae* in Costa Rica. *Plant Dis* 82:1171. <https://doi.org/10.1094/PDIS.1998.82.10.1171D>
- Ozeki J, Takahashi S, Komatsu K, Kagiwada S, Yamashita K, Mori T, Hirata H, Yamaji Y, Ugaki M, Namba S (2006) A single amino acid in the RNA-dependent RNA polymerase of *Plantago asiatica mosaic virus* contributes to systemic necrosis. *Arch Virol* 151:2067–2075. <https://doi.org/10.1007/s00705-006-0766-3>
- Pájtli É, Eke S, Palkovics L (2015) First report of the *Plantago asiatica mosaic virus* (PLAMV) incidence on *Lilium* sp. in Hungary. *Plant Dis* 99:1288. <https://doi.org/10.1094/PDIS-01-15-0107-PDN>
- Parrella G, Greco B, Pasqualini A, Nappo G (2015) *Plantago asiatica mosaic virus* found in protected crops of lily hybrids in Southern Italy. *Plant Dis* 99:1289. <https://doi.org/10.1094/PDIS-03-15-0281-PDN>
- Purcifull DE, Edwardson JR, Christie RG (1966) Electron microscopy of intracellular aggregates in pea (*Pisum sativum*) infected with clover yellow mosaic virus. *Virology* 29:276–284. [https://doi.org/10.1016/0042-6822\(66\)90034-1](https://doi.org/10.1016/0042-6822(66)90034-1)
- Schneider CA, Rasband WS, Eliceiri KW (2012) NIH image to ImageJ: 25 years of image analysis. *Nat Methods* 9:671–675. <https://doi.org/10.1038/nmeth.2089>
- Solovyev AG, Novikov VK, Merits A, Savenko EI, Zelenina DA, Tyulkina LG, Morozov SY (1994) Genome characterization and taxonomy of *Plantago asiatica mosaic potexvirus*. *J Gen Virol* 75: 259–267. <https://doi.org/10.1099/0022-1317-75-2-259>
- Uga H, Tsuda S (2005) A one-step reverse transcription-polymerase chain reaction system for the simultaneous detection and identification of multiple tospovirus infections. *Phytopathology* 95:166–171. <https://doi.org/10.1094/PHTO-95-0166>
- Vidal AK, Camps R, Besoain X (2016) First report of necrotic streaking of Asiatic lilies caused by *Plantago asiatica mosaic virus* in Chile. *Plant Dis* 100:1799. <https://doi.org/10.1094/PDIS-01-16-0091-PDN>
- Villesen P (2007) FaBox: an online toolbox for fasta sequences. *Mol Ecol Notes* 7:965–968. <https://doi.org/10.1111/j.1471-8286.2007.01821.x>
- Wong SM, Mahtani PH, Lee KC, HH Y, Tan Y, Neo KK, Chan Y, Wu M, Chng CG (1997) *Cymbidium mosaic potexvirus* RNA: complete nucleotide sequence and phylogenetic analysis. *Arch Virol* 142: 383–391. <https://doi.org/10.1007/s007050050084.pdf>
- Xu LF, Ming J, Yuan SX (2017) First report of *Plantago asiatica mosaic virus* in lily hybrids in China. *Plant Dis* 101:263. <https://doi.org/10.1094/PDIS-06-16-0819-PDN>
- Yamaji Y, Kagiwada S, Nakabayashi H, Ugaki M, Namba S (2001) Complete nucleotide sequence of *Tulip virus X* (TVX-J): the border between species and strains within the genus *Potexvirus*. *Arch Virol* 146:2309–2320. <https://doi.org/10.1007/s007050170004.pdf>
- Zettler FW, Hiebert E, Maciel-Zambolim E, Christie RG, El-Nil MMA (1980) A potexvirus infecting *Nandina domestica* ‘Harbor Dwarf’. *Acta Hort* (110):71–77. 10.17660/ActaHortic.1980.110.7