

A detection method based on reverse transcription loop-mediated isothermal amplification for a genetically heterogeneous *Plantago asiatica* mosaic virus

Ken Komatsu¹ · Kensaku Maejima² · Naoko Fujita² · Osamu Netsu² ·
Tatsuya Tomomitsu² · Tsutomu Arie¹ · Tohru Teraoka¹ · Shigetou Namba²

Received: 2 December 2014 / Accepted: 26 January 2015 / Published online: 13 May 2015
© The Phytopathological Society of Japan and Springer Japan 2015

Abstract A reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay was developed to detect *Plantago asiatica* mosaic virus (PIAMV), one of the most damaging lily-infecting viruses and a member of the genus *Potexvirus* in the family *Alphaflexiviridae*. A set of six primers was designed based on the central core region of the coat protein gene of the Li1 isolate of PIAMV, which detected the isolate most efficiently at 65 °C. The RT-LAMP assay specifically detected several PIAMV isolates with a high level of genetic and biological variation, but not potato virus X (another virus species in the same *Potexvirus* genus). The sensitivity of the RT-LAMP was tenfold higher than that of conventional RT-PCR. Moreover, with a simple method using a toothpick, PIAMV was directly detected from infected lily leaves using the RT-LAMP assay without RNA extraction. This simple and highly sensitive method can be used for rapid surveys for PIAMV.

Keywords *Plantago asiatica* mosaic virus (PIAMV) · Reverse transcription loop-mediated isothermal amplification (RT-LAMP) · Detection

Introduction

Plantago asiatica mosaic virus (PIAMV) is a member of the genus *Potexvirus* in the family *Alphaflexiviridae* (Komatsu et al. 2008). PIAMV has a single-stranded RNA genome of positive polarity and is approximately 6100 nucleotides long. The PIAMV genomic RNA contains five open reading frames (ORFs); ORF1 encodes an RNA-dependent RNA polymerase (RdRp), ORF2 to ORF4 encode triple gene block proteins 1–3 (TGBp1–3), and ORF5 encodes a coat protein (CP). Since PIAMV was first isolated from *Plantago asiatica* in Russia in 1994 (Solovyev et al. 1994), several isolates from different types of plant species have been reported including Li isolates from the monocotyledon lily (*Lilium leichtlinii*), a Pr isolate from the dicotyledon primrose (*Primula sieboldii*), and an Na isolate from the woody plant *Nandina domestica* (Hughes et al. 2005; Komatsu et al. 2008). The genomic RNA of this virus is also highly variable among isolates (Komatsu et al. 2008). This high level of genetic and biological variation makes PIAMV difficult to detect. Because PIAMV causes severe necrosis in lilies in the Netherlands, a rapid and highly sensitive diagnostic tool for this virus is needed (EPPO 2011).

At this time, several diagnostic methods for PIAMV have been developed, including enzyme-linked immunosorbent assay (ELISA), direct tissue blotting, macroarray hybridization and RT-PCR (Chen et al. 2013; Ozeki et al. 2006; Sugiyama et al. 2008). However, these techniques involve time-consuming steps and require

Electronic supplementary material The online version of this article (doi:10.1007/s10327-015-0599-6) contains supplementary material, which is available to authorized users.

✉ Shigetou Namba
anamba@mail.ecc.u-tokyo.ac.jp

¹ Laboratory of Plant Pathology, Tokyo University of Agriculture and Technology (TUAT), 3-5-8 Saiwaicho, Fuchu, Tokyo 183-8509, Japan

² Laboratory of Plant Pathology, Department of Agricultural and Environmental Biology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo, Tokyo 113-8657, Japan

specialized laboratory equipment, such as a hybridization oven or a thermal cycler, limiting their use in the field.

Recently, a loop-mediated isothermal amplification (LAMP) assay was developed and has been applied to the diagnosis of many animal and plant pathogens (Mori and Notomi 2009). LAMP assays have also been adapted to rapidly detect plant RNA viruses by adding reverse transcriptase to the reaction, referred to as reverse-transcription LAMP (RT-LAMP) (Fukuta et al. 2003; Nie 2005; Varga and James 2006). LAMP assays are highly specific because the primers recognize at least six independent target regions. In addition, LAMP can isothermally amplify the target sequence in approximately 1 h using a water bath at a constant temperature of 60–65 °C. Moreover, in LAMP assays, the turbidity caused by an insoluble byproduct, white magnesium pyrophosphate precipitate, allows the visualization of target gene amplification. Based on these characteristics, LAMP assays are suitable for on-site diagnostic use. Furthermore, real-time monitoring of the LAMP reaction can be performed using a fluorescent indicator.

In the present study, we developed a LAMP-based detection system for PIAMV. Using a fluorescent indicator, we optimized the assay conditions and evaluated its sensitivity and specificity. Our assay is also capable of detecting PIAMV from infected leaves, which facilitates detection of the virus under practical conditions.

Materials and methods

Plant material and virus inoculation

All viruses used in this study [PIAMV-Li1, PIAMV-Pr, Li1-NaCP and potato virus X (PVX)-OS] were used to inoculate *Nicotiana benthamiana* using agroinfiltration as described previously (Maejima et al. 2014). Infected plants were maintained in a growth chamber at 25 °C with 16-h light/8-h dark. Lily plants used for direct detection of PIAMV were obtained from a commercial grower.

Agroinfectious clones of virus variants for infection of plants

The binary vector of PIAMV-Li1, pLi1, for inoculation was described previously (Ozeki et al. 2006). Binary vectors of PIAMV-Pr and PVX-OS for inoculation were constructed as pLi1; full-length cDNA of each virus, which was controlled by the cauliflower mosaic virus 35S promoter, was cloned into pCAMBIA1301 (Kagiwada et al. 2002; Komatsu et al. 2008). To construct pLi1-NaCP, an artificially synthesized CP gene of the Na isolate of PIAMV (Eurofins MWG Operon, Ebersberg, Germany) was inserted into the corresponding region (nt 5362–5985 of the Li1 isolate) of

pLi1 by replacing the original CP gene using the Gibson Assembly Kit (New England Biolabs, Ipswich, MA, USA). All binary vectors for inoculation were used to transform *Agrobacterium tumefaciens* strain EHA105 and used for agroinfiltration, as described above.

RNA extraction and RT-PCR

Total RNA was extracted from the upper leaves of virus-infected *N. benthamiana* plants using ISOGEN reagent (Nippon Gene, Tokyo, Japan), according to the manufacturer's instructions at 10 days after inoculation. After total RNA was dissolved in RNase-free distilled water to make a solution of approximately 500 ng/μL, 1 μL of the total RNA solution was used as a template for RT-LAMP and RT-PCR. RT-PCR was performed with the SuperScript III One-Step RT-PCR System with Platinum Taq (Life Technologies, Carlsbad, CA, USA) using Li-F3 and Li-B3, the PIAMV-specific external primers of the LAMP reaction (see Table 1 for their sequences). RT-PCR was performed in a total volume of 25 μL under the following conditions: RT reaction at 50 °C for 30 min; denaturation at 94 °C for 2 min; 35 cycles of 94 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s; followed by a final extension at 72 °C for 7 min. A total of 2 μL of the RT-PCR products was analyzed using 1 % (w/v) agarose gel electrophoresis.

Primer design

A primer set for the RT-LAMP reaction was designed based on the coat protein gene of the PIAMV Li1 isolate using the PrimerExplorer V4 software (<http://primerexplorer.jp/>). The set includes two inner primers [FIP (F1c + F2) and BIP (B1c + B2)], two outer primers (F3 and B3), and two loop primers (Loop F and Loop B), whose sequences and positions are shown in Fig. 1. Details of the primers are provided in Table 1.

RT-LAMP

The RT-LAMP reaction was performed as described previously (Sugawara et al. 2012), except that total RNA was used as template instead of DNA and 1.25 U of AMV reverse transcriptase (Nippon Gene) was included in the LAMP reaction mixture (total 25 μL). Unless otherwise stated, the mixture was incubated at 65 °C for 30 min followed by incubation at 80 °C for 5 min to inactivate DNA polymerase. For real-time monitoring of RT-LAMP reactions, fluorescent detection reagent (Eiken Kagaku, Tokyo, Japan) was added to the reaction mix, and fluorescence was detected with the Genie I instrument (OptiGene, Horsham, UK). DNA amplification was also

Table 1 Primers used for RT-LAMP for plantago asiatica mosaic virus (PIAMV) in this study

Primer name	Sequence (5′–3′)	Length
PIAMV-F3	CGCCAAAATCATCTGGAACG	20
PIAMV-B3	GACCTTGCCGTCTCGTTG	18
PIAMV-FIP	AGCGGTGCTCGAACTTGATGTTGACTCTCCAGAAACCTCCA	42
PIAMV-BIP	TCTTCGATGGCCTTCTCAACCCTGACTTCGTCGGGTGTGG	40
PIAMV-loop F	TGCCCGGGCGAAACCC	16
PIAMV-loop B	CGGCTCTCGAACCCATCGG	19

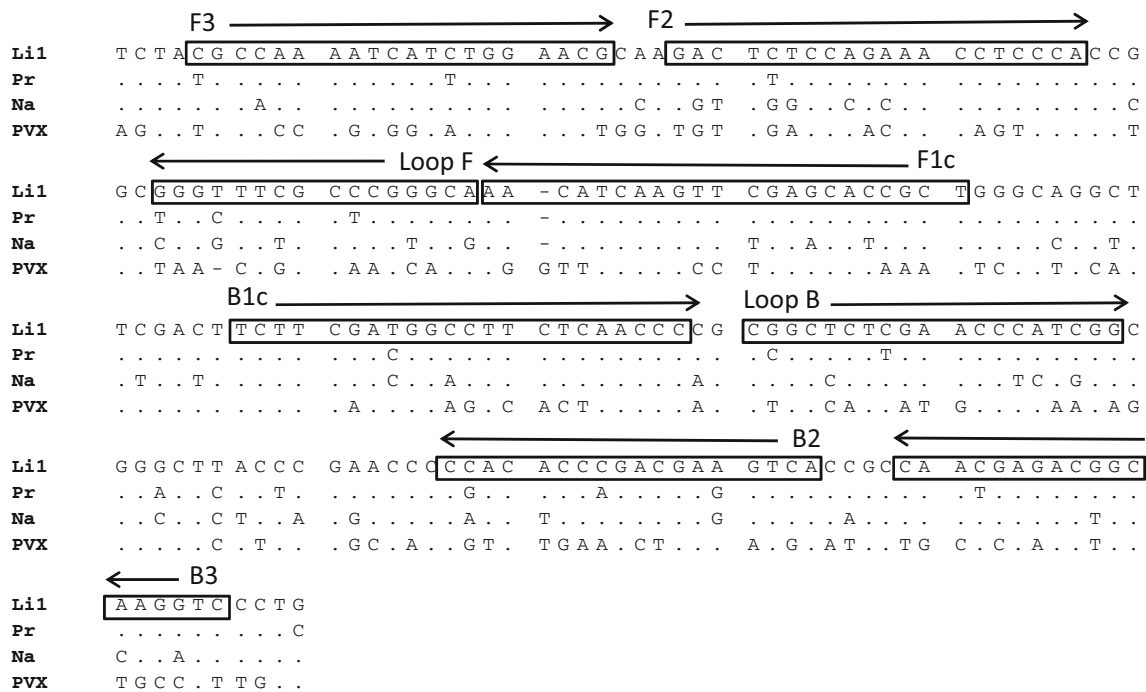


Fig. 1 Alignment of the nucleotide sequences of a 210-nucleotide coat protein-coding region of plantago asiatica mosaic virus isolates (Li1, AB360790; Pr, AB360796; NaMV, AY800279) and potato virus

X (PVX, OS isolate, AB056718). Nucleotides identical to Li1 are marked as periods; gaps are indicated by dashes. The sequences used for RT-LAMP primers are boxed and indicated by an arrow line

detected based on the fluorescence intensity under a 254-nm ultraviolet light.

Results

RT-LAMP detection of PIAMV and its reaction conditions

To develop a specific, rapid and highly sensitive RT-LAMP method for detecting PIAMV, we first designed five primer sets for the CP gene of the Li1 isolate, which were used for RT-LAMP. In this assay, we used total RNA extracted from Li1-infected *N. benthamiana* leaves as template. One primer set produced nonspecific amplification, and three other primer sets yielded insufficient

amplification. As a result, the primer set depicted in Fig. 1 and Table 1 was considered suitable for PIAMV detection because it resulted in efficient amplification with no non-specific products (data not shown).

To examine the optimal thermal conditions of the RT-LAMP assay using this primer set, we performed RT-LAMP reactions at temperatures of 59–66 °C for 30 min, with the aforementioned total RNA as a template. As shown in Fig. 2, RT-LAMP detected the Li1 isolate of PIAMV at a wide range of temperatures, while at lower incubation temperatures the increase in fluorescence generally began after a longer incubation. At 65 °C, the final fluorescence intensity was highest, and the fluorescence started to increase as rapidly as at 66 °C (approximately 7 min). Therefore, an incubation temperature of 65 °C was used for all subsequent RT-LAMP assays.

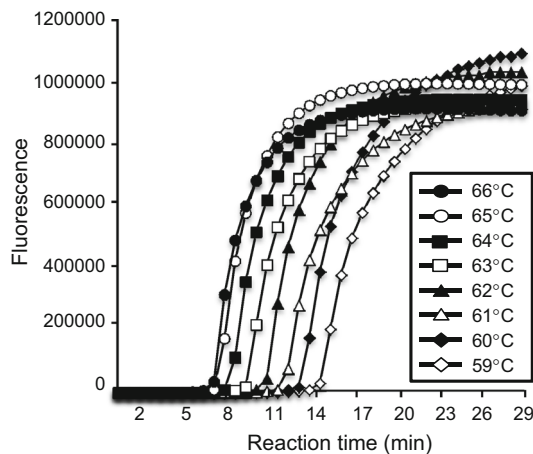


Fig. 2 Optimization of RT-LAMP reaction temperature for plantago asiatica mosaic virus. RT-LAMP amplification curves with the primer set listed in Table 1, using 100 ng of total RNA isolated from a Li1-infected *Nicotiana benthamiana* plant as a template, are shown. RT-LAMP reactions were run at incubation temperatures from 59 to 66 °C for 30 min. Similar results were obtained in two independent experiments. Time interval between each symbol is 45 s

Specificity of PIAMV detection by RT-LAMP

Because the primer set for RT-LAMP in this study was designed based on the Li1 isolate of PIAMV (Fig. 1), it remains unknown whether this primer set can be used for the detection of other PIAMV isolates, including Pr and Na. However, the Na isolate of PIAMV is not available. Thus, we constructed chimeric virus variants of Li1, which contains the CP gene of the Na isolate of PIAMV, and determined whether they could be detected using the RT-LAMP developed in this study. This strategy, in which a chimeric virus with the CP gene of unavailable virus isolates was constructed to express the CP of interest in plants, was successfully used to determine whether the plum pox virus (PPV) detection kit based on immunochromatography could detect minor PPV strains (Maejima et al. 2014).

When *N. benthamiana* was agroinfiltrated with a chimeric Li1 variant, Li1-NaCP, that possesses the CP gene of Na isolate of PIAMV, the chimeric virus systemically infected the host and caused weak necrosis on the upper leaves (data not shown). We also confirmed by RT-PCR and direct sequencing of the amplified products that Li1-NaCP retained the chimeric sequence in the upper leaves. We obtained similar results with two other chimeric clones, which confirmed stability of the chimeric virus.

We extracted total RNA from the upper leaves of *N. benthamiana* plants infected with Li1, Pr, Li1-NaCP, and PVX (isolate OS) (Kagiwada et al. 2002), and used these total RNA samples for RT-LAMP reactions. Among these four viruses, our RT-LAMP assay could detect three

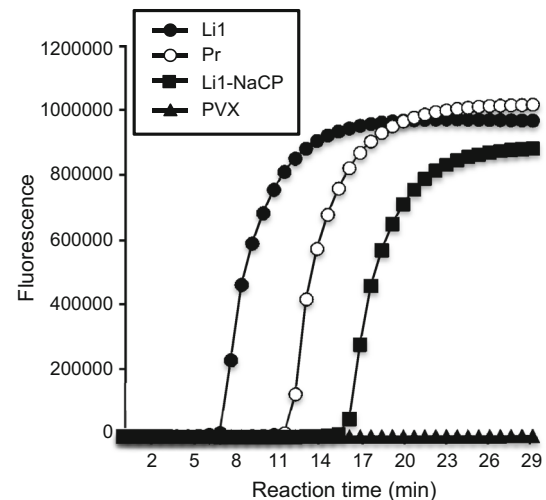


Fig. 3 Specificity of the RT-LAMP primer set for detecting plantago asiatica mosaic virus using total RNA from a plant of *Nicotiana benthamiana* infected with Li1, Pr, Li1-NaCP or potato virus X (PVX) as the template. RT-LAMP reaction was run for 30 min at 65 °C; shown is a representative experiment of two independent experiments with similar results. Time interval between each symbol is 45 s

PIAMV isolates, but not PVX (Fig. 3). Consistent with the nucleotide sequence identities of the CP genes with Li1, which are 93, 78, and 37 % for Pr, Na and PVX, respectively, the fluorescence started to increase at later times with decreasing CP nucleotide sequence identities with Li1. These results demonstrated that the RT-LAMP assay developed in this study could detect different PIAMV isolates.

Sensitivity of PIAMV detection using RT-LAMP compared with RT-PCR

To compare the sensitivity of our RT-LAMP assay with that of RT-PCR, total RNA extracted from a Li1-infected *N. benthamiana* leaf was diluted serially up to 10^9 -fold, and each sample was subjected to RT-LAMP and RT-PCR simultaneously. RT-LAMP successfully detected PIAMV from as little as 10 fg of total RNA, whereas RT-PCR could amplify the CP gene of PIAMV from as little as 100 fg of total RNA (Fig. 4a, c). Note that even when we used primers specifically designed for RT-PCR in the previous report (CP-gene-specific primers CP-F [5'-ATG GCA CTC AAC CAA GCT CCG AC-3'] and CP-R [5'-GTC GGA GGG GGA GGG GAG GAA TT-3']; Ozeki et al. 2006) instead of the external primers for our RT-LAMP, the sensitivity of these primers was not as high as that of the primers used in Fig. 4c (data not shown). Amplification of the PIAMV-CP gene by RT-LAMP was also confirmed based on visual observation of the reaction tubes under UV light (Fig. 4b). These results suggest that, to

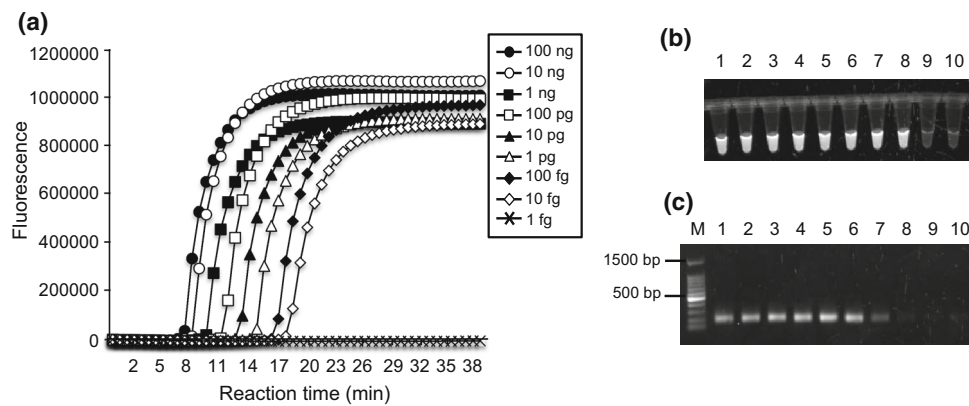


Fig. 4 Relative sensitivity of RT-LAMP and one-step RT-PCR to detect plantago asiatica mosaic virus. **a** RT-LAMP amplification curves using serial tenfold dilutions of total RNA (100 ng to 1 fg) from a Li1-infected plant of *Nicotiana benthamiana* as the template. RT-LAMP reaction was run for 40 min at 65 °C. The time interval between each symbol is 45 s. **b** Visual detection of the RT-LAMP using ultraviolet light. Tubes 1–9 100 ng to 1 fg total RNA preparations from a Li1-infected plant of *N. benthamiana*. Tube 10

indicates the nontemplate control. **c** Agarose gel electrophoresis of one-step RT-PCR using the same dilutions of total RNA as in **a** with primers PIAMV-F3 and –B3. Lane 1 is the 100-bp DNA ladder (New England Biolabs); lanes 2–9 are amplified products using 100 ng to 1 fg of total RNA from a Li1-infected plant as template. Lane 10 indicates the nontemplate control. Similar results were obtained in two independent experiments using total RNA extracted from two different Li1-infected plants as template

detect PIAMV, RT-LAMP was about tenfold more sensitive than RT-PCR, with a detection limit of 10 fg of total RNA extracted from virus-infected leaves.

Direct detection of PIAMV from infected lily leaves

To examine whether our RT-LAMP assay could detect PIAMV from infected leaves without extracting total RNA, we employed a simple method using a toothpick. After pricking the infected leaf with a toothpick several times, we dipped the toothpick into the LAMP reaction mix prepared in a PCR tube and performed RT-LAMP reactions. When we pricked Li1-infected leaves of *N. benthamiana* and dipped the toothpick into a reaction tube, a rapid increase in fluorescence was observed (Fig. 5; sample no. 8). In the same experiment, PIAMV was successfully detected from rust-coloured and necrotic lily leaves from five independent plants (Fig. 5b; sample nos. 1–5), although real-time monitoring of the LAMP reaction revealed that the reaction time needed for sufficient fluorescence differed depending on samples (Fig. 5a; approximately 25–65 min). In contrast, no virus was detected from healthy, symptomless lily leaves after a RT-LAMP reaction of 70 min (Fig. 5). RNA extraction and subsequent conventional RT-PCR and sequencing of these lily leaves confirmed that the lily leaves with rust-coloured symptoms used in Fig. 5a, b were infected with PIAMV, while symptomless lily leaves were not infected (Fig. 5c). Therefore, the toothpick method was applicable for the direct detection of PIAMV from infected lily leaves.

Discussion

Lily is one of the most popular and important ornamental plants in the world. Because most lilies are propagated vegetatively by bulbs, they are susceptible to virus infection and accumulate viruses during propagation. Several viruses, including cucumber mosaic virus, lily symptomless virus, lily mottle virus, and PIAMV are known to infect lily plants (Kwon et al. 2013). Among these, PIAMV is one of the most damaging threats to lily production because it can cause necrosis in the whole lily plant, reducing the yield and quality of cut flowers (EPPO 2011). Considering the growing global trade of ornamental flowers, including lilies, rapid and highly sensitive diagnostic tools are critical for controlling outbreaks of PIAMV.

Currently available techniques for PIAMV detection include ELISA and RT-PCR, but these are time-consuming and require specialized equipment, which is impractical for field use. In contrast, the RT-LAMP assay developed in this study takes only 30 min to detect PIAMV isolates from total RNA extracted from infected plants. Despite the effect of incubation temperature on such assays, our RT-LAMP assay successfully detected PIAMV at a wide range of temperatures (59–66 °C), which renders it appropriate for field diagnostics because the reaction can easily be performed in a water bath. Moreover, we found that the RT-LAMP exhibits tenfold higher sensitivity compared with RT-PCR in accordance with other studies showing that RT-LAMP is more sensitive than conventional RT-PCR (Fukuta et al. 2013). In addition, our RT-LAMP assay can directly detect PIAMV using a toothpick without

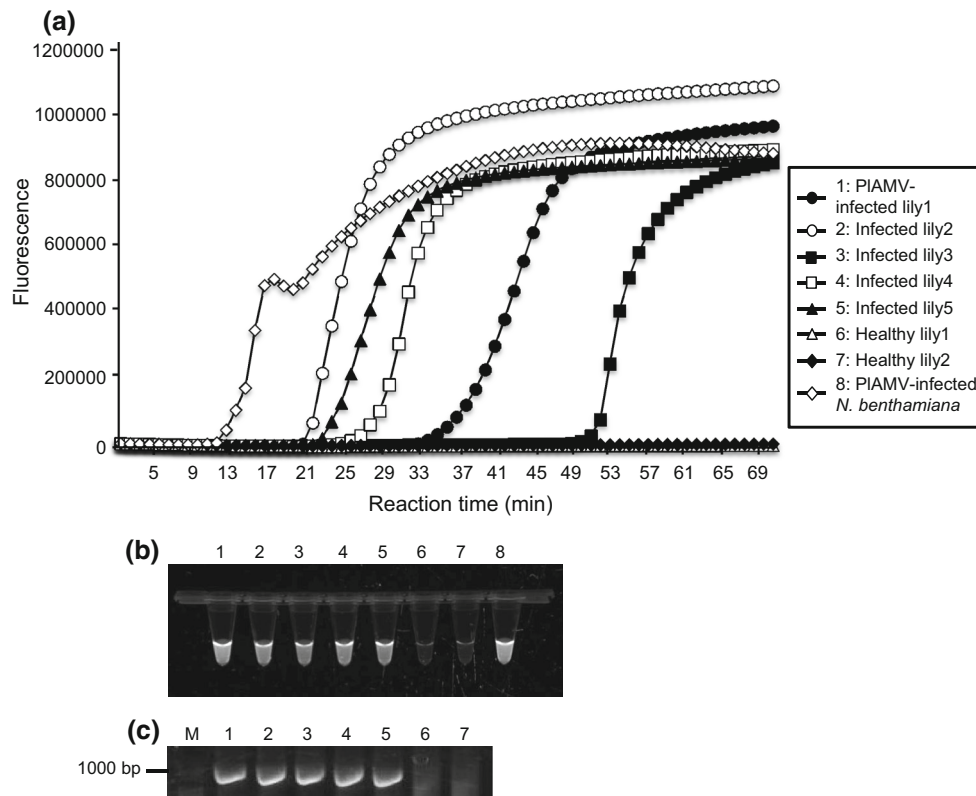


Fig. 5 Direct detection of plantago asiatica mosaic virus (PIAMV) from infected leaves using a toothpick dipped into the RT-LAMP reaction mixtures without templates before the reaction. The toothpick had been used to prick PIAMV-infected lily leaves from five independent plants (nos. 1–5), healthy lily leaves (nos. 6, 7), or a PIAMV-infected leaf of *Nicotiana benthamiana* (no. 8). RT-LAMP reaction was run for 70 min at 65 °C. **a** RT-LAMP amplification curves using the three toothpick-pricked leaf samples as templates. Time interval between each symbol is 60 s. **b** Visual detection of the

RT-LAMP using ultraviolet light. Samples are numbered as in **a**. **c** Agarose gel electrophoresis of PIAMV-specific, one-step RT-PCR using total RNA extracted from the lily leaves used in RT-LAMP as a template. Samples are numbered as in **a**. Primer set used (different than in Fig. 4): the reverse primer polydT-ClaNot (5'-AAA GCG GCC GCA TCG ATT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTV-3') and the forward primer ru-preTGBp2-F (5'-GTC GGA CTC TAC ATC AGC CT-3')

extracting total RNA from infected lily and *N. benthamiana* plants. This feature allows for efficient diagnosis of field samples.

However, in our direct RT-LAMP assay using a toothpick, the time when fluorescence starts to increase (approximately 21–50 min) appears to be later than that needed to increase the limit of detection of total RNA, which was demonstrated in Fig. 4 (approximately 18 min for the last amplified dilution). Although we cannot exclude the possibility that our primer set did not perfectly anneal to PIAMV isolates from lilies, which contributes to a delay in amplification, the delay is most likely due to an inhibitory effect of lily sap in RT-LAMP reaction mixture. Indeed, when we pricked healthy lily leaves using a toothpick and dipped it into the LAMP reaction mixture with total RNA extracted from Li1-infected *N. benthamiana* leaves as a template, amplification time was delayed (Fig. S1). In the future, studies to determine the exact detection limit of PIAMV using a toothpick and direct RT-

LAMP assay should be done to increase the confidence in the direct RT-LAMP method for PIAMV.

To our knowledge, RT-LAMP has been developed to detect only two viruses in the genus *Potexvirus*; namely, *Cymbidium mosaic virus*, which causes severe damage to orchids, and *Pepino mosaic virus* (PepMV), an emerging virus that infects tomato plants worldwide (Hasiów-Jaroszewska and Borodynko 2013; Lee et al. 2011; Ling et al. 2013). Recently, for the detection of PepMV (which has a high level of within-species genetic diversity), a genotype-specific RT-LAMP was developed (Ling et al. 2013). This system is useful and allows tomato growers to determine the genetic diversity of the virus in their own fields. However, we cannot adapt this genotype-specific detection system to PIAMV, a genetically heterogeneous potexvirus species similar to PepMV, because insufficient sequence information is available for genotype classification of this virus. Instead, in the case of PIAMV, it is important to develop a system that can detect a wide variety of isolates.

To this end, we designed the RT-LAMP primers for PIAMV targeting the central core region of the *CP* gene. Because this primer target region is relatively conserved within the genus *Potexvirus*, our RT-LAMP assay can detect PIAMV isolates with high genomic sequence diversity. In contrast, our assay cannot detect PVX, which is a less closely related species of the same genus. This relatively broad spectrum of the RT-LAMP developed in this study can be adapted to detect unreported PIAMV isolates and can be used for the early detection of emerging viruses that infect lilies and affect their quality. Future studies should determine the detection range of the RT-LAMP and the lower limit of potexvirus nucleotide sequence identity that can be detected. Based on our results, whether other PIAMV isolates are detected or not using our RT-LAMP assay remains unclear.

Acknowledgments We thank Mr. T. Moriyama for providing lily plants. This work was supported in part by Grants-in-Aid for Young Scientists (B) (26850231) from the Japan Society for the Promotion of Science.

References

- Chen CC, Jhang YL, Lin BY, Chiang FL, Cheng YH, Deng TC (2013) Serological reagent preparation and improvement of serological method for the detection of *Plantago asiatica mosaic virus* in lily. *J Taiwan Agric Res* 62:268–279
- EPPO (2011) New pest records in EPPO member countries: *Plantago asiatica mosaic virus* (*Potexvirus*, PIAMV) found on *Lilium* spp. in the Netherlands. EPPO Reporting Service 2011/082. http://www.eppo.int/PUBLICATIONS/reporting/reporting_service.htm. Accessed 09 Aug 2014
- Fukuta S, Kato S, Yoshida K, Mizukami Y, Ishida A, Ueda J, Kanbe M, Ishimoto Y (2003) Detection of tomato yellow leaf curl virus by loop-mediated isothermal amplification reaction. *J Virol Methods* 112:35–40
- Fukuta S, Tamura M, Maejima H, Takahashi R, Kuwayama S, Tsuji T, Yoshida T, Itoh K, Hashizume H, Nakajima Y, Uehara Y, Shirako Y (2013) Differential detection of *Wheat yellow mosaic virus*, *Japanese soil-borne wheat mosaic virus* and *Chinese wheat mosaic virus* by reverse transcription loop-mediated isothermal amplification reaction. *J Virol Methods* 189:348–354
- Hasiów-Jaroszewska B, Borodynko N (2013) Detection of Pepino mosaic virus isolates from tomato by one-step reverse transcription loop-mediated isothermal amplification. *Arch Virol* 158:2153–2156
- 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
- Kagiwada S, Yamaji Y, Nakabayashi H, Ugaki M, Namba S (2002) The complete nucleotide sequence of *Potato virus X* strain OS: the first complete sequence of a Japanese isolate. *J Gen Plant Pathol* 68:94–98
- 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
- Kwon JY, Ryu KH, Choi SH (2013) Reverse transcription polymerase chain reaction-based system for simultaneous detection of multiple lily-infecting viruses. *Plant Pathol J* 29:338–343
- Lee MS, Yang MJ, Hseu YC, Lai GH, Chang WT, Hsu YH, Lin MK (2011) One-step reverse transcription loop-mediated isothermal amplification assay for rapid detection of *Cymbidium mosaic virus*. *J Virol Methods* 173:43–48
- Ling KS, Li R, Bledsoe M (2013) *Pepino mosaic virus* genotype shift in North America and development of a loop-mediated isothermal amplification for rapid genotype identification. *Virol J* 10:117
- Maejima K, Himeno M, Netsu O, Ishikawa K, Yoshida T, Fujita N, Hashimoto M, Komatsu K, Yamaji Y, Namba S (2014) Development of an on-site plum pox virus detection kit based on immunochromatography. *J Gen Plant Pathol* 80:176–183
- Mori Y, Notomi T (2009) Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. *J Infect Chemother* 15:62–69
- Nie X (2005) Reverse transcription loop-mediated isothermal amplification of DNA for detection of *Potato virus Y*. *Plant Dis* 89:605–610
- 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
- Solovyev AG, Novikov VK, Merits A, Savenkov EI, Zelenina DA, Tyulkina LG, Morozov SYu (1994) Genome characterization and taxonomy of *Plantago asiatica mosaic potexvirus*. *J Gen Virol* 75:259–267
- Sugawara K, Himeno M, Keima T, Kitazawa Y, Maejima K, Oshima K, Namba S (2012) Rapid and reliable detection of phytoplasma by loop-mediated isothermal amplification targeting a house-keeping gene. *J Gen Plant Pathol* 78:389–397
- Sugiyama S, Masuta C, Sekiguchi H, Uehara T, Shimura H, Maruta Y (2008) A simple, sensitive, specific detection of mixed infection of multiple plant viruses using microarray and microtube hybridization. *J Virol Methods* 153:241–244
- Varga A, James D (2006) Use of reverse transcription loop-mediated isothermal amplification for the detection of *Plum pox virus*. *J Virol Methods* 138:184–190