Journal of Virological Methods 265 (2019) 71–76

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Short communication

Development of a LAMP assay with a portable device for real-time detection of begomoviruses under field conditions

Fariha Wilisiani[a], Aika Tomiyama[b], Hiroshi Katoh[c], Sedyo Hartono[d], Yutaro Neriya[b], Hisashi Nishigawa[a,b], Tomohide Natsuaki[a,b,*]

[a] United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan
[b] Plant Pathology Laboratory, School of Agriculture, Utsunomiya University, 350 Mine-machi, Utsunomiya, Tochigi 321-8505, Japan
[c] Center for Bioscience Research and Education, Utsunomiya University, 350 Mine-machi, Utsunomiya, Tochigi 321-8505, Japan
[d] Virology Laboratory, Faculty of Agriculture, Gadjah Mada University, Bulaksumur, Yogyakarta, 55281, Indonesia

A R T I C L E I N F O

Keywords:
Begomovirus
Detection
LAMP
Solanaceae
Cucurbitaceae
Portable device

A B S T R A C T

The emergence of begomovirus infection is one of the most important problems affecting production of a variety of vegetable crops worldwide. Infection by begomoviruses has been detected and spread rapidly on *Cucurbitaceae* and *Solanaceae* plants in Indonesia. A rapid and simple detection assay for begomoviruses under field conditions for routine sampling of plants is needed. Primers for a loop-mediated isothermal amplification (LAMP) assay were designed based on the sequences of three Indonesian begomoviruses, namely *Tomato leaf curl New Delhi virus* (ToLCNDV), *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), and *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV), infecting *Cucurbitaceae* and *Solanaceae* plants. LAMP assays using a Genelyzer™ III portable fluorometer with a toothpick method successfully detected these begomoviruses in infected melon, pepper, and eggplant samples. LAMP assays conducted during a field survey for detection of the three begomoviruses on 104 fresh leaves indicated that most of the samples were positive; the findings were confirmed by PCR using universal primers of begomovirus as a common detection method. These results demonstrate that this simple and rapid LAMP assay using a fluorometer portable device may be used to achieve real-time detection of begomoviruses under field conditions.

The emergence of begomovirus infection is one of the most important problems affecting production of vegetable crops worldwide. Viruses of the genus *Begomovirus* (family *Geminiviridae*) have a circular single-stranded DNA genome encapsidated in geminate particles. Begomoviruses have either a monopartite or bipartite genome known as DNA-A and DNA-B, respectively, each of which is approximately 2.7-kb long. The DNA-A genome encode the AV1 and AV2 open reading frame (ORF) in the virion-sense orientation and AC1, AC2, AC3, and AC4 ORFs in the complementary-sense orientation, whereas the DNA-B genome contains the BV1 ORF in the virion-sense orientation, and the BC1 ORF in the complementary-sense orientation (Brown et al., 2015; Zaidi et al., 2017). Begomoviruses infect a wide range of dicotyledonous plants, mostly in tropical and subtropical regions, and are transmitted by the whitefly *Bemisia tabaci.* The symptoms of begomovirus infection are typically mosaic, yellowing, curling on leaves, and stunted growth on plants. Infection of begomoviruses has been detected and spread rapidly on *Solanaceae* and *Cucurbitaceae* plants in Indonesia, such as *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) and *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV) (De Barro et al., 2008; Jamsari and Pedri, 2013; Kenyon et al., 2014; Kintasari et al., 2013) from *Solanaceae* plants, and *Squash leaf curl China virus* (SLCCNV) and *Tomato leaf curl New Delhi virus* (ToLCNDV) from *Cucurbitaceae* plants (Mizutani et al., 2011; Septariani et al., 2014; Wiratama et al., 2015).

The most commonly used detection method for begomoviruses is PCR using universal primers. However, this method requires equipment for thermal cycling, and is unsuitable for use under field conditions because of the lack of convenient portable instruments (Parida et al., 2008). Moreover, the total running time for detection by PCR, including the DNA extraction step and electrophoresis, requires several hours. Therefore, a rapid and simple detection assay for routine sampling of viruses infecting plants is needed. In recent years, a loop-mediated isothermal amplification (LAMP) assay has been increasingly used for detection of plant pathogens. LAMP is a novel nucleic acid amplification assay that relies on auto-cycling strand displacement DNA synthesis catalyzed by *Bst* DNA polymerase (Notomi et al., 2000; Parida et al., 2008). The LAMP technique provides several advantages over

* Corresponding author at: Plant Pathology Laboratory, School of Agriculture, Utsunomiya University, 350 Mine-machi, Utsunomiya, Tochigi 321-8505, Japan
E-mail address: natsuaki@cc.utsunomiya-u.ac.jp (T. Natsuaki).

https://doi.org/10.1016/j.jviromet.2018.10.005
Received 27 April 2018; Received in revised form 7 August 2018; Accepted 12 October 2018
Available online 12 October 2018
0166-0934/

other DNA amplification methods. A LAMP assay is performed using 4–6 primers that recognize several distinct regions on the target gene, thus it is a highly specific amplification assay. The amplification of nucleic acids by a LAMP assay is performed under isothermal conditions, meaning that a thermal cycling machine is not required.

The LAMP procedure has been applied for detection of begomoviruses using species-specific primers, including *Tomato yellow leaf curl virus* (TYLCV) (Almasi et al., 2013; Fukuta et al., 2003), *Squash leaf curl virus* (SLCV) (Kuan et al., 2010), *Tomato leaf curl Bangalore virus* (ToLCBaV) (Arutselvan et al., 2017), *Tomato leaf curl New Delhi virus-[potato]* (ToLCNDV-[potato]) (Jeevalatha, et al., 2018), *Tomato leaf curl Sinaloa virus* (ToLCSiV), *Tomato yellow mottle virus* (TYMoV), and *Potato yellow mosaic Panama virus* (PYMPV) (Herrera-Vásquez et al., 2018). To our knowledge, no LAMP assay has been reported using a primer set that can detect multiple begomoviruses simultaneously. Furthermore, no real-time LAMP assay using a portable isothermal amplification and fluorescence detection device for detecting pathogens in the field with limited infrastructure has been developed. For plant virus detection, a RT-LAMP assay using a portable device was reported for three wheat viruses, namely *Wheat yellow mosaic virus* (WYMV), *Japanese soil-borne mosaic virus* (JSBWMV) and *Chinese wheat mosaic virus* (CWMV), belonging to the genus *Furovirus* (Fukuta et al., 2013), and also for *Chrysanthemum stem necrosis virus* (CSNV) belonging to the genus *Tospovirus* (Suzuki et al., 2016). To our knowledge, a LAMP assay using this portable device has not previously been reported for begomoviruses. In this study, we developed and evaluated the effectiveness of a LAMP assay using a portable device and a primer set for detection of several begomoviruses simultaneously under field conditions. This assay will be useful for rapid and simple detection of plant viruses during routine field surveys.

A primer set for begomovirus detection by means of a LAMP assay was designed based on the nucleotide sequences of Indonesian isolates of ToLCNDV (LC335722), PepYLCIV (AB267834), and TYLCKaV (KF446663), especially from the AC1 ORF region of DNA-A as the amplification target (Fig. 1), using the PrimerExplorer V5 software (http://primerexplorer.jp/lamp4.0.0/index.html) and modified manually. These primers consisted of forward and backward outer primers (F3 and B3), and forward and backward inner primers (FIP and BIP; Table 1). The AC1 gene of DNA-A was previously a target region for begomovirus detection by PCR using universal primers (Briddon and Markham, 1994; Rojas et al., 1993). The amplification target for detection of *Tomato leaf curl Sinaloa virus* (ToLCSiV) and *Tomato yellow mottle virus* (TYMoV) by a LAMP assay was also located in this region (Herrera-Vásquez et al., 2018).

The LAMP assay was performed with Isothermal Master Mix (OptiGene, Horsham, UK). Initially, dried reagent of the Isothermal Master Mix containing DNA polymerase, thermostable inorganic pyrophosphatase, optimized reaction buffer, $MgSO_4$, dNTPs, and double-stranded-DNA binding dye was dissolved with resuspension buffer and was ready to use directly as the reaction mixture for LAMP. The LAMP assay was tested using a toothpick method, whereby the sample tissue was pierced with a toothpick three to five times and the tip of the toothpick was dipped into the LAMP reaction mixture, as the DNA extraction process (Fukuta et al., 2005; Suzuki et al., 2016). The reaction mixture contained 15 µl Isothermal Master Mix, 5 pmol of each outer primer and 20 pmol of each inner primer (Katoh et al., 2016 with modifications), and 7 µl distilled water to make up the 25 µl final reaction volume. The LAMP amplification was run on a real-time fluorometer (Genelyzer™ FIII, Canon Medical Systems, Tochigi, Japan). All LAMP assays were run under isothermal conditions at 63 °C for 30 min, followed by post-amplification annealing (98–80 °C, 0.05 °C/s). Results data from the fluorometer were stored and analyzed using the Genie® Explorer v2.0.6.3 software (OptiGene). Initially, the LAMP assay was tested in the laboratory using samples of melon, pepper, and eggplant that were known to be naturally infected with begomoviruses of Indonesian isolates of ToLCNDV, PepYLCIV, and TYLCKaV, respectively. Furthermore, this LAMP assay was used directly in the field for begomovirus detection from melon, pepper, eggplant, and tomato plants with typical begomovirus symptoms during a field survey in Indonesia in 2017. To confirm the LAMP results, total DNA was extracted from all leaf samples using the Cica Geneus® DNA Prep Kit (for Plant) (Kanto Reagents, Tokyo, Japan) and amplified by PCR using the begomovirus universal primers UPV1 (Briddon and Markham, 1994) and PAV1c715 (Rojas et al., 1993), which amplified approximately 1.5-kbp fragment of the DNA-A begomovirus.

Appropriate design of the primers is a critical factor for development of a LAMP assay (Boonham et al., 2008; Parida et al., 2008). The primer set used for the LAMP assay in the present study consisted of two outer primers (F3 and B3) that recognized two distinct regions and two inner primers (FIP and BIP) that recognized four distinct regions of the target sequence. Although optional primers, namely loop primers, can accelerate the LAMP reaction (Nagamine et al., 2002), we could not find suitable sequences for the design of loop primers in the alignment of three begomoviruses sequences in the current study. The recognition of six distinct sequences by outer and inner primers in the target DNA is a strict requirement in a LAMP assay (Notomi et al., 2000). All of the primer sequences used in this study matched fully with the ToLCNDV sequence genome. A few mismatches existed between primers and sequences of PepYLCIV and TYLCKaV, but all of the mismatches were located far from the 3′ end sequences of the primers (Table 1).

The LAMP assay conducted in the laboratory using samples that were confirmed to be naturally infected with begomoviruses showed that, for all infected samples, specific amplification products were generated during the amplification reaction, whereas no amplification products were detected from the healthy melon sample (Fig. 2). These results indicated that the primer set used in this study can detect three begomoviruses simultaneously, namely Indonesian isolates of ToLCNDV, PepYLCIV, and TYLCKaV. A LAMP assay using a primer set that can detect phytoplasma group has been reported (De Jonghe et al., 2017; Hodgetts et al., 2011; Sugawara et al., 2012). However to our knowledge, this study is the first report of a LAMP assay using a primer set for detection of three begomoviruses simultaneously. In addition, the amplification product of each sample was observed in less than 15 min that might be affected by the concentration of the samples (Fig. 2), thus the assay procedure facilitates rapid detection of three begomoviruses tested in this study. The annealing curve showed that the annealing temperature (T_a) of the amplified LAMP product during the annealing step was not clearly detected in LAMP using samples with the toothpick method in this study (data not shown). However, the T_a of each sample was clearly detected for the total DNA extracted from infected plant samples used in the LAMP assay (data not shown). This result suggested that components of plant tissue samples might influence the annealing step in this assay. The annealing temperature of all samples was similar (85.0–85.5 °C), thus indicating that the GC content in the target region of DNA-A in the three begomoviruses tested in this study might be similar. The LAMP assay using a primer set for detection of phytoplasma group showed a similar T_a (~86 °C) for all tested samples (De Jonghe et al., 2017). The concentration of the samples cannot be measured due to the LAMP assay was tested using a toothpick method as the DNA extraction process. Therefore, the sensitivity test of LAMP assay was conducted using total DNA from infected leaf samples serially diluted in healthy leaf DNA (10^{-1} to 10^{-6} dilutions containing 1 ng to 0.001 pg of infected leaf total DNA in 40–50 ng of healthy leaf total DNA) and then used 2 µL of dilutions per reaction (2 ng to 0.002 pg) as templates for LAMP and PCR assays. Both LAMP and PCR assays could detect up to 10^{-7} dilutions (0.002 pg DNA). The LAMP assay in this study was as sensitive as LAMP for ToLCNDV-[potato] detection (Jeevalatha et al., 2018).

The reliability of LAMP assays for detection of three begomoviruses under field conditions was tested in four regions, namely Bantul (Yogyakarta), Magelang (Central Java), East Lombok (West Nusa Tenggara), and Denpasar (Bali), Indonesia (Fig. 3A–D). The detection of

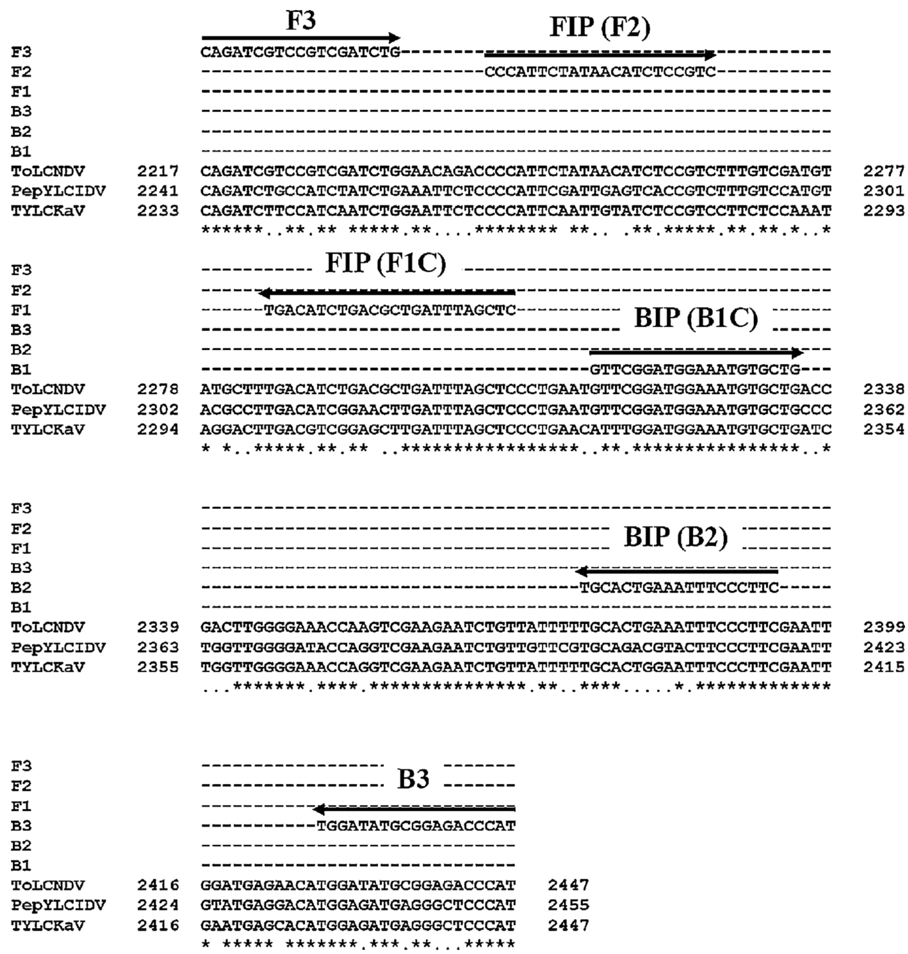

Fig. 1. Alignment of consensus sequences of Indonesian isolates of *Tomato leaf curl New Delhi virus* (ToLCNDV; LC335722), *Pepper yellow leaf curl Indonesia virus* (PepYLCIV; AB267834), *Tomato yellow leaf curl Kanchanaburi* virus (TYLCKaV; KF446663), and the LAMP primer sequences. The inner FIP primer consists of the reverse sequence of F1 followed by that of the F2 sequence. The inner BIP primer consists of the B1 sequence followed by that of the reverse B2 sequence. Mismatches to the primer sequences are indicated by a dot. Direction of primers are indicated by arrows.

Table 1
LAMP primers used for detection of three begomoviruses of *Tomato leaf curl New Delhi virus* (ToLCNDV), *Pepper yellow leaf curl Indonesia virus* (PepYLCIV), and *Tomato yellow leaf curl Kanchanaburi virus* (TYLCKaV).

Primer name	Type	Length (nt)	Sequence (5′–3′)
F3	LAMP forward outer	19	CAGATCGTCCGTCGATCTG
B3	LAMP reverse outer	19	ATGGGTCTCCGCATATCCA
FIP	LAMP forward outer (<u>F1C</u> + F2)	46	<u>GAGCTAAATCAGCGTCAGATGTCA</u>CCCATTCTATAACATCTCCGTC
BIP	LAMP reverse outer (<u>B1C</u> + B2)	39	<u>GTTCGGATGGAAATGTGCTG</u>GAAGGGAAATTTCAGTGCA

Underline indicates the complementary sequences of the F1 and B1 primers.

begomoviruses in a total of 104 fresh symptomatic leaves of melon, pepper, eggplant, and tomato plant samples, by the LAMP assay showed that most of the collected samples were infected by begomoviruses (Table 2). In addition, for several samples the LAMP assay showed very rapid detection of the begomoviruses that might be affected by the concentration of the samples (Fig. 4A). In addition, LAMP assay developed in this study can detect the three begomoviruses (ToLCNDV, PepYLCIV, and TYLCKaV) simultaneously and not distinguish individual viruses or even a co-infection. Detections by LAMP assays for all of the samples were confirmed by PCR in the laboratory using universal primers for begomoviruses UPV1 (Briddon and Markham, 1994) and PAV1c715 (Rojas et al., 1993). Comparison of both methods indicated that all of the samples that were positive for begomovirus detection by the LAMP assay also were positive for detection by PCR (Fig. 4A&B). However, three melon samples from Bantul, Yogyakarta and one tomato sample from Denpasar, Bali showed a negative result for begomovirus detection by both LAMP and PCR.

To develop a LAMP assay for use under field conditions, the toothpick method proved to be successful in the present study. Compared with DNA extraction methods that are expensive, time consuming, and also need complex equipment, the present method is simpler and can be applied under field conditions. Given that the *Bst* DNA polymerase used in the LAMP assay is less sensitive to inhibitors present in plant than *Taq* DNA polymerase used in PCR (Francois et al., 2011; Kaneko et al., 2007), the toothpick method can be used for DNA extraction in the LAMP procedure. LAMP assays using the toothpick method are reported to be successful for *Tomato yellow leaf curl virus* (*Begomovirus*) (Fukuta et al., 2005) and *Chrysanthemum stem necrosis*

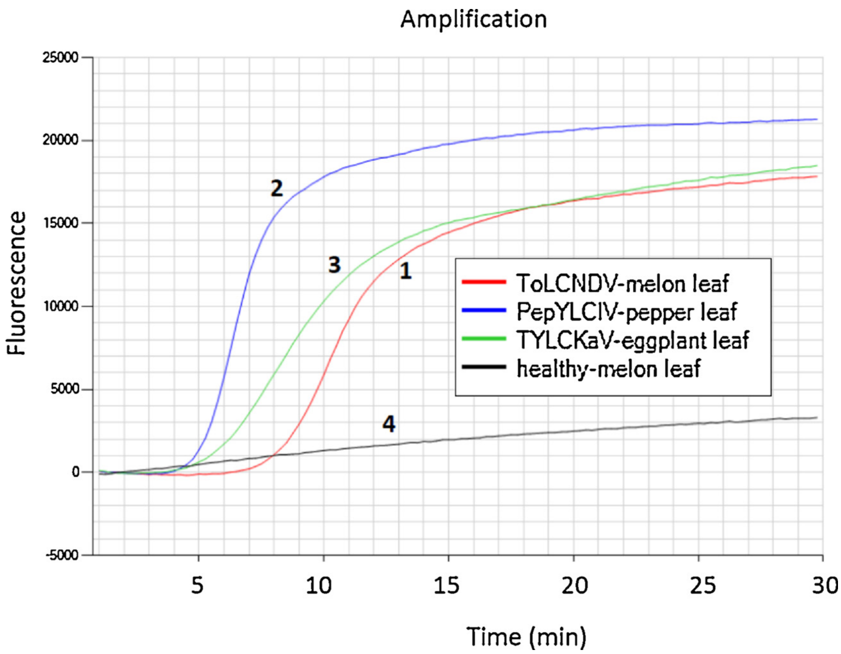

Fig. 2. Time-course of fluorescence during the LAMP reaction of begomovirus-infected samples. The LAMP assay was run under isothermal conditions at 63 °C for 30 min. The bold numbers 1 to 3 indicate the melon (red), pepper (blue), and eggplant (green) samples that were infected with Indonesian isolates of the begomoviruses ToLCNDV, PepYLCIV, and TYLCKaV, respectively. The bold number 4 indicates the healthy melon sample (black), included as a negative control.

Fig. 3. LAMP assay for detection of begomoviruses during a field survey in a melon field, Indonesia. A) Location of assay at the side of a farmhouse adjacent to the melon field. B) Toothpick method of DNA extraction from melon leaf. C) Dipping the toothpick into the LAMP reaction mixture. D) Setting the tubes in the Genelyzer™ III fluorometer for the LAMP assay.

virus (*Tospovirus*) detection (Suzuki et al., 2016). Samples of sap extract obtained from an infected leaf were also successfully used in a LAMP assay for begomovirus detection (Herrera-Vásquez et al., 2018). In addition, a LAMP assay with template DNA that was prepared using microwave irradiation was demonstrated to be a simple tool for detection of anthracnose-causing fungus *Colletotrichum gloeosporioides* in infected strawberry plants (Katoh et al., 2016).

In the present study, the LAMP assay was conducted using the dried and optimized Isothermal Master Mix commercial kit, which renders the procedure easy and simple to use under field conditions. Furthermore, the assay can be performed using a portable device that is battery-powered, user-friendly, hand-held, and weather-proof, and thus is suitable for detection under field conditions. This device can also accommodate the isothermal reaction process for eight simultaneous samples in one heating block, and no additional specialized equipment is required in this LAMP assay. A LAMP assay using this device is also capable of real-time monitoring of fluorescence. Thus, the assay demonstrated in this study is a real-time LAMP assay that is simple, rapid, and suitable for routine detection.

Table 2
Confirmation of LAMP assay with PCR methods for detection of begomoviruses during a field survey in Indonesia.

Location	Sample	Total no. of samples	No. of samples positive for infection by begomovirus	
			LAMP	PCR
Bantul, Yogyakarta	Melon	23[a]	20	20
	Pepper	4	4	4
Magelang, Central Java	Melon	5	5	5
	Eggplant	7	7	7
	Pepper	15	15	15
	Tomato	8	8	8
East Lombok, West Nusa Tenggara	Pepper	4	4	4
Denpasar, Bali	Pepper	32	32	32
	Tomato	6[a]	5	5

[a] Three melon samples and one tomato sample were negative for infection by three Indonesian begomoviruses as detected using both LAMP and PCR methods in this study.

In conclusion, the LAMP assay using a primer set developed in this study successfully detected Indonesian isolates of the begomoviruses ToLCNDV, PepYLCIV, and TYLCKaV simultaneously. Moreover, the LAMP assay using a portable device detected plant viruses rapidly and simply under field conditions for routine survey.

Ethical standards

This study did not include experiments with human participants or animals performed by any of the authors.

Conflict of interest

The authors declare that they have no conflict of interest.

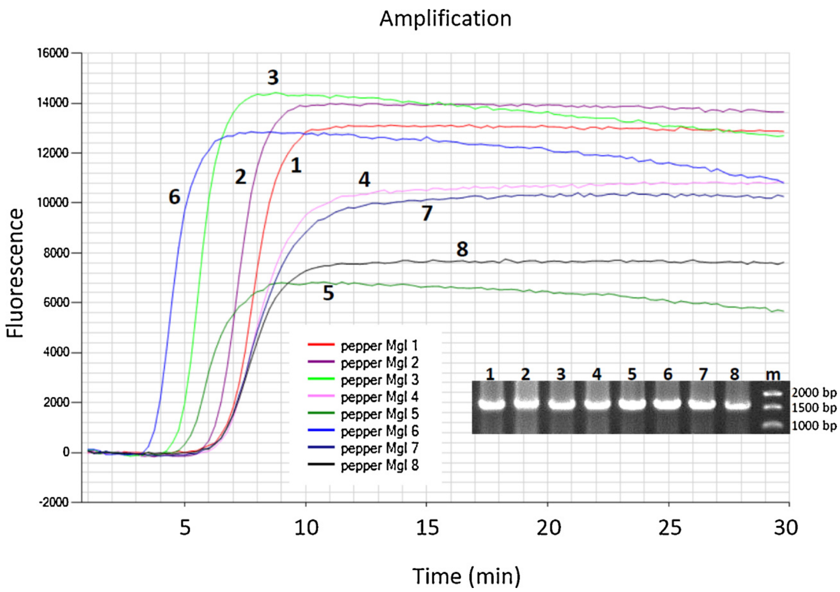

Fig. 4. Example of LAMP and PCR detections of samples. Time-course of fluorescence in the LAMP reaction during a field survey in Indonesia. The bold numbers 1 to 8 indicate the amplification curve of individual fresh-leaf pepper samples collected in one field in Magelang, Indonesia. Begomovirus detection using universal primer of UPV1 (Briddon and Markham, 1994) and PAV1c715 (Rojas et al., 1993) by PCR. The amplicon product of 1.5 bp were shown for positive sample.

Acknowledgments

This study was supported in part by JSPS KAKENHI (Grant-in-Aid for Scientific Research (B)) Grant numbers 26304023 & 17H04617. We thank Robert McKenzie, PhD, from Edanz Group (www.edanzediting.com/ac), for editing a draft of this manuscript.

References

Almasi, M.A., Ojaghkandi, M.A., Hemmatabadi, A., Hamidi, F., Aghael, S., 2013. Development of colorimetric loop-mediated isothermal amplification assay for rapid detection of the Tomato yellow leaf curl virus. J. Plant Pathol. Microb. 4, 153. https://doi.org/10.4172/2157-7471.1000153.

Arutselvan, R., Krishna Reddy, M., Makeshkumar, T., 2017. Rapid detection of *Tomato leaf curl Bengaluru virus* through loop mediated isothermal amplification assay. Virus Dis. 28, 303–308. https://doi.org/10.1007/s13337-017-0385-5.

Boonham, N., Glover, R., Tomlinson, J., Mumford, R., 2008. Exploiting generic platform technologies for the detection and identification of plant pathogens. Eur. J. Plant Pathol. 121, 355–363. https://doi.org/10.1007/s10658-008-9284-3.

Briddon, R.W., Markham, P.G., 1994. Universal primers for the PCR amplification of dicot-infecting geminiviruses. Mol. Biotech. 1, 202–205. https://doi.org/10.1007/BF02921559.

Brown, J.K., Zerbini, F.M., Navas-Castillo, J., Moriones, E., Ramos-Sobrinho, R., Silva, J.C.F., Fiallo-Olivé, E., Briddon, R.W., Hernández-Zepeda, C., Idris, A., Malathi, V.G., Martin, D.P., Rivera-Bustamante, R., Ueda, S., Varsani, A., 2015. Revision of *Begomovirus* taxonomy based on pairwise sequence comparisons. Arch. Virol. 160, 1593–1619. https://doi.org/10.1007/s00705-015-2398-y.

De Barro, P.J., Hidayati, S.H., Frohlich, D., Subandiyah, S., Ueda, S., 2008. A virus and its vector, *Pepper yellow leaf curl virus* and *Bemisia tabaci*, two new invaders of Indonesia. Biol. Invasions 10, 411–433. https://doi.org/10.1007/s10530-007-9141-x.

De Jonghe, K., De Roo, I., Maes, M., 2017. Fast and sensitive on-site isothermal assay (LAMP) for diagnosis and detection of three fruit tree phytoplasmas. Eur. J. Plant Pathol 147, 749–759. https://doi.org/10.1007/s10658-016-1039-y.

Francois, P., Tangomo, M., Hibbs, J., Bonetti, E.-J., Boehme, C.C., Notomi, T., Perkins, M.D., Schrenzel, J., 2011. Robustness of a loop-mediated isothermal amplification reaction for diagnostic applications. FEMS Immunol. Med. Microbiol. 62, 41–48. https://doi.org/10.1111/j.1574-695X.2011.00785.x.

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. https://doi.org/10.1016/S0166-0934(03)00187-3.

Fukuta, S., Anai, N., Kato, M., Yoshimura, Y., Fukaya, M., Yabe, K., Oya, T., Kanbe, M., 2005. Simplification of template DNA preparation for the loop-mediated isothermal amplification (LAMP) method to detect Tomato yellow leaf curl virus. Ann. Rept. Kansai. Pl. Prot. 47, 37–41. https://doi.org/10.4165/kapps.47.37.

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 loot-mediated isothermal amplification reaction. J. Virol. Methods. 189, 348–354. https://doi.org/10.1016/j.jviromet.2013.03.005.

Herrera-Vásquez, J.A., Puchades, A.V., Elvira-González, L., Jaén-Sanjur, J.N., Carpino, C., Rubio, L., Galipienso, L., 2018. Fast detection by loop-mediated isothermal amplification (LAMP) of the three begomovirus species infecting tomato in Panama. Eur. J. Plant Pathol. 151, 243–250. https://doi.org/10.1007/s10658-017-1358-7.

Hodgetts, J., Tomlinson, J., Boonham, N., Gonzalez-Martin, I., Nikolić, P., Swarbrick, P., Yankey, E.N., Dickinson, M., 2011. Development of rapid in-field loop-mediated isothermal amplification (LAMP) assays for phytoplasmas. Bull. Insectol. (Suppl.) 64, S41–S42.

Jamsari, J., Pedri, J., 2013. Complete nucleotide sequence of DNA A-like genome and DNA-β of monopartite *Pepper yellow leaf curl virus*, a dominant *Begomovirus* infecting *Capsicum annuum* in West Sumatera Indonesia. Asian J. Plant Pathol. 7, 1–14. https://doi.org/10.3923/ajppaj.2013.1.14.

Jeevalatha, A., Kaundal, P., Kumar, R., Raigond, B., Kumar, R., Sharma, S., Chakrabarti, S.K., 2018. Optimized loop-mediated isothermal amplification assay for *Tomato leaf curl New Delhi virus-[potato]* detection in potato leaves and tubers. Eur. J. Plant. Pathol. 150, 565–573. https://doi.org/10.1007/s10658-017-1300-z.

Kaneko, H., Kawana, T., Fukushima, E., Suzutani, T., 2007. Tolerance of loop-mediated isothermal amplification to a culture medium and biological substances. J. Biochem. Biophys. Methods 70, 499–501. https://doi.org/10.1016/j.jbbm.2006.08.008.

Katoh, H., Fukuda, T., Nishigawa, H., Natsuaki, T., 2016. Rapid detection of *Colletotrichum gloeosporioides* in infected strawberry plants using loop-mediated isothermal amplification. J. Gen. Plant Pathol. 82, 190–198. https://doi.org/10.1007/s10327-016-0665-8.

Kenyon, L., Tsai, W.-S., Shih, S.-L., Lee, L.-M., 2014. Emergence and diversity of begomoviruses infecting solanaceous crops in East and Southeast Asia. Virus. Res. 186, 104–113. https://doi.org/10.1016/j.virusres.2013.12.026.

Kintasari, T., Septariani, D.W.N., Sulandari, S., Hidayati, S.H., 2013. *Tomato yellow leaf curl Kanchanaburi virus* associated with yellow mosaic disease of eggplant in Java. Phytopathol. Indonesia J. 9, 127–131. https://doi.org/10.14692/jfi.9.4.127.

Kuan, C., Wu, M., Lu, Y., Huang, H., 2010. Rapid detection of *Squash leaf curl virus* by loop-mediated isothermal amplification. J. Virol. Methods. 169, 61–65. https://doi.org/10.1016/j.jviromet.2010.06.017.

Mizutani, T., Daryono, B.S., Ikegami, M., Natsuaki, K.T., 2011. First report of *Tomato leaf curl New Delhi virus* infecting cucumber in Central Java. Indonesia. Plant Dis. 95, 1485. https://doi.org/10.1094/PDIS-03-11-0196.

Nagamine, K., Hase, T., Notomi, T., 2002. Accelerated reaction by loop-mediated isothermal amplification using loop primers. Mol. Cell. Probes 16, 223–229. https://doi.org/10.1006/mcpr.2002.0415.

Notomi, T., Okayama, H., Masubuchi, T., Yonekawa, T., Watanabe, K., Amino, N., Hase, T., 2000. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res. 28, e63. https://doi.org/10.1093/nar/28.12.e63.

Parida, M., Sannarangaiah, S., Dash, P.K., Rao, P.V.L., Morita, K., 2008. Loop mediated isothermal amplification (LAMP): a new generation of innovative gene amplification technique; perspectives in clinical diagnosis of infectious diseases. Rev. Med. Virol. 18, 407–421. https://doi.org/10.1002/rmv.593.

Rojas, M.R., Gilbertson, R.L., Russell, D.R., Maxwell, D.P., 1993. Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. Plant Dis. 77, 340–347. https://doi.org/10.1094/PD-77-0340.

Septariani, D.N., Hidayat, S.H., Nurhayati, E., 2014. Identification of the causal agent of yellow leaf curl disease on cucumbers. J.H.P.T. Tropika 14, 80–86. http://jhpttropika.fp.unila.ac.id/index.php/jhpttropika/article/view/313/306.

Sugawara, K., Himeno, M., Keima, T., Kitazawa, Y., Maejima, K., Oshima, K., Namba, S., 2012. Rapid and reliable detection of phytoplasma by loop-meidated isothermal amplification targeting a housekeeping gene. J Gen. Plant Pathol. 78, 389–397. https://doi.org/10.1007/s10327-012-0403-9.

Suzuki, R., Fukuta, S., Matsumoto, Y., Hasegawa, T., Kojima, H., Hotta, M., Miyake, N., 2016. Development of reverse transcription loop-mediated isothermal amplification assay as a simple detection method of *Chrysanthemum stem necrosis virus* in chrysanthemum and tomato. J. Virol. Methods. 236, 29–34. https://doi.org/10.1016/j.jviromet.2016.07.005.

Wiratama, D.M.P., Wirya, G.N.A.S., Adnyani, N.N.P., Nyana, D.N., Suastika, G., 2015. First report on *Begomovirus* infection on cucumber in Bali. Phytopathol. Indonesia J. 11, 175–178. https://doi.org/10.14692/jfi.11.5.175.

Zaidi, S.S.-E.-A., Martin, D.P., Amin, I., Farooq, M., Mansoor, S., 2017. *Tomato leaf curl New Delhi virus*: a widespread bipartite begomovirus in the territory of monopartite begomoviruses. Mol. Plant Pathol. 18, 901–911. https://doi.org/10.1111/mpp.12481.