European Journal of Plant Pathology

# Fast detection by loop-mediated isothermal amplification (LAMP) of the three begomovirus species infecting tomato in Panama

--Manuscript Draft--

| | |
|---|---|
| **Manuscript Number:** | EJPP-D-17-00438R1 |
| **Full Title:** | Fast detection by loop-mediated isothermal amplification (LAMP) of the three begomovirus species infecting tomato in Panama |
| **Article Type:** | Short Communication |
| **Keywords:** | PYMPV; ToLCSiV; TYMoV; Begomovirus; LAMP, Solanum lycopersicum |
| **Corresponding Author:** | Luis Galipienso<br>Instituto Valenciano de Investigaciones Agrarias<br>Moncada, Valencia SPAIN |
| **Corresponding Author Secondary Information:** | |
| **Corresponding Author's Institution:** | Instituto Valenciano de Investigaciones Agrarias |
| **Corresponding Author's Secondary Institution:** | |
| **First Author:** | Jose Herrera-Vásquez |
| **First Author Secondary Information:** | |
| **Order of Authors:** | Jose Herrera-Vásquez |
| | Andrés Puchades |
| | Laura Elvira-González |
| | Jose Jaén-Sanjur |
| | Caterina Carpino |
| | Luis Rubio |
| | Luis Galipienso |
| **Order of Authors Secondary Information:** | |
| **Funding Information:** | Secretaría Nacional de Ciencia, Tecnología e Innovación (SENACYT) of Panama (FID14-020) — Dr Jose Herrera-Vásquez |
| | Instituto Valenciano de Investigaciones Agrarias — Mr Andrés Puchades |
| | Università degli Studi di Palermo — Miss Caterina Carpino |
| **Abstract:** | Potato yellow mosaic Panama virus (PYMPV), Tomato leaf curl Sinaloa virus (ToLCSiV) and Tomato yellow mottle virus (TYMoV) of genus Begomovirus (family Geminiviridae) are the only three begomovirus species detected infecting tomato (Solanum lycopersicum L.) in Panama. PYMPV, ToLCSiV and TYMoV induce symptoms of stunting, yellowing, curling, distortion of leaves and reduction of fruit size and cause important economic loses. A loop-mediated amplification under isothermal conditions (LAMP) assay was developed for the individual detection of these three begomovirus species by using a set of three primer pairs specific per each one of them. Amplification products were visualized by gel electrophoresis or direct Gel-Red staining of DNA into the reaction tube. PYMPV, ToLCSiV and TYMoV were detected in total DNA extracts obtained from different plant tissues such as leaves, stems, flowers, fruits and roots of infected tomato plants collected in different production regions of Panama. LAMP sensitivity was similar to that of conventional PCR but, the first procedure was faster and cheaper than the last one. Moreover, all three viruses were successfully detected by LAMP and not by conventional PCR from sap extracts |

| | |
|---|---|
| | obtained from leaf tissues of infected tomato plants which were embedded into 3MM Whatman paper and stored several days, facilitating the samples processing as well as the material movement among different laboratories. Therefore, LAMP is a specific, rapid and cheap procedure to detect all three begomoviruses infecting tomato in Panama and it is suitable for field surveys and sanitation programs. |
| **Response to Reviewers:** | Dear Editor,<br><br>All changes proposed by reviewers have been included in the reviewed version of the manuscript.<br><br>We would like to thank the reviewers for careful and thorough reading of this manuscript and for the thoughtful comments and constructive suggestions, which help to improve the quality of this manuscript. We enclose a detailed list of responses to all reviewer´s comments (author replies are in bold)<br><br>Reviewer´s comments and author´s replies<br><br>Reviewer #1: The manuscript describes a method based on LAMP for the detection of three begomovirus species in tomato from Panama. The three viruses, PYMPV, ToLCSiV and TYMoV, produce symptoms in leaves, and affect fruit production.<br>The description of the method involves determination of reliability and specificity of detection, comparison of sensitivity with conventional PCR, comparison of detection in different parts of plants (the distribution), and the robustness. In addition the authors show that positive results can be obtained also from leaf tissues of infected tomato plants which were embedded into 3MM Whatman paper and stored several days.<br>The text is well written, and the discussion adequately compares the results with those in the literature.<br>Some minor corrections in the text need to be introduced:<br><br>Line 63: (…) was similar to that…<br>Line 96: (…) genomes are not predominant…<br>Line 97: (…) genomes code for the AV2…<br>Line 105: (…) sanitation programs it is essential…<br>Line 107 (…) TYMoV based on a loop-mediated…<br>Line 237: (…) can be stored and MAILED to laboratories far away from THE SITES OF SAMPLE COLLECTION for further analysis.<br>Line 243: (…) could be used for epidemiological studies.<br>Line 244: (…) as well as in sanitation programs.<br><br>Author´s reply: All these minor corrections have been included in the reviewed version of the manuscript<br><br>Reviewer #2: The MS is written well and contains useful information though could use little more editing to clarify some points and/or present the data easier to follow by readers. I made some comments and indicated points that were not clear to me in attached PDF file for authors' consideration. I am still curious though whether this LAMP assay will be able to distinguish the other begomoviruses that share relatively high sequence homology. Table 2 was very confusing and I was not able to follow. I believe it can be revised to make it more reader friendly. I encourage authors to pay attention to all my comments/suggestions that I made attached PDF file.<br><br>Author´s reply: All changes have been done<br><br>Line 58: “primers” has been replaced by “primer”<br>Line 92: “sense strand” has been removed<br>Line 119: “polymerases” has been replaced by “polymerase”<br>Line 128: “ground to a fine powder” has been replaced by “homogenized”<br>Line 183: information about the primer set used to detect PYMPV, ToLCSiV and TYMoV by conventional PCR (primers names, nucleotide sequences, amplicon sizes and virus target genes as published in the Herrera-Vázquez et al., 2016) has been included in the table nº1<br>Line 236: percentage data of positive detections obtained by LAMP from sap extracts |

| | |
|---|---|
| | of virus infected tomato plants embedded into Whatman papper and time of sample storage as well has been included in the reviewed version of the manuscript<br><br>Table 2: We have included in the reviewed version of the manuscript a new table 2 with the number and also the percentages of plants with single and mixed infections. We hope this new table shows the information more friendlily than table of the original manuscript<br><br>Figure 2: LAMP and PCR negative controls corresponding to non-infected tomato plants have been included in the agarose gel electrophoresis (lane 5)<br><br>We hope the new data and corrections incorporated to the paper enable it to be published in European Journal of Plant Pathology |

# Fast detection by loop-mediated isothermal amplification (LAMP) of the three begomovirus species infecting tomato in Panama

**J. A. Herrera-Vásquez, A. V. Puchades, L. Elvira-González, J. N. Jaén-Sanjur, C. Carpino, L. Rubio, L. Galipienso**

J. A. Herrera-Vásquez, J. N. Jaén-Sanjur

Grupo de Investigación de Protección Vegetal (GIPV), Centro de Investigación Agropecuaria Central (CIAC), Instituto de Investigación Agropecuaria de Panamá (IDIAP), Ctra. Panamericana, Los Canelos, Santa María, Estafeta de Divisa, 0619 Herrera, Panama

A. V. Puchades, L. Elvira-González, C. Carpino, L. Rubio, L. Galipienso

Instituto Valenciano de Investigaciones Agrarias (IVIA), 46113 Moncada, Valencia, Spain

J. N. Jaén-Sanjur

Escuela de Biología, Facultad de Ciencias Naturales, Exactas y Tecnología, Ciudad Universitaria Dr. Octavio Méndez Pereira, Universidad de Panamá (UP), Estafeta Universitaria, 3366 Panama 4, Panama

C. Carpino

Department of Agricultural and Forestry Science, University of Palermo, Piazza Marina, 61, 90133 Palermo, Italy

L. Galipienso

Departamento de Biotecnología, Escuela Técnica Superior de Ingeniería Agronómica y del Medio Natural, Universitat Politècnica de València (UPV), Camí de Vera, s/n, 46022 València, Spain

Corresponding author. E-mail address: galipienso.lui@gva.es

Phone number: +34 963 42 40 00

**Running head** Detection of begomoviruses by LAMP

**Abstract** *Potato yellow mosaic Panama virus* (PYMPV), *Tomato leaf curl Sinaloa virus* (ToLCSiV) and *Tomato yellow mottle virus* (TYMoV) of genus *Begomovirus* (family *Geminiviridae*) are the only three begomovirus species detected infecting tomato (*Solanum lycopersicum* L.) in Panama. PYMPV, ToLCSiV and TYMoV induce symptoms of stunting, yellowing, curling, distortion of leaves and reduction of fruit size and cause important economic loses. A loop-mediated amplification under isothermal conditions (LAMP) assay was developed for the individual detection of these three begomovirus species by using a set of three primer pairs specific per each one of them. Amplification products were visualized by gel electrophoresis or direct Gel-Red staining of DNA into the reaction tube. PYMPV, ToLCSiV and TYMoV were detected in total DNA extracts obtained from different plant tissues such as leaves, stems, flowers, fruits and roots of infected tomato plants collected in different production regions of Panama. LAMP sensitivity was similar to that of conventional PCR but, the first procedure was faster and cheaper than the last one. Moreover, all three viruses were successfully detected by LAMP and not by conventional PCR from sap extracts obtained from leaf tissues of infected tomato plants which were embedded into 3MM Whatman paper and stored several days, facilitating the samples processing as well as the material movement among different laboratories. Therefore, LAMP is a specific, rapid and cheap procedure to detect all three begomoviruses infecting tomato in Panama and it is suitable for field surveys and sanitation programs.

**Keywords** PYMPV, ToLCSiV, TYMoV, LAMP, Begomovirus, *Solanum lycopersicum*

Tomato (*Solanum lycopersicum* L.) is one of the most important horticultural crops worldwide, with a total harvested area of approximately 5 million hectares and a production around 170 million tons in 2014 (FAOSTAT 2017). Tomato originated in western South America and it was probably domesticed in Central America (Kimura and Sinha 2008). In Panama, tomato has been increasing due mainly to the interest of food transformation industry to process local products. Since 2000, emerging viruses of the genus *Begomovirus* have enhanced the tomato production losses as consequence of the increase of their transmission vectors populations, the whitefly *Bemisia tabaci* Gennadius (*Hemiptera: Aleyrodidae*) (Engel et al. 1998; Hull 2013).

Begomoviruses have a circular ambi-sense and single-stranded DNA genome (ssDNA) of one or two genomic components, named DNA A and DNA B, each one of 2.5–2.6 kb in size (Brown 1997; Varma and Malathi 2003). The DNA A sense strand encodes for the AV1 and AV2 proteins which are the viral coat and movement proteins, respectively, while the anti-sense DNA strand encodes for the AC1, AC2, AC3 and AC4 proteins which are the viral replication (Rep), transcriptional activator (TrAP), replication enhancer (Ren) and host cell-cycle control proteins, respectively. DNA B encodes for the BV1 and BC1 proteins with nuclear shuttle (NSP) and viral movement functions, respectively. Most begomovirus species present in the Americas (New World begomoviruses) have bipartite genomes lacking the AV2 protein, whereas in Africa, Asia, Australasia and Europe, begomovirus species (Old World begomoviruses) with bipartite genomes are not predominant and only the species with monopartite genomes code for the AV2 protein (Brown et al. 2012, 2015).

Three New World begomoviruses with bipartite genomes, *Potato yellow mosaic Panama virus* (PYMPV), *Tomato leaf curl Sinaloa virus* (ToLCSiV) and *Tomato yellow mottle virus* (TYMoV) are the only begomovirus species infecting tomato crops in

Panama. Infected plants with these viruses show symptoms of stunting, yellowing, curling, distortion of leaves and reduction of fruit size (Engel et al. 1998; Herrera-Vásquez et al. 2015, 2016). To determine the spread, incidence and impact of PYMPV, ToLCSiV and TYMoV in tomato crops as well as to implement quarantine and sanitation programs it is essential to dispose of specific and sensitive detection methods. In this work, we have developed a fast and sensitive method for specific and fast detection of PYMPV, ToLCSiV and TYMoV based on a loop-mediated amplification under isothermal conditions (LAMP) (Mori and Notomi 2009). To our knowledge, only two other begomoviruses such as *Tomato yellow leaf curl virus* (TYLCV) and *Tomato leaf curl Bangalore virus* (ToLCBaV) has been successfully detected by using this technique (Fukuta et al. 2003; Almasi et al. 2013; Arutselvan et al. 2017). LAMP is increasingly used for detecting different human and plant pathogens (Fukuta et al. 2003; Parida et al. 2008; Tomlinson et al. 2010; Bühlmann et al. 2013). LAMP has been reported to be at least as sensitive as the conventional PCR and has the advantage of avoiding the use of expensive thermal cycling instruments since the nucleic acid amplification is performed under isothermal conditions (Kuan et al. 2010), enabling its application in developing countries where sophisticated laboratory equipment is not available (Boonham et al., 2014). Moreover, LAMP is much less sensitive to nucleic acid polymerase inhibitors than conventional PCR, and can be used with non- processed sap extracts facilitating sample processing (Francois et al. 2011; Elvira-González et al. 2017).

Viral DNA was purified from dry or fresh (50 mg) tissues of PYMPV, ToLCSiV or TYMoV -infected or non- infected tomato plants of different commercial (DRD 8539, Liro 42, Miramar, SUN 7705 and Tointer) and local (Entero Grande, IDIAP T-8 and IDIAP T-9) cultivars following the methodology described by Dellaporta et al.

(1983). Plant material were placed in a plastic bag containing 600 µl of extraction buffer (100 mM Tris-HCl, 50 mM EDTA, pH 8, 500 mM NaCl, 10 mM β-mercaptoethanol, 10% SDS) and homogenized with a pestle. Then, DNAs were precipitated by adding 1.25 M potassium acetate and 300 µl of isopropanol. The mix was centrifuged at 14,000 rpm for 10 min and DNA was resuspended in 50 µl of ultrapure sterile water. The tomato plants used in this study were collected in plots of two main production areas of Panama (provinces of Chiriquí and Herrera). The presence of PYMPV-, ToLCSiV- or TYMoV infections was previously determined by conventional PCR (Herrera-Vásquez et al. 2016). Healthy tomato plants (Var. Mariana) were grown at 25 $^{o}$C in a temperature and light controlled insect-proof chamber to be used as negative amplification controls. Tomato plants infected with TYLCV or with *Tomato leaf curl New Delhi virus* (ToLCNDV), two widespread begomoviruses (kindly provided by Dr. M. I. Font-San-Ambrosio) were used as amplification specificity controls. TYMoV has been always detected in mixed infections with other begomoviruses and all tomato plants infected with this virus used in this study were also infected with PYMPV.

Nucleotides sequences of PYMPV, ToLCSiV and TYMoV isolates from Panama, Costa Rica and Nicaragua were retrieved from GenBank (accession numbers Y15034, NC_002048, KP313716, KP313717 for PYMPV; NC_009606, KP318651, KP318652, AJ608286, AJ508778 to AJ508780, AF131213 for ToLCSiV; and NC_019946, KC176780, AF112981, KP318653, KP318654 for TYMoV) and aligned with the Clustal W program (Larkin et al. 2007) to identify conserved sequence stretches. Nucleotide identities among isolates from the same viral species were very high and the CP (for PYMPV) and Rep (for ToLCSiV and TYMoV) regions were chosen as the amplification targets. A set of three primer pairs specific for each PYMPV, ToLCSiV and TYMoV- was designed by using the LAMP Designer 1.12 sofware (Premier

Biosoft, Palo Alto, CA) (Table 1). LAMP reaction was performed by adding 1 µl of nucleic acid extract in an eppendorf tube to a final reaction volume of 25 µl containing 1 x Isotermal Amplification Buffer (20 mM Tris-HCl, 10 mM $(NH_4)_2$ $SO_4$, 50 mM KCl, 2 mM $MgSO_4$, 0.1% Tween 20), 4 mM $MgSO_4$, 1.4 mM dNTPs mix (0.35 mM of each dATP, dTTP, dCTP and dGTP), 160 U/ml Bst DNA polymerase (New England Biolabs, UK) and a mix of the three primer pairs (0.2 µM F3 and B3, 1.6 µM FIP and BIP, 0.4 µM LoopF and LoopR). PYMPV, ToLCSiV and TYMoV were detected in separate reaction tubes by using the set of three primer pairs specific for each of them. The mixture was incubated at 60 ◦C for 40 min in a water bath and heated at 80 ◦C for 10 min to stop the reaction. The amplification products obtained were analyzed by electrophoresis in 2% agarose gels and visualized by using Gel Red (Biotium Inc., Fremont) under UV light. To simplify the procedure, 5 µl of Gel Red was added in the reaction mix and the fluorescence was visualized directly under UV.

The reliability and specificity of PYMPV, ToLCSiV or TYMoV detection by LAMP was assessed with total DNA extracts obtained from tomato leaf tissues of infected tomato plants. Total DNA extracts obtained from leaf tissues of TYLCV- and ToLCNDV- infected and non-infected tomato plants were used as negative amplification controls. LAMP amplification products were only observed by agarose electrophoresis analysis in samples corresponding to PYMPV-, ToLCSiV- or TYMoV-infected tomato plants but not in those corresponding to healthy or TYLCV- or ToLCNDV-infected plants (Fig. 1). TYMoV- infected tomato plants used in this study were co-infected with PYMPV, so amplification products were observed with the primer sets specific for both viruses (Fig 1a and c, lanes 1 and 2). However, amplification products were only observed from DNA extracts of tomato plants infected with only PYMPV with the corresponding primer set (Fig 1a, Lane 4). Fluorescence

under UV light after adding the Gel Red in the reaction was observed in the eppendorf tubes containing amplification products and not in those corresponding to healthy or TYLCV- or ToLCNDV- infected tomato plants (Fig. 1).

To compare the sensitivities of LAMP and conventional PCR, 10-fold serial dilutions from total DNA extracts obtained from leaf tissue of a PYMPV-, ToLCSiV- or TYMoV-infected tomato plant and adjusted to the concentration of 100 ng/µl were amplified by both methods: LAMP was carried out as described above and the conventional PCR as previously described Herrera-Vásquez et al. (2016) using a set of primers specific for each virus (Table 1). LAMP and conventional PCR had identical sensitivities allowing the detection of all three begomoviruses up to the dilution $10^{-2}$ of total DNA extracts, equivalent to the concentration of 1 ng/µl (Fig. 2). Also, LAMP sensitivity for detection of PYMPV, ToLCSiV or TYMoV was similar to those reported for begomoviruses TYLCV and ToLCBaV and other plant viruses such as *Potato virus Y* (PVY), *Prunus necrotic ringspot virus* (PNRSV), *Tomato torrado virus* (ToTV) and *Southern tomato virus* (STV) (Fukuta et al. 2003; Almasi et al. 2013; Zong et al. 2014; Przewodowska et al. 2015; Budziszewska et al. 2016; Elvira-González et al. 2017; Arutselvan et al. 2017).

The distribution of PYMPV, ToLCSiV and TYMoV within the infected plants was examined by LAMP from total DNA extracts of different tomato tissues such as leaf, stem, flower, fruit and root. Equivalent extracts from non-infected tomato plants were used as negative controls. Amplification products were observed in agarose gels after electrophoresis or directly in eppendorf tubes under UV light in all tissues of tomato plants infected with the three begomoviruses, but not in the equivalent extracts of non-infected plants. The results were identical for the three begomoviruses and Fig. 3

200 shows those for PYMPV. Wide virus dispersion within the plant has been also reported
201 for other bipartite begomovirus, *Squash leaf curl virus* (SLCV) (Kuan et al. 2010).
202 The robustness of PYMPV, ToLCSiV and TYMoV detection by LAMP was
203 assayed by analyzing 94 tomato plants collected in eight tomato plots of two main
204 production areas of Panama (provinces of Chiriquí and Herrera) during the period
205 2011–2012, which were previously analyzed for these viruses by conventional PCR
206 (Herrera-Vásquez et al. 2016). Results obtained showed a 100% of coincidence between
207 both techniques (Table 2). Single PYMPV, ToLCSiV and TYMoV infections were
208 detected in 48 (51.1%), 40 (42.6%) and 2 (2.1%) samples, respectively. PYMPV was
209 found in all plots of the two provinces, while ToLCSiV was only found in plots of
210 Chiriquí province and TYMoV in plots of Herrera province. Most samples showed
211 double infections (64.8%), being prevalent the combination PYMPV/ToLCSiV
212 (49.2%). None of the samples showed ToLCSiV/TYMoV infection (Herrera-Vásquez et
213 al. 2016).
214 To facilitate the processing of samples, virus DNA amplification was assayed by
215 conventional PCR and LAMP from sap extracts of dry leaf tissues of five PYMPV and
216 ToLCSiV-infected tomato plants and the only two available TYMoV-infected tomato
217 plants. Around 50-100 mg of dry leaf tissue was put into an eppendorf tube containing
218 600 µl of extraction buffer (100 mM Tris-HCl, 50 mM EDTA, pH 8, 500 mM NaCl)
219 and homogenized with a plastic cone. Then, 20 µl of sap extracts were applied into
220 3MM Whatman paper and stored at room temperature until LAMP analysis. Equivalent
221 extract of a non-infected tomato plants was used as negative control. After several days
222 (43 days in the case of samples showed in the Fig. 4), embedded 3MM paper was put
223 into a 1.5 ml eppenforf containing 150 µl of water, incubated at room temperature for
224 10 min and then centrifuged at 10,000 rpm for 20 min. LAMP was carried out from 1 µl

of eluted sap extracts with the sets of specific primers for these begomoviruses as described above, and amplification products were visualized by both electrophoresis in 2% agarose gels or fluorescence of reaction mix in eppendorf tubes under UV light. Conventional PCR was performed following the protocol described by Herrera-Vásquez et al. (2016). As show the Fig. 4, specific amplification products in agarose gels and fluorescence in eppendorf tubes were observed in samples corresponding to sap extracts of PYMPV, ToLCSiV and TYMoV infected plants amplified by LAMP but not in those of equivalent samples amplified by conventional PCR, probably due to presence of nucleic acid polymerse inhibitors (Francois et al. 2011; Elvira-González et al. 2017). Positive detections were obtained from the 100% of Whatman paper eluted samples after 43 days of paper storage containing the embedded samples at room temperature.

In addition, no amplification products or tube fluorescence was observed on samples corresponding to sap extracts from non-infected plants. Therefore, this method could facilitate the processing of a large number of samples as well as the material movement among different laboratories. Whatman paper with the sap extracts can be stored (almost 43 days for PYMPV, ToLCSiV and TYMoV) and mailed to laboratories far away from the sites of sample collection for further analysis.

In conclusion, the LAMP assay developed in this study is a specific, sensitive, rapid and cost-effective method for the detection of the three begomovirus species (PYMPV, ToLCSiV and TYMoV) infecting tomato crops in Panama. Virus detection from sap extracts following the procedure describe above enable the "*in situ*" sample processing at the collection place storing it into 3MM Whatman paper until further LAMP analysis. Therefore, this technique could be used for epidemiological studies of these viruses on field surveys, as well as in sanitation programs of governmental institutions and plant suppliers.

**Acknowledgements** This work was supported by the project FID14-020 of the Secretaría Nacional de Ciencia, Tecnología e Innovación (SENACYT) of Panama. Dr. J.A. Herrera-Vázquez was recipient of a National Research Investigator award (SNI) from the SENACYT for a training stage in the Virology laboratory of IVIA under Dr. L. Galipienso's supervision. A. Puchades was recipient of a specialization fellowship from IVIA and C. Carpino was recipient of a predoctoral fellowship from the University of Palermo, Sicily (Italy). We thank Dr. M. I. Font-San-Ambrosio (UPV, Valencia, Spain) for providing the TYLCV and ToLCNDV isolates used in this work.

**Compliance with ethical standards** All authors in this manuscript have read and approved current version of the article. The part of the paper corresponding to detection of PYMPV, ToLCSiV and TYMoV by conventional PCR from tomato field samples which was previously published has been referenced in the manuscript. No conflict of interest exits in the submission of this manuscript.

## References

Almasi, M.A., Ojaghkandi, M.A., Hemmatabadi, A., Hamidi, F., Aghaei, S. (2013). Development of colorimetric loop-mediated isothermal amplification assay for rapid detection of the *Tomato yellow leaf curl virus*. *Journal of Plant Pathology and Microbiology*, *4(1)*, 153, doi: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 Disease*, doi:10.1007/s13337-017-0385-5

Boonham, N., Kreuze, J., Winter, S., Vlugt, R. van der., Bergervoet, J., Tomlinson, J., et al. (2014). Methods in virus diagnostics: from ELISA to next generation sequencing. *Virus Research, 186*, 20–31.

Brown, J. K. (1997). The biology and molecular epidemiology of the Geminiviridae subgroup III. In G. Stacey & N. T. Keen (Eds.), *Plant-Microbe Interactions, 2* (pp. 125–195). New York: Chapman & Hall.

Brown, J. K., Fauquet, C. M., Briddon, R. W., Zerbini, M., Moriones, E., & Navas-Castillo J. (2012). Geminiviridae. In A. M. Q. King, M. J. Adams, E. B. Carstens & E. J. Lefkowitz (Eds), *Virus taxonomy, IXth Report of the ICTV* (pp. 351–373). London: Elsevier Academic Press.

Brown, J.K., Zerbini, F.M., Navas-Castillo, J., Moriones, E., Ramos-Sobrinho, R., Silva, J.C., et al. (2015). Revision of Begomovirus taxonomy based on pairwise sequence comparisons. *Archives of Virology, 160(6)*, 1593–1619.

Budziszewska, M., Wieczorek, P., & Obrępalska-Stęplowska, A. (2016). One-step reverse transcription loop-mediated isothermal amplification (RT-LAMP) for detection of *Tomato torrado virus*. *Archives of Virology, 161(5)*, 1359–1364.

Bühlmann, A., Pothier, J. F., Rezzonico, F., Smits, T. H., Andreou, M., Boonham, N., et al. (2013). *Erwinia amylovora* loop-mediated isothermal amplification (LAMP) assay for rapid pathogen detection and on-site diagnosis of fire blight. *Journal of Microbiological Methods, 92(3)*, 332–339.

Dellaporta, S., Woods, H., & Hicks, J. (1983). A plant DNA minipreparation: version II. *Plant Molecular Biology Reporter, 1(4)*, 19–21.

Elvira-González, L., Puchades, A. V., Carpino, C., Alfaro-Fernández, A., Font-San-Ambrosio, M. I., Rubio, L., et al. (2017). Fast detection of *Southern tomato virus*

by one-step transcription loop-mediated isothermal amplification (RT-LAMP). *Journal of Virological Methods, 241*, 11–14.

Engel, M., Fernández, O., Jeske, H., & Frischmuth, T. (1998). Molecular characterization of a new whitefly-transmissible bipartite geminivirus infecting tomato in Panama. *Journal of General Virology, 79(10)*, 2313–2317.

FAOSTAT. (2017). FAO Statistical Database. http://faostat3.fao.org/faostat-gateway/go/to/download/Q/QC/E. Accessed 19 March 2017.

Francois, P., Tangomo, M., Hibbs, J., Bonetti, E. J., Boehme, C. C., Notomi T., et al. (2011). Robustness of a loop-mediated isothermal amplification reaction for diagnostic applications. *FEMS Immunology* and *Medical Microbiology*, *62(1)*, 41–48.

Fukuta, S., Kato, S., Yoshida, K., Mizukami, Y., Ishida, A., Ueda J., et al. (2003). Detection of *Tomato yellow leaf curl virus* by loop-mediated isothermal amplification reaction. *Journal of Virological Methods*, *112(1–2)*, 35–40.

Herrera-Vásquez, J. A., Ortega, D., Romero, A. B., Davino, S., Mejía, L. C., Panno, S., et al. (2016). Begomoviruses infecting tomato crops in Panama. *Journal of Phytopathology, 164(2)*, 102–113.

Herrera-Vásquez, J. A., Ortega, D., Romero, A. B., Davino, S., Mejía, L.C., Panno, S., et al. (2015). First report of *Tomato leaf curl Sinaloa virus* infecting tomato crops in Panama. *New Disease Reports 31*, 30.

Hull, R. (2013). Plant Virology, 5th ed. London: Elsevier Academic Press.

Kimura, S., & Sinha, N. (2008). Tomato (*Solanum lycopersicum*): a model fruit-bearing crop. *Cold Spring Harbor Protocols*, doi:10.1101/pdb.emo105

Kuan, C. P., Wu, M. T., Lu, Y. L., & Huang, H. C. (2010). Rapid detection of *Squash leaf curl virus* by loop-mediated isothermal amplification. *Journal of Virological Methods, 169(1)*, 61–65.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McGettigan, P. A., et al. (2007). Clustal W and Clustal X version 2.0. *Bioinformatics, 23(21)*, 2947–2948.

Mori, Y., & Notomi, T. (2009). Loop-mediated isothermal amplification (LAMP): a rapid, accurate, and cost-effective diagnostic method for infectious diseases. *Journal of Infection and Chemotherapy, 15(2)*, 62–69.

Parida, M., Sannarangaiah, S., Dash, P. K., Rao, P., & Morita, K. (2008). Loop mediated isothermal amplification (LAMP): a new generation of innovative gene amplification technique; perspectives in clinical diagnosis of infectious diseases. *Reviews in Medical Virology, 18(6)*, 407–421.

Przewodowska, A., Zacharzewska, B., Chołuj, J., & Treder, K. (2015). A one-step, real-time reverse transcription loop mediated isothermal amplification assay to detect *Potato virus Y*. *American Journal of Potato Research, 92(3)*, 303–311.

Tomlinson, J., Dickinson, M., & Boonham, N. (2010). Detection of *Botrytis cinerea* by loop-mediated isothermal amplification. *Letters in Applied Microbiology, 51(6)*, 650–657.

Varma, A., & Malathi, V. G. (2003). Emerging geminivirus problems: a serious threat to crop production. *Annals of Applied Biology, 142(2)*, 145–164.

Zong, X., Wang, W., Wei, H., Wang, J., Chen, X., Xu, L., et al. (2014). Rapid detection of *Prunus necrotic ringspot* virus using magnetic nanoparticle-assisted reverse transcription loop-mediated isothermal amplification. *Journal of Virological Methods, 208*, 85–89.

346 **Figures and tables**

347

348 **Fig. 1** Analysis of LAMP amplification products by electrophoresis in a 2% agarose gel
349 (at the top) or by direct visualization of fluorescence under UV light in eppendorf tubes
350 (at the bottom) obtained by using a set of specific primers for PYMPV (panel a),
351 ToLCSiV (panel b) and TYMoV (panel c). LAMP was performed with total DNA
352 extracts obtained from leaf tissues of two tomato plants carrying a PYMPV/TYMoV
353 mixed infection (lanes 1–2) and two tomato plants carrying a ToLCSiV or PYMPV
354 single infections (lanes 3 and 4, respectively). Lanes 5 and 6, correspond to LAMP
355 from total DNA extracts obtained from leaf tissues of the TYLCV and ToLCNDV-
356 infected tomato plants, respectively, and lane 7 to the non-infected tomato plant. Lane
357 M corresponds to 1Kb Plus DNA molecular weight marker (Thermo Fisher Scientific,
358 Waltham, MA).

359

360 **Fig. 2** Electrophoresis in 2% agarose gels of amplification products obtained by LAMP
361 and conventional PCR from total DNA extracts (100 ng/µl) (lane 1) and 10-fold serial
362 dilutions (lanes 2–4) of leaf tissue of a PYMPV (panel a), ToLCSiV (panel b) and
363 TYMoV (panel c)-infected tomato plant by using the specific primers set for each virus.
364 Lane 5 corresponds to LAMP and conventional PCR amplifications from total DNA
365 extracts (100 ng/µl) of non-infected tomato plants used as negative controls. Lane M
366 corresponds to 1Kb Plus DNA molecular weight marker (Thermo Fisher Scientific,
367 Waltham, MA).

368

369 **Fig. 3** Analysis of amplification products by electrophoresis in 2% agarose gel (at the
370 top) or by direct visualization of fluorescence under UV light in reaction mix tubes (at

the bottom) obtained by LAMP from total DNA extracts of leaf, stem, flower, fruit and root tissues of a PYMPV-infected tomato plant (lanes 1–5, respectively) and the equivalent extracts of a non-infected tomato plant (lanes 6–10, respectively). Lane M corresponds to 1Kb Plus DNA molecular weight marker (Thermo Fisher Scientific, Waltham, MA).

**Fig. 4** Electrophoresis in 2% agarose gels (at the top) or direct visualization of fluorescence under UV light in eppendorf tubes (at the bottom) of amplification products obtained by LAMP and conventional PCR from sap of leaf tissues of five PYMPV and ToLCSiV-infected tomato plants (panels a and b, respectively; lanes 1-5) and two TYMoV-infected tomato plants (panel c, lanes 1-2) by using the specific primers set for each virus. Sap extracts were applied into 3MM Whatman paper and stored at room temperature for 43 days until LAMP analysis. Lanes 6 in panels a and b and lane 3 in panel c correspond to LAMP and PCR from equivalent sap extracts of a non-infected tomato plant. Lane M corresponds to 1Kb Plus DNA molecular weight marker (Thermo Fisher Scientific, Waltham, MA).

**Table 1** Set of primers used for detection by LAMP and PCR of *Potato yellow mosaic Panama virus* (PYMPV), *Tomato leaf curl Sinaloa virus* (ToLCSiV) and *Tomato yellow mottle virus* (TYMoV).

| Virus | Primer name | Type | Sequence (5`–3`) | Amplicon size (bp) | Genomic Region |
|---|---|---|---|---|---|
| PYMPV | F3 | LAMP Forward outer | CAGGAATTGGGCCAAGAATA | | CP |
| | B3 | LAMP Reverse outer | CGACACGGTGGGTAATAC | | |
| | FIP | LAM Forward inner (FIC + TTTT + F2) | GGACCTTACAAGGCCCTTCACATCTAT CGGACGTTGAGGA | | |
| | BIP | LAMP Reverse inner (BIC + TTTT + B2) | AGTCATTCGAGCAGCGACATGCGTGT AACGTCAGATATACACA | | |
| | Loop F | LAMP Forward loop | ACCTCTTGGAACATCAGGC | | |
| | Loop R | LAMP Reverse loop | ACATCTTACACACTGGTAAGGT | | |
| | PYMPVF | PCR Forward | CTATCTTTAACTCAAAATGCC | 743 | |
| | PYMPVR | PCR Reverse | ATCCGAATCTTAAGCGTTGC | | |
| ToLCSiV | F3 | LAMP Forward outer | TGTTGTGGTTGTTCTTCCC | | |
| | B3 | LAMP Reverse outer | CAAGTTCCGACGTCAAGT | | Rep |
| | FIP | LAMP Forward inner (FIC + TTTT + F2) | CAAACGACACATATGCCAAGGCTGAT TCGCATTGCTTCGT | | |
| | BIP | LAMP Reverse inner (BIC + TTTT + B2) | TCTGGCAGATCTACCGTCGATCGACA AGGATGGAGATACAA | | |
| | Loop F | LAMP Forward loop | GTTGAATGCAGCATGTGCA | | |
| | Loop R | LAMP Reverse loop | CTGGAATTGTCCCCATTCGA | | |
| | ToLCSiVF | PCR Forward | GAAATGTTGTGGTTGTTCTTCCCG | 580 | |
| | ToLCSiVR | PCR Reverse | GTCCCAAAACTCCATAGAGAG | | |
| TYMoV | F3 | LAMP Forward outer | TTAACATCTGAGCTGCTCTTAG | | |
| | B3 | LAMP Reverse outer | TTCATTCGTGTCGCAAGG | | Rep |
| | FIP | LAMP Forward inner (FIC + TTTT + F2) | CCAGAAACTCTTCGACCTCAGTCCTGA ATGTTCGGATGGAA | | |
| | BIP | LAMP Reverse inner (BIC + TTTT + B2) | CTGGGCTTTACCTTCGAGTTGTCTCCA CGAAGATGGGGAA | | |
| | Loop F | LAMP Forward loop | CCAACCAGGTCAGCACAT | | |
| | Loop R | LAMP Reverse loop | ATAAGCACATGGAGGTGAGG | | |
| | TYMoVF | PCR Forward | TCTGTAGTGCAGATTCAGTAGACCC | 479 | |
| | TYMoVR | PCR Reverse | GGAACCCAATTGGTGTTACGCTC | | |

408

409

410 **Table 2** LAMP detection of *Potato yellow mosaic Panama virus* (PYMPV), *Tomato*

411 *leaf curl Sinaloa virus* (ToLCSiV) and *Tomato yellow mottle virus* (TYMoV) from field

412 tomato plants collected in different production areas of Panama.

413

| **Location** | **Nº Samples** | **Single infections** | | | **Mixed infections** | | **% single infections[a]** | **% mixed infections[a]** |
|---|---|---|---|---|---|---|---|---|
| | | **PYMPV** | **ToLCSiV** | **TYMoV** | **PYMPV/ToLCSiV** | **PYMPV/TYMoV** | | |
| Plot 1: Palmarito (province of Chiriquí) | 10 | 2 | 0 | 0 | 1 | 0 | 66.7 | 33.3 |
| Plot 2: La Unión (province of Chiriquí) | 11 | 3 | 0 | 0 | 2 | 0 | 60 | 40 |
| Plot 3: Miraflores (province of Chiriquí) | 12 | 2 | 0 | 0 | 7 | 0 | 22.2 | 77.8 |
| Plot 4: Alto Quiel (province of Chiriquí) | 11 | 1 | 2 | 0 | 7 | 0 | 30 | 70 |
| Plot 5: Caisán Abajo (province of Chiriquí) | 18 | 0 | 2 | 0 | 11 | 0 | 15.4 | 84.6 |
| Plot 6: Caisán Centro (province of Chiriquí) | 12 | 0 | 1 | 0 | 7 | 0 | 12.5 | 87.5 |
| Plot 7: La Arenita (province of Herrera) | 10 | 3 | 0 | 0 | 0 | 1 | 75 | 25 |
| Plot 8: La Trinidad (province of Herrera) | 10 | 0 | 0 | 0 | 0 | 1 | 0 | 100 |
| Total | 94 | 11 | 5 | 0 | 35 | 2 | 35.2 | 64.8 |

414 [a] Percentage was calculated over the total number of virus-infected plants

**Fig. 1**

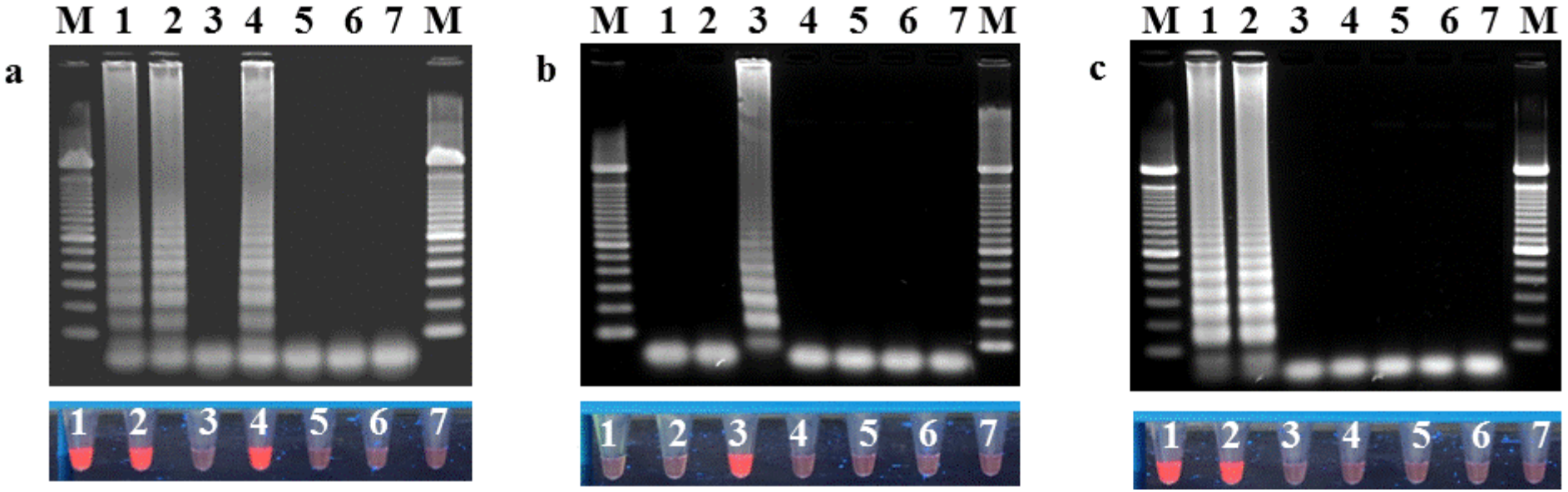

a
M 1 2 3 4 5 6 7 M
1 2 3 4 5 6 7
b
M 1 2 3 4 5 6 7 M
1 2 3 4 5 6 7
c
M 1 2 3 4 5 6 7 M
1 2 3 4 5 6 7

**Fig. 2**

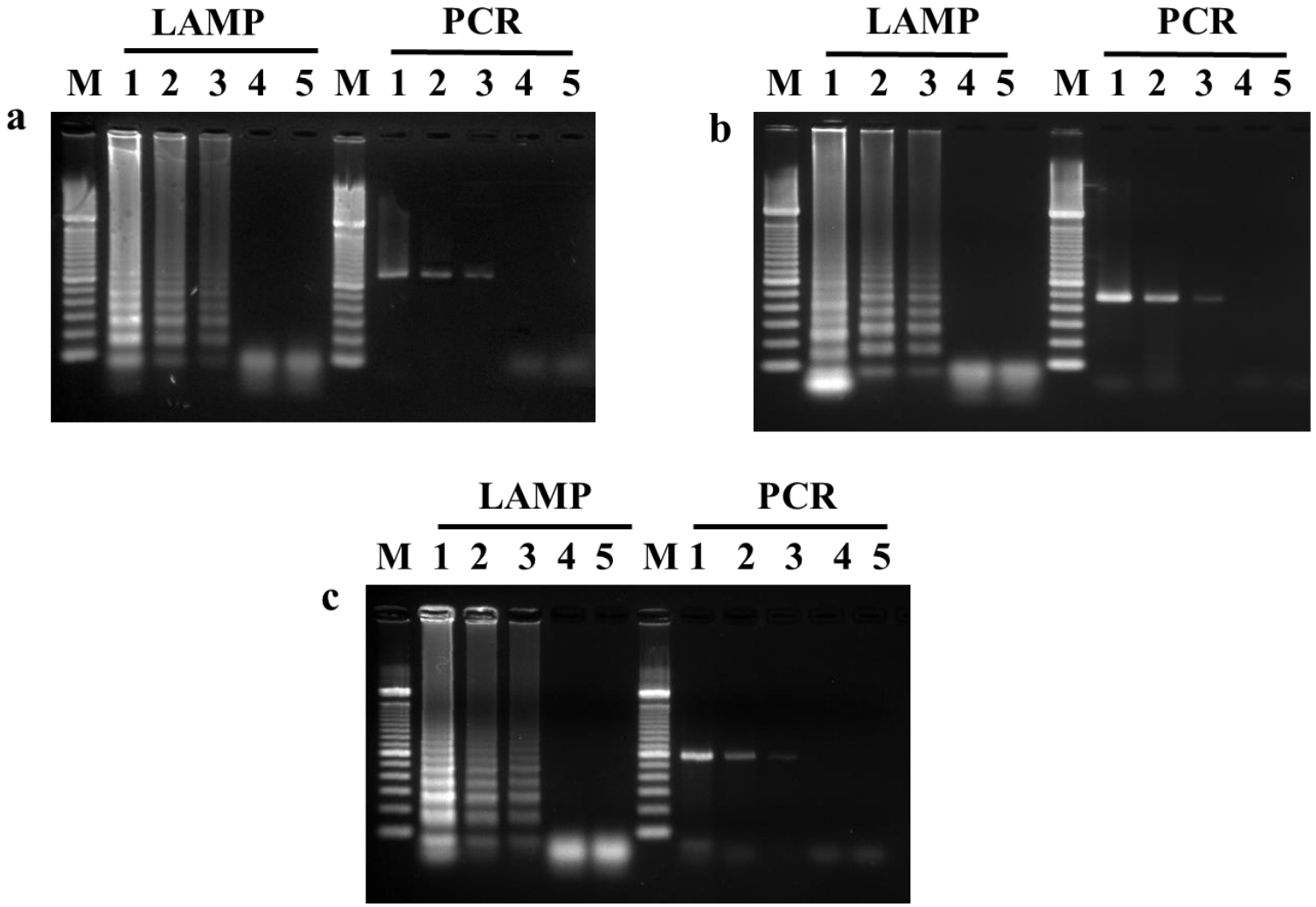


**Fig. 3**

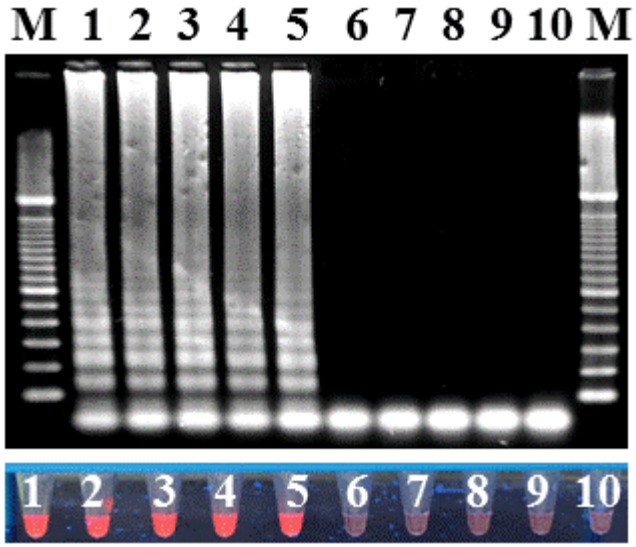
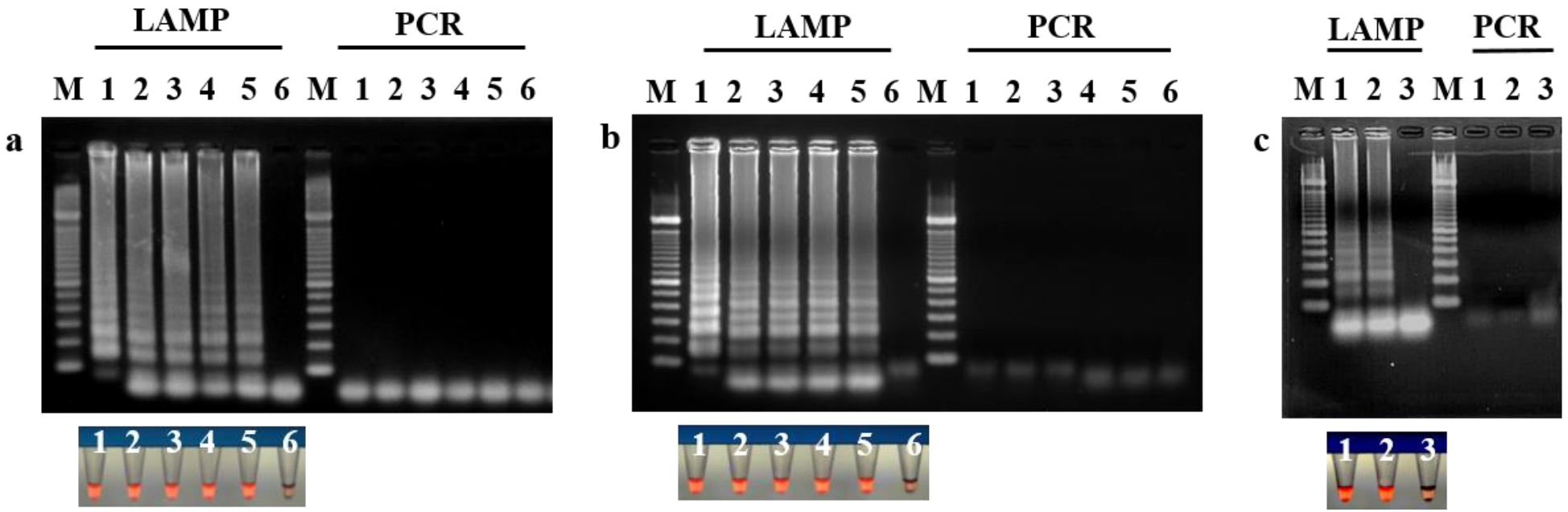


**Fig. 4**


LAMP
PCR
M 1 2 3 4 5 6 M 1 2 3 4 5 6
a
1 2 3 4 5 6
LAMP
PCR
M 1 2 3 4 5 6 M 1 2 3 4 5 6
b
1 2 3 4 5 6
LAMP
PCR
M 1 2 3 M 1 2 3
c
1 2 3