Application of the LAMP Assay as a Diagnostic Technique for Rapid Identification of *Thrips tabaci* (Thysanoptera: Thripidae)

LIDA FEKRAT,[1,2] MOHAMMAD ZAKI AGHL,[1] and VAHID TAHAN[1]

J. Econ. Entomol. 108(3): 1337–1343 (2015); DOI: 10.1093/jee/tov099

ABSTRACT Rapid and accurate identification of potentially invasive taxa that may cause high economic losses or environmental damage is of critical importance. The onion thrips, *Thrips tabaci* Lindeman, ranks as one of the world's most destructive agricultural pests and commonly found in imported agricultural products and field samples, but is prone to undetected transport because of its minute size as well as cryptic behavior. Although traditional taxonomic methods are pretty useful in straightforward assignment of specimens to the genus *Thrips*, identification in the species level is much more difficult and requires expertise, knowledge, and experience. Furthermore, it is often difficult or impossible to identify or distinguish this species from other thrips by using material from other stages of development. Based on the foregoings, use of a molecular technique known as loop-mediated isothermal amplification (LAMP) as a rapid and robust alternative species diagnostic tool would be valuable. In this study, a relatively quick and simple method was used to detect the presence of onion thrips DNA rapidly and discriminate it from other species, by using material from different stages of development. Not only LAMP itself required less than 1 h to complete but also amounts of DNA as little as that recovered from a single specimen were adequate for the detection. Another advantage of this identification system is that nonspecialists will be able to make faster and cheaper identifications.

KEY WORDS LAMP, loop-mediated isothermal amplification, molecular identification, rapid diagnostic tool, *Thrips tabaci*

Onion thrips, *Thrips tabaci* Lindeman, is one of the world's most economically important pests with worldwide distribution (Liu and Spark 2003, Jenser and Szénási 2004). This species is commonly found in imported agricultural products and field samples. There is no doubt that traditional detection procedure that utilizes morphological characteristics has been the gold standard method to identify thrips species such as onion thrips; however, morphological identification of a species is often difficult when there are some intra- and interpopulation morphological variation of shape, color, or size among the individuals like those seen in onion thrips. Moreover, morphological identification requires expertise taxonomists and the method itself is extremely time-consuming. Furthermore, not only is it hard to identify species of thrips juveniles but also most of the taxonomic keys are limited to adult thrips, mainly because many of the morphological characteristics are not well-developed in the larval stages. This is especially problematic for quarantining protocols because crop infestations detected at ports of entry often include larval stage material. For these reasons, the development of a rapid and robust method for species identification that can be applied to material from any of the life stages is necessary.

Various molecular methods have already been used to augment or supplement the use of morphological characters for species identification purposes for many thrips species (Moritz et al. 2000, Brunner et al. 2002, Toda and Komazaki 2002; Walsh et al. 2005; Rugman-Joneset et al. 2006, Hoddle et al. 2008, Glover et al. 2010). Although these methods are informative to discriminate various Thysanoptera species, most of them are costly, time-consuming, or need various equipments.

Loop-mediated isothermal amplification (LAMP; Notomi et al. 2000) as a relatively new method could provide a valuable alternative molecular diagnostic tool. LAMP is a one-step process that amplifies a target DNA at a single temperature using specifically designed pairs of primers and a DNA polymerase. A total of eight distinct regions of the target DNA can be recognized using a set of six primers including forward inner primer (FIP) and backward inner primer (BIP) as inner primers, backward outer primer (B3) and forward outer primer (F3) as outer primers, and backward loop primer and forward loop primer as the loop primers. Annealing of the FIP initiates synthesis of the first-strand of the target sequence. Afterwards, the synthesized first strand was hybridized and displaced by the F3, which is a few bases shorter and lower in concentration than FIP. However, unlike the template, there is additional sequence corresponding to the FIP primer at the 5′ end of the newly synthesized first strand. The formation of a loop structure was facilitated

[1] Department of Plant Protection, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
[2] Corresponding author, e-mail: fekrat@ferdowsi.um.ac.ir.

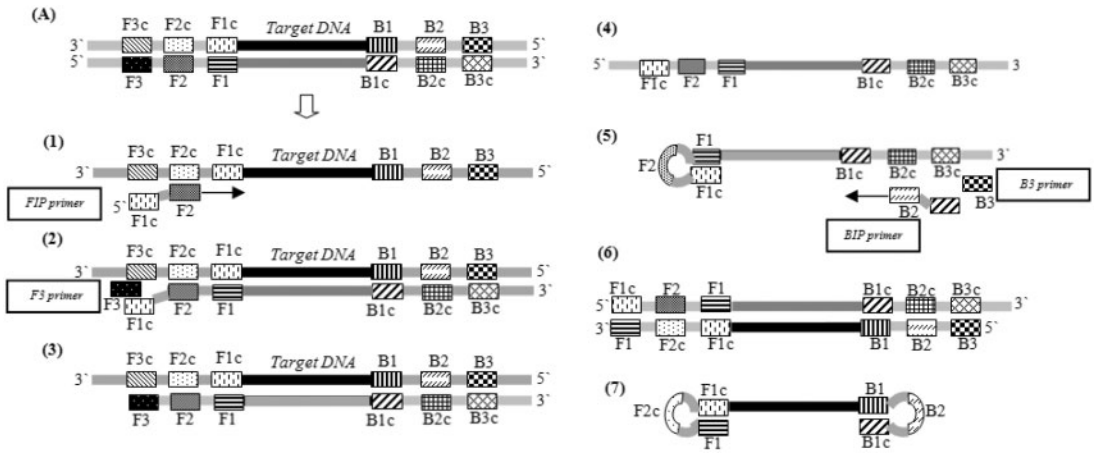

Fig. 1. Schematic representation of generation of stem loop DNA with dumbbell-shaped structure at both ends, which is ready to enter into amplification step of LAMP reaction (modified from Parida et al. 2008).

by a sequence of FIP primer, which is complementary to the F1 region of the target strand. This single-stranded DNA serves as template for BIP-initiated DNA synthesis and subsequent hybridization and displacement by B3 primer, which leads to creation of a final template with sequences that are complementary to F1 at one end and B1 at the other. Folding back and annealing of these complementary regions to the F1 and B1 regions of the target sequence results in formation of a dumbbell-shaped structure, which is quickly converted to a stem-looped DNA. This structure acts as a template for subsequent amplification (Fig. 1). Furthermore, two additional loop primers (forward loop [LF] and backward loop [LB]) enhance the reaction efficiency of the LAMP procedure, thereby reducing the amplification time (Notomi et al. 2000, Ravindran et al. 2012).

The LAMP method is rapid, robust, specific, and labor-saving; it requires only a simple thermostat-based instrument for operation under isothermal conditions. Moreover, use of six primers that recognize eight regions in the target DNA increases LAMP specificity compared with classical and nested polymerase chain reaction (PCR) in which specificity is limited to the recognition of two and four regions in the target DNA, respectively (Ravindran et al. 2012, Faggion et al. 2013).

Lamp has been already used in a broad spectrum of applications, ranging from detection, identification, and quantification of pathogenic microorganisms to sex determination in embryos (Iwamoto et al. 2003, Hong et al. 2004, Fukuta et al. 2004, Hirayama et al. 2004, Parida et al. 2005, Poon et al. 2006, Ohtsuki et al. 2008, Fu et al. 2011, Gonçalves et al. 2014). However, up to now, less than a handful of studies have been considered the efficiency of this method for rapid identification of insects or insect pests (Itakura et al. 2006, Huang et al. 2009). In this study, we describe the design of a six-primer set as well as the optimization of a simple protocol to develop a rapid and robust method for the onion thrips LAMP detection that can be used in less well-equipped laboratories, at quarantine ports of entry, and even at field sites. It is hoped that this system can be extended to other economically important members of the Thysanoptera.

Materials and Methods

Samples. Thrips specimens were collected from onion fields in Shirhesar, Khorasan-e-Razavi province by shaking and beating onion plants onto a white plastic tray. Thrips picked up using a moistened fine brush and stored in vials containing 96% ethanol.

For rapid and economical extraction of DNA from onion thrips material that can be used in LAMP, we just ground one thripid specimen in 1.5-ml Eppendorf tube containing 20 µl of distilled water or 10 mM Tris-HCl (pH = 8). This sample solution was used as a DNA source.

Primer Design for LAMP. To design primers for the LAMP reactions, mitochondrial gene sequence representing the cytochrome oxidase I (COI) region, as described in GenBank accession no. AB262444.1, was used. The Primer Explorer program version 4 (http://primerExplorer.jp) was used to design three pairs of primers. The sequences of the primers are given in Table 1.

LAMP Reaction. A temperature of 60°C and a time of 60 min were used to optimize the reaction components (Tomita et al. 2008), i.e., the betaine concentrations (0, 0.5, 1 M) and the calcein concentrations (0, 1, 2, and 4 µM). Moreover, the reaction temperature (55 and 60°C) and the amplification time (15, 30, 60, and 120 min) were evaluated to optimize the LAMP conditions.

After optimization reaction components and conditions, LAMP was performed in a total volume of 30 µl, containing 3 µl of the 10× BSM reaction Buffer (Thermoscientific-Fermentas, Vilnus, Lithuania), 0.33 µM each of primers F3 and B3, 1.3 µM each of primers FIP and BIP, 0.67 µM each of primers LF and LB along with 1 M betaine, 1.6 mM dNTPs. The mixture was first

Table 1. Sequences of LAMP primers designed from the onion thrips mtCOI sequence (see text for explanation)

Primer	Sequence (5′ → 3′)
F3	5′-TGATCAGTTATTTTAACAGCCAT-3′
B3	5′-TGAGAAATTAGTCCAAATCCTG-3′
FIP	5′-AGAGGTATTTAAGTTTCGGTCAGTTCTTCTTCTTTTATCTTTACCAGTGT-3′
BIP	5′-TTGACCCTAGAGGAGGAGGGAAATGTAAACTTCTGGGTGAC-3′
LF	5′-AAAAGTATTGTGATAGCTCCCGCT-3′
LB	5′-GACCCTGTTTTATATCAACACCTT-3′

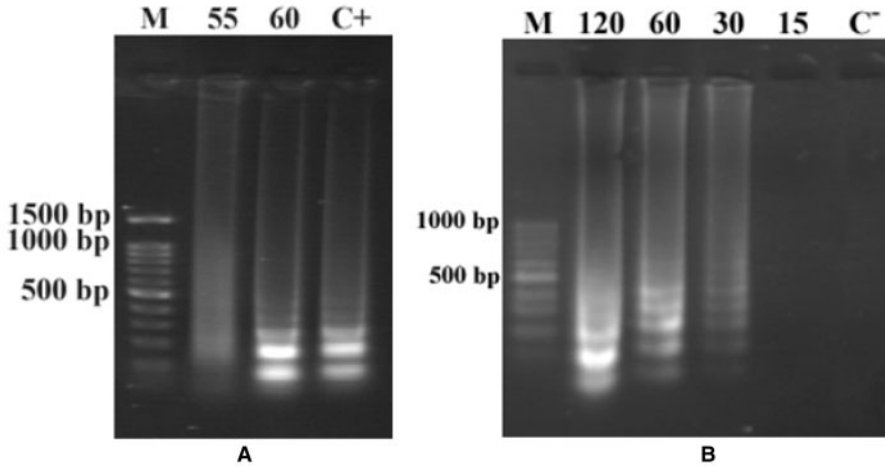

Fig. 2. Optimization of LAMP conditions. (A) Electrophoretic patterns of LAMP products carried out at 55 and 60°C. (B) Electrophoretic patterns of LAMP products obtained from different reaction times (0, 15, 30, 60, and 120 min), as indicated. Lane M, 100-bp ladder size marker. All the products were examined on 2% agarose gels and stained with DNA green viewer.

incubated at 95°C for 5 min, followed by cooling on ice for 1 min, then 8 U of the *Bsm* DNA polymerase (Thermoscientific-Fermentas, Vilnus, Lithuania) was added and incubated at 60°C for 60 min, then the temperature was raised to 80°C for 10 min to terminate the reaction.

To confirm that the amplified LAMP product had the expected size (210 bp), restriction digestion was performed using digestion with *Mbo*I (Thermoscientific-Fermentas, Vilnus, Lithuania), according to the conditions recommended by the manufacturer. The products were subjected into 2% agarose gels for electrophoresis.

Direct Analysis of LAMP Products. LAMP amplicons were detected directly in the reaction tubes via the addition of 1 mM MnCl2 and 50 μM calcein (Sigma) to the LAMP reaction products corresponding to different incubation times (Tomita et al. 2008). The florescence intensities produced were compared under visible light and UV light. Positive reactions were indicated by yellowish green fluorescence. Results obtained from different reaction times were further confirmed via gel electrophoresis on a 2% agarose gel.

Detection of Onion Thrips. LAMP reactions were performed on different life cycle stages of onion thrips specimens, including first- and second-instar larvae as well as adults by adding material from the grounded specimens directly into the LAMP reactions.

Species Discrimination. Single adult specimens of the onion thrips and western flower thrips were grounded in separate tubes containing 20 μl of distilled water and used in LAMP reactions. Results were analyzed by gel electrophoresis.

Sensitivity of LAMP. To test the sensitivity of a designed primer set, LAMP was carried out on a dilution series of DNA extracted from a single onion thrips specimen. The DNA sample was diluted from 200 ng to 200 pg, and the presence of a LAMP product was determined via direct visualization and electrophoresis on a 2% agarose gel.

In another experiment, the sensitivity was evaluated by grounding 1, 3, 6, and 10 thripid specimens in separate Eppendorf tubes each contained 20 μl of distilled water, and used them as templates. Material from each of these reactions also was examined using agarose gel electrophoresis.

Results

DNA Extraction and Specificity of LAMP Reaction. The LAMP reactions worked successfully for *T. tabaci* via simply grounding the specimens (Fig. 2). Evaluation of amplification time showed that a LAMP product initially being formed after 30 min (Fig. 2). No amplification was observed after 15 min (Fig. 2). No amplification was observed at 55°C, but amplification was successfully being formed at 60°C (Fig. 2). The optimal concentrations of betaine and

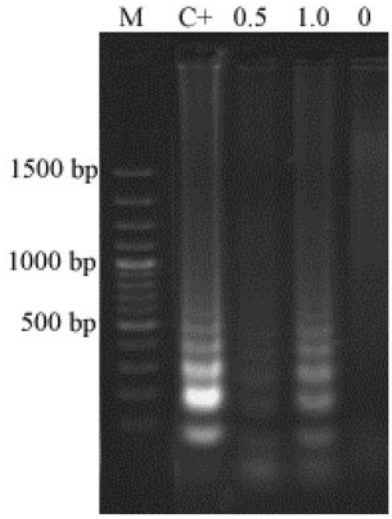

Fig. 3. Optimization of LAMP conditions. Electrophoretic patterns of LAMP products at different concentrations of betaine (0, 0.5, and 1 M), as indicated. Lane M, 100-bp ladder size marker.

calcein were found to be 1 M (Fig. 3) and 2 μM (Fig. 4), respectively.

Addition of $MnCl_2$ and calcein produced a yellowish green fluorescence during DNA synthesis, which was more apparent under UV light (Fig. 4). The LAMP products were also detected using naked eye by observing the color change during the reaction (Fig. 4). In gel electrophoresis, LAMP reactions produce similar ladder-like pattern of many different-sized bands. In the absence of calcein, the LAMP reaction was performed successfully (Fig. 4, Lane 0), but because of the absence of a florescent dye, the results were not visible in natural light and under UV light (Fig. 4A and B). Based on the florescence intensities under UV light as well as the color intensities in natural light, 2 μM was chosen as the optimal concentration in the LAMP reactions (Fig. 4). A higher concentration of calcein (4 μM) acts as an inhibitor of LAMP reactions (Fig. 4).

Products also were digested using the restriction enzyme *Mbo*I to confirm that the correct sequences were being amplified. As expected, this digestion collapsed the ladder-like pattern into one main band of the predicted size (210 bp; Fig. 5). Some faint bands also were visible, probably representing partial digest products (Fig. 5).

LAMP Reactions on Material From Different Stages of the Onion Thrips. The LAMP reactions on material from different developmental stages of onion thrips (first- and second-larval instars) worked successfully when these materials added directly to the lamp reactions. Gel electrophoresis showed that the onion thrips can be detected by LAMP using material from virtually different stages of its life cycle (Fig. 6).

Sensitivity of LAMP. The LAMP sensitivity testing was conducted using a 10-fold dilution series from 200 ng to 20 pg of DNA. LAMP bands were clearly

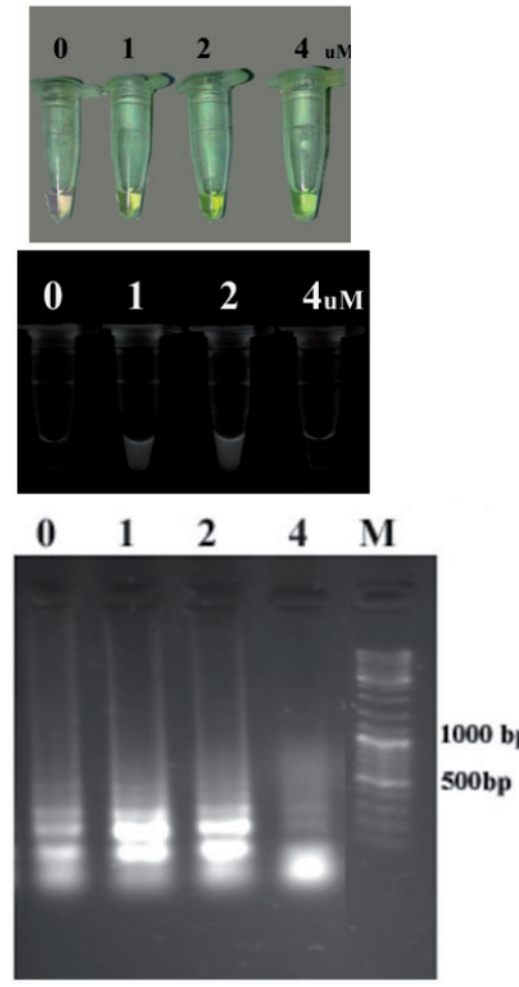

Fig. 4. Visualization of LAMP products at different concentrations of calcein (0, 1, 2, and 4 μM), as indicated (A) under normal light, (B) under UV light (black and white image), and (C) electrophoretic patterns of LAMP products at different concentrations of calcein (0, 1, 2, and 4 μM), as indicated. Lane M, 100-bp ladder size marker.

visible for samples containing 200 ng to 200 pg of DNA (Fig. 7A).

The sensitivity of LAMP was also evaluated by grounding 1, 3, 6, and 10 thripid specimens in separate Eppendorf tubes each containing 20 μl distilled water, and used them as templates (Fig. 7B). Bands were quite clearly visible for samples containing 3, 6, and 10 thripid individuals and also were also visible for samples containing only one thripid specimen (Fig. 7B).

These data suggest that LAMP was very sensitive in detecting *T. tabaci* using these designed primers.

Specificity of Detection. The specificity of LAMP using the designed primer set to amplify only "*T. tabaci*" species was tested using DNA samples from another widespread and economically important thripid species of family Thripidae, *Frankliniella occidentalis* (Pergande) (Fig. 8). As expected, the results presented

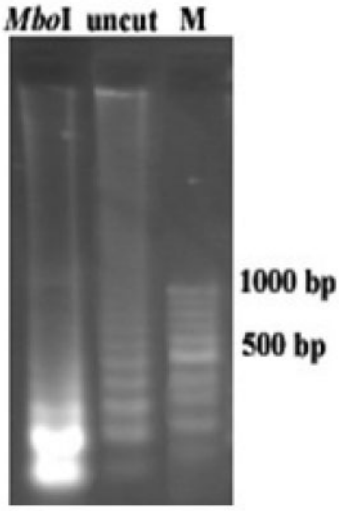

Fig. 5. LAMP products digested by *Mbo*I restriction enzyme. Lane M, 100-bp ladder size marker.

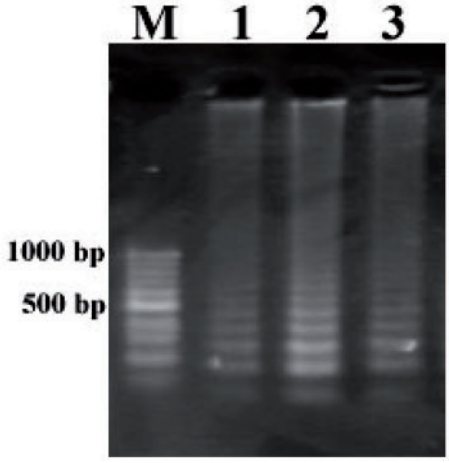

Fig. 6. Electrophoresis of LAMP products from different developmental stages of onion thrips: 1, first instar; 2, second instar; and 3, adult stages, respectively.

at Fig. 6 show that material from onion thrips specifically detected by LAMP. In other words, primer set amplified products from the "onion thrips" samples, but not from western flower thrips.

Discussion

Precise identification of many thrips species is difficult not only because of their minute size, color morphs, and complex life cycles but also because of the presence of unusual morphological forms of species on different hosts as well as coexistence of different species on the same host (Rebijith et al. 2014). There are no reliable keys for identification of immature stages of Thysanoptera, so adults are required for morphological examination of them to species level. Moreover, because of insufficient phenotypic variation, the classical morphological taxonomy for many thripid species specifically preadult life stages has become practically useless (Brunner et al. 2004).

In this regard, development a rapid and reliable method for identification of the pest species causing infestations by using materials from any stage of the life cycle can be an added advantage. In an effort to achieve this goal, a series of experiments have been carried out to evaluate the LAMP efficiency to determine the minimum incubation time required to detect LAMP products (Fig. 2), detect material from various onion thrips life stages (Fig. 6), and test the ability of this method to discriminate between materials from various thrips species (Fig. 8).

Generally speaking, LAMP seems to be less sensitive to the presence of nontarget DNA or reaction inhibitors compared with other amplification methods (Notomiet al. 2000, Soliman and El-Matbouli 2005). Our results support the use of grounding the specimens for DNA extraction because it is very easy, fast, and also inexpensive. By using crashed material, our reactions were successful whether or not the DNA was quantified before use in LAMP.

This may be most relevant in situations where the rapid detection of pest infestations is required in implementing quarantine procedures in a timely manner. Furthermore, using three pairs of primers designed to amplify specific regions of mitochondrial COI (mtCOI) DNA of onion thrips, we were able to successfully carry out the LAMP reactions in as little as 30 min (Fig. 2). This is a reduction by approximately one-quarter of the reaction time required for other PCR-based molecular techniques.

We have also shown that onion thrips material can be detected by LAMP by using material from various stages of the life cycle. Only a single specimen was sufficient for detection of thrips material. To further demonstrate the utility of this approach, the calcein dye also was used for detection of LAMP reaction products. Reaction products stained with calcein were clearly detected using gel electrophoresis. The incorporation of this dye, which results in the formation of a fluorescent yellowish green metal indicator, allows for direct visualization of reaction products not only by naked eye in natural light but also in the presence of UV light.

It seems that compared with other molecular applications that need much more detailed analysis to allow for differentiation between thrips species, LAMP reaction has the potential to be extremely useful for the rapid detection and identification of onion thrips material. Furthermore, this method could discriminate onion thrips from another potentially infesting thrips species in <1 h. Using LAMP, we showed that onion thrips material was discriminated from material derived from another economically important pest species in the family Thripidae, i.e., the western flower thrips, *F. occidentalis*. Using the designed onion thrips primers, after a 30-min time interval, LAMP reaction products were clearly observed only when the onion thrips material was used as the template (Fig. 8).

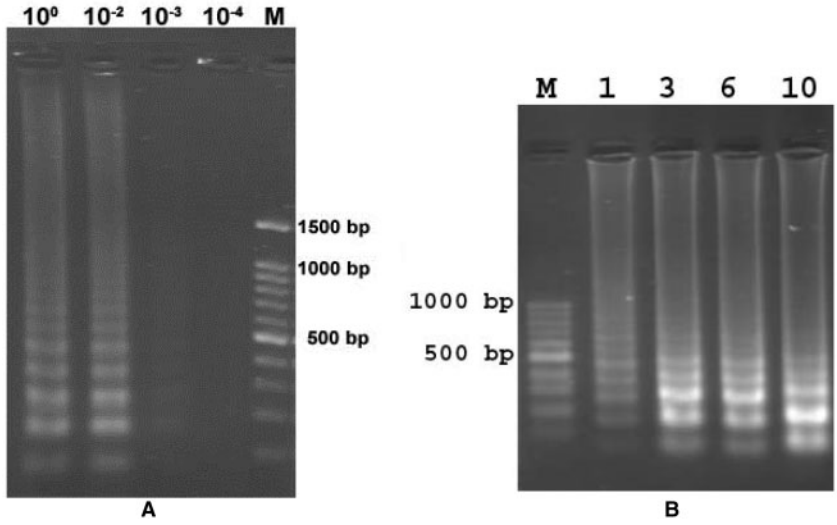

Fig. 7. Sensitivity of LAMP (A) using a 10-fold dilution series from 200 ng (10^{0}) to 20 pg (10^{-4}) of DNA, and (B) using 1, 3, 6, and 10 thripid specimens as templates. Lane M, 100-bp ladder size marker.

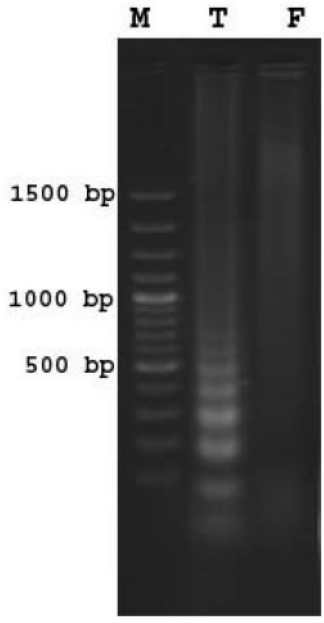

Fig. 8. LAMP specificity using DNA sample from another thripid species, *F. occidentalis*. T, *Thrips tabaci*; F, *F. occidentalis*.

Further checking at later time point (60 min) also confirmed that products were seen only in the onion thrips (not shown). Therefore, for species identification purposes, it will be suitable to obtain the results of the LAMP reactions within a 30–120-min time window.

In conclusion, LAMP as a rapid, precise, and simple diagnostic procedure proved to be an effective tool that can be used for the identification of onion thrips of different life stages. This identification approach could be used not only for the species presently studied but also, in the future, for other pest thrips species of agricultural, horticultural, and forestry interestand importance. This will, in turn, help in elucidation of the epidemiology of tospoviruses, their management, and serve as a potentially valuable tool in quarantine at ports of entry. Another merit of this approach is its requirement for relatively simple equipment for isothermal amplification and analysis of the target material. Our results also showed that by just grounding a single specimen, the sufficient amounts of material from the onion thrips to successfully LAMP detection was provided. Results of LAMP also can be observed using either gel electrophoresis or visual detection when calcein is incorporated. Lastly, a relatively short time is needed for completion of the entire process from the extraction of DNA to the detection of the presence of a particular species, so developing and using this technique for other species, especially pests of economic or quarantine importance, would be enormously precious in maintaining a pest-free status, especially in the areas subject to infestations of the quarantine pests where quick decisions about implementations of quarantine measures could be crucially important.

Acknowledgment

We would like to appreciate the financial support of Ferdowsi University of Mashhad.

References Cited

Brunner, P. C., C. Fleming, and J. E. Frey. 2002. A molecular identification key for economically important thrips species (Thysanoptera: Thripidae) using direct sequencing and a PCR-RFLP-based approach. Agric. For. Entomol. 4: 127–136.

Brunner, P. C., E. K. Chatzivassilious, N. I. Katis, and J. E. Frey. 2004. Host-associated genetic differentiation in *Thrips*

tabaci (Insecta: Thysanoptera), as determined from mtDNA sequence data. Heredity 93: 364–370.

Faggion, S. A., A. R. Salvador, K. L. Jacobino, L. F. B. Bortolotto, M. B., Lopes, M. Silva, E. V. Santos, A. L. Fachin, S. C. França, and M. Marins. 2013. Loop-mediated isothermal amplification assay for the detection of *Ehrlichia canis* DNA in blood samples from dogs. Arch. Med. Vet. 45: 197–201.

Fu, S., G. Qu, S. Guo, L. Ma, N. Zhang, S. Zhang, and S. S. Z. Gao. 2011. Applications of loop-mediated isothermal DNA amplification. Appl. Biochem. Biotechnol. 163: 845–850.

Fukuta, S., K. Ohishi, K. Yoshida, Y. Mizukami, A. Ishida, and M. Kanbe. 2004. Development of immunocapture reverse transcription loop-mediated isothermal amplification for the detection of tomato spotted wilt virus from chrysanthemum. J. Virol. Methods 121: 49–55.

Gonçalves, D. S. da, A. P. A. Cassimiro, C. D. de Oliveira, N. B. Rodrigues, and L. A. Moreira. 2014. *Wolbachia* detection in insects through LAMP: loop mediated isothermal amplification. Parasit. Vectors 7: 228.

Glover, R. H., D. W. Collins, K. Walsh, and N. Boonham. 2010. Assessment of loci for DNA barcoding in the genus *Thrips* (Thysanoptera: Thripidae). Mol. Ecol. Resour. 10: 51–59.

Hoddle, M. S., J. M. Heraty, P. F. Rugman-Jones, L. A. Mound, and R. Stouthamer. 2008. Relationships among species of *Scirtothrips* (Thysanoptera: Thripidae, Thripinae) using molecular and morphological data. Ann. Entomol. Soc. Am. 101: 491–500.

Hong, T. C., Q. L. Mai, D. V. Cuong, M. Parida, H. Minekawa, T. Notomi, F. Hasebe, and K. Morita. 2004. Development and evaluation of a novel loop-mediated isothermal amplification method for rapid detection of severe acute respiratory syndrome coronavirus. J. Clin. Microbiol. 42: 1956–1961.

Huang, C. G., J. C. Hsu, D. S. Haymer, G. C. Lin, and W. J. Wu. 2009. Rapid Identification of the Mediterranean Fruit Fly (Diptera: Tephritidae) by Loop-Mediated Isothermal Amplification. J. Econ. Entomol. 102: 1239–1246.

Itakura, S., T. Kankawa, H. Tanaka, and A. Enoki. 2006. Identification of two subterranean termite species (Isoptera: Rhinotermitidae) using the loop-mediated isothermal amplification (LAMP) method. Sociobiology 47: 99–113.

Iwamoto, T., T. Sonobe, and K. Hayashi. 2003. Loop-mediated isothermal amplification for direct detection of *Mycobacterium tuberculosis complex, M. avium*, and *M. intracellulare* in sputum samples. J. Clin. Microbiol. 41: 2616–2622.

Jenser, G., and Á. Szénási. 2004. Review of the biology and vector capability of *Thrips tabaci* Lindeman (Thysanoptera: Thripidae). Acta Phytopathol. Entomol. Hung. 39: 137–155.

Liu, T. X. and A. N. Sparks. 2003. Injury and distribution of onion thrips (Thysanoptera: Thripidae) in red cabbage heads. Southwest. Entomol. 28: 77–79.

Moritz, G., C., Delker, M. Paulsen, L. A. Mound, and W. Burgermeister. 2000. Modern methods for identification of Thysanoptera. Bull. OEPP 30: 591–593.

Notomi, T., H. Okayma, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino, and T. Hase. 2000. Loop mediated isothermal amplification of DNA. Nucleic Acids Res. 28: e63.

Ohtsuki, R., K. Kawamoto, Y. Kato, M. M. Shah, T. Ezaki, and S. I. Makino. 2008. Rapid detection of *Brucella* spp. by the loop-mediated isothermal amplification method. J. Appl. Microbiol. 104: 1815–1823.

Parida, M., K. Horioke, H. Ishida, P. K. Dash, P. Saxena, A. M. Jana, M. A. Islam, S. Inoue, N. Hosaka, and K. Morita. 2005. Rapid detection and differentiation of dengue virus serotypes by a real-time reverse transcription-loop-mediated isothermal amplification assay. J. Clin. Microbiol. 43: 2895–2903.

Parida, M., S. Sannarangaiah, P. Kumar Dash, P. V. L. Rao, and K. Morita. 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.

Poon, L. L., B. W. Wong, E. H. Ma, K. H. Chan, L. M. Chow, W. Abeyewickreme, N. Tangpukdee, K. Y. Yuen, Y. Guan, S. Looareesuwan, and J. S. Peiris. 2006. Sensitive and inexpensive molecular test for falciparum malaria: detecting *Plasmodium falciparum* DNA directly from heat-treated blood by loop-mediated isothermal amplification. Clin. Chem. 52: 303–306.

Ravindran, A., J. Levy, E. Pierson, and D. C. Gross. 2012. Development of a loop mediated isotherman amplification procedure as a sensitive and rapid method for detection of *Candidates liberibacter solanacearum* in potatoes and psyllids. Phytopathology 102: 899–907.

Rebijith, K. B., R. Asokan, V. Krishna, H. H. Ranjitha, N. K. Krishna Kumar, and V. V. Ramamurthy. 2014. DNA barcoding and elucidation of cryptic diversity in thrips (Thysanoptera). Fla. Entomol. 97: 1328–1347.

Rugman-Jones, P. F., M. S. Hoddle, and L. A. Mound. 2006. Molecular identification key for pest species of *Scirtothrips* (Thysanoptera: Thripidae). J. Econ. Entomol. 99: 1814–1819.

Soliman, H. and M. El-Matbouli. 2005. An inexpensive and rapid diagnostic method of koi herpesvirus (KHV) infection by loop-mediated isothermal amplification. Virol. J. 2: 83.

Toda, S., and S. Komazaki. 2002. Identification of thrips species (Thysanoptera: Thripidae) on Japanese fruit trees by polymerase chain reaction and restrictionfragment length polymorphism of the ribosomalITS2 region. Bull. Entomol. Res. 92: 359–363.

Tomita, N., Y. Mori, H. Kanda, and T. Notomi. 2008. Loop-mediatedisothermal amplification (LAMP) of gene sequences and simple visual detection of products. Nat. Protoc. 3: 877–882.

Walsh, K., N. Boonham, I. Barker, and D. W. Collins. 2005. Development of a sequence-specific real-time PCR to the melon thrips *Thrips palmi* (Thysan., Thripidae). J. Appl. Entomol. 129: 272–279.

Received 4 March 2015; accepted 8 April 2015.