



A loop-mediated isothermal amplification (LAMP) assay for the rapid detection of toxigenic *Fusarium temperatum* in maize stalks and kernels

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ABSTRACT

Fusarium temperatum is an emerging maize pathogen that causes maize ear and stalk rot diseases and produces various mycotoxins including moniliformin, beauvericin, enniatins and fumonisin B1, which poses a potential risk to the human food or animal feed supply chains. Early detection of *F. temperatum* is crucial to prevent its derived mycotoxins from entering the food chain, and is also a useful tool in disease management practices. Here, we describe a loop-mediated isothermal amplification (LAMP) assay for rapid diagnosis of *F. temperatum*. The 28S ribosomal DNA sequences (28S rDNA) of *F. temperatum* were used to design a set of six primers. The reaction conditions were optimized for developing a fast assay with high specificity and sensitivity, and were able to detect the presence of less than 10 pg of target DNA per reaction within 60 min. Furthermore, the resulting amplicons were visualized by adding SYBR Green I to the reaction tubes. Suspected *F. temperatum* infected maize stalk samples collected from Yunnan province, China were identified using the developed LAMP assay. In conclusion, the method not only provides a rapid and specific screening for the existence of *F. temperatum* in a bulk of maize samples without using sophisticated equipment, but also is potentially useful for other agriculturally important toxigenic fungi.

1. Introduction

Fusarium is a large and diverse genus of fungi with significant agricultural and economic importance, including a large number of species that are important pathogens of maize and other cereal crops (Ma et al., 2013). *Fusarium* spp. also produce a wide range of biologically active secondary metabolites in infected plants or in stored grains, including mycotoxins which are harmful to animals and humans consuming the grain (Desjardins and Proctor, 2007). In China, many *Fusarium* species are associated with ear and stalk rot diseases of maize, which resulted in significant yield loss and mycotoxin contamination problems in the past (Zhang et al., 2015). The most abundant *Fusarium* species isolated from maize are *Fusarium verticillioides*, *F. graminearum*, *F. meridionale* and *F. temperatum* (Duan et al., 2016; J.H. Wang et al., 2014). *F. temperatum* is an important maize pathogen and has been described as a new species by Scaufaire et al. (2011). Until now, it is widespread in Europe, Asia as well as North and South America (Boutigny et al., 2017; Fumero et al., 2015; Lanza et al., 2016; Robles-Barrios et al., 2015; Shin et al., 2014; Varela et al., 2013; Venturini

et al., 2016; J.H. Wang et al., 2014). *F. temperatum* is able to produce various mycotoxins including moniliformin, beauvericin, enniatins and fumonisin B1 (Fumero et al., 2015; Scaufaire et al., 2012). Due to the production of a variety of mycotoxins, *F. temperatum* could represent a toxicological risk for maize production from pre-harvest to storage stage. Therefore, new diagnostic techniques for the rapid identification of *F. temperatum* would be valuable for use in the food and feed industry.

Conventionally, identification of *Fusarium* species has been mainly based on morphological characteristics and differences in DNA sequences of housekeeping genes. However, morphological identification to the species level is very difficult, time-consuming and needs in-depth knowledge of taxonomy. In addition, PCR-based amplification methods are still expensive because they require dedicated laboratory equipment and well-trained staff. These factors impede the use of PCR-based analysis in on-site applications e.g. agriculture, food and feed production (Niessen and Vogel, 2010). As an alternative approach, loop-mediated isothermal amplification (LAMP) is a novel method of nucleic acid amplification that is catalyzed by Bst DNA polymerase with strand

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displacement activity and occurs under isothermal condition at a temperature range from 60 to 65 °C (Notomi et al., 2000). A simple heating block is sufficient to fulfill the requirements for a LAMP assay, eliminating the need for costly equipment such as thermal cyclers. Furthermore, the temperature can be maintained by an exothermal reaction linked to specifically engineered phase-change materials to perform the analysis without any instruments (Sema et al., 2015). In addition, the LAMP assay can be easily monitored by the following ways: (i) agarose gel electrophoresis; (ii) measuring turbidity of magnesium pyrophosphate in the LAMP reactions; (iii) measuring fluorescence using SYBR green or calcein; and (iv) by color change using a metal ion-binding indicator dye such as hydroxynaphthol blue (HNB) (Das et al., 2012). In this study, we aimed to develop an effective, sensitive, accurate, reliable and rapid diagnostic method for *F. temperatum*, to be used in the field or in storage facilities. The developed visualization method for LAMP products, in the reaction tube, demonstrates the potential for application to improve quality management in a cost-effective manner in the cereal industry.

2. Materials and methods

2.1. Source of strains

The *Fusarium* strains were obtained from diseased maize stalks and kernels collected from different provinces of China from 2015 to 2017 (Table 1). All strains were identified based on their morphological observation and sequencing of the translation elongation factor 1a (EF-1 α) sequencing and maintained in our laboratory. Non-*Fusarium* strains including *Pythium acanthophoron*, *Bipolaris zeicola*, *Alternaria alternata*, *Nigrospora* sp., *Trichoderma* sp., *Pestalotiopsis* sp., *Bipolaris* sp., *Dothideomycete* sp., *Phoma* sp., *Glomerella graminicola* and *Verticillium dahliae* used in this study were also maintained in our laboratory (Table 1).

2.2. Culture conditions and preparation of genomic DNA

Fusarium and non-*Fusarium* strains were cultured on potato dextrose

Table 1

Strains of fungi used for screening the specificity of the designed LAMP primers in this study. Results of amplification by the LAMP assay were shown.

Species	Number	Host	Sources in China	LAMP
<i>Fusarium</i> strains (n = 34)				
<i>F. temperatum</i>	15	Maize	Liaoning, Yunnan	+
<i>F. graminearum</i>	3	Maize	Henan, Liaoning, Yunnan	–
<i>F. meridionale</i>	3	Maize	Yunnan	–
<i>F. verticillioides</i>	3	Maize	Henan, Shandong, Liaoning	–
<i>F. boothii</i>	1	Maize	Yunnan	–
<i>F. proliferatum</i>	1	Maize	Liaoning	–
<i>F. fujikuroi</i>	1	Maize	Henan	–
<i>F. asiaticum</i>	1	Maize	Yunnan	–
<i>F. cerealis</i>	1	Maize	Yunnan	–
<i>F. brachygybosum</i>	1	Maize	Henan	–
<i>F. oxysporum</i>	1	Maize	Yunnan	–
<i>F. incarnatum</i>	1	Maize	Yunnan	–
<i>F. equiseti</i>	1	Maize	Yunnan	–
<i>F. avenaceum</i>	1	Maize	Yunnan	–
Non- <i>Fusarium</i> strains (n = 11)				
<i>Pythium acanthophoron</i>	1	Maize	Yunnan	–
<i>Bipolaris zeicola</i>	1	Maize	Yunnan	–
<i>Alternaria alternata</i>	1	Maize	Yunnan	–
<i>Nigrospora</i> sp.	1	Maize	Yunnan	–
<i>Trichoderma</i> sp.	1	Maize	Yunnan	–
<i>Pestalotiopsis</i> sp.	1	Maize	Yunnan	–
<i>Bipolaris</i> sp.	1	Maize	Yunnan	–
<i>Dothideomycete</i> sp.	1	Maize	Yunnan	–
<i>Phoma</i> sp.	1	Maize	Yunnan	–
<i>Glomerella graminicola</i>	1	Maize	Yunnan	–
<i>Verticillium dahliae</i>	1	Cotton	Henan	–

agar (PDA) medium at 25 °C for at least 5 days. The mycelia were harvested by scratching the agar surface using 2 ml ddH₂O. Then, the suspensions were filtrated through Whatman's filter paper and frozen at –80 °C. Mycelial genomic DNA was isolated using a DNasecure Plant Kit (Tiangen, Beijing, China) according to the manufacture's protocol and stored at –20 °C. Quantification of genomic DNA was performed using the optical density of DNA (260/280 nm) measured by a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc. Wilmington, DE, USA). The DNA samples were diluted by ddH₂O to 100 ng/ μ l.

2.3. LAMP primer design

The 28S ribosomal DNA sequences (28S rDNA) were identified in *Fusarium* spp. and have been used as a valuable phylogenetic marker for species identification in the *Fusarium* genus (Mulè et al., 1997). After comparison of sequences, among strains of *F. temperatum* and other similar species in the National Center for Biotechnology Information (NCBI) website, 28S rDNA was selected as the target gene for this study. A number of primers were designed to target the 28S rDNA of *F. temperatum* using LAMP Primer Explorer V5 software (<https://primerexplorer.jp/>). The LAMP primers and the loop primers used in this study are presented in Fig. 1.

2.4. Determination of conditions for the LAMP assay

The LAMP assay was performed in a 25 μ l reaction mixture containing 2.5 μ l 10 \times ThermoPol Buffer (200 mM Tris-HCl pH 8.8, 100 mM KCl, 100 mM (NH₄)₂SO₄, 20 mM MgSO₄, 1% Triton X-100), 2 μ l MgSO₄ (100 mM), 3.5 μ l dNTPs (10 mM), 4 μ l betaine (5 M), 1 μ l each of FIP and BIP (40 mM), 0.5 μ l each of F3 and B3 (10 mM), 1 μ l each of LF and LB (10 mM), 1 μ l Bst DNA Polymerase Large Fragment (8 U/ μ l, New England Biolabs), 2 μ l DNA template and 5 μ l of ddH₂O. The mixture was incubated at 62.0, 62.4, 63.3, 64.5, 66.0, 67.3 and 68.0 °C. Time-specific amplification was carried out for 30, 45 and 60 min with serial 10 fold dilution from 100 fg/ μ l to 100 ng/ μ l. The amplified products were detected by adding 0.25 μ l 10,000 \times SYBR Green I (Solarbio, Beijing) or 2% agarose gel electrophoresis.

2.5. Specificity of the LAMP assay

The specificity of the LAMP primers was evaluated by comparing mycelial DNA of *F. temperatum* strains from various locations, other *Fusarium*, and non-*Fusarium* fungal species as well as *Oomycetes* (Table 1). LAMP reactions were carried out at 65 °C for 60 min. After the reaction, the LAMP products were visually detected by color change methods using SYBR Green I.

2.6. Detection of *Fusarium temperatum* in diseased maize stalks and kernels

To diagnose *F. temperatum* in diseased maize stalks and kernels, 20 μ l of a conidial suspension of *F. temperatum* at a concentration of 10⁶/ml was inoculated into the maize stalks at the ten-leaf stage or sprayed on 5 kernels. The diseased tissues were excised, and DNA was extracted using the DNasecure Plant Kit (Tiangen, Beijing, China) according to the manufacturer's protocol. Samples were stored at –20 °C if not used immediately. The DNA extracted from maize stalks and kernels inoculated with ddH₂O served as a negative control.

3. Results

3.1. Target selection, primer design and optimization of the LAMP assay

Several genes were selected as potential target sequences for LAMP primer design using primer design software LAMP Primer Explorer V5 (<https://primerexplorer.jp/>), including the translation elongation

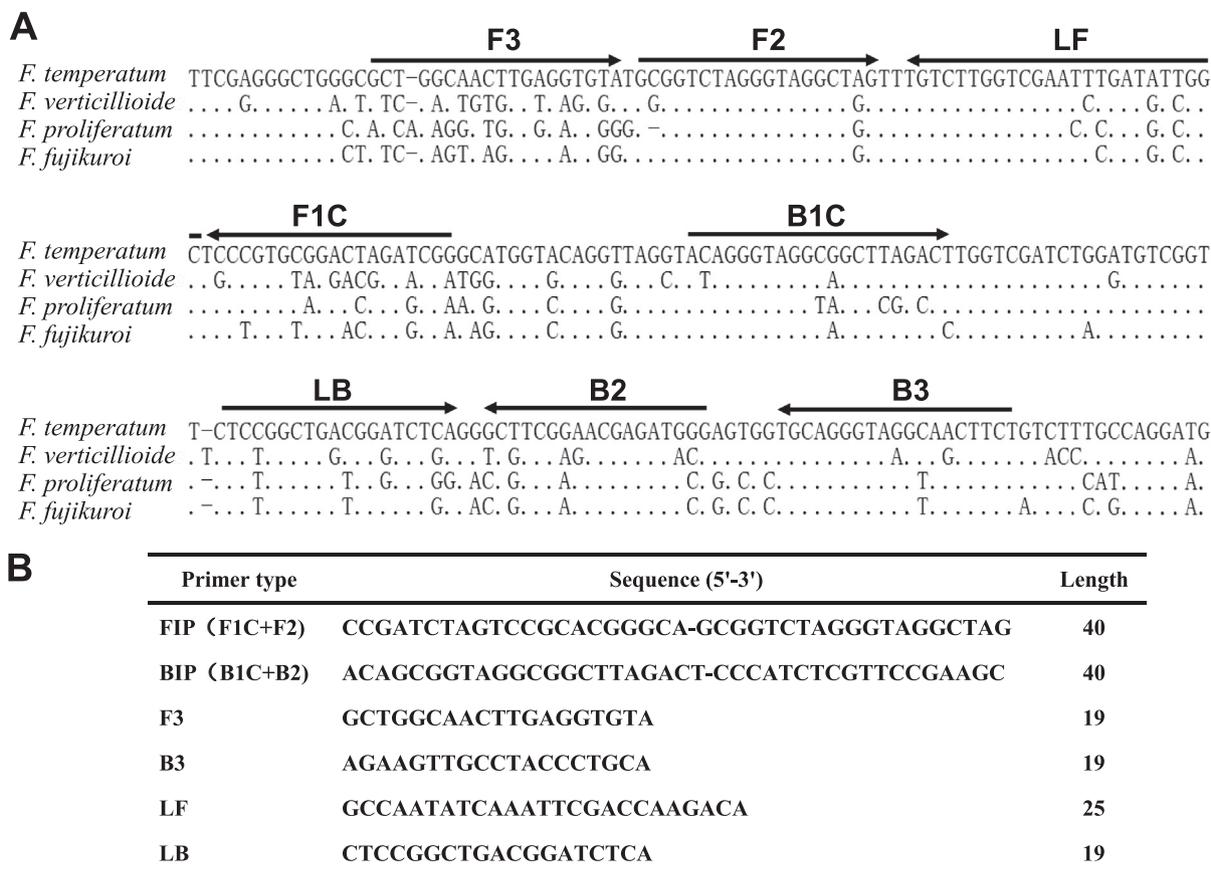


Fig. 1. Position and sequences of the loop-mediated isothermal amplification (LAMP) primers used for detection and identification of *F. temperatum*. (A) Multiple nucleotide sequence alignment of the target region of 28S rDNA used to design the LAMP primers. The forward inner primer (FIP) is composed of two binding regions, F1C and F2. The backward primer (BIP) consists of two binding regions, B1C and B2. F3 means a forward outer primer and B3 means a backward outer primer. LF means a loop forward primer and LB means a loop backward primer, which are used to accelerate or enhance the sensitivity of the LAMP assay. Annealing sites of primers are labeled. (B) The primer sequences used for each of the six LAMP primers.

factor (*EF1-α*) gene, largest subunit of the RNA polymerase gene (*RPB1*) and second largest subunit of the RNA polymerase gene (*RPB2*), the 28S rDNA and internally transcribed spacer region (ITS). Considering the GC content, annealing factors, in silico species specificity and potential sensitivity, the 28S rDNA of *F. temperatum* was selected as the target region for the LAMP assay in this study. The primer sequences are presented in the Fig. 1.

The optimal LAMP reaction temperature was evaluated using a temperature gradient ranging from 62 to 68 °C. After the reaction, the LAMP products demonstrated ladder-like banding pattern on agarose gel, with sizes ranging from 150 bp and larger (Fig. 2A). There were more intense ladder-like bands in lanes 4 and 5 (Fig. 2A). Similar results were observed by visual detection with diluted SYBR Green I (Fig. 2B). Thus, 65 °C was selected as the standard reaction temperature.

The limit of DNA detection of the LAMP assay was also tested at 30, 45 and 60 min with 10-fold serially diluted *F. temperatum* genomic DNA (100 ng/μl to 100 fg/μl). The results showed that the limit of DNA detection occurred at a 10⁻⁵ dilution or 10 pg (reaction time for 60 min) of total DNA template, 100 pg (45 min) and 100 ng (30 min), respectively, (Fig. 3). The results of LAMP by visual detection with diluted SYBR Green I agreed with those of gel electrophoresis, and UV light (wavelength 365 nm) increased the difference between positive and negative tubes (Fig. 3). Therefore, the LAMP amplification conditions were optimized for 60 min at 65 °C and the color change methods of SYBR Green I to detect the LAMP products under both natural and UV light.

3.2. Specificity analysis of the LAMP assay

The specificity of the LAMP assay was initially tested by detecting mycelial DNA of different *F. temperatum* strains, which were isolated from diseased stalks or kernels of maize from Liaoning, Henan, Shandong and Yunnan province of China (Table 1). Positive or negative results were easily determined by SYBR Green I. In the LAMP assay, the color of the reaction mixture changed to yellowish green for positive amplification, whereas the original orange color was observed in the negative control (Fig. 4A). Moreover, intense bright-green fluorescence was observed under UV light for the positive amplification, whereas no fluorescence was observed in the negative control (Fig. 4A). Furthermore, mycelial DNA of other *Fusarium* and non-*Fusarium* fungal species as listed in Table 1 were tested for specificity analysis. No color changes were observed in the tubes of other non-*F. temperatum* fungal species after LAMP amplification, indicating the LAMP assay developed in this study can easily distinguish *F. temperatum* from other plant pathogens (Fig. 4B).

3.3. Diagnostic analysis of maize stalks and kernels infected with *F. temperatum*

To demonstrate the feasibility of early detection of *F. temperatum* in infected maize stalks and kernels, total DNA was extracted from diseased maize stalks and kernels that were artificially inoculated with *F. temperatum* at days 7 and 5 after inoculation, respectively (Fig. 5A and B). The LAMP assay of the inoculated maize tissues developed a yellowish color under natural light, and bright green fluorescence under

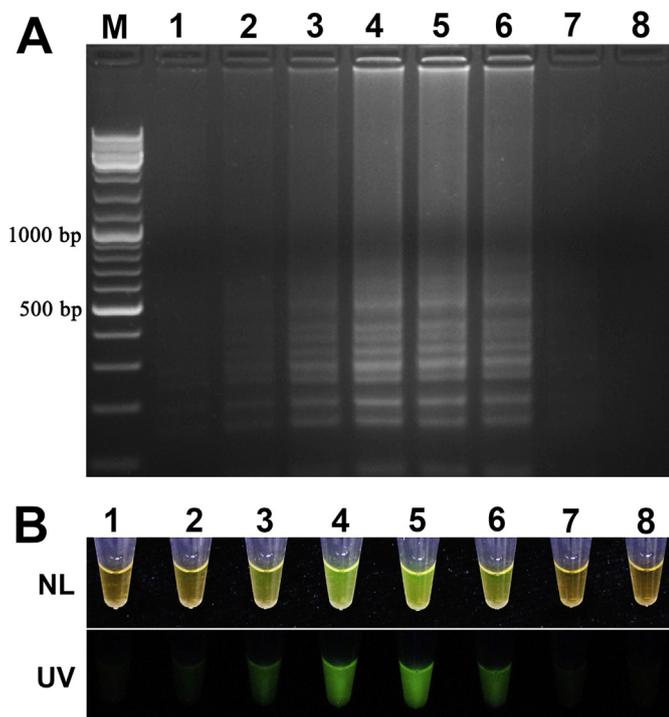


Fig. 2. Optimization of reaction temperature for the LAMP assay. (A) Visualization of LAMP products by agarose gel electrophoresis. (B) Visualization of LAMP products in reaction tubes under natural light (NL) and UV light (UV). M, molecular size marker (1 kb DNA ladder, ranging from 100 bp to 10 kb). Lanes and tubes 1 to 7 show results for incubation at 62.0, 62.4, 63.3, 64.5, 66.0, 67.3 and 68.0 °C, respectively. Lanes and tubes 8 is the negative control (ddH₂O).

UV light in the reaction tubes, while the healthy maize tissues showed no color change or fluorescence (Fig. 5C and D). Results show that the assay has no cross reaction with background maize DNA.

Furthermore, in order to confirm the reliability and specificity of the developed technique, we used diseased maize stalks and kernel samples collected from Yunnan province, China to validate the developed LAMP protocol in this study again. *F. temperatum* was identified in 34 symptomatic maize samples from a total of 166 analyzed (Table 2). In addition, *F. temperatum* was isolated from most of the LAMP-positive symptomatic samples, and not from the LAMP-negative symptomatic samples. These results indicate that *F. temperatum* is one of the major causal agents of *Fusarium* ear and stalk rot disease of maize in this province. These results also demonstrate that the LAMP assay developed in the present study can be used to rapidly diagnose maize ear and stalk rot diseases caused by *F. temperatum*.

4. Discussion

Maize (*Zea mays* L.) is an important crop for human consumption, animal feed and industrial processing. Diseases caused by *Fusarium* spp. were among the most important factors affecting the yield and grain quality of maize in the worldwide maize-growing regions. Many different *Fusarium* species which have varied pathogenicity and mycotoxin production potentials can infect an individual maize plant. *F. temperatum* was first identified in Europe and has already been detected in different maize-growing regions of China (Scaufilaire et al., 2011; J.H. Wang et al., 2014). Mycotoxin profiles showed that *F. temperatum* is able to produce moniliformin, beauvericin, enniatins and fumonisin B1 (J.H. Wang et al., 2014). *F. temperatum* represents a considerable risk for disease development in maize fields and mycotoxin contamination in maize-derived commodities. Hence, accurate identification of *F. temperatum* has become one of the preconditions to enable reasonable measures for the reduction of the disease occurrence and mycotoxin contamination. However, identification of *Fusarium* species through conventional approaches was somewhat difficult due to their similar

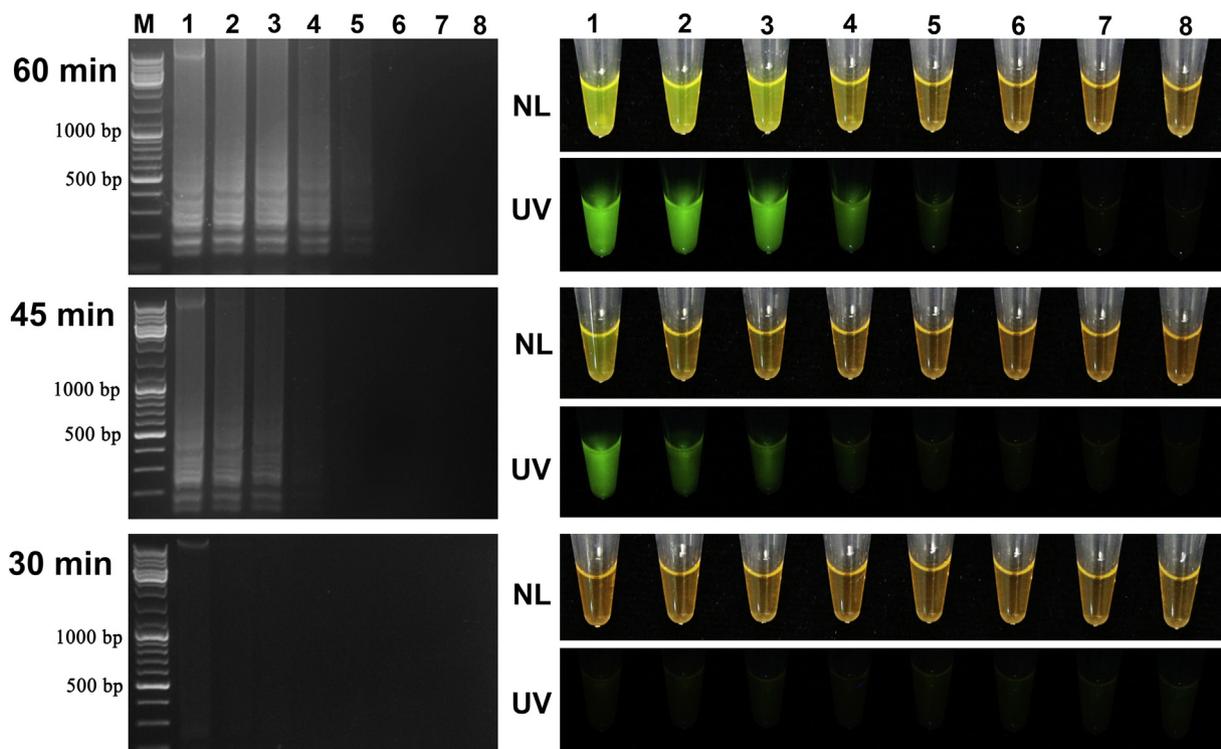


Fig. 3. Optimal reaction time and sensitivity of detection for the LAMP assay. Lanes and tubes 1 to 7 show results for genomic DNA at 100 ng, 10 ng, 1 ng, 100 pg, 10 pg, 1 pg and 100 fg, respectively. Lanes and tubes 8 is the negative control (ddH₂O). M, molecular size marker. WL, white light. UV, UV light.

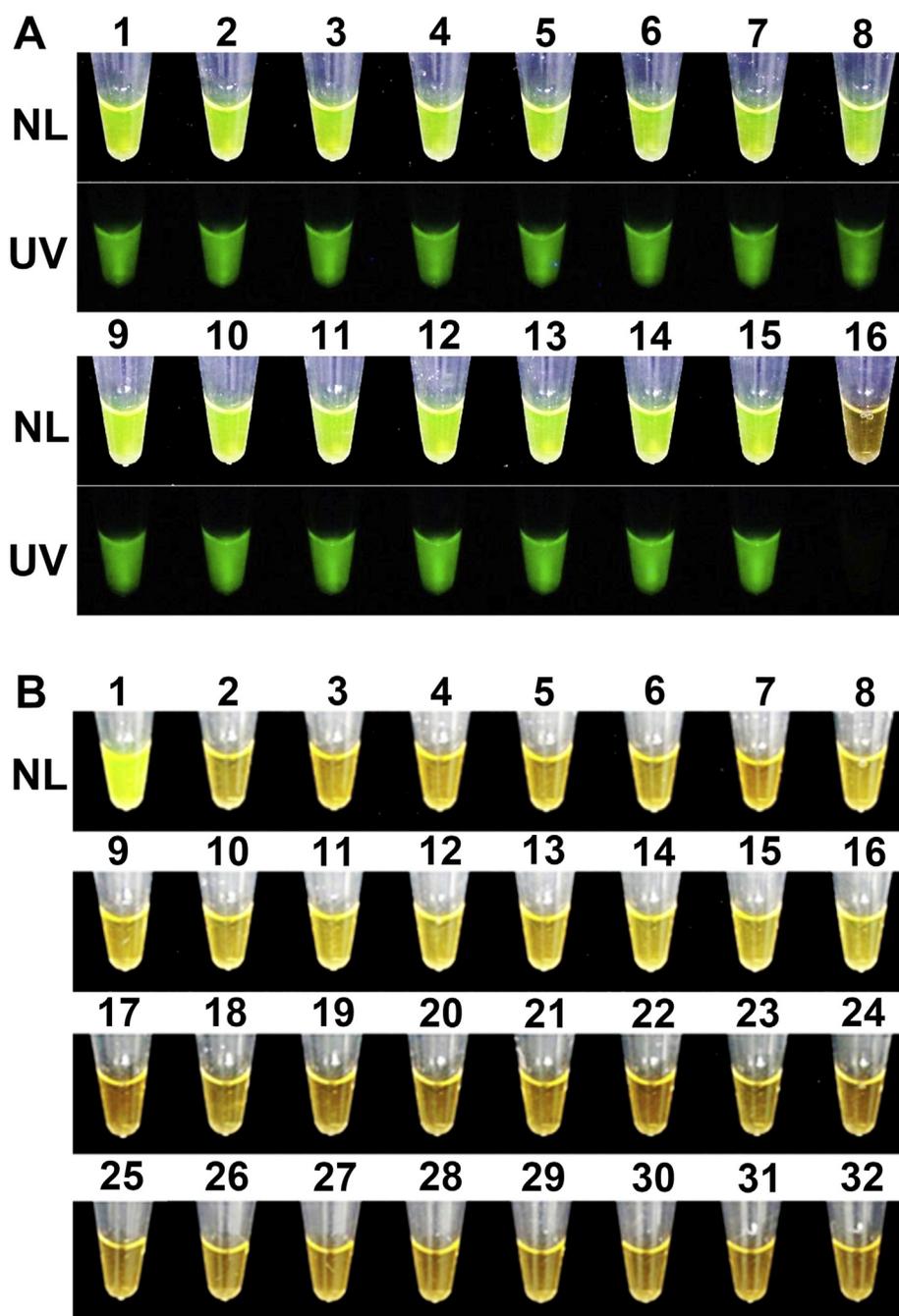


Fig. 4. Specificity of detection for the LAMP assay. (A) Lanes 1 to 15, *F. temperatum* from various locations. Lane 16 is the negative control. (B) LAMP assay for *Fusarium* spp. and other pathogens. Lane 1, *F. temperatum* (positive control); lane 2 to 4, *F. graminearum*; lane 5 to 7, *F. meridionale*; lane 8 to 10, *F. verticillioides*; lane 11, *F. boothii*; lane 12, *F. proliferatum*; lane 13, *F. fujikuroi*; lane 14, *F. asiaticum*; lane 15, *F. cerealis*, lane 16, *F. brachyglabrum*; lane 17, *F. oxysporum*; lane 18, *F. incarnatum*; lane 19, *F. equiseti*; lane 20, *F. avenaceum*; lane 21, *Pythium acanthophoron*; lane 22, *Bipolaris zeicola*; lane 23, *Alternaria alternata*; lane 24 *Nigrospora* sp.; lane 25, *Trichoderma* sp.; lane 26, *Pestalotiopsis* sp.; lane 27, *Bipolaris* sp.; lane 28, *Dothideomycete* sp.; lane 29, *Phoma* sp.; lane 30, *Glomerella graminicola*; lane 31, *Verticillium dahliae*; lane 32, negative control.

and overlapping morphological traits that complicate their differentiation (Kvas et al., 2009). Although several PCR-based assays or LAMP-based assays have been reported for rapid diagnosis of *F. graminearum* and *F. culmorum* in wheat and soybean, no attempt has been made to use LAMP assay for detection and identification of *F. temperatum*, which is an emerging threat to maize production.

In this study, we developed a specific, rapid and robust LAMP method for detection of *F. temperatum* from diseased maize stalk and kernel samples. Present study represents most of the taxa commonly prevailing in maize tissue in the field or under storage conditions. DNA from other related *Fusarium* spp. or from other genera was never amplified with the primer set used in this study. The optimum conditions of the developed LAMP assay to detect *F. temperatum* were 65 °C run for 60 min. However, detection can be easily completed within 45 min at 65 °C according to the results from visual inspection of SYBR Green I and agarose gel electrophoresis, though these conditions are less

sensitive. These results demonstrated that the developed LAMP assay for *F. temperatum* was highly specific and can be used worldwide for diagnostic purposes. However, one point needs to be mentioned, though the designed primer set can theoretically differentiate *F. temperatum* from *F. subglutinans*, we didn't test the specificity by assaying DNA for these species because we didn't identify any *F. subglutinans* in our collected samples. We could not determine if the developed LAMP assay can distinguish *F. temperatum* from *F. subglutinans* because *F. temperatum* corresponds to *F. subglutinans* Group 1 previously described by Steenkamp et al. (2002).

As compared to other PCR-based diagnostic methods, e.g. conventional PCR and real-time quantitative PCR (qPCR) assay, LAMP assay doesn't require expensive instrumentation and specialized training for operation and data analysis. LAMP assay is also highly specific, as it uses up to six target-specific primers that recognize eight distinct regions on the target template (Notomi et al., 2000). In some instances,

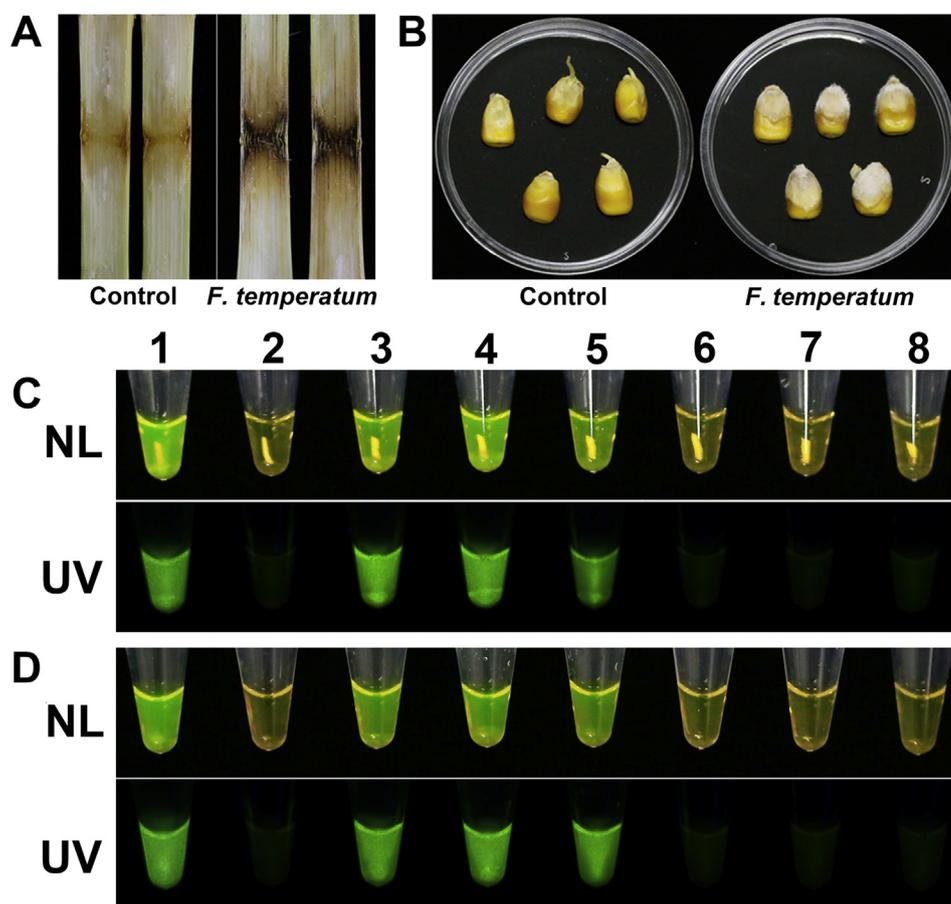


Fig. 5. Detection of *F. temperatum* in diseased maize tissues using the developed LAMP assay. (A) Maize stalks cut longitudinally showing necrotic lesions around the insertion point at 7 days post-inoculation. (B) Symptoms of maize kernels inoculated by *F. temperatum* at 5 days post-inoculation. No symptoms were observed in the control (A and B). Detection and identification of *F. temperatum* in diseased maize stalks (C) and kernels (D) using the developed LAMP assay. Lane 1, 100 ng genomic DNA of *F. temperatum* as positive control; lane 2 ddH₂O as the negative control; lane 3 to 5, genomic DNA extracted from maize stalks (C) and kernels (D) inoculated by *F. temperatum*; lane 6 to 8, genomic DNA extracted from maize stalks (C) and kernels (D) mock-inoculated by ddH₂O.

Table 2

Detection of *Fusarium temperatum* in diseased maize samples in the field collected from Yunnan province of China using LAMP assay.

Collection	Longitude	Latitude	Suspect samples	Number of positive
Lincang	100°10′	23°89′	48	13
Qujing	103°80′	25°50′	46	5
Zhaotong	103°72′	27°35′	48	14
Chuxiong	102°09′	25°16′	8	1
Kunming	102°49′	24°93′	16	1
Total			166	34

amplification by LAMP may be faster than by PCR-based methods, because there is no time needed in cycling between different temperatures. From this point of view, several reports have already shown that sensitivity of the LAMP assay was higher than, or similar to that of PCR-based assays for the detection of various pathogens, including viruses, bacteria and fungi (James et al., 2010; Niessen and Vogel, 2010; F. Wang et al., 2014). In addition, LAMP has been shown to be tolerant to naturally occurring inhibitors in biological samples (Le and Vu, 2017). Therefore, in combination with its speed, specificity and sensitivity, the LAMP assay presented here has been a robust tool in the screening of large numbers of samples that can also be used as a tool in quality inspection procedures.

In conclusion, the LAMP method developed in this study has the potential to be extremely useful for rapid diagnosis of *F. temperatum* in the field without specialized equipment. The assay can also be a significant tool, for enhancing the efficiency, to evaluate the quality of raw materials to be processed into food and feed products, and has the potential to be expanded for use in the rapid diagnosis of other plant pathogens.

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Authors' contributions

WG conceived and designed the experiments. LYS and HAH performed the experiments. JZ, DDZ and WG collected samples in the field. XFD and WG analyzed the data. DPJ and WG wrote the manuscript.

References

- Boutigny, A.L., Scauflaire, J., Ballois, N., Ioos, R., 2017. *Fusarium temperatum* isolated from maize in France. *Eur. J. Plant Pathol.* 148, 997–1001.
- Das, A., Babiuk, S., McIntosh, M.T., 2012. Development of a loop-mediated isothermal amplification assay for rapid detection of *Capripoxviruses*. *J. Clin. Microbiol.* 50, 1613–1620.
- Desjardins, A.E., Proctor, R.H., 2007. Molecular biology of *Fusarium* mycotoxins. *Int. J. Food Microbiol.* 119, 47–50.
- Duan, C., Qin, Z., Yang, Z., Li, W., Sun, S., Zhu, Z., Wang, X., 2016. Identification of pathogenic *Fusarium* spp. causing maize ear rot and potential mycotoxin production in China. *Toxins* 8, 186.
- Fumero, M., Reynoso, M., Chulze, S., 2015. *Fusarium temperatum* and *Fusarium subglutinans* isolated from maize in Argentina. *Int. J. Food Microbiol.* 199, 86–92.
- James, H.E., Ebert, K., McGonigle, R., Reid, S.M., Boonham, N., Tomlinson, J.A., Hutchings, G.H., Denyer, M., Oura, C.A., Dukes, J.P., et al., 2010. Detection of African swine fever virus by loop-mediated isothermal amplification. *J. Virol. Methods* 164, 68–74.
- Kvas, M., Marasas, W.F.O., Wingfield, B.D., Wingfield, M.J., Steenkamp, E.T., 2009. Diversity and evolution of *Fusarium* species in the *Gibberella fujikuroi* complex. *Fungal Divers.* 34, 1–21.

- Lanza, F.E., Mayfield, D.A., Munkvold, G.P., 2016. First report of *Fusarium temperatum* causing maize seedling blight and seed rot in North America. *Plant Dis.* 100, 1019.
- Le, D.T., Vu, N.T., 2017. Progress of loop-mediated isothermal amplification technique in molecular diagnosis of plant diseases. *Appl. Biol. Chem.* 60, 169–180.
- Ma, L.J., Geiser, D.M., Proctor, R.H., Rooney, A.P., O'Donnell, K., Trail, F., Gardiner, D.M., Manners, J.M., Kazan, K., 2013. *Fusarium* pathogenomics. *Annu. Rev. Microbiol.* 67, 399–416.
- Mulè, G., Logrieco, A., Stea, G., Bottalico, A., 1997. Clustering of trichothecene-producing *Fusarium* strains determined from 28S ribosomal DNA sequences. *Appl. Environ. Microbiol.* 63, 1843–1846.
- Niessen, L., Vogel, R.F., 2010. Detection of *Fusarium graminearum* DNA using a loop-mediated isothermal amplification (LAMP) assay. *Int. J. Food Microbiol.* 140, 183–191.
- Notomi, T., Okayama, H., Masubuchi, H., Yonekawa, T., Watanabe, K., Amino, N., Hase, T., 2000. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* 28, e63.
- Robles-Barrios, K.F., Medina-Canales, M.G., Rodriguez-Tovar, A.V., Perez, N.O., 2015. Morphological and molecular characterization, enzyme production and pathogenesis of *Fusarium temperatum* on corn in Mexico. *Can. J. Plant Pathol.* 37, 495–505.
- Scaufaire, J., Gourgue, M., Munaut, F., 2011. *Fusarium temperatum* sp. nov. from maize, an emergent species closely related to *Fusarium subglutinans*. *Mycologia* 103, 586–597.
- Scaufaire, J., Gourgue, M., Callebaut, A., Munaut, F., 2012. *Fusarium temperatum*, a mycotoxin-producing pathogen of maize. *Eur. J. Plant Pathol.* 133, 911–922.
- Sema, M., Alemu, A., Bayih, A.G., Getie, S., Getnet, G., Guelig, D., Burton, R., LaBarre, P., Pillai, D.R., 2015. Evaluation of non-instrumented nucleic acid amplification by loop-mediated isothermal amplification (NINA-LAMP) for the diagnosis of malaria in Northwest Ethiopia. *Malar. J.* 14, 44.
- Shin, J.H., Han, J.H., Lee, J.K., Kim, K.S., 2014. Characterization of the maize stalk rot pathogens *Fusarium subglutinans* and *F. temperatum* and the effect of fungicides on their mycelial growth and colony formation. *Plant Pathol. J.* 30, 397–406.
- Steenkamp, E.T., Wingfield, B.D., Desjardins, A.E., Marasas, W.F., Wingfield, M.J., 2002. Cryptic speciation in *Fusarium subglutinans*. *Mycologia* 94, 1032–1043.
- Varela, C.P., Casal, O.A., Padin, M.C., Martinez, V.F., Oses, M.J.S., Scaufaire, J., Munaut, F., Castro, M.J.B., Vazquez, J.P.M., 2013. First report of *Fusarium temperatum* causing seedling blight and stalk rot on maize in Spain. *Plant Dis.* 97, 1252–1253.
- Venturini, G., Toffolatti, S.L., Passera, A., Pilu, R., Quaglino, F., Casati, P., 2016. First report of *Fusarium temperatum* causing ear rot on maize in Italy. *J. Plant Pathol.* 98, 686.
- Wang, F., Yang, Q., Qu, Y., Meng, J., Ge, B., 2014. Evaluation of a loop-mediated isothermal amplification suite for the rapid, reliable, and robust detection of Shiga toxin-producing *Escherichia coli* in produce. *Appl. Environ. Microbiol.* 80, 2516–2525.
- Wang, J.H., Zhang, J.B., Li, H.P., Gong, A.D., Xue, S., Agboola, R.S., Liao, Y.C., 2014. Molecular identification, mycotoxin production and comparative pathogenicity of *Fusarium temperatum* isolated from maize in China. *J. Phytopathol.* 162, 147–157.
- Zhang, W.W., Deng, X.D., Yu, X.P., Pei, X.F., Fu, G.M., Wang, X.L., Li, B.B., Wang, L.Y., 2015. The recent *Fusarium mycotoxin* situation in grain and feed in China. *World Mycotoxin J.* 8, 545–551.