Early and Rapid Detection of Tomato Gray Mold Utilizing Loop - Mediated Isothermal Amplification (LAMP) Technology

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Abstract [**Objectives**] To develop methods for the early and rapid detection of tomato gray mold. [**Methods**] Utilizing the *ACTIN* gene of *Botrytis cinerea* as the target, a set of specific primers for loop-mediated isothermal amplification (LAMP) was designed and screened. Subsequently, the reaction system and conditions were optimized to achieve rapid isothermal amplification of *B. cinerea*. [**Results**] Through agarose gel electrophoresis and SYBR Green I visualization analysis, the optimal dosages of Bst II DNA polymerase and dNTPs, as well as the optimal ratio of internal to external primers, were determined to be 0.6 U/μL, 1.25 mmol/L, and 2 ∶ 1, respectively. The specific detection of *B. cinerea* was accomplished at 61 ℃ for 40 min, achieving a sensitivity of 100 ag/μL, which is 10^6 times greater than that of conventional PCR detection. When this method was applied to the detection of tomato diseases, the detection limit for *B. cinerea* spores reached 20 spores/mL. Furthermore, the pathogen was detectable in tomato leaves that had been infected for 4 d, despite the absence of visible phenotypic symptoms of gray mold. [**Conclusions**] This method is suitable for the early, rapid, sensitive, and visual detection of tomato gray mold, thereby offering technical support for its early diagnosis, prevention, and control.

Key words Tomato, *Botrytis cinerea*, Gray mold, Loop-mediated isothermal amplification (LAMP), *ACTIN* gene

1 Introduction

Botrytis cinerea, a globally prevalent fungal pathogen, is responsible for a widespread plant disease characterized by high rates and significant yield reductions[1]. This pathogen is noted for its rapid infection process, broad host range, and prolonged period of damage[2]. In greenhouse vegetable production, *B. cinerea* has become a major limiting factor for tomato (*Solanum lycopersicum* L.) cultivation, severely impacting both yield and fruit quality[3]. The intensive cultivation of greenhouse vegetables creates an environment conducive to the emergence and proliferation of plant diseases. When outbreaks occur on a large scale, they are challenging to manage and frequently result in significant losses. Consequently, the establishment of an early disease warning system and the implementation of preventive interventions prior to disease onset are crucial for effectively controlling disease spread. Such measures hold substantial importance and offer promising applications for the sustainable and safe production of vegetables.

The essential factor in achieving early disease warning is the rapid, accurate, and timely detection and diagnosis of pathogenic bacteria. In recent years, technologies for identifying pathogenic microorganisms have undergone continuous advancement and refinement. These developments have progressed from traditional methods based on morphological characterization of pathogens to variable-temperature nucleic acid amplification techniques grounded in conventional PCR, and subsequently to isothermal nucleic acid amplification technologies. Consequently, the detection and identification of pathogenic microorganisms have become increasingly precise and expedited. Loop-mediated isothermal amplification (LAMP) was first introduced by Japanese researchers Notomi *et al.*[4] in 2000 as an isothermal nucleic acid amplification technique characterized by high sensitivity, rapid reaction time, minimal equipment requirements, and easily observable results. This method overcomes the limitations associated with variable-temperature nucleic acid amplification, rendering it particularly suitable for rapid detection at the grassroots level and in the field. Consequently, LAMP has rapidly emerged as a prominent focus in nucleic acid detection research and is extensively utilized across diverse domains, including disease diagnosis, epidemiological surveillance, food safety testing, animal pathogen detection, transgenic organism identification, and plant disease diagnosis[5-8]. LAMP detection technology systems have been successfully established for the identification of various agricultural pathogenic microorganisms, including *Fusarium oxysporum* and *Colletotrichum* spp. in strawberry[9], *Pseudomonas syringae* pv. *actinidiae* in kiwifruit[10], *Fusarium solani* in *Medicago sativa*[11], *Botryosphaeria dothidea* in apple[12], *Plasmopara viticola* in grape[13], *Ustilago hordei* and *Pyrenophora graminea* in *Hordeum vulgare*[14-15], *Leptosphaeria biglobosa* and *Leptosphaeria maculans* in oilseed rape[16], *Phytophthora parasitica* in tobacco[17], *Bipolaris sorokiniana* in maize[18], and *Sphaerotheca aphanis* in strawberry[19]. The application of LAMP technology in *B. cinerea* primarily focuses on the detection and identification of drug-resistant strains of *B. cinerea*[20-21]. However, its use for the early detection of *B. cinerea*

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infection in plants and the early diagnosis of gray mold disease has not yet been documented. To this end, LAMP-specific primers targeting the *ACTIN* gene were designed to develop a rapid LAMP detection system for *B. cinerea*. The detection limits for *B. cinerea* spores, the temporal detection threshold for tomato infection, and the correlation with the phenotypic manifestation of tomato disease were systematically analyzed, in order to provide technical support for the early diagnosis and management of tomato gray mold.

2 Materials and methods

2.1 Materials The strains tested in this study included *B. cinerea*, *Corynespora cassiicola*, *Botryosphaeria dothidea*, *Penicillium expansum*, *Alternaria alternata*, *Pseudomonas syringae* DC3000, *Pectobacterium carotovorum*, *Gibberella zeae*, and *Bipolaris* species, all of which were obtained from the Biology Institute, Hebei Academy of Sciences. *Fusarium pseudograminearum* was sourced from Hebei University of Science and Technology, while *Fusarium oxysporum* and *Rhizoctonia solani* were acquired from Hebei Agricultural University. All fungal strains were cultured on potato dextrose agar (PDA) solid medium, whereas bacterial strains were cultured in Luria-Bertani (LB) medium at 28 ℃.

Bst II DNA polymerase, Bst buffer, and dNTP mixture were all obtained from Beijing Biomed Gene Technology Co., Ltd. The genomic DNA extraction kit was procured from Mei5 Biotechnology Co., Ltd. SYBR Green I was sourced from Shanghai Ruichu Biotechnology Co., Ltd. The super clean bench was acquired from Suzhou Antai Airtech Co., Ltd., the benchtop high-speed refrigerated centrifuge from Hunan Xiangyi Laboratory Instrument Development Co., Ltd., the constant temperature metal bath from CoYoTebio Company, and the biochemical incubator from Shanghai Yiheng Technology Instrument Co., Ltd.

2.2 Primer design The *ACTIN* gene (accession No.: AJ000335.1) of *B. cinerea* was retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov). Gene fragments exhibiting low homology to other pathogenic fungi were identified through sequence alignment and selected as target sequences for primer design using the PrimerExplorer V5 platform (http://primerexplorer.jp). A set of LAMP primers was designed targeting the B1, B2, B3, F1c, F2c, and F3c regions of the target sequence (Fig. 1). This set included a pair of external primers (F3/B3) and a pair of internal primers (FIP/BIP). Primer synthesis was performed by Sangon Biotech (Shanghai) Co., Ltd. The primer sequences are presented in Table 1.

Table 1 LAMP-H2 primer sequences

Primer name	Primer sequence (5′→3′)
F3-H2	CCCCTGCATTCTACGTCTCT
B3-H2	GCAGTGGTGGAGAAAGTGTA
FIP-H2	ACGTGAGTAACTCCGTCACCGGCTCTCCCTTTACGCTTCCG
BIP-H2	CTCTCTTCCTCACGCCATTGCTCGCTCAGCCAAGATCTTCA

2.3 Establishment of reaction conditions for LAMP According to the manufacturer's instructions for Bst II DNA polymerase (Biomed), the initial LAMP reaction system was established with a total volume of 20 μL. The reaction mixture comprised 2.0 μL of 10 × Bst II reaction buffer (containing 8 mmol/L $MgSO_4$), 3.5 μL of dNTPs (10 mmol/L), 0.5 μL each of the external primers F3-H2 and B3-H2 (10 μmol/L), 1 μL each of the internal primers FIP-H2 and BIP-H2 (10 μmol/L), 1 μL of Bst II DNA polymerase (8 U/μL), and 1 μL of DNA template, with the volume adjusted to 20 μL using sterile ultrapure water. The LAMP reaction was conducted by incubating the mixture at 65 ℃ for 60 min, followed by enzyme inactivation at 85 ℃ for 5 min.

Based on the initial LAMP reaction system, single-factor screening was performed to optimize key reaction components, including Bst II DNA polymerase, dNTPs, the ratio of internal to external primers, as well as reaction temperature and duration. The experimental conditions for each individual factor were established as follows: the final concentrations of Bst II DNA polymerase were set at 0 (control), 0.2, 0.4, 0.6, and 0.8 U/μL; the final concentrations of dNTPs were 0 (control), 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, and 2.0 mmol/L. The ratios of internal to external primers were 1∶1, 1∶2, 1∶3, 2∶1, and 3∶1, with a control group lacking both internal and external primers. Reaction temperatures were varied at 57, 59, 61, 63, 65, 67, 69, and 71 ℃, with room temperature serving as the control. Reaction durations were set at 0 (control), 10, 20, 30, 40, 50, 60, and 70 min. The LAMP reactions were conducted by altering one factor at a time while maintaining all other component dosages and conditions constant. Upon completion of the reaction, the optimal LAMP reaction conditions for the primer set were established based on the outcomes of gel electrophoresis and fluorescent dye detection.

2.4 Specificity analysis of LAMP primers LAMP amplification was performed using the DNA of *B. cinerea* and the control strain as templates, employing the aforementioned LAMP reaction system. Sterile ultrapure water served as the negative control. The amplification products were analyzed via gel electrophoresis and fluorescent dye detection to assess the specificity of the LAMP primers. The control strains comprised *C. cassiicola*, *B. dothidea*, *P. expansum*, *A. alternata*, *P. syringae*, *P. carotovorum*, *G. zeae*, *Bipolaris* species, *F. oxysporum*, *R. solani*, and *F. pseudograminearum*.

2.5 Sensitivity analysis of LAMP reaction Genomic DNA from *B. cinerea* was extracted, and its concentration was quantified using a NanoDrop spectrophotometer. The DNA samples were subsequently diluted 10-fold with bacteria-free ultrapure water. Template DNA concentrations of 1 ng/μL, 100 pg/μL, 10 pg/μL, 1 pg/μL, 100 fg/μL, 10 fg/μL, 1 fg/μL, 100 ag/μL, 10 ag/μL, and 1 ag/μL were prepared for LAMP and PCR assays. Sterile ultrapure water was used as a negative control (CK) to assess the sensitivity of the LAMP assay. The PCR protocol consisted of an initial denaturation at 95 ℃ for 5 min, followed by 30 cycles of denaturation at 95 ℃ for 30 sec, annealing at 53 ℃ for 30 sec, and extension at 72 ℃ for 30 sec, with a final extension step at 72 ℃ for 10 min. The upstream primer sequence was 5′-GCCATTGCTCGTGTTGA-3′, and the downstream primer sequence was 5′-GGCAGTGGTGGAGAAAGT-3′.

2.6 Preparation of spore suspensions of *B. cinerea* and determination of the detection limit for spore concentration *B. cinerea* was cultured on PDA medium until sporulation occurred. Spores were then collected by scraping with an inoculation needle into a 5% Tween-80 solution, followed by filtration through sterile gauze. Spore concentration was determined using a hemocytometer. A stock spore suspension was prepared at a concentration of 2×10^6 spores/mL and subsequently diluted in a 10-fold serial gradient. This resulted in a series of spore suspensions with concentrations of 2×10^6, 2×10^5, 2×10^4, 2×10^3, 2×10^2, 2×10^1, 2×10^0, 2×10^{-1}, and 2×10^{-2} spores/mL for subsequent analyses. For the LAMP assay, 1 μL of each spore suspension concentration were used as templates. The lowest spore concentration yielding a positive LAMP result was defined as the detection limit of this method.

2.7 Cultivation of aseptic tomato seedlings and analysis of the detection limit for pathogen infection time The tomato seeds were initially soaked in sterile water for 5 min, followed by immersion in 75% ethanol for 30 sec. Subsequently, they were rinsed three times with sterile water, soaked in 10% sodium hypochlorite for 4 min, and finally washed five times with sterile water. The treated seeds were then transferred into culture flasks containing Murashige and Skoog (MS) medium, sealed with sealing film, and incubated in a light-controlled environment at (25 ± 2) ℃ under a 16 h light/8 h dark photoperiod.

After the tomato aseptic seedlings developed 3 to 4 leaves, seedlings exhibiting similar growth conditions were selected and inoculated with varying concentrations of *B. cinerea* spore suspensions as previously described. The control group was not inoculated with the pathogen. After 16 h of dark incubation, the seedlings were transferred to a light incubator maintained at (25 ± 2) ℃ for further cultivation, during which phenotypic changes were monitored. At multiple time points post-inoculation (days 1 to days 5), leaves were harvested from the tomato plants. After surface sterilization with 75% ethanol, genomic DNA was extracted from the leaves for LAMP assay. The earliest time point at which a positive LAMP reaction was observed was considered the detection limit for pathogen infection using this method.

2.8 Determination of LAMP reaction results The results of the LAMP reaction were evaluated using two methods: 1.5% agarose gel electrophoresis and SYBR Green I fluorescence staining. Positive electrophoresis results were characterized by the presence of distinct trapezoidal bands, whereas no such bands appeared in negative reactions. Prior to the reaction, 1 μL of SYBR Green I fluorescent staining solution was applied to the inner surface of the tube cap. Following the reaction, the staining solution was mixed thoroughly with the reaction mixture by inverting the tube, and the resulting color change was observed visually. A positive reaction was indicated by a green coloration, while a negative reaction exhibited an orange coloration.

3 Results and analysis

3.1 Specific identification of LAMP primer sets of *B. cinerea* The specificity of primer amplification is a fundamental prerequisite for the successful identification of pathogenic bacteria using the LAMP method. The LAMP-H2 primer sets (F3-H2, B3-H2; FIP-H2, BIP-H2), designed based on the *ACTIN* gene sequence of *B. cinerea*, were employed to amplify the genomic DNA of the target pathogen *B. cinerea* as well as that of non-target pathogens via LAMP, in order to assess the specificity and applicability of these primer sets. As illustrated in Fig. 2, only the amplification result for *B. cinerea* No. 2 was positive. Electrophoresis revealed a distinct trapezoidal band, and SYBR Green I staining exhibited a green coloration. In contrast, the negative control and other non-target pathogenic bacteria showed no amplification bands and were stained orange. These findings demonstrated that the LAMP-H2 primer set possessed high specificity, enabling the specific detection of *B. cinerea* without cross-reactivity to other non-target pathogenic bacteria, thereby validating its suitability for LAMP-based detection of *B. cinerea*.

3.2 Establishment of LAMP detection conditions for *B. cinerea*

3.2.1 LAMP reaction system. As illustrated in Fig. 3, the LAMP reaction conducted with varying concentrations of Bst II DNA polymerase yielded positive amplification results at enzyme concentrations ranging from 0.4 to 0.8 U/μL. Electrophoretic analysis revealed distinct trapezoidal bands, and SYBR Green I staining exhibited a green coloration. In contrast, no amplification bands were observed in the control (0 U/μL) and 0.2 U/μL enzyme concentrations, with the staining appearing orange (Fig. 3A). LAMP reaction was conducted using varying concentrations of dNTPs. Positive amplification results were observed at dNTP concentrations of 1 – 2 mmol/L. Electrophoretic analysis revealed distinct trapezoidal bands, and SYBR Green I staining exhibited a green coloration. In contrast, no amplification bands were detected, and the staining appeared orange in the control (0 mmol/L) and dNTP concentrations of 0.5 and 0.75 mmol/L (Fig. 3B). The LAMP reaction was conducted using varying ratios of internal to external primers. Positive amplification was observed across all tested primer ratios. Electrophoretic analysis revealed distinct trapezoidal bands, and SYBR Green I staining exhibited a green coloration. The control showed no amplification bands and was stained orange (Fig. 3C). Considering both amplification efficiency and cost comprehensively, the optimal concentrations of Bst II DNA polymerase and dNTPs, along with the optimal ratio of internal to external primers, were determined to be 0.6 U/μL, 1.25 mmol/L, and 2∶1, respectively. A 20 μL LAMP reaction system for *B. cinerea* was developed, comprising 1 × Bst II buffer, 8 mmol/L Mg^{2+}, 0.6 U/μL Bst II DNA polymerase, 1.25 mmol/L dNTPs, 0.5 μmol/L each of FIP-H2 and BIP-H2 primers, 0.25 μmol/L each of F3-H2 and B3-H2 primers, and 1 μL of DNA template, with the final volume adjusted to 20 μL using sterile ultrapure water.

3.2.2 LAMP reaction procedure. The aforementioned LAMP reaction system was utilized to amplify *B. cinerea* at various reaction temperatures (57, 59, 61, 63, 65, 67, 69, and 71 ℃) and reaction time (10, 20, 30, 40, 50, 60, and 70 min) to determine the optimal LAMP reaction conditions for *B. cinerea*. As illustrated in Fig. 4, positive amplification results were observed within the temperature range of 57 – 67 ℃. Electrophoresis revealed distinct

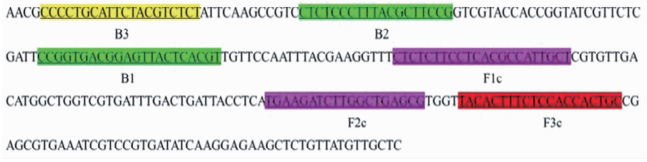
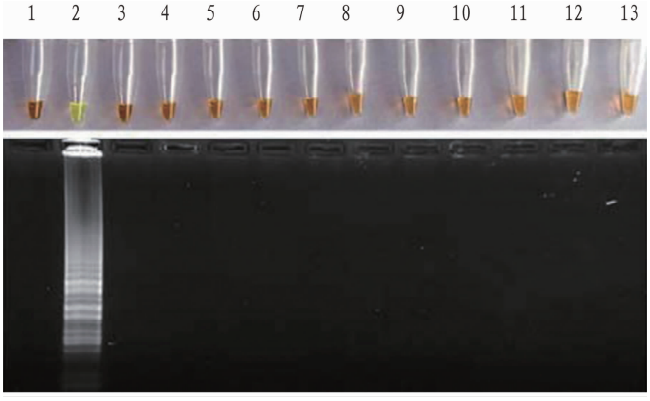


Fig. 1 Target gene sequence of *Botrytis cinerea*



NOTE 1. Negative control; 2. *B. cinerea*; 3. *B. dothidea*; 4. *C. cassiicola*; 5. *P. expansum*; 6. *A. alternata*; 7. *F. oxysporum*; 8. *R. solani*; 9. *G. zeae*; 10. *Bipolaris*; 11. *F. pseudograminearum*; 12. *P. syringae*; 13. *P. carotovorum*.

Fig. 2 Specificity of LAMP detection method for *Botrytis cinerea*

trapezoidal bands, and SYBR Green I staining exhibited a green coloration. No amplification bands were detected in the control or at 69 and 71 ℃, where the staining appeared orange (Fig. 4A). The amplification yielded positive results within 30 – 70 min of reaction. Electrophoretic analysis revealed distinct trapezoidal bands, and SYBR Green I staining exhibited a green coloration. In contrast, the control and reaction for 10 and 20 min showed no amplification bands, with staining appearing orange (Fig. 4B). Considering both amplification efficiency and cost factors comprehensively, the LAMP reaction protocol for *B. cinerea* was established as follows: incubation at 61 ℃ for 40 min, followed by inactivation at 85 ℃ for 5 min.

3.3 Sensitivity of LAMP detection method for *B. cinerea*

As illustrated in Fig. 5, the minimum template mass concentration yielding a positive reaction was 100 ag/μL, indicating that the detection sensitivity for *B. cinerea* was 100 ag/μL. In contrast, when the target gene fragment of *B. cinerea* was amplified using PCR, the minimum positive template mass concentration was 100 pg/μL, which was six orders of magnitude higher than the detection sensitivity of the LAMP assay. These results demonstrate that the established LAMP detection method exhibits exceptionally high sensitivity.

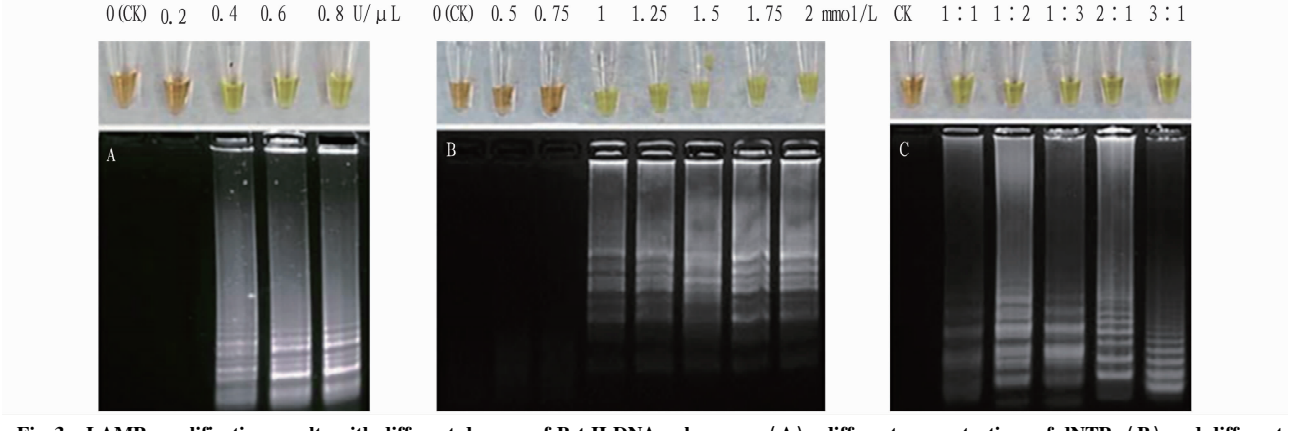


Fig. 3 LAMP amplification results with different dosages of Bst II DNA polymerase (A), different concentrations of dNTPs (B) and different ratios of internal to external primers (C)

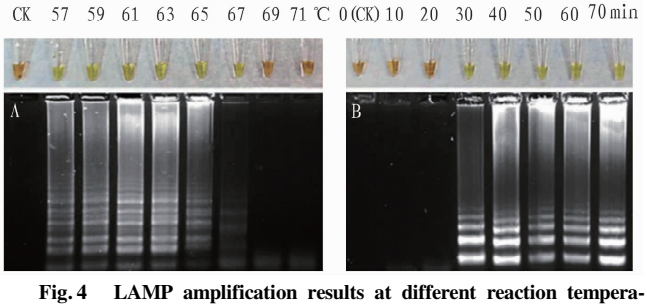


Fig. 4 LAMP amplification results at different reaction temperatures (A) and different reaction time (B)

3.4 Detection limit of spore concentration of *B. cinerea*

As illustrated in Fig. 6, the spore concentration of *B. cinerea* ranged from 2×10^6 to 2×10^1 spores/mL. The agarose gel electrophoresis

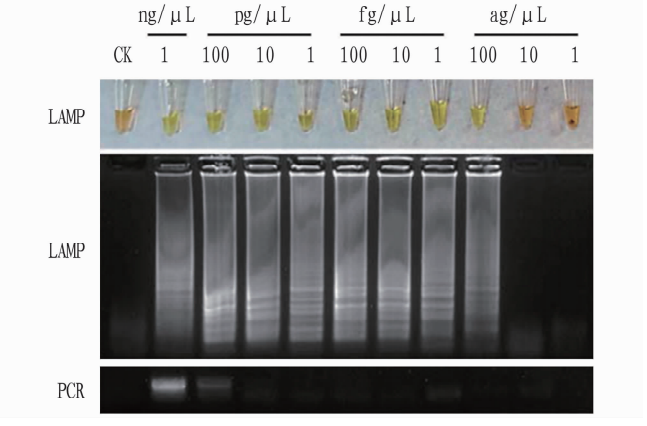
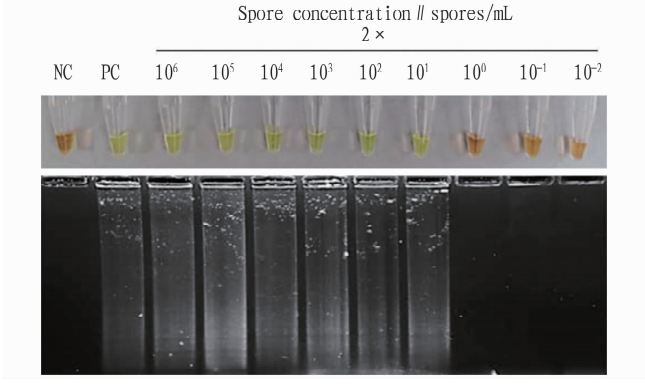


Fig. 5 Sensitivity of LAMP detection method for *Botrytis cinerea*

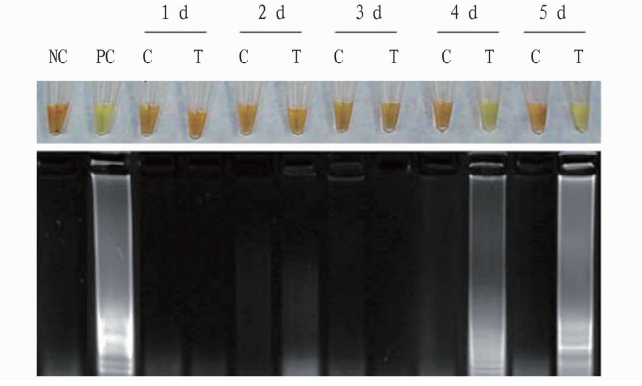
of the LAMP reaction products exhibited diffuse bands accompanied by a green color change, indicating a positive result. When the spore concentration of *B. cinerea* was below 2×10^1 spores/mL, the LAMP reaction product exhibited no amplification band and presented an orange color reaction, indicating a negative result. These findings demonstrate that the minimum detectable spore concentration of *B. cinerea* in this reaction system is 2×10^1 spores/mL, equivalent to 20 spores/mL.



NOTE NC. Negative control; PC. Positive control (genomic DNA of *B. cinerea*).

Fig. 6 LAMP detection of varying spore concentrations of *Botrytis cinerea*

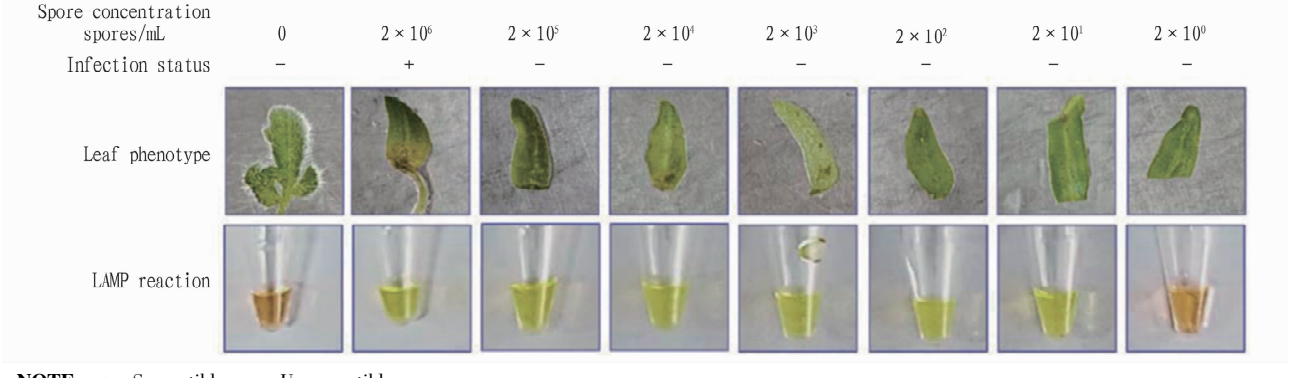
3.5 Detection limit for the infection time by *B. cinerea* in tomatoes A suspension containing 2×10^1 spores/mL of *B. cinerea* was inoculated onto aseptic tomato seedlings and cultured for varying durations (1, 2, 3, 4, and 5 d). Subsequently, tomato leaves were collected for LAMP detection, with the results presented in Fig. 7. From the 1st to the 3rd day post-inoculation, the LAMP reaction products exhibited no amplification bands, accompanied by an orange color change, and the results were negative, indicating the absence of pathogenic fungi in the tomato leaves. However, on the 4th and 5th days, electrophoresis of the LAMP reaction products revealed the presence of diffuse bands, accompanied by a green color change, yielding positive results. These findings demonstrate that *B. cinerea* can be detected in tomato leaves as early as 4 d after infection.



NOTE NC. Negative control; PC. Positive control (genomic DNA of *B. cinerea*); C. Untreated tomato leaves; T. Tomato leaves inoculated with *B. cinerea*.

Fig. 7 LAMP detection of *Botrytis cinerea* infection in tomatoes at different time

3.6 Corresponding relationship between the detection results of *B. cinerea* and the phenotype of tomato gray mold To investigate the relationship among pathogen load, tomato disease susceptibility, and LAMP detection, sterile tomato seedlings were inoculated with varying concentrations of *B. cinerea* spore suspensions (2×10^6, 2×10^5, 2×10^4, 2×10^3, 2×10^2, 2×10^1, and 2×10^0 spores/mL). Following 4 d of cultivation, LAMP detection was performed, and the plant phenotypes were assessed. As illustrated in Fig. 8, when the spore concentration of *B. cinerea* was 2×10^6 spores/mL, the tomato leaves exhibited susceptible phenotypes, and the LAMP assay yielded positive results. At spore concentrations ranging from 2×10^5 to 2×10^1 spores/mL, the tomato leaves did not display obvious infection symptoms, although the LAMP assay remained positive. When the spore concentration was 2×10^0 spores/mL, the tomato leaves did not exhibit susceptibility to the disease, and the LAMP assay was negative. The results demonstrate that this LAMP detection method is capable of identifying the presence of pathogenic fungi in tomatoes at the early stages of infection by *B. cinerea*, even when the pathogen load is extremely low and no disease symptoms are observable. This method can be utilized for the early diagnosis and early warning in the prevention and control of tomato gray mold.



NOTE +. Susceptible; −. Unsusceptible.

Fig. 8 Corresponding relationship between LAMP detection results and pathogen load as well as the phenotype of tomato susceptibility

4 Conclusions and discussion

The detection and identification of pathogenic microorganisms primarily involve traditional morphological feature analysis and molecular biological techniques. Traditional morphological identification of pathogenic bacteria is time-consuming and labor-intensive, necessitating the expertise of skilled professionals, which constrains the accuracy of disease diagnosis[22]. Molecular detection and identification techniques based on PCR technology—including PCR-RFLP, nested PCR, multiplex PCR, real-time quantitative PCR, and immuno-PCR—enable accurate, rapid, and quantitative identification of pathogens through the amplification of specific gene fragments of pathogens. However, these methods necessitate costly thermal cyclers and specialized expertise, thereby restricting their use to laboratory settings[23]. LAMP technology enables nucleic acid amplification at a constant temperature through the activity of strand displacement DNA polymerase, thereby overcoming the limitations associated with temperature-dependent nucleic acid amplification methods. This advancement allows the use of simple equipment, such as a constant-temperature water bath, to complete the amplification process[4]. LAMP can achieve nucleic acid amplification of 10^9 to 10^{10} fold within 30 to 60 min, demonstrating sensitivity that is 2 to 5 orders of magnitude greater than that of conventional PCR techniques[23]. Furthermore, by incorporating fluorescent dyes or acid-base indicators, the amplification results can be visualized in real time with the naked eye[7-10], facilitating simple, rapid, and efficient detection of pathogenic microorganisms. This capability significantly enhances the feasibility of grassroots and on-site diagnostic applications.

The primary challenge in LAMP detection technology resides in the selection of target gene sequences and the design of specific primers. Target genes must possess sequences that are highly conserved across different strains within a species, while simultaneously exhibiting sufficient divergence between species[23]. The internal transcribed spacer (*ITS*) sequences within ribosomal DNA (rDNA), commonly employed in the classification and identification of fungal species, are frequently utilized as target genes for LAMP assays targeting plant pathogens[9,12-17,19]. Additionally, the translation elongation factor *EF*-1α[11,24] and the rDNA intergenic spacer (*IGS*)[9] exhibit characteristics such as high intraspecific conservation and interspecific variation, making them suitable target genes for LAMP detection. In this study, the *ITS*, *EF*-1α, and *ACTIN* gene sequences of various pathogenic fungi were compared to design and screen a set of LAMP primers targeting the variable region of the *ACTIN* gene specific to *B. cinerea*. Subsequently, a rapid visual LAMP detection method for tomato gray mold disease was developed. This method enables the specific amplification of *B. cinerea* within 40 min, demonstrating an exceptionally high sensitivity of up to 100 ag/μL. In comparison, the sensitivity of most previously reported LAMP assays typically ranges from the pg to fg level[9,11-17,19]. Conventional PCR assays exhibit sensitivities at the ng to pg level[9,15-17,19]. Furthermore, the reaction outcomes can be directly observed with the naked eye through SYBR Green I staining. These findings indicate that this method constitutes a rapid, sensitive, specific, and reliable approach for the detection of gray mold disease.

Considering that *B. cinerea* is an airborne pathogen primarily disseminated through spores, this study employed this approach to analyze the detection limit of *B. cinerea* spores, the detection limit of infection time in tomatoes, and the correlation between detection outcomes and the phenotypic expression of tomato disease. The results demonstrated that the minimum detection limit of this method for *B. cinerea* spores was 20 spores/mL. Spores were detectable in tomato leaves 4 d post-infection, a stage at which the leaves had not yet exhibited any symptoms of infection. These findings indicate that the LAMP detection method is capable of identifying the presence of the pathogen during the early stages of *B. cinerea* infection in tomatoes, even when the pathogen load is minimal and no disease phenotype is apparent. This is critically important for the early monitoring and warning in the prevention and control of tomato gray mold. It holds substantial practical value for ensuring tomato quality and yield, reducing pesticide usage, lowering disease management costs, and increasing farmers' income. Moreover, it has broad application prospects in the green and safe production of vegetables.

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