



# OPEN Rapid on-site differentiation of two invasive whitefly cryptic species using LAMP

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Loop-mediated isothermal amplification (LAMP) is a promising technique for detecting pest species used on the spot due to its simplicity and rapidity. The whitefly is regarded as a species complex encompassing over 44 cryptic species. These species are morphologically indistinguishable but exhibit notable differences in their biological traits. Among them, *Bemisia tabaci* MEAM1 and MED are the most invasive and devastating members. Accurately and quickly distinguishing between these two invasive whiteflies in the field directly affects the implementation of control measures. However, it is often difficult to directly distinguish between the two when they occur together in the field and require extensive specialist knowledge or instrumentation. However, one of the limitations of the current LAMP is that the reaction mixture requires cold chain, which is not ideal for the detection on the spot. To promote its actual application of LAMP on the spot, the LAMP methods to differentiate *B. tabaci* MEAM1 and MED were developed in the laboratory, and *Trialeurodes vaporariorum* was used as a negative control. Then the effect of the preservation condition (room temperature and low temperature) of the reaction mixture on the efficiency of LAMP was compared. The study showed that the application of *B. tabaci* MED- and MEAM1- LAMP reaction mixture can differentiate *B. tabaci* MEAM1, *B. tabaci* MED, and *T. vaporariorum* after low-temperature preservation for more than 24 h. Finally, we validated the method with temperature-controlled hot-water cup in the field and proved its effectiveness when applied to the field. The results demonstrated that the low-temperature preservation of reaction mixture provides available technical support for the application of LAMP on the spot.

**Keywords** Loop-mediated isothermal amplification, Cryptic species, On-site diagnostics, Reaction mixture, Low-temperature preservation

Early detection and identification of crop pests is an important task to control pests, avoid economic losses, and reduce pesticide costs. The early detection and identification of pests are crucial for intercepting and preventing them from becoming established in new areas. Unfortunately, sometimes, the visual identification of pest species is difficult. The sweet potato whitefly, *Bemisia tabaci* (Gennadius), is regarded as a species complex encompassing over 44 cryptic species<sup>1,2</sup>. These species are morphologically indistinguishable but exhibit notable differences in their biological traits. Among them, *B. tabaci* MEAM1 and MED are the most invasive and devastating members<sup>3,4</sup>. These two invasive species differ in many biological traits, including host adaptability, insecticide resistance, and virus transmission ability<sup>5–8</sup>. For example, *B. tabaci* MED has a higher host adaptability and selectivity, insecticide resistance, and heat tolerance than *B. tabaci* MEAM1<sup>9</sup>. The virus acquisition and retention capacity of *B. tabaci* MED was higher than that of *B. tabaci* MEAM1. The transmission frequency was also significantly higher than that of *B. tabaci* MEAM1<sup>11</sup>. On the other hand, *B. tabaci* MEAM1 has lower mortality, higher fecundity, and faster development than *B. tabaci* MED<sup>12</sup>. Since 2008, the *B. tabaci* MED species has become dominant in China<sup>13</sup>. It is imperative to differentiate between these two invasive whitefly species precisely and expeditiously on the spot, as this directly impacts the implementation of control measures.

The development of field identification and monitoring techniques is essential. Nevertheless, the necessity of DNA extraction, the lack of experimental instruments, the requirement of temperature, the need for refrigerated

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storage of reagents, and many other issues restrict the identification of pests in field<sup>14</sup>. Until now, many insects mainly depend on the mitochondrial cytochrome oxidase subunit I (mtCOI) sequencing or other PCR-based molecular markers<sup>15–17</sup>. The efficacy and precision of these methods in detecting insects in laboratory settings is undisputed. However, their use necessitates the deployment of costly apparatus, and the process is both laborious and time-consuming. As the need for field identification of pests gradually increases, these methods are deemed unsuitable for large-scale implementation. Conversely, an optimal approach to detection would encompass a rapid, accurate, and straightforward methodology while also offering cost-effectiveness<sup>18</sup>. Therefore, developing an alternative methodology that does not necessitate specialized instrumentation while maintaining the capacity to discern insect specimens would be advantageous. LAMP (Loop-mediated isothermal amplification) can overcome many of the current problems in field identification.

LAMP was first described by Notomi et al.<sup>19</sup> and is regarded as a promising technique for detecting pest species used on the spot due to its simplicity and rapidity<sup>20</sup>. LAMP offers a relatively rapid amplification reaction at one temperature, and the products are visualized<sup>21–24</sup>. But it has some drawbacks of its own. The LAMP reaction mixture requires cold chain to maintain the enzymatic activity of Bst DNA polymerase, and the prepared reaction mixture will quickly fail at room temperature<sup>25</sup>. The necessity for a cold chain to store the LAMP reagents significantly constrains the applicability of numerous LAMP assays that have been previously described, and kits are particularly appealing if they do not require a cold chain<sup>26</sup>. Some researchers have elected to utilize lyophilized LAMP to obviate the necessity for cold storage. However, a disadvantage of lyophilization is the necessity for sophisticated equipment, such as a rotary or manifold freeze-dryer. Using a freeze-dryer may result in alterations to the material structure, which could potentially impact the test's sensitivity and specificity of the test<sup>14</sup>. Furthermore, freeze-dryers are costly and not widely accessible in many laboratories or in resource-limited settings<sup>14</sup>. Lee et al.<sup>14</sup> indicated that the LAMP reagent system can be refined by using sucrose as stabilizer, but we experimented by adding it to *B. tabaci* MED- and MEAM1-LAMP reaction mixture, and it was no longer possible to distinguish between *B. tabaci* MED and MEAM1 at room temperature for 6 h.

The study aimed to rapidly identify *B. tabaci* MEAM1, *B. tabaci* MED, and *Trialeurodes vaporariorum* in the field, and to demonstrate that the low-temperature preservation of reaction mixture can improve the field application of LAMP. In this study, specificity was verified using tissue, and sensitivity was verified using homogenate with the published *B. tabaci* MEAM1-LAMP primers and the newly designed *B. tabaci* MED-LAMP primers. Next, the effect of two different preservation conditions (room temperature and low temperature) on LAMP results was explored. Finally, combining low-temperature preservation and a temperature-controlled hot-water cup developed a new field-friendly method. The method facilitates the greater feasibility of LAMP for field surveillance in resource-limited settings.

## Materials and methods

### Sample collection and DNA extraction

Adult *B. tabaci* MED and MEAM1 samples were collected from Jinan, Shandong Province. All sampled insects were soaked in 100% alcohol and stored at -20°C for subsequent analysis. Genomic DNA was extracted from the insect tissue samples using the TIANamp Genomic DNA Kit (Tiangen, China) according to the manufacturer's instructions. The extracted DNA was used immediately for LAMP assays or stored at -20°C for further experiments.

### Selection of species-specific primers from the published *Bemisia tabaci* MED- and MEAM1-LAMP primers using tissue

Because the DNA extraction is a limited factor for using LAMP on the spot, we want to develop the method using insect tissues. Therefore, the published primers of LAMP for *B. tabaci* MEAM1 or MED<sup>27,28</sup> were selected using whitefly tissues. All primer information is summarized in **Table S1** and **Table S2**. The primers were synthesized by Beijing Qingke Biotech, China. The results showed that only one set of these primers was specific for *B. tabaci* MEAM1. Therefore, we designed a new LAMP primer as the candidate *B. tabaci* MED-specific primer using tissues.

### Specificity determination of newly designed *Bemisia tabaci* MED-LAMP primer using genomic DNA and tissue

The mtCOI sequences of *B. tabaci* MED and MEAM1 were downloaded from the NCBI database: *B. tabaci* MED (AJ517769.1) and *B. tabaci* MEAM1 (AJ517768.1). The method to design the LAMP primers for *B. tabaci* MED was followed by Yang et al.<sup>29</sup> Briefly, the sequences were aligned using CLUSTAL W, and specific points were marked with ESPrnt3.0 software. Specific primers for *B. tabaci* MED COI were designed using PrimerExplorer V5. Finally, five sets of primers for *B. tabaci* MED were selected and then synthesized by Beijing Qingke Biotech (Beijing, China) for subsequent experimental verification.

The 25-μL polymerase chain reaction (PCR) system included 12.5μL 2×LAMP master mix (Shenggong Biotechnology, Shanghai, China), 0.5μL each F3/B3 primers (10μM), 4μL each FIP/BIP primers (10μM), 0.5μL DNA polymerase (Shenggong Biotechnology, Shanghai, China), 1μL template DNA, and sterilized double distilled water (ddH<sub>2</sub>O; Shenggong Biotechnology) to 25μL. A movable diaphragm was placed at the upper end of the reaction tube. It was not in contact with the reaction system, thus preventing the nucleic acid dye from mixing with the reaction system in advance, which often causes inaccurate results<sup>29</sup>. Before amplification, a 0.5–1μL drop of SYBR Green I (Solarbio) nucleic acid dye was placed in the cap of the reaction tube. After amplification, the reaction tube was briefly centrifuged, or the SYBR Green I dye briefly shaken into the bottom of the tube and mixed with the reaction solution.

The specificity of the LAMP primers for *B. tabaci* MED was verified using the DNA of *B. tabaci* MED, while the DNA of *B. tabaci* MEAM1 was used as a non-targeting control. The sterile distilled water (ddH<sub>2</sub>O) without

nuclease was used as a non-template control (CK). All samples were tested in at least three independent runs. The LAMP products were separated by 1% agarose gel electrophoresis. The specificity of the selected *B. tabaci* MED specific-LAMP primer was further testified by LAMP reaction using tissue.

### Determination of sensitivity of *Bemisia tabaci* MEAM1- and MED-LAMP primer using homogenate and its application

The sensitivity of the primers was tested using a series of concentrations of *B. tabaci* MEAM1 homogenate. The whitefly individual was taken in the tube and ground directly in it, and then 30  $\mu\text{L}$  of  $\text{ddH}_2\text{O}$  was added and dissolved thoroughly to obtain homogenate. The homogenate was used as the template stock solution. Then, 3  $\mu\text{L}$  of stock solution was used to prepare a ten-fold dilution series ( $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ , and  $10^{-5}$ ) by sequentially adding 27  $\mu\text{L}$  of sterile  $\text{ddH}_2\text{O}$ . The different homogenate concentrations were used for LAMP reactions, with sterile  $\text{ddH}_2\text{O}$  as the blank control (CK). After amplification, the reaction tube was briefly centrifuged, or the SYBR Green I dye was briefly shaken into the bottom of the tube and mixed with the reaction solution. Meanwhile, the sensitivity of the selected *B. tabaci* MED specific-LAMP primer was tested using a series of concentrations of *B. tabaci* MED homogenate, which followed the method described the same as MEAM1-LAMP primer.

The visual method based on *B. tabaci* MED- and MEAM1-specific LAMP primers in the present study can be used to determine the purity of the *B. tabaci* population on the spot or the laboratory. To detect the purity of the population, six whitefly individuals, including *B. tabaci* MED and MEAM1, in the ratios of 6:0, 5:1, 4:2, 2:4, 1:5, and 0:6 were ground, respectively.

### Validation of effectiveness of storage *Bemisia tabaci* MED- and MEAM1-LAMP reaction mixture at room temperature and using sucrose as a stabilizer, respectively

The MED- and MEAM1-LAMP reaction mix was placed in a room-temperature environment for 6 h. The *B. tabaci* MED was verified using the tissue of *B. tabaci* MED, while the tissue of *B. tabaci* MEAM1 was used as a non-targeting control. The sterile distilled water ( $\text{ddH}_2\text{O}$ ) without nuclease was used as a non-template control (CK) and vice versa.

To testify the validation of effectiveness of using 8% (w/v) sucrose as stabilizer for *B. tabaci* MED- and MEAM1-LAMP reaction mixture, the sucrose (CAS 57-50-1, molecular biology grade, catalog No. S0389, Sigma-Aldrich) was added to the premixed LAMP reagent of the *B. tabaci* MED and MEAM1, then left at room temperature for 6 h before experiments were performed. Insect tissue of *B. tabaci* MED and MEAM1 were used as a template in LAMP reactions. The sterile distilled water ( $\text{ddH}_2\text{O}$ ) without nuclease was used as a non-template control (CK).

### Validation of effectiveness of low-temperature preservation of *Bemisia tabaci* MED- and MEAM1-LAMP reaction mixture

Because the prepared reaction mixture will quickly fail at room temperature, and using sucrose as a stabilizer for *B. tabaci* MED- and MEAM1-LAMP reaction mixture is failed. Therefore, we use low-temperature preservation of reaction mixture to maintain the enzymatic activity of Bst DNA polymerase (Fig. 3A-C). Due to the similarity of the distribution areas and hosts of the *B. tabaci* and *T. vaporariorum*, and their morphological similarity, so we validated the *T. vaporariorum* together as a non-targeting control.

Low-temperature preservation of MED- and MEAM1-LAMP reaction mixture was used. After freezing the reaction mixture for 6 h, 8 h, 12 h and 24 h, respectively, remove it to validate the effectiveness using *B. tabaci* MED, MEAM1, and *T. vaporariorum*. The sterile distilled water ( $\text{ddH}_2\text{O}$ ) without nuclease was used as a non-template control (CK).

### Verification of effectiveness of frozen *Bemisia tabaci* MED- and MEAM1-LAMP reaction mixture in field

The campaign was carried out on a sunny day to find better conditions for collecting *B. tabaci*. We carried a portable, insulated, temperature-controlled hot-water cup (USB smart cup, model TXJ-U1 with adjustable temperature, Tan Xun Jia Co), which meets the constant temperature required by LAMP; we were also equipped with a specialized instrument case.

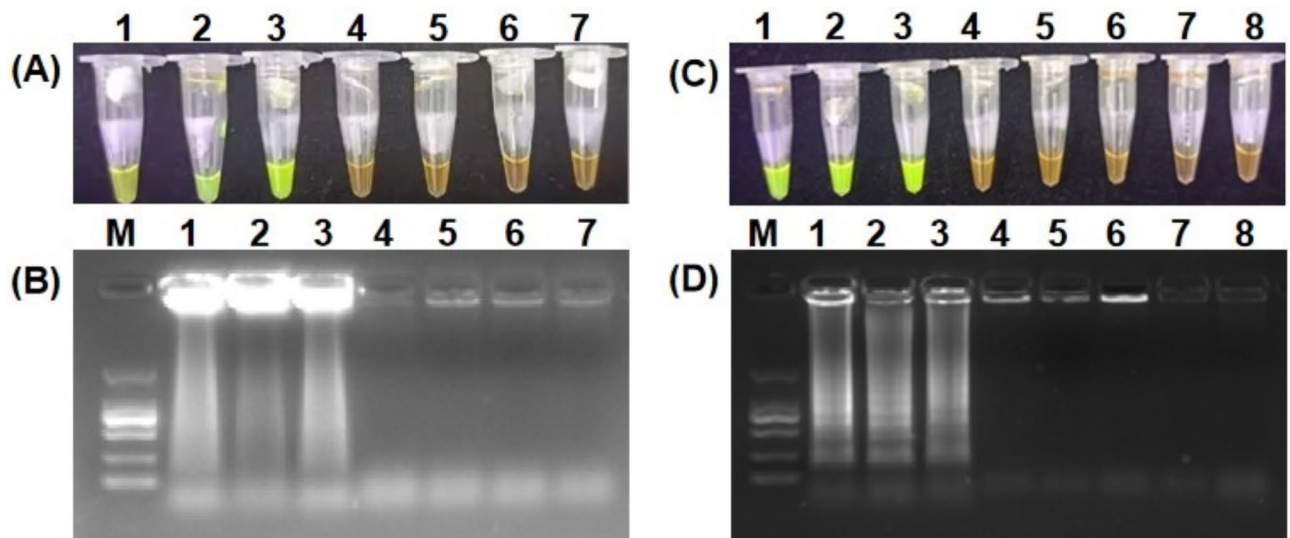
The case contained gloves and respirators, test tube rack, sterile 0.2 mL tubes (for whitefly storage), portable charger, float (for water-bath heating), experimental tin foil, puncher, forceps, sterile forceps, alcohol burner and lighters, sealing film, timer, marker pen, a rubber tubing (for catching whitefly), a pipette set (P10, P200) with respective sterile filter tips, the LAMP reaction mixture in a tube of low-temperature preservation.

Insect tissue was directly used as a template in LAMP reactions. Individual whiteflies were divided in half and added to the two reaction mixtures. The sterile distilled water ( $\text{ddH}_2\text{O}$ ) without nuclease was used as a non-template control (CK). The fourteen reactions were simultaneously run in the temperature-controlled hot-water cup. The water temperature in the cup was controlled through a USB-C interface using the manufacturer's application installed on a cellular phone. Individual reaction tubes were placed in a circular float within the cup. All other aspects of the assay remained the same.

## Results

### Selection of species-specific primers selected from published *Bemisia tabaci* MED- and MEAM1-LAMP primers using tissue

Among the six published primers, only one *B. tabaci* MEAM1-LAMP primer (primer B2 in Table S1) had an amplification efficiency of 100% using *B. tabaci* MEAM1 tissue and no amplification using *B. tabaci* MED tissue (Fig. 1A). Agarose gel electrophoresis revealed that the products of positive LAMP reactions showed a typical



**Fig. 1.** Specificity of published *Bemisia tabaci* MEAM1-LAMP primers (A and B) and *Bemisia tabaci* MED-LAMP primers designed (C and D) in the present study using tissue. (A) primer B2: *B. tabaci* MEAM1 (1–3), *B. tabaci* MED (4–6), and sterile ddH<sub>2</sub>O (7). (B) primer B2: 2000 bp DNA ladder marker (M, TaKaRa), *B. tabaci* MEAM1 (1–3), *B. tabaci* MED (4–6), and sterile ddH<sub>2</sub>O (7). (C) *B. tabaci* MED (1–3), *B. tabaci* MEAM1 (4–6), and sterile ddH<sub>2</sub>O (7–8). (D) 2000 bp DNA ladder marker (M, TaKaRa), *B. tabaci* MED (1–3), *B. tabaci* MEAM1 (4–6), and sterile ddH<sub>2</sub>O (7–8).

ladder-like appearance. No amplification was observed in the negative control with *B. tabaci* MED tissue or sterile ddH<sub>2</sub>O (Fig. 1B). All *B. tabaci* MED-LAMP primers failed to amplify using *B. tabaci* MED tissue (Fig. S1). All samples were tested in at least three independent runs, which produced comparable results. Therefore, we used this specific primer for *B. tabaci* MEAM1 (primer B2 in Table S1) in all subsequent work.

#### Specificity of *Bemisia tabaci* MED-LAMP primer using genomic DNA and tissue

Among the five candidate *B. tabaci* MED-specific primers, only one set had an amplification effect of 100% for *B. tabaci* MED and no amplification efficiency for *B. tabaci* MEAM1. The primer information is summarized below: primer F3-1 (5'-ATTTTACCAGGGTTTGGAATT-3'), B3-1 (5'-CCTGTAGGAACGGCAAT A-3'), FIP-1 (5'-TAGCATAAATTATCCCCAACCTTCCTTCTCATTTAATTAGCAGCGAG-3'), and BIP-1 (5'-TCTTAGGGTTTATTGTTTGAGGACAGCTGAAGTGAATAAGCTCGA-3'). The final processing time used in this experiment is 63°C for 25 min and then 80°C for 10 min. Agarose gel electrophoresis revealed that the products of positive LAMP reactions showed a typical ladder-like appearance. No amplification was observed in the negative control with extracted DNA of *B. tabaci* MEAM1 or sterile ddH<sub>2</sub>O (Fig. S2).

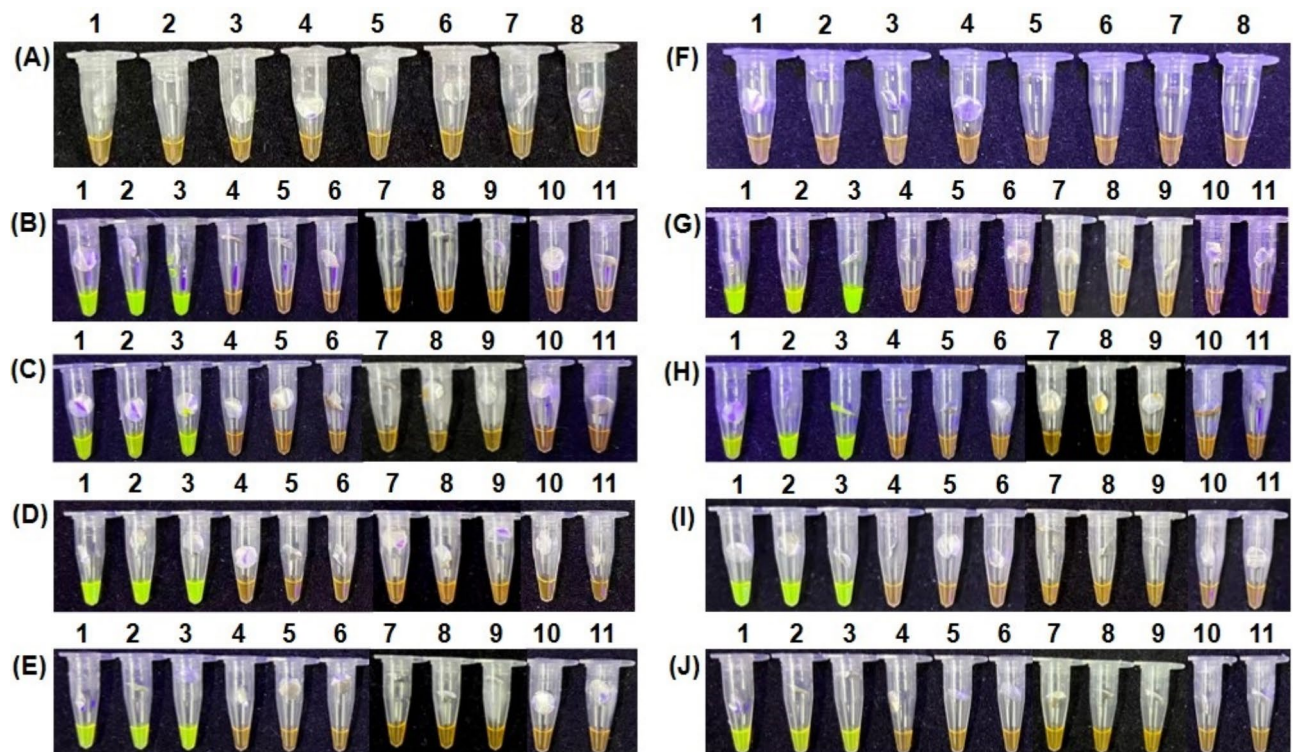
The tissue was used as the template to differentiate *B. tabaci* MEAM1 and MED. The reaction using tissue of *B. tabaci* MED showed visible green fluorescence, while tubes using tissue of *B. tabaci* MEAM1 appeared orange (Fig. 1C). Agarose gel electrophoresis revealed that the products of positive LAMP reactions showed a typical ladder-like appearance. No amplification was observed in the negative control using tissue of *B. tabaci* MEAM1 or sterile ddH<sub>2</sub>O (Fig. 1D).

#### Sensitivity of *Bemisia tabaci* MEAM1- and MED-LAMP primer using homogenate and its application

To assess the sensitivity of *B. tabaci* MEAM1-LAMP primer (primer B2 in Table S1), the LAMP assay was performed using a 10-fold dilution series of *B. tabaci* MEAM1 homogenate. Reaction solutions using homogenate from stock solution showed green fluorescence, whereas the others with higher dilution were orange (Fig. S3). This suggests that the lowest detection limit of the LAMP assay for *B. tabaci* MEAM1 was homogenate. To assess the sensitivity of *B. tabaci* MED-LAMP primer, the LAMP assay was performed using a 10-fold dilution series of *B. tabaci* MED homogenate. Reaction solutions with 10- or 100-fold diluted homogenate exhibited green fluorescence, whereas those with higher dilution were orange (Fig. S4). This suggests that the lowest detection limit of the LAMP assay for *B. tabaci* MED was 100-fold diluted homogenate.

The results of the *B. tabaci* MEAM1-specific LAMP primer with the tissue showed that the mixture of *B. tabaci* MED and MEAM1 in the ratios of 5:1, 4:2, 2:4, 1:5, and 0:6 had visible green fluorescence, while the reaction system without *B. tabaci* MEAM1 tissues were still orange (Fig. S5A). The results of *B. tabaci* MED-specific LAMP primer with the tissue showed the mixture of *B. tabaci* MED and MEAM1 with the ratio of 6:0, 5:1, 4:2, 2:4, and 1:5 have visible green fluorescence, while the reaction system with only *B. tabaci* MED tissues was still orange (Fig. S5B).





**Fig. 2.** Validation of the effectiveness of using sucrose as a stabilizer stored at room temperature (A and F) or using low-temperature preservation for *Bemisia tabaci* MEAM1- and MED-LAMP reaction mixture (B-E; G-J). (A) Using the MEAM1-LAMP primer: *B. tabaci* MEAM1 (1–3), *B. tabaci* MED (4–6), and sterile ddH<sub>2</sub>O (7–8). (B–E) MEAM1-LAMP reaction mixture storage for 6, 8, 12 and 24 h, respectively: *B. tabaci* MEAM1 (1–3), *B. tabaci* MED (4–6), *T. vaporariorum* (7–9) and sterile ddH<sub>2</sub>O (10–11). (F) Using the MEAM1-LAMP primer: *B. tabaci* MED (1–3), *B. tabaci* MEAM1 (4–6), and sterile ddH<sub>2</sub>O (7–8). (G–J) MED-LAMP reaction mixture storage for 6, 8, 12 and 24 h, respectively: *B. tabaci* MED (1–3), *B. tabaci* MEAM1 (4–6), *T. vaporariorum* (7–9) and sterile ddH<sub>2</sub>O (10–11).

### Validation of effectiveness of storage *Bemisia tabaci* MED- and MEAM1-LAMP reaction mixture at room temperature and using sucrose as a stabilizer

The insect tissue was directly used as the template to differentiate *B. tabaci* MEAM1 and MED using MED- and MEAM1-LAMP reaction mixture placed at room temperature for 6 h. In the two experiments, all tubes using tissue of *B. tabaci* MED and *B. tabaci* MEAM1 appeared orange. No amplification was observed in the sterile ddH<sub>2</sub>O (Fig. S6). The reaction mixture lost its effectiveness after 6 h at room temperature.

Using the reaction solution with sucrose at room temperature for 6 h to differentiate *B. tabaci* MEAM1 and MED. At the end of the experiment, all the tubes appeared orange. No amplification was observed in the sterile ddH<sub>2</sub>O. The experimental results showed that the premixed LAMP reagent could no longer differentiate between *B. tabaci* MED and MEAM1 (Fig. 2A and F).

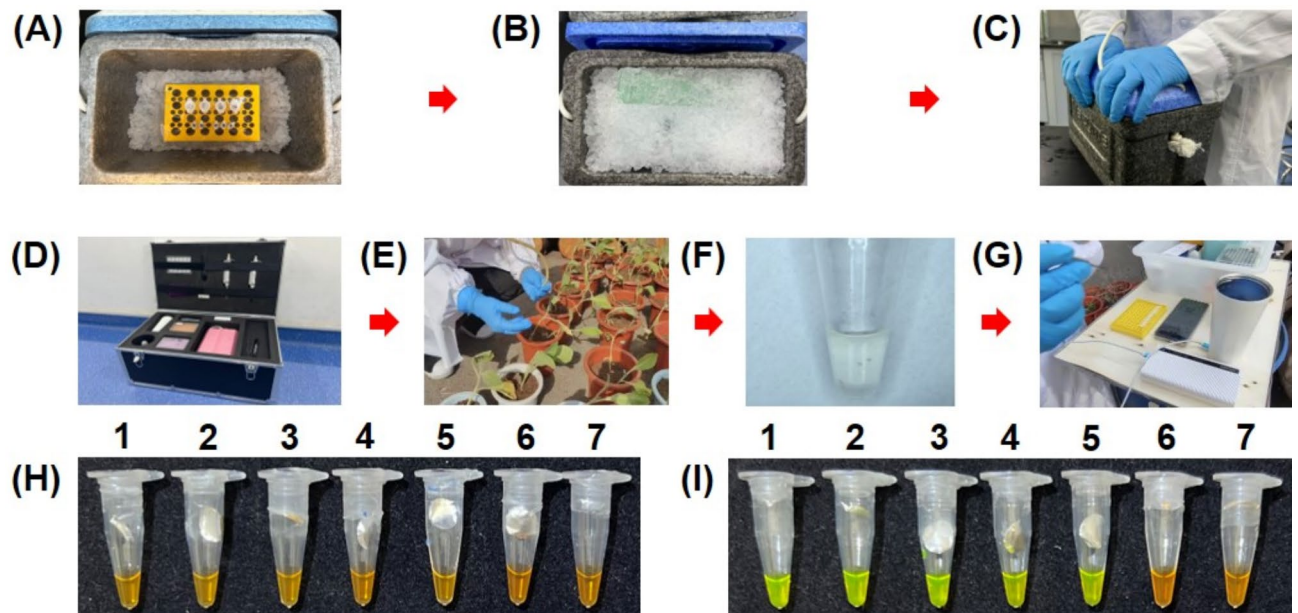
### Validation of effectiveness of low-temperature preservation of *Bemisia tabaci* MED- and MEAM1-LAMP reaction mixture

The insect tissue was directly used as the template to differentiate *B. tabaci* MEAM1, *B. tabaci* MED and *T. vaporariorum* using MEAM1-LAMP reaction mixture were frozen for 6 h, 8 h, 12 h and 24 h. The reaction using tissue of *B. tabaci* MEAM1 showed visible green fluorescence, while tubes using tissue of *B. tabaci* MED and *T. vaporariorum* appeared orange. No amplification was observed in the sterile ddH<sub>2</sub>O (Fig. 2B–E).

The experiments using the MED-LAMP reaction mixture were frozen for 6 h, 8 h, 12 h and 24 h. The reaction using tissue of *B. tabaci* MED showed visible green fluorescence, while tubes using tissue of *B. tabaci* MEAM1 and *T. vaporariorum* appeared orange. No amplification was observed in the sterile ddH<sub>2</sub>O (Fig. 2G–J). Both MED- and MEAM1-LAMP reaction mixtures are still effective after low-temperature preservation for 24 h. Low-temperature preservation of reaction mixture greatly improves the effective duration; this is beneficial for the on-site application of LAMP.

### Verification of effectiveness of the frozen *Bemisia tabaci* MED- and MEAM1-LAMP reaction mixture on the spot

We conducted the experiments using MED- and MEAM1-LAMP reaction mixture that have been frozen for 6 h. Individual whiteflies were divided in half and added to each MED- and MEAM1-LAMP reaction mixtures, then



**Fig. 3.** Method of low-temperature preservation of reaction mixture and using low-temperature preservation of *Bemisia tabaci* MEAM1- and MED-LAMP reaction mixture for 6 h to test in field with tissue. (A–C) Assembly steps for low-temperature preservation facility. (D) Low-temperature preservation of reaction mixture and instrument case. (E) Sample collection. (F) LAMP premixes containing insect tissue. (G) Temperature-controlled hot-water cup. (H) MEAM1-LAMP reaction mixture: Samples collected on site (1–5), and sterile ddH<sub>2</sub>O (6–7). (I) MED-LAMP reaction mixture: Samples collected on site (1–5), and sterile ddH<sub>2</sub>O (6–7).

the whitefly tissue was directly used as the template to experiment. The reaction using whitefly tissue of MED-LAMP reaction mixture showed visible green fluorescence, while tubes using whitefly tissue of MEAM1-LAMP reaction mixture appeared orange. No amplification was observed in the sterile ddH<sub>2</sub>O (Fig. 3H–I). According to the experimental results, all the collected whiteflies were identified as *B. tabaci* MED. From this, it can be seen that both MED- and MEAM1-LAMP reaction mixtures are still effective after low-temperature preservation for 6 h. The results of this study indicate that it is feasible to conduct straightforward molecular tests in settings with constrained resources.

## Discussion

Molecular marker methods play an important role in accurately identifying species<sup>23</sup>, especially in identifying cryptic species, which are morphologically indistinguishable<sup>30</sup>. But these molecular marker methods are difficult to completely detach from the laboratory environment and quickly obtain results on the spot. The use of LAMP for the identification of insects offers numerous advantages, particularly in terms of its high sensitivity and specificity. Additionally, the technique requires only minute quantities of the target in a sample for a positive diagnosis, which is a significant benefit. The sensitivity of this technique is superior to, or at least comparable to, that of other molecular techniques. It allows for detecting the target in quantities ranging from picograms to femtograms, depending on the protocol employed<sup>31</sup>. Furthermore, this approach is an appealing option in contexts where resources are scarce. Its affordability and minimal requirements for specialized equipment or expertise make it a potentially viable alternative in such settings<sup>32</sup>.

Using tissue samples, we evaluated published LAMP primers for *B. tabaci* MEAM1 and successfully designed primers specific to MED<sup>27,28</sup>. These two species-specific LAMP primers also can be used to determine the purity of the whitefly population (Fig. S5). For instance, maintaining a homogeneous whitefly population in a controlled laboratory setting is crucial when studying insecticide resistance or investigating its biological characteristics. The direct use of tissue as a template, without DNA extraction, markedly enhanced the efficiency of the experiment. This process is rapid, and does not require a DNA extraction kit. The findings substantiated that insect tissue can be employed in the LAMP methodology for the purpose of species identification<sup>29,33</sup>. Accordingly, using insect tissue as a direct template represents a viable alternative to conventional DNA extraction methodologies, obviating the necessity for costly reagents and specialized laboratory facilities. The simplicity of the sample preparation process may facilitate future applications of LAMP assays for real-time and on-site identification of insects, thereby reducing the costs associated with the analysis<sup>31</sup>.

In the present study, we used room temperature and low-temperature preservation of the reaction mixture for LAMP. The LAMP reaction, which utilized premixed reagents that had been stored at room temperature for six hours, did not yield amplification products, presumably because the activity of the Bst DNA polymerase had been lost in the premixed reagents. Our study demonstrated that by placing the reaction mixture at room

temperature for 6 h before testing, the results showed that it did not have specificity. However, applying *B. tabaci* MED- and MEAM1- LAMP reaction mixture can differentiate *B. tabaci* MEAM1 and MED without DNA extraction after low-temperature preservation for more than 24 h. This result indicates that the LAMP assay, which combined low-temperature preservation of reaction mixture and a temperature-controlled hot-water cup, fully applies to insect detection (Fig. 3D–G). We accomplish these experiments aimed to achieve molecular results directly on the spot, and the volume and weight of the equipment are portable. And the prices of these required equipment are not expensive. To our knowledge, this is the first published demonstration of detecting *B. tabaci* MEAM1 and MED using the LAMP assay in a field setting. Combined with similar observations from previous reports<sup>29</sup>, these results showed that this assay has great simplicity and can effectively address various limitations associated with DNA extraction, including the necessity for a laboratory setting. Furthermore, this illustrates the suitability of LAMP as a highly sensitive assay for identifying species in field conditions. The collection of field samples and their immediate grinding insect tissue can be conducted, after which the samples can be tested for the identification of pests using the user-friendly and efficient LAMP method. This process can be completed in 60 min. The specific and quick diagnosis of LAMP is required for prediction and management control strategy selection in the field. On aggregate, the experiments evidenced the actual suitability and reliability of the LAMP approach for in-field molecular investigations<sup>20</sup>.

## Conclusion

In conclusion, the LAMP protocol described in this study offers a new, cost-effective, highly sensitive, specific, and rapid approach to identifying insects. Compared to PCR and other assays, LAMP does not require strict reaction conditions, complicated technical operation, or special equipment. Instead, this method requires only low-temperature preservation of the reaction mixture and a water bath. The test results show that the low-temperature preservation increases the effective duration of the reaction mixture. This protocol provides an important diagnostic tool for spot setting. These all prove that in-field LAMP is no longer a conceptual or potential tool that might provide useful and immediate information in a wide range of studies.

## Data availability

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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## Author contributions

D. C. and Y. J. Z. designed the project. Y. G. L., L. F. Y., Y. L. L. and Y. C. performed the experiments and analyzed the data. Y. G. L. wrote the manuscript. All authors read and approved the final manuscript.

## Declarations

## Competing interests

The authors declare no competing interests.

## Informed consent

We hereby attest that we have informed consent from all persons figuring in images included in this manuscript to be shown in online open-access publications.

## Additional information

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