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A Colorimetric RT-LAMP Assay for Rapid Detection of *Soybean mosaic* Virus SC15

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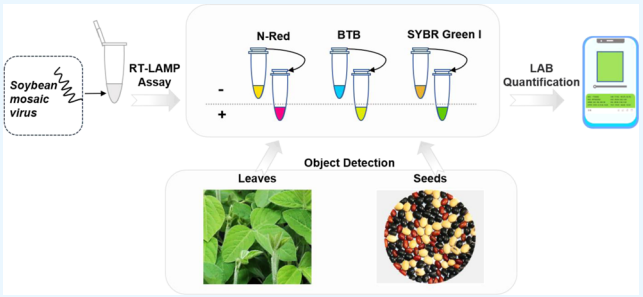
ABSTRACT: *Soybean mosaic virus* (SMV) represents one of the most devastating viral diseases affecting soybeans worldwide. Among its strains, SMV-SC15 is notable for its virulence, predominance, and widespread occurrence. Rapid and on-site diagnosis is important for controlling the spread of SMV-SC15. In this study, we proposed a colorimetric reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay for the detection of SMV-SC15 using three color indicators for visual interpretation: Neutral Red (N-Red), Bromothymol Blue (BTB), and SYBR Green I. The SMV-SC15 in the soybean tissue was detected with remarkable sensitivity and specificity within 30 min, achieving a detection limit as low as 10^{-4} ng/μL. 200 soybean leaf samples from the field were analyzed by the colorimetric RT-LAMP assays, holding significant potential for rapid screening of SMV-SC15-resistant cultivars, thereby contributing to effective SMV control.



INTRODUCTION

Soybean mosaic virus (SMV) infection is considered as one of the most severe viral diseases affecting soybeans worldwide, especially in China. This disease has caused massive production losses and seed quality deterioration.[1−3] SMV isolates have been classified into diverse strains based on virus symptoms in different hosts.[4] In China, twenty-two strains (SC1-SC22) have been identified according to the SMV symptoms in ten different soybean cultivars.[5−7] Among these strains, SMV-SC15 can infect all differential hosts and is prevalent in most soybean-producing regions.[8−10] Therefore, SMV-SC15 represents the most virulent, predominant, and widespread strain worldwide. However, management options for SMV-SC15 are limited, especially in remote settings. The most effective and environmentally friendly strategies for SMV disease management include the development of SMV-SC15-resistant soybean varieties and early detection of the virus in soybean materials such as seeds and vegetative parts.

Biological assays are widely used for the detection and diagnosis of SMV.[11,12] However, they are time-consuming, labor-intensive, and insensitive. In addition, it was reported that many soybean plants and seeds infected with SMV at an early stage are asymptomatic, making it difficult to detect.[11−13] In general, soybean plants or seeds are coinfected by SMV-SC15 and other strains in the field, making phenotypic observation alone insufficient for accurate diagnosis. In contrast, the assays based on nucleic acids have higher specificity, sensitivity, and efficiency.[14,15] With the development of sequencing techniques, the genome of SMV strains has been revealed,[16] and single-nucleotide polymorphisms (SNPs) in the CP genes of SMV have been found among different SMV strains, which makes it possible to specifically identify SMV-SC15 by SNP detection at the genomic level. Based on the genomic sequences of viruses, the reverse transcription-polymerase chain reaction (RT-PCR) and real-time fluorescence quantitative RT-PCR (RT-qPCR) have been used to detect plant viruses.[17−19] RT-qPCR is considered the gold standard for the diagnosis of multiple pathogens in laboratories and clinics.[20−22] However, these assays rely on specialized instrumentation, which is not conducive to on-site detection.

Loop-mediated isothermal amplification (LAMP) is a molecular diagnostic technique working under isothermal conditions, holding promise as an alternative to RT-qPCR. Compared with RT-PCR, LAMP is highly compatible with point-of-care analysis.[23,24] This feature significantly simplifies the procedure and reduces the requirements for equipment, endowing it with the potential to improve the diagnosis in plant protection. In addition, it has the advantages of rapidity,

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simplicity, high specificity, low detection limit, and so on. Moreover, LAMP results can be detected by various methods, among which colorimetric LAMP can provide direct visual result interpretation.[25−28]

Herin, we developed a colorimetric RT-LAMP assay for the CP gene of SMV-SC15 by specifically amplifying and directly reading the results with N-Red, BTB, and SYBR Green I, respectively. Then, the leaves collected from 28 cultivars on different days after inoculation were analyzed with the proposed method. We evaluated the RT-LAMP assay in the seeds of 15 soybean varieties, including Zhonghuang 70, to further investigate the seed transmission rate of SMV-SC15. In addition, the leaf samples of 200 soybean varieties in the field were analyzed with the established method, and the reliability of the RT-LAMP assay was tested and compared with RT-qPCR in detecting SMV-SC15.

MATERIALS AND METHODS

Materials. The virulent strains SMV-SC3, SMV-SC7, SMV-SC15, and SMV-SC18 were provided by the National Centre for Soybean Improvement (NCSI).

For early stage screening of SMV-SC15-resistant varieties, 28 soybean accessions, including Fendou 78, Fendou 92, Handou 14, Jindou 29, Kefeng NO.1, Lu 99−2, SZ-20, SZ-54, SZ-188, SZ-218, SZ-221, SZ-244, SZ-288, SZ-292, SZ-319, SZ-341, SZ-565, SZ-609, SZ-613, SZ-615, SZ-834, SZ-840, SZ-843, SZ-869, SZ-1058, Yuandou NO.1, Zhonghuang 13, and Zhonghuang 70, were cultivated in pots in a greenhouse. Each of the 28 soybean cultivars was planted with 30−40 healthy seeds, and 20 plants with similar growth were screened for SMV-SC15 inoculation. The SMV-SC15 strain was mechanically inoculated onto plants after the pair of primary leaves had fully unfolded.[29,30] The first three leaves from each plant of the twenty-eight cultivars were then collected for phenotypic observations at 2, 3, 4, 6, 8, 10, 12, 15, 18, 21, and 24 days postinoculation and then stored at −80 °C.

To investigate the seed transmission of SMV-SC15, 15 soybean cultivars, including Fendou 78, Fendou 92, Handou 14, Jinda 70, SZ-23, SZ-108, SZ-319, SZ-419, SZ-869, SZ-1232, SZ-1249, SZ-1250, SZ-1286, SZ-1293, and Zhonghuang 70, were planted at the Experimental Station of the College of Agronomy of Shanxi Agricultural University in 2022, and the seeds were collected after harvest for analysis. Specifically, 100 grains of Zhonghuang 70 were planted in pots, and the first three-leaf samples were collected 30 days after planting for further examination of seed transmission.

To screen SMV-SC15-resistant varieties in the field, 200 soybean cultivars (Table S3) were planted at the experimental site (planting soybeans for 6 years in a row) in 2023 and leaf samples of 5−6 leaves were collected at the seedling stage only and then stored at −80 °C.

RNA Extraction and cDNA Synthesis. Total RNA was extracted from soybean leaves and seeds using the EZ-10 away RNA Mini-Preps Kit (BBI, China). Viral cDNA synthesis was performed utilizing the GoScript Reverse Transcription System (Promega).

RT-LAMP Primers Design. The CP genome sequences of SMV-SC3, SMV-SC7, and SMV-SC15 were obtained from the NCBI Genbank database, with accession numbers MH919384.1, MH919385.1, and MH919386.1, respectively. These sequences were aligned using MEGA7 software. Based on SNPs identified within the CP genomic sequences of these SMV strains, a set of genome-specific primers was designed for the amplification of the CP segment of SMV-SC15 using PRIMER EXPLORER V5 (http://primerexplorer.jp/e/.). This set comprised six primers: two outer primers SC15-F3/SC15-B3 (SC15-F3: 5′-AGGGAGTTAGCCCGTTAT-3′, SC15-B3: 5′-CCCAA AAGAGAATGCATGTT-3′), two inner primers SC15-FIP (F1C−F2)/SC15-BIP (B1C−B2) (SC15-FIP: 5′-AGAGCTGCAGCCTTCATTTGTGGTCACCTCCAAAA-CACC G-3′, SC15-BIP: 5′-CGGGAGTTAACAA-CAAGCTGTTTGCATCTCTTGCAGTGT GCC-3′), and two loop primers SC15-LF/SC15-LB (SC15-LF: 5′-TATTGCCTCTC TTGCCCTG-3′, SC15-LB: 5′-CATCTC-GACCAACTCCGAA-3′). These primers were synthesized by Sangon Biotech (Shanghai).

Construction of RT-LAMP System for SMV-SC15. Optimization experiments for an RT-LAMP system targeting SMV-SC15 were performed using the CFX Duet Real-Time PCR System (USA) with a real-time fluorescence quantification assay. This study evaluated the effects of four parameters: temperature (at six levels: 69.0, 67.6, 65.0, 60.4, 54.9, and 50.5 °C), Bst DNA polymerase concentration (0, 1.6, 3.2, 4.8, 6.4, and 8 U), inner/outer primer ratio (3:1, 4:1, 5:1, 6:1, and 7:1), and Mg^{2+} concentration (2, 3, 4, 5, and 6 mM) on RT-LAMP reaction efficiency. Outer primer concentrations remained fixed throughout, and the ratio of inner to outer primers was predefined. Optimal reaction conditions were determined by analyzing amplification curves, with each assay replicated 3 times for reliability.

The optimized RT-LAMP assay was performed in a 20.0 μL system containing 4 mM $MgSO_4$ (New England Biolabs), 0.8 M betaine (Solarbio, China), 2.5 μL 10× isothermal amplification buffer (New England Biolabs) (1× buffer contains 2 mM $MgSO_4$), 0.75 μM dNTPs (Solarbio, China), 1.2 μM of each inner primer (SC15-FIP/SC15-BIP), 0.2 μM of each outer primer (SC15-F3/SC15-B3), 0.3 μM of each loop primer (SC15-LF/SC15-LB), 4.8 U Bst 2.0 WarmStart DNA Polymerase (New England Biolabs), and 1 μL of 100 ng/μL template cDNA, with the remainder supplemented with ddH_2O. The reaction was performed at 65 °C for 30 min, followed by analysis of the amplification products by 2% agarose gel electrophoresis. In addition, the RT-LAMP process could be monitored on a real-time fluorescence quantitative instrument by incorporating 0.6× SYBR Green I, allowing direct assessment of the amplification curve.

Colorimetric RT-LAMP Assay. In the colorimetric RT-LAMP assay, 100 μM N-Red or 60 μM BTB was added to the tubes before amplification, while 1 μL of 1000× SYBR Green I was added to the tubes after amplification. The results were read by observing the color change as reported by Guan et al.,[31] Nopwinyuwong et al.,[32] and Tanner et al.,[33] as follows: pink, yellow-green, and fluorescent green were observed in the successful RT-LAMP reaction, while yellow, cyan, and orange were observed in the negative response.

RT-PCR Assay. RT-PCR amplification was performed using the *TransStart* FastPfu DNA Polymerase kit (TransGen Biotech, China). The final RT-PCR reaction contained 15 μL of 3.0 μL 5× FastPfu buffer, 0.4 μL dNTPs (2.5 μM), 0.3 μL of each outer primer (SC15-F3/SC15-B at 10 μM), 0.3 μL FastPfu DNA polymerase (2.5 U/μL), and 2.0 μL templates (50 ng/μL). The remainder was supplemented with ddH_2O. The thermal cycling conditions were as follows: an initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 15

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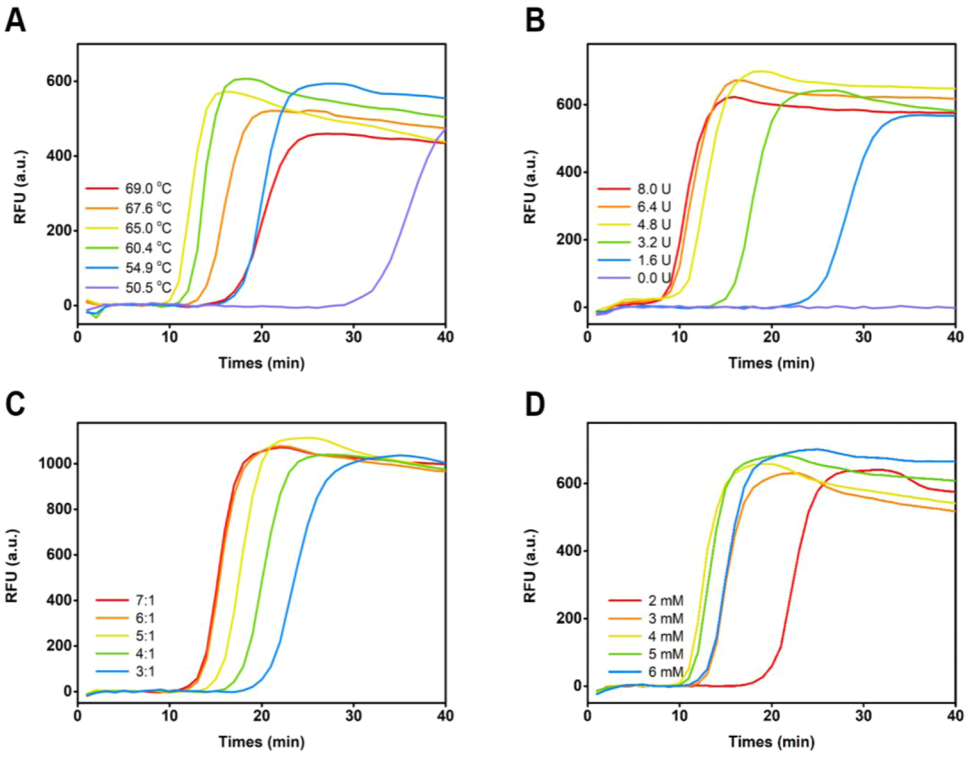

Figure 1. Optimization of the RT-LAMP assay under different conditions, including (A) temperature, (B) Bst DNA polymerase amount, (C) the ratio of inner to outer primers, and (D) Mg^{2+} concentration.

s. The RT-PCR results were visualized in a 1% agarose gel electrophoresis.

RT-qPCR Assay. The RT-qPCR amplification was performed using the *TransStart* Tip Green qPCR SuperMix kit (TransGen Biotech, China). The RT-qPCR final reaction contained a 20 μL system: 10.0 μL 2× Tip Green qPCR SuperMix, 0.4 μL each of out primers (SC15-F3/SC15-B3) (10 μM), and 2.0 μL 50 ng/μL templates. The remainder was supplemented by ddH_2O. The qPCR cycling conditions were initiated at 94 °C for 30 s, followed by 45 cycles of 94 °C for 5 s and 60 °C for 30 s. RT-qPCR results were assessed through the fluorescence amplification curve.

Specificity and Sensitivity of RT-LAMP Assay for SMV-SC15. To assess the specificity of the RT-LAMP assay for SMV-SC15, the cDNA of SMV-SC3, SMV-SC7, SMV-SC15, and SMV-SC18 were selected as the target templates for the RT-LAMP amplification reaction.

The SMV-SC15 cDNA concentrations were prepared in a gradient from 10^1 to 10^{-6} ng/μL for analysis. Concentrations ranging from 10^1 to 10^{-5} ng/μL were utilized to evaluate the sensitivity of the colorimetric RT-LAMP assay. Similarly, the same concentration gradient was applied in RT-PCR assays. For the sensitivity analysis of the RT-qPCR assay, concentrations of 10^0 to 10^{-6} ng/μL were used as input samples.

The outcomes of the RT-LAMP assay were assessed using 2% agarose gel electrophoresis, real-time fluorescence, and colorimetric RT-LAMP analysis. Additionally, colorimetric RT-LAMP results were conducted through spectrophotometric analysis to acquire absorption spectrum curves.

Application of the Colorimetric RT-LAMP Assay for SMV-SC15. The colorimetric RT-LAMP assay was developed to detect SMV-SC15 using leaves of Nannong 1138−2 infected with SMV-SC3, SMV-SC7, SMV-SC15, and SMV-SC18. The collected leaves were subjected to RNA extraction and cDNA synthesis according to the instructions of the kits to obtain samples for the RT-LAMP assay. The reliability of the colorimetric RT-LAMP assay to detect SMV-SC15 was tested on 28 soybean cultivars inoculated with SMV-SC15. In addition, seeds from 15 soybean cultivars were tested to determine if they were infected with the SMV-SC15 strain, of which Zhonghuang 70 was selected to further investigate seed transmission of SMV-SC15 and its 100 plants were detected. For screening of SMV-SC15-resistant varieties, leaves from 200 soybean cultivars were collected in the field and analyzed using the colorimetric RT-LAMP assay.

In the colorimetric RT-LAMP assay, N-Red, BTB, and SYBR Green I were used for analysis. Reaction solutions were photographed under identical conditions. These photographs were processed using color recognition software to convert the colors to the LAB color model. The LAB comprises two color channels, denoted by a^* and b^*. The a^* value signifies the color shift from green to red and the b^* value indicates the color shift from blue to yellow. The chromogenic bias of distinct indicators determines whether the channels are a^* or b^*. RT-qPCR assays were performed simultaneously for comparative analysis.

RESULTS

RT-LAMP Primers Designed for the Detection of SMV-SC15. SMV, which belongs to the genus Potyvirus in the family Potyviridae, has a single-stranded and positive RNA genome of about 9.6 kb in length.[34] It consists mainly of 11 proteins, including P1, HC-Pro, CP, etc.[35,36] Among them, the CP genes are particularly conserved among different SMV strains, and the C-terminus of SMV-CP is even more conserved.[37,38] Therefore, we designed six RT-LAMP primers targeting the C-terminal region of SMV-CP sequences to

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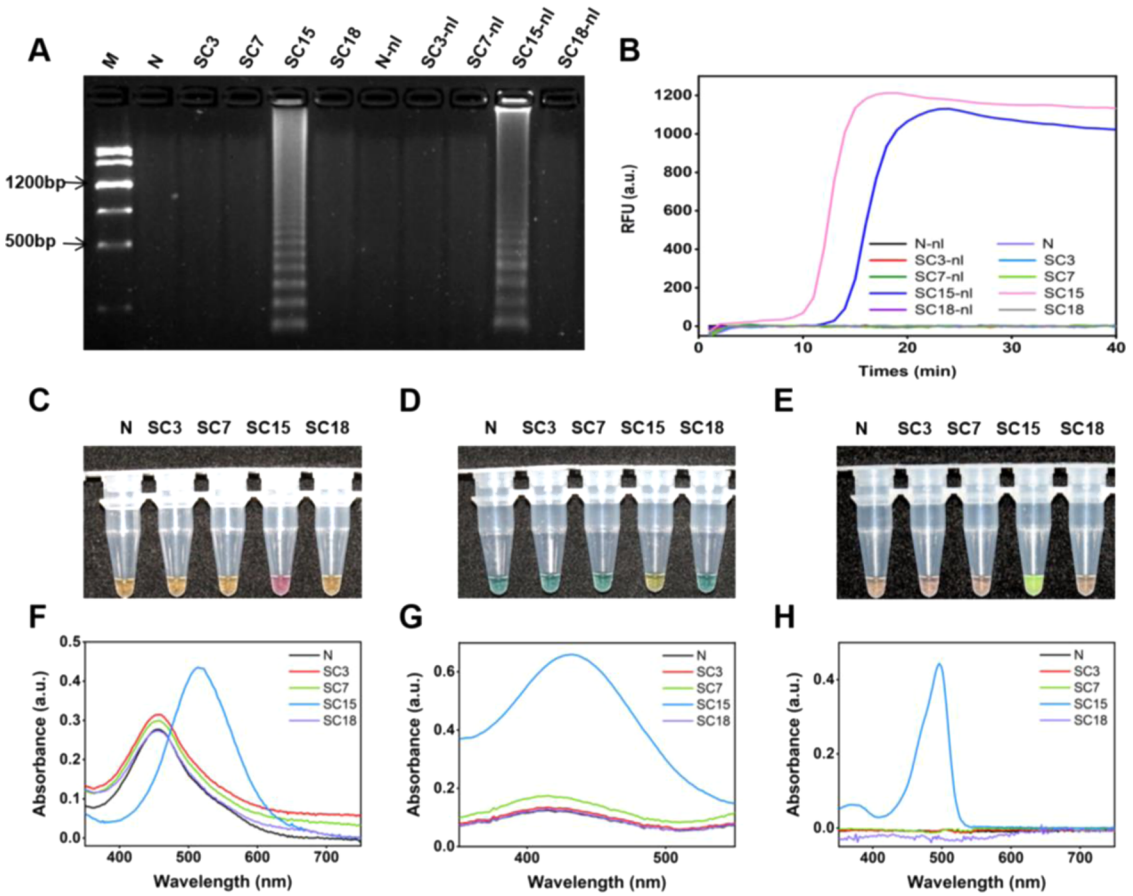

Figure 2. Evaluation of the RT-LAMP assay specificity for SMV-SC15. The cDNA of SMV-SC3, SMV-SC7, SMV-SC15, and SMV-SC18 was used to test the specificity of the RT-LAMP assay. (A) RT-LAMP results obtained from a 2% agarose gel. (B) Specificity evaluation of real-time RT-LAMP. Specificity assessment of colorimetric RT-LAMP stained by (C) N-Red, (D) BTB, and (E) SYBR Green I, respectively. The UV−vis absorption spectra of the colorimetric RT-LAMP stained by (F) N-Red, (G) BTB, and (H) SYBR Green I, respectively. nl: non loop primers; M: DNA Marker III; N: negative control.

specifically amplify SMV-SC15, incorporating SNP sites at the 3′ end of the forward inner primer (SC15-FIP) and loop primers (SC15-LF/SC15-LB) to further refine specificity (Figure S1).

Optimization of RT-LAMP Reaction for Detection of SMV-SC15. Initial temperature optimization experiments using temperature gradients showed that the optimal reaction temperature range of the RT-LAMP reaction, as indicated by the fluorescence amplification curves, was 60.4 to 65 °C and reached its plateau fastest at 65 °C, establishing this as the optimal reaction temperature (Figure 1A). Subsequent tests to determine the optimal amount of Bst DNA polymerase in a 20 μL RT-LAMP reaction system showed an increase in amplification efficiency with enzyme amounts ranging from 1.6 to 8.0 U. Notably, amplification efficiency peaked with no significant difference at enzyme amounts of 4.8, 6.4, and 8 U (Figure 1B), leading to the selection of 4.8 U as the optimal concentration in the 20 μL RT-LAMP system, balancing cost and reaction efficiency. Further refinements were made by adjusting the ratio of inner/outer primers used in the reaction to increase amplification efficiency. It was found that RT-LAMP amplification efficiency was improved progressively as the ratio of inner to outer primers increased from 3:1 to 6:1. In particular, the amplification efficiency peaked at a ratio of 6:1, and a further increase to a ratio of 7:1 did not result in a significant improvement in efficiency. Therefore, we determined that the concentration ratio of inner to outer primers in the RT-LAMP system was 6:1 (Figure 1C). In addition, Mg^{2+} plays a critical role in influencing nucleic acid amplification in RT-LAMP reactions. Analysis of the fluorescence amplification curves showed that the RT-LAMP reaction reached its plateau phase most rapidly when the Mg^{2+} concentration was set at 4 mM (Figure 1D). Based on these findings, a concentration of 4 mM Mg^{2+} was identified as the optimal condition for the RT-LAMP reaction.

The finalized RT-LAMP reaction parameters were thus established: 4.8 U of Bst DNA polymerase, 6:1 ratio of inner (SC15-FIP/SC15-BIP, 1.2 μM) to outer primers (SC15-F3/SC15-B3, 0.2 μM), and 4 mM Mg^{2+} within a 20 μL reaction volume.

Specificity Evaluation of the RT-LAMP Assay for SMV-SC15. As the SMV-SC15 strain generally coinfects soybean plants or seeds with other strains in the field, the specificity of the RT-LAMP amplification reaction is recognized as a critical requirement for the successful detection of SMV-SC15. In this context, cDNA from the SMV-SC3, SMV-SC7, SMV-SC15, and SMV-SC18 strains was used to evaluate the specificity of the assay. Distinct ladder-like electrophoretic patterns and S-shaped curves were observed exclusively in the samples from the SMV-SC15 strain, which differed from the negative control, and the samples from SMV-SC3, SMV-SC7, and SMV-SC18 (Figure 2A,B). These results indicated that the

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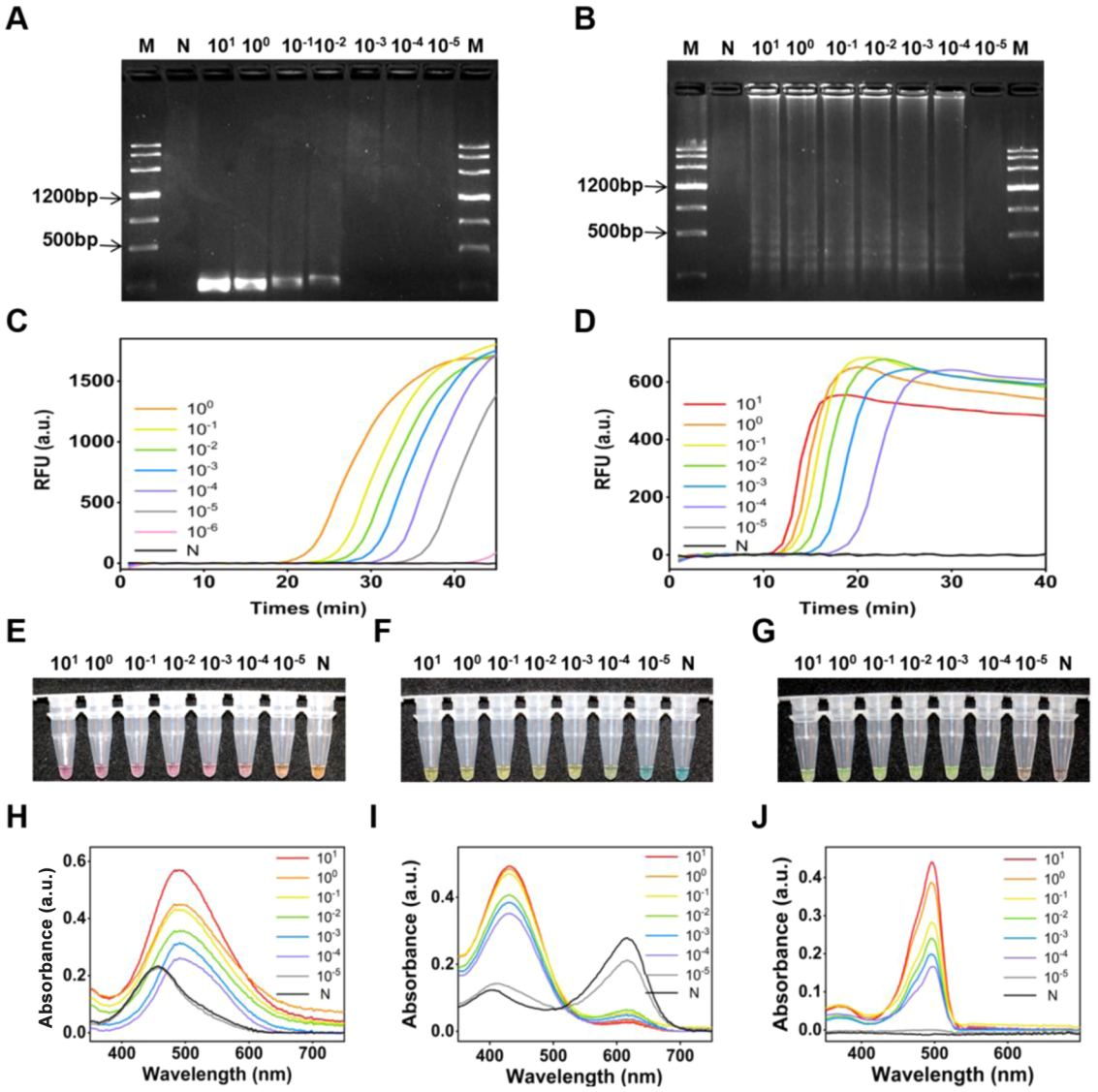

Figure 3. Evaluation of the sensitivity of the RT-LAMP assay. The SMV-SC15 cDNA was diluted in serial 10-fold gradients to obtain a series of testing templates. (A) Agarose gel electrophoresis results of RT-PCR sensitivity. (B) Agarose gel electrophoresis results of the RT-LAMP sensitivity for SMV-SC15 detection. (C) Real-time RT-PCR sensitivity assessment. (D) Real-time RT-LAMP sensitivity assessment. Visual results of the colorimetric RT-LAMP assays stained by (E) N-Red, (F) BTB, and (G) SYBR Green I, respectively. UV−vis absorption spectra of the colorimetric RT-LAMP assays stained by (H) N-Red, (I) BTB, and (J) SYBR Green I, respectively. Lanes and tubes 1−7: Concentrations of SMV-SC15 cDNA templates of 10^{1}, 10^{0}, 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5} ng/μL. M: DNA Marker III; N: negative control.

inner primers (SC15-FIP/SC15-BIP) contributed significantly to the specificity of the RT-LAMP assay for the SMV-SC15 strain. Furthermore, the inclusion of loop primers (SC15-LF/SC15-LB) was found to facilitate the optimization of the RT-LAMP reaction time to 20 min (Figure 2B). This result was further verified by agarose gel electrophoresis analysis (Figure S2).

The ability of the colorimetric RT-LAMP system to specifically detect the SMV-SC15 strain was then evaluated. It was found that among the tubes containing RT-LAMP amplification products, a clear color change was observed only in the samples containing SMV-SC15 strains, in contrast to the tubes without SMV-SC15 cDNA, which remained unchangeable (Figure 2C−E). To verify the accuracy of the visual RT-LAMP results, further analysis was performed using an ultraviolet−visible (UV−vis) spectrophotometer. The analysis revealed that the samples containing SMV-SC15 showed distinct peaks at approximately 510, 430, and 500 nm, respectively, whereas the control samples showed either weak absorption or no significant peaks in these regions (Figure 2F−H). The agreement between the results of the colorimetric RT-LAMP assay and both the electrophoresis patterns and the fluorescence amplification curves confirms the high specificity of the developed assay for the detection of the SMV-SC15 strain.

Sensitivity Evaluation of the RT-LAMP Assay for SMV-SC15. Serial 10-fold dilutions of SMV-SC15 cDNA were used to assess the sensitivity of the RT-LAMP assay. In the RT-PCR reaction, a minimum template concentration of 10^{-2} ng/μL could be detected by electrophoresis (Figure 3A). Sensitivity analysis was further performed using the RT-qPCR system with specificity (Figure S3). The amplification curve of the RT-qPCR assay showed that the SMV-SC15 concentrations could be detected as low as 10^{-5} ng/μL (Figure 3C). It was found

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Table 1. Corresponding Periods of SMV-SC15 Infection Detected by Phenotype Identification and Colorimetric RT-LAMP Assay[a]

Variety	Phenotype	RT-LAMP	Variety	Phenotype	RT-LAMP
Fendou 78	15d	4d	SZ-319	18d	8d
Fendou 92	21d	8d	SZ-341	10d	4d
Handou 14	18d	8d	SZ-565	12d	4d
Jindou 29	12d	4d	SZ-609	15d	6d
Kefeng NO. 1		12d	SZ-613	15d	4d
Lu 99−2			SZ-615	15d	6d
SZ-20	10d	4d	SZ-834	21d	6d
SZ-54	21d	10d	SZ-840	12d	4d
SZ-188	10d	4d	SZ-843	10d	4d
SZ-218	21d	8d	SZ-869	18d	6d
SZ-221	18d	10d	SZ-1058	12d	6d
SZ-244	12d	6d	Yuandou NO. 1	12d	6d
SZ-288	21d	10d	Zhonghuang 13	12d	6d
SZ-292	18d	6d	Zhonghuang 70	21d	8d

[a]No phenotype or RT-LAMP test was negative for SMV-SC15 infection.

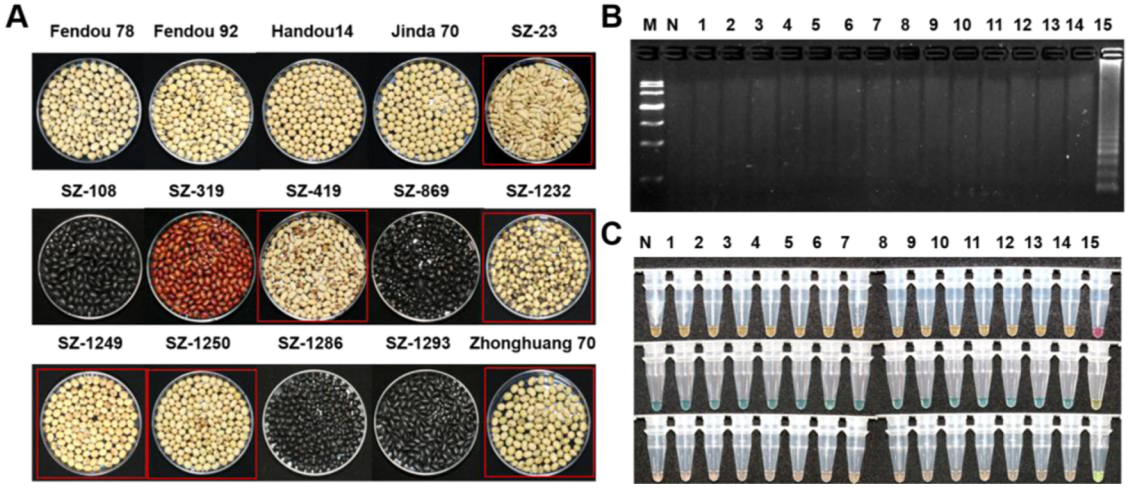

Figure 4. RT-LAMP detection of SMV-SC15 infection in soybean seeds. (A) Phenotype observation of different varieties of soybean seeds. The mottled seeds were labeled with a red frame. (B) Gel electrophoresis analysis of the RT-LAMP products. (C) Chromogenic analysis of the colorimetric RT-LAMP using N-Red, BTB, and SYBR Green I. Tubes 1−15: Fendou 78, Fendou 92, Handou 14, Jinda 70, SZ-23, SZ-108, SZ-319, SZ-419, SZ-869, SZ-1232, SZ-1249, SZ-1250, SZ-1286, SZ-1293, and Zhonghuang 70. M: DNA Marker III. N: negative control.

that the RT-LAMP method was able to efficiently amplify SMV-SC15 cDNA, requiring only 20 min for detection at levels as low as 10^{-4} ng/μL (Figure 3B,D). The sensitivity analysis was also performed on the colorimetric RT-LAMP based on N-Red, BTB, and SYBR Green I. The LAMP assay solutions exhibited color changes, transitioning to pink, yellow-green, and fluorescent green, respectively, upon treatment with N-red, BTB, and SYBR Green I over a concentration range of 10^{-4} to 10^{1} ng/μL of SMV-SC15 cDNA solutions (Figure 3E−G). Notably, in agreement with the positive color results of the colorimetric RT-LAMP system, the absorption spectral peaks at 510, 430, and 500 nm were observed at template concentrations as low as 10^{-4} ng/μL (Figure 3H−J). It was verified that the incorporation of an optimal amount of dyes into the RT-LAMP system did not significantly affect its detection capability. The results obtained from the electrophoresis, fluorescence, visualization, and UV−vis absorption spectra analyses of the RT-LAMP assay all showed that the lowest template concentration can be down to 10^{-4} ng/μL, and the sensitivity of the colorimetric RT-LAMP assay is sufficient to meet the requirements for practical detection.

RT-LAMP Testing of SMV-SC15 in Soybean Leaves after Inoculation. The biological assay is currently the most common approach used by many breeders to screen for SMV-SC15-resistant varieties.[39] 28 soybean varieties were selected for inoculation to observe their phenotypes. These varieties showed different levels of resistance to SMV-SC15 (Figure S4 and Table 1). Notably, Lu 99−2 and Kefeng NO.1 showed no signs of SMV disease phenotype after 24 days of inoculation. In contrast, other soybean varieties began to show pathological phenotypes 10 days or more after inoculation with SMV-SC15. For example, SZ-188 showed a mosaic phenotype 10 days after inoculation, while Zhonghuang 70 developed leaf curling 21 days after inoculation.

To further investigate the effect of SMV-SC15 infection on different soybean cultivars, the colorimetric RT-LAMP assay was used to test the leaves of 28 soybean cultivars inoculated with SMV-SC15 (Figure S5). The results for the Lu 99−2 variety were the same as those for the negative control conditions, showing weak colors in yellow, cyan, and orange when stained with N-Red, BTB, and SYBR Green I, respectively. These results indicated that Lu 99−2 was not

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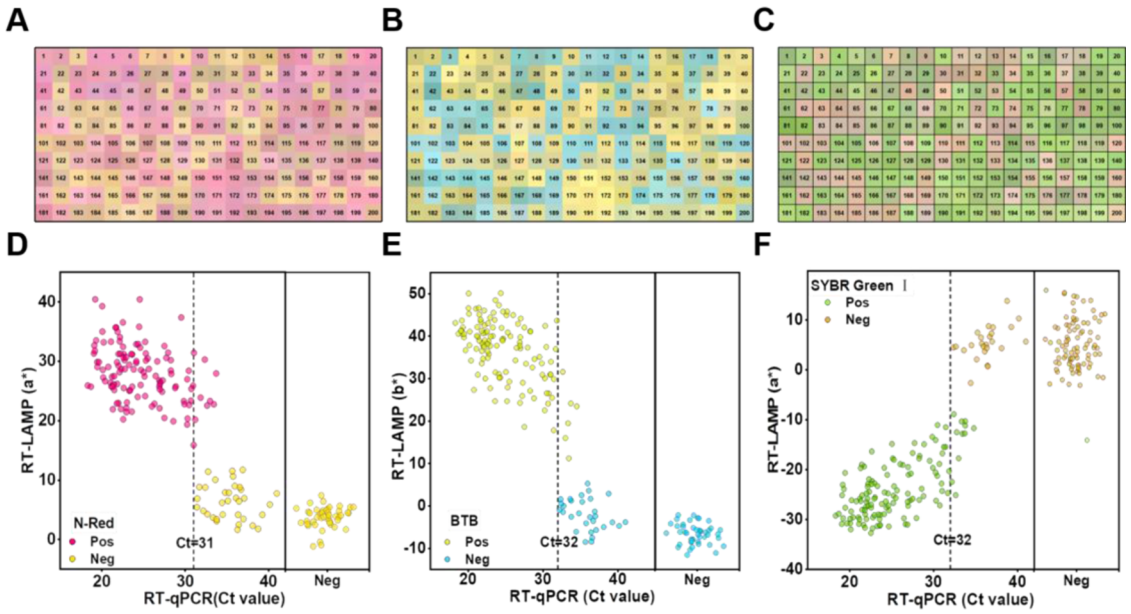

Figure 5. Detection of the SMV-SC15 in 200 soybean leaves with diverse varieties from the field. Color blocks obtained from colorimetric RT-LAMP reaction solutions based on (A) N-Red, (B) BTB, and (C) SYBR Green I. The color blocks can be captured and quantitatively identified by a smartphone installed with color recognizer software. Scatter plots showing the colorimetric RT-LAMP results of 200 soybean leaves based on (D) N-Red, (E) BTB, and (F) SYBR Green I compared to RT-qPCR, respectively. Each dot represented one sample. The *Y*-axis of each scatter represents the *a** or *b** value of the colorimetric RT-LAMP quantized result, and the *X*-axis represents the *Ct* value of the RT-qPCR result.

infected by SMV-SC15, indicating its resistance to SMV-SC15. However, although Kefeng NO.1 did not show any pathological phenotype during the observation period, it was found to be infected with SMV-SC15 12 days after inoculation using the colorimetric RT-LAMP assay. SMV-SC15 infection was also detected in the remaining 26 cultivars at various times after inoculation. The ability of the colorimetric RT-LAMP assay to detect SMV-SC15 before the appearance of typical symptoms underlines its potential for early detection of infection (Table 1). For example, in Fendou 78, SMV-SC15 was detected by the colorimetric RT-LAMP on day 4, whereas typical symptoms were not observed until day 15 after inoculation. This supports the efficacy of the colorimetric RT-LAMP assay in identifying SMV-SC15 at the early stages.

RT-LAMP Detection of the Seed Transmission Rate of SMV-SC15. Seed transmission is one of the main routes for the spread of *Soybean mosaic* virus. Fifteen soybean seeds with diverse varieties were selected to diagnose whether they were infected with SMV-SC15 by observing symptoms and detecting SMV-SC15 by colorimetric RT-LAMP assays. The results showed mottled coats on the seeds of SZ-23, SZ-419, SZ-1232, SZ-1249, SZ-1250, and Zhonghuang 70 (Figure 4A). The ambiguity regarding their SMV-SC15 infection status necessitated the use of the RT-LAMP assay to detect these 15 soybean seed samples. The electrophoresis analysis showed that only Zhonghuang 70 (lane 15) showed obvious step-shaped bands (Figure 4B). Similarly, only Zhonghuang 70 showed a pronounced color shift, distinguishing it from the negative control (Figure 4C). The results indicated that Zhonghuang 70 seeds might carry SMV-SC15, while other varieties with mottled seed coats might be caused by other viruses.

To assess the seed transmission rate of SMV-SC15 in Zhonghuang 70 seeds, they were cultivated and the leaves of 100 plants were diagnosed by the colorimetric RT-LAMP. A significant observation was that 9 out of these 100 samples showed color variations different from the negative control (Figure S6), demonstrating the presence of seed transmission for SMV-SC15 in Zhonghuang 70 with a transmission rate of 9%.

Testing Field Soybean Samples Using the Colorimetric RT-LAMP Assays. Investigating the resistance of soybean varieties to SMV-SC15 under field conditions is crucial for mosaic disease management. Based on the specific amplification of SMV-SC15 cDNA, the colorimetric RT-LAMP assays were used to assess SMV-SC15 infection in 200 soybean leaves collected in the field (Table S4). Of these, 60.5% (121/200), 63% (126/200), and 65.5% (131/200) samples showed strong positive color reactions—pink, yellow-green, and fluorescent green, respectively (Figures 5A–C, and S7). Conversely, 39.5% (79/200), 37% (74/200), and 34.5% (69/200) samples showed weak negative color reactions in yellow, cyan, and orange detected with N-Red, BTB, and SYBR Green I, respectively. The colorimetric RT-LAMP results based on these three indicators were consistently negative in 69 soybean variety leaves, identifying them as resistant to SMV-SC15 out of the 200 tested leaves.

To assess the applicability of colorimetric RT-LAMP using N-Red, BTB, and SYBR Green I for the detection of SMV-SC15, its performance was compared with the RT-qPCR assay (Figure 5 and Table S1). The colorimetric RT-LAMP assay results were quantified by converting color responses to LAB color space values (*a** or *b**) using a smartphone equipped with color recognition software, which facilitates quantitative analysis. Scatter plots of LAB values versus *Ct* values obtained from RT-qPCR were then plotted for comparison (Figure 5D–F).

All RT-qPCR positive samples with *Ct* values <31, 32, and 32 were consistently positive in the colorimetric RT-LAMP assays based on N-Red, BTB, and SYBR Green I, whereas almost all samples with *Ct* > 31, 32, and 32 were predominantly negative, with only a few exceptions (9 positives

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out of 88, 7 positive out of 81, and 12 positive out of 81), highlighting the sensitivity of the colorimetric RT-LAMP assays. In addition, the overall specificity of the colorimetric RT-LAMP based on N-Red, BTB, and SYBR Green I was 100, 100, and 98.5%, respectively (Tables S2 and S3).

As shown in Figure S6, BTB had the widest range of LAB quantitative values, facilitating easy differentiation of color results. Note that BTB is known for its low cost as a pH dye. In conclusion, the BTB-based colorimetric RT-LAMP assay was the optimal detection method for soybean samples in the field due to its better applicability and higher efficiency.

DISCUSSION

Soybean mosaic disease, which is frequently caused by SMV, is recognized as a widespread viral disease of soybean that significantly affects both the quantity and quality of seeds.[40] Of the 22 SMV strains, SMV-SC15 has been identified as the most virulent strain with the greatest prevalence and universality throughout China. Point-of-care testing (POCT) is an important diagnostic technique for SMV disease management in different settings, especially in remote areas. Here, a colorimetric RT-LAMP method based on N-Red, BTB, and SYBR Green I was developed for the rapid and visual detection of SMV-SC15 strain in infected soybean tissues directly from the field. This method aimed to facilitate early detection of soybean mosaic disease, allowing timely intervention.

Detection of LAMP products can be achieved by either colorimetric reporting systems or electrophoresis, while the latter requires specialized equipment. It has been documented that a variety of visual reporting systems, including DNAzyme,[41] modified nanoenzyme,[42] immunosorbent assays,[43] nanoparticles,[44] fluorescent dyes,[45] pH-sensitive dyes,[33] and metal ion indicators[46,47] have been used to detect LAMP reactions. In the present study, the indicators including N-Red, BTB, and SYBR Green I were employed to construct the RT-LAMP assays to achieve high sensitivity and specificity. The efficacy of the colorimetric RT-LAMP assays for SMV-SC15 was evaluated by quantifying the colorimetric responses in the LAB color space (a^* or b^* values) using a smartphone equipped with color recognition software. The results showed that BTB significantly aided in differentiating the colorimetric results. Since BTB is known for its low cost as a pH indicator, the BTB-based colorimetric LAMP assay could be used for the POCT detection of the SMV-SC15 strains due to its portability, cost-effectiveness, and independence from expensive equipment.

SMV is mainly spread by aphids in a nonpersistent manner and through seeds.[48] It has been observed that infections at the seedling stage have a significant impact on soybean yield and quality, whereas infections occurring after flowering or at incidences below 25% have minimal impact.[49] In addition, many studies have shown that soybean plants and seeds infected with SMV at an early stage often remain asymptomatic.[11−13] Therefore, early detection of SMV in soybean materials, such as seeds and vegetative parts, is one of the most effective and environmentally friendly strategies to prevent or reduce the spread of the virus for SMV disease management. In this study, the colorimetric RT-LAMP method could detect the SMV-SC15 strains in leaves prior to the appearance of typical symptoms, consistent with findings related to SMV-SC7.[13] In addition, the use of RT-LAMP assays for field screening of soybean varieties has been shown to facilitate early diagnosis of diseases associated with the SMV-SC15 strains. These assays are considered promising for rapid detection of SMV-SC15 infection, particularly in remote areas, allowing farmers to remove infected plants promptly.

Seed transmission has been identified as an important factor in the epidemiology of SMV in China.[50] Due to its capacity for seed transmission, SMV is found in soybean-growing regions worldwide. Therefore, planting virus-free soybean seeds and cultivars is the most effective strategy to reduce losses associated with SMV. It has been documented that seed transmission rates can vary from 0 to 64%, depending on the virus strain and soybean cultivar.[51−53] However, reports on seed transmission rates for individual SMV strains are lacking. In this study, 15 seeds of soybean varieties were tested for SMV-SC15 infection using a colorimetric RT-LAMP assay. The results showed that Zhonghuang 70 was infected by SMV-SC15, with a seed transmission rate of 9%.

Screening for SMV-resistant soybean germplasm resources has been identified as crucial for disease control with soybean-resistant varieties. Observation of phenotypes for the diagnosis of SMV-resistant soybean germplasm resources has been successfully applied and is widely used by breeders. However, it typically takes about a month for significant phenotypes to manifest after inoculation with SMV strains. In addition, the presence of SMV in soybean materials, such as seeds, stem, and leaf parts, varies significantly with SMV strains, soybean varieties, disease stages, and regional climates, posing a major challenge to the screening of SMV-resistant soybean varieties and the formulation of disease control strategies. After 24 days of inoculation, Lu 99−2 and Kefeng NO.1 showed no signs of the SMV disease phenotype. However, using the RT-LAMP colorimetric assay, SMV-SC15 was not detected in Lu 99−2, while Kefeng NO.1 was found to be infected 12 days after inoculation. These results suggest that Lu 99−2 and Kefeng NO.1 have different resistance mechanisms to SMV-SC15.

Field screening for disease-resistant germplasm represents the main strategy used by breeders. The mixing of the SMV-SC15 strain with other strains in the field complicates the direct identification of specific SMV strains due to the closely related genome sequences among different SMV strains, posing a significant challenge to the detection of these strains. In the current study, a series of RT-LAMP primers were designed for the specific detection of SMV-SC15 strain based on the cDNA variations within the genomic sequences of the CP genes among SMV strains. The colorimetric RT-LAMP assay was used to assess SMV-SC15 infection in leaves of 200 soybean cultivars collected at the seedling stage from fields grown with soybean for six consecutive years. As a result, 69 soybean varieties were found to be resistant to SMV-SC15, representing potential candidates for future breeding efforts to improve SMV resistance.

There are also several limitations to our study. First, the testing criteria for the individuals may have influenced the group characteristics, thus causing some deviation in our results. Second, it is not clear how well viral load shown by *Ct* values from RT-qPCR informs about the degree of infectivity in SMV-SC15-infected individuals. So, this study self-defined a *Ct* value less than 35 as the classification standard of its positive samples based on the following reasons: it has been determined that samples with *Ct* values greater than 36 indicate less than 10 viral RNA molecules[54] (low loads), and samples with *Ct* values greater than 35 in this experiment yield all negative results through colorimetric RT-LAMP assay based on different indicators. In further research, the simplified

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sample handling methods and tools can be integrated with the established RT-LAMP assay,[55−57] which will make it more promising for direct field detection. Moreover, it can also be combined with CRISPR/Cas technology, which will help develop more accurate and sensitive detection platforms.

CONCLUSIONS

This research reports a colorimetric RT-LAMP assay for the detection of SMV-SC15. The comparison testing results have revealed that the BTB-based RT-LAMP has superior performance. Under this system, a closed-tube visual detection of cDNA samples can be achieved, effectively avoiding amplicon contamination. Moreover, this assay is capable of detecting SMV-SC15 before the appearance of typical symptoms, thus reducing potential yield losses. Through the detection and screening of field samples, 69 soybean varieties resistant to SMV-SC15 were identified, providing valuable resources for breeding work.

In summary, RT-LAMP is the first method for the specific detection of SMV-SC15 at molecular levels. Compared with previously reported molecular detection techniques for crop viruses/viroids, this method has many superior analytical features (the details are shown in Table S5). It is characterized by fast detection speed, high sensitivity, visual results, no dependence on equipment, and low cost. RT-LAMP is suitable for the early diagnosis of SMV-SC15 and can thus be used as an useful tool for resistance breeding, which is of great significance for the prevention and control of soybean SMV diseases.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c03372.

Schematic diagram of RT-LAMP primers design; electrophoresis assay under different reaction times of the RT-LAMP; the RT-qPCR assay specificity for SMV-SC15; the phenotypes of 28 soybean leaves after inoculation with SMV-SC15; chromogenic response of 28 soybean varieties of different periods in colorimetric RT-LAMP reaction solution; analysis of the seed transmission rate of SMV-SC15; chromogenic response 200 soybean leaves of different varieties in colorimetric RT-LAMP reaction solution; LAB values of the color results based on the colorimetric RT-LAMP; summary of colorimetric RT-LAMP results; sensitivity of the colorimetric RT-LAMP assay; specificity of the colorimetric RT-LAMP assay; statistics of 200 field leaves testing data; comparison of the method features (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Hill, J. H.; Bailey, T. B.; Benner, H. I.; Tachibana, H.; Durand, D. P. *Soybean mosaic* virus: effects of primary disease incidence on yield and seed quality. *Plant Dis.* **1987**, *71*, 237−239.

(2) Wrather, J. A.; Anderson, T. R.; Arsyad, D. M.; Tan, Y.; Ploper, L. D.; Porta-Puglia, A.; Ram, H. H.; Yorinori, J. T. Soybean disease loss estimates for the top ten soybean-producing countries in 1998. *Can. J. Plant Pathol.* **2001**, *23*, 115−121.

(3) Yang, Y.; Lin, J.; Zheng, G.; Zhang, M.; Zhi, H. Recombinant *Soybean mosaic* virus is prevalent in Chinese soybean fields. *Arch. Virol.* **2014**, *159*, 1793−1796.

(4) Conover, R. A. Studies of two viruses causing mosaic diseases in soybean. *Phytopathology* **1948**, *38*, 724−735.

(5) Wang, Y.; Zhi, H.; Guo, D.; Gai, J.; Chen, Q.; Li, K.; Li, H. Classification and distribution of strain groups of *Soybean mosaic* virus in Northern China spring planting soybean region. *Soybean Sci.* **2004**, *24*, 263−268.

(6) Zhan, Y.; Zhi, H.; Yu, D.; Gai, J. Identification and distribution of SMV strains in Huang-Huai Valleys. *Sci. Agric. Sin.* **2006**, *39* (10), 2009−2015.

(7) Wang, D.; Li, H.; Zhi, H.; Tian, Z.; Hu, C.; Hu, G.; Huang, Z.; Zhang, L. Identification of strains and screening of resistance resources to *Soybean mosaic* virus in Anhui Province. *Chin. J. Oil Crop Sci.* **2014**, *36*, No. 374.

(8) Guo, D.; Zhi, H.; Wang, Y. Identification and distribution of *Soybean mosaic* virus strains in Middle and Northern Huang Huai Region of China. *Chin. J. Oil Crop Sci.* **2005**, *27*, 64−68.

(9) Li, K.; Yang, Q.; Zhi, H.; Gai, J. Identification and distribution of *Soybean mosaic* virus strains in Southern China. *Plant Dis.* **2010**, *94* (3), 351−357.

(10) Li, K.; Xia, Y.; Wang, D.; Yang, Y.; Ren, R.; Gao, L.; Zhang, K.; Zhi, H. Analysis of dynamic change of *Soybean mosaic* virus strains in Heilongjiang province of China. *Soybean Sci.* **2014**, *33*, 880−884.

(11) Kwak, H. R.; Hong, S. B.; Byun, H. S.; Park, B.; Choi, H. S.; Myint, S. S.; Kyaw, M. M. Incidence and molecular identification of begomoviruses infecting tomato and pepper in Myanmar. *Plants* **2022**, *11* (8), No. 1031, DOI: 10.3390/plants11081031.

(12) Lozano, I.; Leiva, A. M.; Jimenez, J.; Fernandez, E.; Carvajal-Yepes, M.; Cuervo, M.; Cuellar, W. J. Resolution of cassava-infecting alphaflexi viruses: molecular and biological characterization of a novel group of potexviruses lacking the TGB3 gene. *Virus Res.* **2017**, *241*, 53−61.
(13) Wu, W.; Yin, C.; Yue, A.; Niu, J.; Du, W.; Liu, D.; Zhao, J. Rapid and visual detection of *Soybean mosaic* virus SC7 with a loop-mediated isothermal amplification strategy. *Sens. Actuators, B* **2022**, *373*, No. 132733, DOI: 10.1016/j.snb.2022.132733.
(14) Zhao, C. L.; Zhu, Q.; Mu, X. Q.; Geng, C.; Tian, Y. P.; Yuan, X. F.; Yan, Z. Y.; Li, X. D. Epitope mapping and a cocktail of monoclonal antibodies to achieve full detection coverage of potato virus Y. *Plant Biotechnol. J.* **2023**, *21* (9), 1725−1727.
(15) Mrkvová, M.; Hančinský, R.; Grešíková, S.; Kaňuková, Š.; Barilla, J.; Glasa, M.; Hauptvogel, P.; Kraic, J.; Mihálik, D. Evaluation of new polyclonal antibody developed for serological diagnostics of tomato mosaic virus. *Viruses* **2022**, *14* (6), No. 1331, DOI: 10.3390/v14061331.
(16) Zhou, G. C.; Shao, Z. Q.; Ma, F. F.; Wu, P.; Wu, X. Y.; Xie, Z. Y.; Yu, D. Y.; Cheng, H.; Liu, Z. H.; Jiang, Z. F.; Chen, Q. S.; Wang, B.; Chen, J. Q. The evolution of *Soybean mosaic* virus: An updated analysis by obtaining 18 new genomic sequences of Chinese strains/isolates. *Virus Res.* **2015**, *208*, 189−198.
(17) Hoffmeisterová, H.; Kratochvílová, K.; Čeřovská, N.; Slavíková, L.; Dušek, J.; Muller, K.; Fousek, J.; Plchová, H.; Navrátil, O.; Kundu, J. K.; Moravec, T. One-Enzyme RTX-PCR for the detection of RNA viruses from multiple virus genera and crop plants. *Viruses* **2022**, *14* (2), No. 298, DOI: 10.3390/v14020298.
(18) Nabi, S. U.; Mir, J. I.; Yasmin, S.; Din, A.; Raja, W. H.; Madhu, G. S.; Parveen, S.; Mansoor, S.; Chung, Y. S.; Sharma, O. C.; Sheikh, M. A.; Al-Misned, F. A.; El-Serehy, H. A. Tissue and time optimization for real-time detection of apple mosaic virus and apple necrotic mosaic virus associated with mosaic disease of apple (*Malus domestica*). *Viruses* **2023**, *15* (3), No. 795, DOI: 10.3390/v15030795.
(19) Chen, H.; Zhou, Y.; Wang, X.; Zhou, C.; Yang, X.; Li, Z. Detection of *citrus yellow vein clearing virus* by quantitative real-time RT-PCR. *Hortic. Plant J.* **2016**, *2* (4), 188−192.
(20) Mahmoud, S. A.; Ganesan, S.; Ibrahim, E.; Thakre, B.; Teddy, J. G.; Raheja, P.; Zaher, W. A. Evaluation of six different rapid methods for nucleic acid detection of SARS-2 virus. *J. Med. Virol.* **2021**, *93* (9), 5538−5543.
(21) Deng, J.; Tian, F.; Liu, C.; Liu, Y.; Zhao, S.; Fu, T.; Sun, J.; Tan, W. Rapid one-step detection of viral particles using an aptamer-based thermophoretic assay. *J. Am. Chem. Soc.* **2021**, *143* (9), 7261−7266.
(22) Ning, B.; Yu, T.; Zhang, S.; Huang, Z.; Tian, D.; Lin, Z.; Niu, A.; Golden, N.; Hensley, K.; Threeton, B.; Lyon, C. J.; Yin, X. M.; Roy, C. J.; Saba, N. S.; Rappaport, J.; Wei, Q.; Hu, T. Y. A smartphone-read ultrasensitive and quantitative saliva test for COVID-19. *Sci. Adv.* **2021**, *7* (2), No. abe3703.
(23) Notomi, T.; Okayama, H.; Masubuchi, T. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* **2000**, *28* (12), No. e63, DOI: 10.1093/nar/28.12.e63.
(24) Nagamine, K.; Hase, T.; Notomi, T. Accelerated reaction by loop-mediated isothermal amplification using loop primers. *Mol. Cell. Probes* **2002**, *16* (3), 223−229.
(25) Shu, R.; Yin, X.; Long, Y.; Yuan, J.; Zhou, H. Detection and control of *pantoea agglomerans* causing plum bacterial shot-hole disease by loop-mediated isothermal amplification technique. *Front. Microbiol.* **2022**, *13*, No. 896567.
(26) Takayama, I.; Nakauchi, M.; Takahashi, H.; Oba, K.; Semba, S.; Kaida, A.; Kubo, H.; Saito, S.; Nagata, S.; Odagiri, T.; Kageyama, T. Development of real-time fluorescent reverse transcription loop-mediated isothermal amplification assay with quenching primer for influenza virus and respiratory syncytial virus. *J. Virol. Methods* **2019**, *267*, 53−58.
(27) Thi, V. L. D.; Herbst, K.; Boerner, K.; Meurer, M.; Kremer, L. P.; Kirrmaier, D.; Freistaedter, A.; Papagiannidis, D.; Galmozzi, C.; Stanifer, M. L.; Boulant, S.; Klein, S.; Chlanda, P.; Khalid, D.; Barreto, M. I.; Schnitzler, P.; Kräusslich, H. G.; Knop, M.; Anders, S. A colorimetric RT-LAMP assay and LAMP-sequencing for detecting SARS-CoV-2 RNA in clinical samples. *Sci. Transl. Med.* **2020**, *12* (556), No. eabc7075, DOI: 10.1126/scitranslmed.abc7075.
(28) Jung, D. H.; Kim, Y.; Cho, H. H.; Lee, B.; Suh, S. J.; Heo, J. H.; Lee, J. H. Automatic quantification of living cells via a non-invasive achromatic colorimetric sensor through machine learning-assisted image analysis using a smartphone. *Chem. Eng. J.* **2022**, *450*, No. 138281.
(29) Bock, K. R.; Guthrie, E. J. Transmission of African cassava mosaic by mechanical inoculation. *Plant Dis. Rep.* **1978**, *62* (7), 580−581.
(30) Hull, R. Mechanical inoculation of plant viruses. *Curr. Protoc. Microbiol.* **2009**, *13*, 16B.6.1−16B.6.4, DOI: 10.1002/9780471729259.mc16b06s13.
(31) Guan, X.; Guo, J.; Shen, P.; Yang, L.; Zhang, D. Visual and rapid detection of two genetically modified soybean events using loop-mediated isothermal amplification method. *Food Anal. Methods* **2010**, *3* (4), 313−320.
(32) Nopwinyuwong, A.; Trevanich, S.; Suppakul, P. Development of a novel colorimetric indicator label for monitoring freshness of intermediate-moisture dessert spoilage. *Talanta* **2010**, *81* (3), 1126−1132.
(33) Tanner, N. A.; Zhang, Y.; Evans, T. C., Jr. Visual detection of isothermal nucleic acid amplification using pH-sensitive dyes. *BioTechniques* **2015**, *58* (2), 59−68.
(34) Zong, T.; Yin, J.; Jin, T.; Wang, L.; Luo, M.; Li, K.; Zhi, H. DnaJ protein that interacts with *Soybean mosaic* virus coat protein serves as a key susceptibility factor for viral infection. *Virus Res.* **2020**, *281*, No. 197870.
(35) Urcuqui-Inchima, S.; Haenni, A. L.; Bernardi, F. Potyvirus proteins: a wealth of functions. *Virus Res.* **2001**, *74* (1−2), 157−175.
(36) Chung, B.-W.; Miller, W. A.; Atkins, J. F.; Firth, A. E. An overlapping essential gene in the potyviridae. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105* (15), 5897−5902.
(37) Kang, S. H.; Lim, W. S.; Hwang, S. H.; Park, J. W.; Choi, H. S.; Kim, K. H. Importance of the C-terminal domain of *Soybean mosaic* virus coat protein for subunit interactions. *J. Gen. Virol.* **2006**, *87*, 225−229.
(38) Seo, J. K.; Vo Phan, M. S.; Kang, S. H.; Choi, H. S.; Kim, K. H. The charged residues in the surface-exposed C-terminus of the *Soybean mosaic* virus coat protein are critical for cell-to-cell movement. *Virology* **2013**, *446* (1−2), 95−101.
(39) Yin, J.; Wang, L.; Jin, T.; Nie, Y.; Liu, H.; Qiu, Y.; Yang, Y.; Li, B.; Zhang, J.; Wang, D.; Li, K.; Xu, K.; Zhi, H. A cell wall-localized NLR confers resistance to *Soybean mosaic* virus by recognizing viral-encoded cylindrical inclusion protein. *Mol. Plant* **2021**, *14* (11), 1881−1900.
(40) Koning, G.; TeKrony, D. M.; Ghabrial, S. A. Soybean seedcoat mottling: Association with *Soybean mosaic virus* and *Phomopsis* spp. seed Infection. *Plant Dis.* **2003**, *87* (4), 413−417.
(41) Xu, H.; Wu, D.; Li, C.; Lu, Z.; Liao, X.; Huang, J.; Wu, Z. Label-free colorimetric detection of cancer related gene based on two-step amplification of molecular machine. *Biosens. Bioelectron.* **2017**, *90*, 314−320.
(42) Zeng, C.; Lu, N.; Wen, Y.; Liu, G.; Zhang, R.; Zhang, J.; Wang, F.; Liu, X.; Li, Q.; Tang, Z.; Zhang, M. Engineering nanozymes using DNA for catalytic regulation. *ACS Appl. Mater.* **2019**, *11* (2), 1790−1799.
(43) Yamanaka, E. S.; Tortajada-Genaro, L. A.; Pastor, N.; Maquieira, A. Polymorphism genotyping based on loop-mediated isothermal amplification and smartphone detection. *Biosens. Bioelectron.* **2018**, *109*, 177−183.
(44) Lu, N.; Zhang, M.; Ding, L.; Zheng, J.; Zeng, C.; Wen, Y.; Liu, G.; Aldalbahi, A.; Shi, J.; Song, S.; Zuo, X.; Wang, L. Yolk-shell nanostructured Fe_3O_4@C magnetic nanoparticles with enhanced peroxidase-like activity for label-free colorimetric detection of H_2O_2 and glucose. *Nanoscale* **2017**, *9* (13), 4508−4515.
(45) Chen, X.; Wang, X.; Jin, N.; Zhou, Y.; Huang, S.; Miao, Q.; Zhu, Q.; Xu, J. Endpoint visual detection of three genetically modified

rice events by loop-mediated isothermal amplification. *Int. J. Mol. Sci.* **2012**, *13* (11), 14421−14433.
(46) Tomita, N.; Mori, Y.; Kanda, H.; Notomi, T. Loop-mediated isothermal amplification (LAMP) of gene sequences and simple visual detection of products. *Nat. Protoc.* **2008**, *3* (5), 877−882.
(47) Goto, M.; Honda, E.; Ogura, A.; Nomoto, A.; Hanaki, K. I. Colorimetric detection of loop-mediated isothermal amplification reaction by using hydroxy naphthol blue. *Biotechniques* **2009**, *46* (3), 167−172.
(48) Hajimorad, M. R.; Domier, L. L.; Tolin, S. A.; Whitham, S. A.; Maroof, M. A. S. *Soybean mosaic* virus: a successful potyvirus with a wide distribution but restricted natural host range. *Mol. Plant Pathol.* **2018**, *19* (7), 1563−1579.
(49) Ren, Q.; Pfeiffer, T. W.; Ghabrial, S. A. *Soybean mosaic* virus incidence level and infection time: Interaction effects on soybean. *Crop Sci.* **1997**, *37* (6), 1706−1711.
(50) Zhang, M.; Lu, W.; Zhong, Z.; Wang, R.; Li, Y. The importance of the diseased seedlings from SMV-infected seeds and the vector of the virus in the epidemic. *Acta Phytopatholgica Sin.* **1986**, *16*, 151−158.
(51) Bowers, G. R.; Goodman, R. M. Strain specificity of *Soybean mosaic* virus seed transmission in soybean. *Crop Sci.* **1991**, *31* (5), 1171−1174.
(52) Domier, L. L.; Steinlage, T. A.; Hobbs, H. A.; Wang, Y.; Herrera-Rodriguez, G.; Haudenshield, J. S.; McCoppin, N. K.; Hartman, G. L. Similarities in seed and aphid transmission among *Soybean mosaic* virus isolates. *Plant Dis.* **2007**, *91* (5), 546−550.
(53) Porto, M. D. M.; Hagedorn, D. Seed transmission of a Brazilian isolate of *Soybean mosaic* virus. *Phytopathology* **1975**, *65*, 713−716, DOI: 10.1094/Phyto-65-713.
(54) Vogels, C. B. F.; Brito, A. F.; Wyllie, A. L.; Fauver, J. R.; Ott, I. M.; Kalinich, C. C.; Petrone, M. E.; Casanovas-Massana, A.; Muenker, M. C.; Moore, A. J.; Klein, J.; Lu, P.; Lu-Culligan, A.; Jiang, X.; Kim, D. J.; Kudo, E.; Mao, T.; Moriyama, M.; Oh, J. E.; Park, A.; Grubaugh, N. D. Analytical sensitivity and efficiency comparisons of SARS-COV-2 qRT-PCR primer-probe sets. *Nat. Microbiol.* **2020**, *5* (10), 1299−1305.
(55) Liao, S. C.; Peng, J.; Mauk, M. G.; Awasthi, S.; Song, J.; Friedman, H.; Bau, H. H.; Liu, C. Smart Cup: A Minimally-instrumented, smartphone-based point-of-care molecular diagnostic device. *Sens. Actuators, B* **2016**, *229*, 232−238.
(56) Li, R. J.; Mauk, M. G.; Seok, Y.; Bau, H. H. Electricity-free chemical heater for isothermal nucleic acid amplification with applications in COVID-19 home testing. *Analyst* **2021**, *146* (13), 4212−4218.
(57) Seok, Y.; Yin, Q.; Li, R.; Mauk, M. G.; Bai, H.; Bau, H. H. Manually-operated, slider cassette for multiplexed molecular detection at the point of care. *Sens. Actuators, B* **2022**, *369*, No. 132353.

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