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Rapid Visual LAMP Method for Detection of Genetically Modified Organisms

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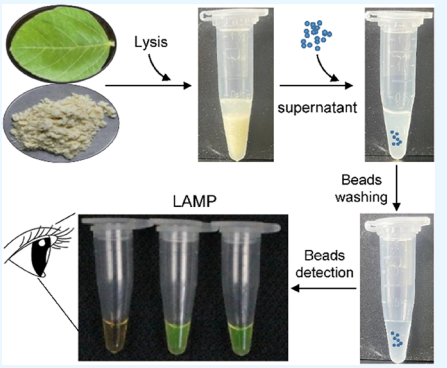
ABSTRACT: We developed a novel loop-mediated isothermal amplification (LAMP) method using DNA captured on polyacrylamide microparticles (PAMMPs) as templates (PAMMPs@DNA-LAMP) for rapid qualitative detection of genetically modified organisms (GMOs). Here, DNA was extracted by a fast and cost-effective method using PAMMPs. Four LAMP primers were designed for the PAMMPs@DNA-LAMP method to detect the cauliflower mosaic virus 35S (CaMV35S) promotor in GMOs. We thus developed this method for rapid extraction of DNA (5−10 min) and fast amplification of DNA within ∼30 min at a constant temperature of 63 °C. Moreover, the DNA captured by PAMMPs (PAMMPs@DNA) could be effectively detected by both conventional and quantitative PCR (qPCR) and LAMP. The PAMMPs@DNA-LAMP method was validated with high specificity, sensitivity, and performance for practical sample analysis. This assay detected 0.01% target sequences, which had a high specificity like qPCR and better than the conventional PCR (cPCR). Furthermore, PAMMPs@DNA-LAMP was successfully used to extract and detect DNA from food samples of the major crops (soybean, maize, rice, etc.). In summary, a novel PAMMPs@DNA-LAMP assay has been developed, which has higher sensitivity and spends less time than the cPCR detection using the conventional DNA extracted process. This method offers a novel approach for rapid detection of GMOs in the field.



1. INTRODUCTION

Since 1996, the first genetically modified (GM) crop was commercially planted, the total planted area of GM crops worldwide has continuous increase from 1.7 million hectares in 1996 to 190 million hectares in 2019.[1] However, some consumers still concern the biosafety of GM food and feed products. To protect consumer's right of knowing information about genetically modified organism (GMO) products, many countries and regions have applied different GMO supervision and legislated labeling rules to manage GMO products.[2] Therefore, the high specificity and sensitivity detection approaches are still needed to be developed.

Molecular diagnosis of the genome is an essential tool in the detection of GMOs.[3] Among these DNA-based approaches, polymerase chain reaction (PCR) and quantitative PCR (qPCR) have been the gold standard for GMO detection. Additionally, digital-PCR (dPCR)[4] and multi-PCR[5,6] have been developed to detect GMOs. However, these methods require expensive instrumentation, technical expertise and can be time-consuming (more than 60 min), which are unsuitable for field applications. In recent years, the isothermal amplification technology known as loop-mediated isothermal amplification (LAMP) has been applied to detect GMOs.[7]

LAMP is a rapid, inexpensive, and isothermal DNA amplification approach.[8] This method uses a set of four or six primers and Bst DNA polymerase with the activity of strand displacement to amplify DNA with high specificity under isothermal conditions within 20−60 min.[9] LAMP products assay can be directly observed with the naked eye by adding SYBR Green I or hydroxy naphthol blue.[10,11] Although the LAMP method has some advantages than PCR-based approaches, such as rapid, cheap instruments, visual analysis, and suits for field testing, yet the detected DNA must be first extracted with conventional methods such as the cetyltrimethylammonium bromide and silicone column extraction. However, extracting DNA from crop seeds and plant/animal samples using these methods is a complicated and speed-limited task. The extracting process is involved in many processing steps requiring special reagents and specialized operation. Therefore, a simple and rapid DNA extraction method is still demanded.

Recently, we fabricated a new kind of polyacrylamide microparticles (PAMMPs) and developed a new method for rapid extraction (3−5 min) of DNA from various samples

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Table 1. Primers for the PAMMPs@DNA-LAMP Assay and Conventional PCR

oligos	sequence (5′ to 3′)	size (bp)	use
35S-F3	TGCCCAGCTATCTGTCACTT	20	LAMP primers
35S-B3	TCCCTTACGTCAGTGGAGAT	20	
35S-FIP	AGGCATCTTCAACGATGGCCTTGTGGAAAAGGAAGGTGGCTC	42	
35S-BIP	CTGCCGACAGTGGTCCCAAAGTTGAAGACGTGGTTGGAACG	41	
35S-F	GCTCCTACAAATGCCATCATTGC	23	PCR primers
35S-R	GATAGTGGGATTGTGCGTCATCCC	24	

including bacteria, mammalian cells, animal tissues, and human blood.[12] In order to decrease the time from sample to detection result, in this study, we develop a novel LAMP assay coupled with PAMMPs@DNA to shorten and simplify the process of DNA extraction and quickly analyze the detection of the CaMV35S promotor that is used with the highest frequency in GMO development as promotor. We validated the specificity, sensitivity, and performance of practical sample analysis of the PAMMPs@DNA-LAMP method. Our work describes an initial development of a PAMMPs@DNA-LAMP assay to detect GMO ingredients for the purposes of rapid and efficient GMO screening.

2. MATERIALS AND METHODS

2.1. Samples. GM maize (MON863, GA21, Bt176, MIR162, and MIR604), GM soybean (A2704-12, GTS 40-3-2, DP356043, MON89788, MON87701), and GM rapeseed (GT73, MS1, and OXY235) with the GM content of 1% (w/w) were purchased from the Institute for Reference Materials and Measurements and the American Oil Chemists' Society. GM rice (Kefeng6, KMD, and TT51-1) powders were supplied by the Center of Science and Technology Development, Ministry of Agriculture of the People's Republic of China. The crude processed food commodities are purchased from local supermarkets and farm product market in Nanjing, China, including wheat flour, rice stick snack, tofu, soybean milk, maize starch, non-GM soybean, non-GM maize, and non-GM rice (labeled with non-GMO).

2.2. Synthesis of PAMMPs. PAMMPs were prepared as previously described.[12] Span 80 (1 mL) and hexane (70 mL) were added to a three-neck flask (250 mL) equipped with a magnetic stirrer and a nitrogen inlet for preparing the oil phase. Acrylamide (264 mg), APMA (25 mg), and MBA (80 mg) were dissolved in 1 mL of deionized water (ddH_2O), to which 1 mL of 10% ε-poly-L-lysine and 80 μL of 20% APS were added to prepare water solution. The water solution was added to the oil phase and stirred continuously for 2 h in a nitrogen atmosphere at 380 rpm. Then, 280 μL of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED) was added and stirred for 2 h. The beads were collected and washed, and finally were resuspended in ddH_2O. The bead solution was added with glutaraldehyde for a final concentration of 0.1% and incubated at 37 °C for 4 h and washed five times with ddH_2O. The PAMMPs were finally resuspended in 10 mL of ddH_2O and stored at room temperature.

2.3. DNA Extraction with PAMMPs. For genomic DNA (gDNA) extraction with PAMMPs from seed powder and food material, 20−50 mg of powder was added in a 1.5 mL tube in the presence of 500 μL of lysis buffer (20 mM Tris, 25 mM NaCl, 2.5 mM EDTA, Protease K, and 0.1% SDS). The lysate was then centrifuged at 4000 *g* for 2 min and 500 μL of supernatant was transferred and added with 100 μL of PAMMPs. The mixture was rotated for 5 min for capturing DNA on PAMMPs (PAMMPs@DNA). The PAMMPs@DNA was washed three times with wash buffer (10 mM Tris, pH 8.0, and 0.1% Tween 20%) and then resuspended in 50 μL of ddH_2O.

For gDNA extraction with PAMMPs from plant tissue, 10−20 mg of leaf tissue was ground in a 1.5 mL tube with a glass pestle in the presence of 200 μL of plant lysis buffer (20 mM Tris, 25 mM NaCl, 2.5 mM EDTA, and 0.05% SDS). Then, the lysate was briefly centrifuged and 200 μL of supernatant were transferred. Subsequently, 100 μL of PAMMPs was added and incubated in a rotator for 5 min. The PAMMPs@DNA was washed three times with wash buffer (10 mM Tris, pH 8.0, and 0.1% Tween 20%). The PAMMPs@DNA was resuspended in 50 μL of deionized water and used as the template for LAMP analyses.

For DNA extraction with a conventional method, the AxyPrep DNA Gel Extraction Kit (Axygen) was used according to the manufacturer's instructions. The concentration and quality of the extracted DNA were evaluated by ultraviolet (UV) absorbance using a NanoVue Plus spectrophotometer (GE Healthcare, USA). The concentration of gDNA was adjusted to 50 ng/μL as the template for LAMP analyses.

2.4. Primers for LAMP and PCR Detections. The development of LAMP primers was based on the sequence of the gene CaMV35S promotor. LAMP primers were designed using the LAMP Designer Software Primer Explorer (v5) (https://primerexplorer.jp/e/) and included the outer forward primer (35S-F3), outer backward primer (35S-B3), forward inner primer (35S-FIP), and backward inner primer (35S-BIP) for amplifying the specific target sequence. Table 1 shows the LAMP and the PCR primers used in this study. The primers were synthesized by Sangon Biotech (Shanghai) Co., Ltd. (Shanghai, China).

2.5. Reaction of the PAMMPs@DNA-LAMP Assay. The PAMMPs@DNA-LAMP detection was conducted in a 25 μL reaction containing 2.5 μL of 10× isothermal amplification buffer, 2.5 μL of $MgSO_4$ (100 mM), 2 μL of betaine (10 M), 2 μL each of 35S-FIP and 35S-BIP (20 mM), 0.5 μL each of 35S-F3 and 35S-B3 (10 mM), 4 μL of dNTPs (10 mM), 1 μL of Bst DNA polymerase large fragment (8 U/μL, New England Biolabs), 3 μL of PAMMPs@DNA template, and 5 μL of double distilled water (ddH_2O). The LAMP reaction was incubated in a thermostatic water bath at 63 °C for 60 min to amplify the target sequence and 80 °C for 10 min to inactivate enzymes. PAMMPs@DNA extracted from non-GMO was used as a control. The amplified products were detected by directly adding 2 μL 1000× SYBR Green I (Solarbio, Beijing) or electrophoresis on 2% agarose gel.

The conventional PCR (cPCR) detection was carried out in a 25 μL reaction containing 3 μL of PAMMPs@DNA, 0.5 μM each primer, and 1× GoTaqGreen Master Mix (Promega, USA). The PCR program is as follows: 95 °C for 5 min; 35

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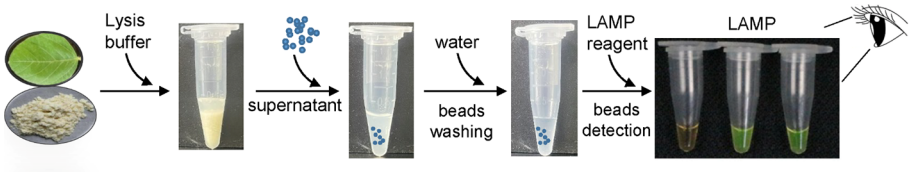

Figure 1. Schematic show of PAMMPs@DNA-LAMP. The DNA is purified by being rapidly captured on PAMMPs, and PAMMPs@DNA is directly detected by LAMP. The LAMP result is visually detected by directly adding SYBR Green I (visual PAMMPs@DNA-LAMP).

cycles at 95 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s; and 72 °C for 10 min. The amplified products were detected by electrophoresis on 2% agarose gel. The qPCR detection was carried out in a 25 μL reaction containing 3 μL of PAMMPs@DNA, 0.5 μM each primer, 0.25 μM probe, and 1× GoTaq Probe qPCR Master Mix (Promega, USA). The qPCR program is as follows: 95 °C for 5 min; 40 cycles at 95 °C for 10 s, and 60 °C for 40 s; the cPCR and qPCR were run on BIO-RAD T100 and LightCycler 96, respectively.

2.6. Specificity of the PAMMPs@DNA-LAMP Assay. The specificity of the PAMMPs@DNA-LAMP and PCR detections were evaluated by comparing different GMO crops with or without the CaMV35S promotor. The GM crops with the CaMV35S promotor include GTS40-3-2, A2704-12, Bt176, MON863, OXY235, and KMD. The GM crops without the CaMV35S promotor include MOM89788, MON87701, DP356043, GA21, MIR604, MIR162, MS1, GT73, and TT51-1.

2.7. Limit of Detection of the PAMMPs@DNA-LAMP Assay. The limit of detection (LOD) was determined by detecting the transgenic soybean GTS 40-3-2 with the CaMV35S promotor. The gDNA of GTS 40-3-2 was extracted using PAMMPs from different contents of GTS 40-3-2 (10, 1, 0.5, 0.1, 0.05, and 0.01%). The PAMMPs@DNA-LAMP detections were performed as described above. Each LAMP reaction was repeated in triplicate. Negative controls contained nuclease-free water in place of template DNA. All reactions were performed three times. The same DNA contents were used to evaluate the LOD of PAMMPs@DNA-LAMP, cPCR, and qPCR.

2.8. Robustness of the PAMMPs@DNA-LAMP Assay. The efficiency of the PAMMPs@DNA-LAMP assay was evaluated by detecting GMOs, 1% GTS40-3-2, 1% MON863, soybean feed, corn feed, wheat flour, rice stick snack, tofu, soybean milk, maize starch, non-GM soybean, and non-GM maize. The non-GM rice was selected and purchased from the Standard Material Resource Platform of China or a local supermarket in Nanjing, China. DNA was extracted using PAMMPs, then PAMMPs@DNA was used as templates for the LAMP assay. At last, PAMMPs@DNA-LAMP result may realize fast-visual-detection for GMOs. At the same time, qPCR is used for verification.

3. RESULTS

3.1. Schematic of the PAMMPs@DNA-LAMP Assay. The detection process of the PAMMPs@DNA-LAMP assay is schematically shown in Figure 1. The samples were first lysed with lysis solution, and then beads were added to the lysate and mixed by inverting the tube several times. After a brief centrifugation, the supernatant was removed and the beads were washed three times with wash buffer. Finally, the beads were added with water. The beads can then be directly used as the template for various detections including cPCR, qPCR, and LAMP.

3.2. Establishment of the PAMMPs@DNA-LAMP Assay. For the development of the PAMMPs@DNA-LAMP assay to detect the CaMV35S promotor, we first validated that DNA can be captured on PAMMPs to form PAMMPs@DNA by directly adding the purified gDNA of transgenic soybean GTS40-3-2 to PAMMPs solution in a tube. After the tube was briefly inverting for several times, PAMMPs were washed and resuspended in ddH_2O for the detection of the CaMV35S promotor with cPCR, qPCR, and LAMP (Figure 2). LAMP

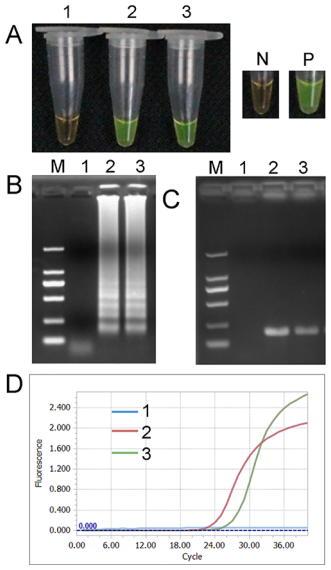

Figure 2. Verification of PAMMPs capturing DNA (PAMMPs@DNA). (A–D) Detection of PAMMPs@DNA template with different assays. PAMMPs@DNA was detected by visual PAMMPs@DNA-LAMP (A), gel electrophoresis-based PAMMPs@DNA-LAMP (B), PAMMPs@ DNA-cPCR (C), and PAMMPs@DNA-qPCR (D), respectively. 1, ddH_2O; 2, GTS40-3-2 gDNA; 3, PAMMPs@GTS40-3-2 DNA.

results can be directly observed by adding SYBR Green I dye (visual PAMMPs@DNA-LAMP) (Figure 2A), which was also confirmed by gel electrophoresis (gel electrophoresis-based PAMMPs@DNA-LAMP) (Figure 2B). PAMMPs@DNA was also successfully detected by cPCR (Figure 2C) and qPCR (Figure 2D), which supported the PAMMPs@DNA-LAMP detection. These results indicated that gDNA can be captured on PAMMPs to form PAMMPs@DNA and the resulting PAMMPs@DNA can be detected by LAMP, cPCR, and qPCR, especially visual PAMMPs@DNA-LAMP.

3.3. DNA Extraction and Detection with the PAMMPs@DNA-LAMP Assay. To verify DNA can be purified from transgenic crops with PAMMPs and detection with PAMMPs@DNA-LAMP, we then tried to extract DNA

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with PAMMPs from leaves and powder of transgenic soybean GTS40-3-2, transgenic corn MON863, transgenic rice Kefeng6, and transgenic rape OXY235, respectively. The CaMV35S promoter was then detected from the obtained PAMMPs@DNA by the visual PAMMPs@DNA-LAMP, gel electrophoresis-based PAMMPs@DNA-LAMP, and cPCR, respectively. The results indicated that gDNA can be extracted from leaves with PAMMPs in a simple one-step capture, and the resulting PAMMPs@DNA can be successfully detected by visual PAMMPs@DNA-LAMP, gel electrophoresis-based PAMMPs@DNA-LAMP, and cPCR, respectively (Figure 3A–C). The results of visual PAMMPs@DNA-LAMP

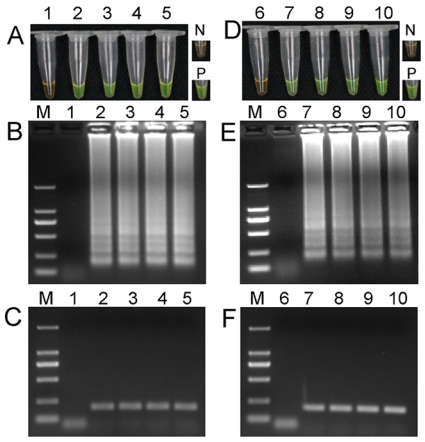

Figure 3. DNA extraction from transgenic leaves and powders with PAMMPs and detection of PAMMPs@DNA with LAMP and cPCR. (A–C) DNA extraction from transgenic leaves with PAMMPs. (D–F) DNA extraction from transgenic powders with PAMMPs. PAMMPs@DNA was, respectively, detected by visual PAMMPs@DNA-LAMP (A, D), gel electrophoresis-based PAMMPs@DNA-LAMP (B, E), and PAMMPs@DNA-cPCR (C, F). 1, ddH_2O; 2–5, leaves of GTS40-3-2, MON863, Kefeng6, and oxygen, respectively; 6, ddH_2O; 2–5, powders of GTS40-3-2, MON863, Kefeng6, and oxygen, respectively.

detection (Figure 3A) were supported by those of gel electrophoresis-based PAMMPs@DNA-LAMP (Figure 3B) and PAMMPs@DNA-cPCR (Figure 3C). The similar results were also obtained with the DNA extraction from transgenic seed powder with PAMMPs and the subsequent detections with visual PAMMPs@DNA-LAMP (Figure 3D), gel electrophoresis-based PAMMPs@DNA-LAMP (Figure 3E), and PAMMPs@DNA-cPCR (Figure 3F). These results indicated that visual PAMMPs@DNA-LAMP was an easy, simple, and rapid assay of GMOs, which was applicable to variant GMO products such as leaves and seed powder.

3.4. Specificity of the PAMMPs@DNA-LAMP Assay. To test the specificity of PAMMPs@DNA-LAMP detection of CaMV35S promotor, DNA was extracted from 14 variants of transgenic crops with PAMMPs and the resulting PAMMPs@DNA was detected by visual PAMMPs@DNA-LAMP and PAMMPs@DNA-cPCR (Figure 4). The results indicated that the positive amplification of the CaMV35S promotor was successfully detected from GTS40-3-2, A2704-12, Bt176, MON863, OXY235, and KMD by visual PAMMPs@DNA-LAMP (color shift from orange to green) (Figure 4A), whereas it was not detected from non-transgenic crops of MOM89788, MON87701, DP356043, MIR604, GA21, MIR162, GT73, MS1, and TT51-1 by visual PAMMPs@DNA-LAMP (no color

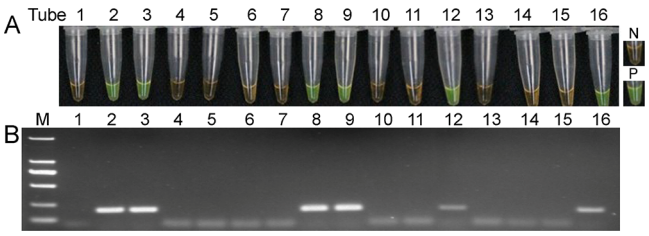

Figure 4. Specificity of PAMMPs@DNA-LAMP. (A) Specificity evaluation by detection of different GM or non-GM crops with PAMMPs@DNA-LAMP. (B) Specificity evaluation by detection of different GM or non-GM crops with PAMMPs@DNA-cPCR. 1, Negative control; 2, positive control (GTS40-3-2); 3–16, A2704-12, MON87701, and MON89788, respectively; DP356043; GA21, MON863, Bt176, MIR162, MIR604, OXY235, MS1, GT73, TT51-1, and KMD, respectively.

shift) (Figure 4A), indicating that the visual PAMMPs@DNA-LAMP can specifically detect the CaMV35S promotor in variant samples. These results were validated by PAMMPs@DNA-cPCR (Figure 4B), indicating the high specificity of the visual PAMMPs@DNA-LAMP assay.

3.5. Sensitivity of the PAMMPs@DNA-LAMP Assay. To evaluate the sensitivity of the PAMMPs@DNA-LAMP assay, DNA was extracted from transgenic soybean GTS40-3-2 of different quantity ratios (10, 1, 0.5%, 0.1, 0.05, and 0.01%) with PAMMPs. The resulted PAMMPs@DNA was detected by visual PAMMPs@DNA-LAMP, gel electrophoresis-based PAMMPs@DNA-LAMP, PAMMPs@DNA-qPCR, and PAMMPs@DNA-cPCR, respectively (Figure 5). The results

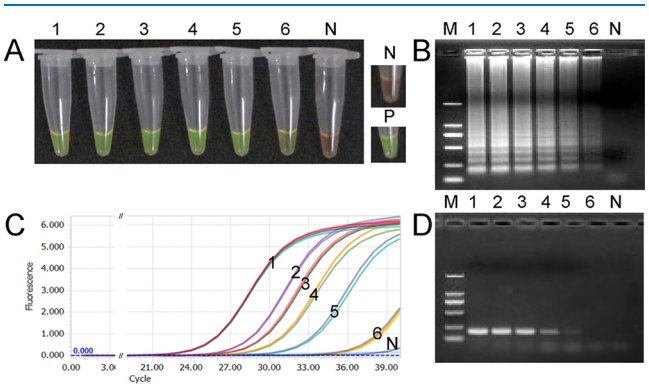

Figure 5. Sensitivity of PAMMPs@DNA-LAMP. (A–D) Detection of the CaMV35S promotor from the PAMMPs@DNA template with different assays. The CaMV35S promotor in different PAMMPs@DNA templates was detected by visual PAMMPs@DNA-LAMP (A), gel electrophoresis-based PAMMPs@DNA-LAMP (B), PAMMPs@DNA-qPCR (C), and PAMMPs@DNA-cPCR (D), respectively.

indicated that transgenic soybean GTS40-3-2 was quantitative detected by both the visual and gel electrophoresis-based PAMMPs@DNA-LAMP (Figure 5A,B), which was validated by PAMMPs@DNA-qPCR and PAMMPs@DNA-cPCR detections (Figure 5C,D). The LOD of PAMMPs@DNA-LAMP was 0.01% (Figure 5A,B), same as that of PAMMPs@DNA-qPCR (0.01%) (Figure 5D) but higher than that of PAMMPs@DNA-cPCR (0.05%) (Figure 5C), indicating that the visual PAMMPs@DNA-LAMP assay has high sensitivity.

3.6. Stability and Reliability of the PAMMPs@DNA-LAMP Assay. To further determine the reproducibility and repeatability of the PAMMPs@DNA-LAMP assay, the

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CaMV35S promotor in DNA extracted from 1% GTS40-3-2 with PAMMPs was detected in triplicate with PAMMPs@DNA-LAMP by three researchers. The results showed that the CaMV35S promotor was reproducibly and repeatably detected by visual PAMMPs@DNA-LAMP (Figure 6A), indicating that

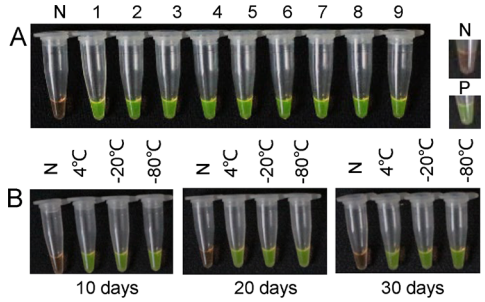

Figure 6. Reproducibility, repeatability, and stability of PAMMPs@DNA-LAMP. (A) Detection of PAMMPs@DNA of 1% GTS 40-3-2 with PAMMPs@DNA-LAMP in triplicate by three individual researchers. 1−3, triplicate by the first researcher; 4−6: triplicates by the second researcher; and 7−9: triplicates by the third researcher; N, negative. (B) LAMP amplification of target genes CaMV35S from PAMMPs@DNA that were kept at different conditions (4, −20, and −80 °C) for various times.

the established visual PAMMPs@DNA-LAMP assay was stable and reliable. This was also supported by successful visual PAMMPs@DNA-LAMP detection of the CaMV35S promotor from PAMMPs@DNA kept at different temperatures (4, −20, and −80 °C) for variant time (from 10 days to 1 month) (Figure 6B).

3.7. Application of the PAMMPs@DNA-LAMP Assay.

To evaluate the practicable application of visual PAMMPs@DNA-LAMP, DNA was extracted from 12 different fields and market products with PAMMPs, and the CaMV35S promotor was detected from the resultant PAMMPs@DNA with PAMMPs@DNA-LAMP. At the same time, 1% PAMMPs@GTS40-3-2 DNA and PAMMPs@MON863 DNA were used as positive controls. The results of both PAMMPs@DNA-LAMP and PAMMPs@DNA-cPCR and PAMMPs@DNA-qPCR detection showed that 1% GTS40-3-2, 1% MON863, soybean feed, and corn feed were CaMV35S promotor positive and the other samples were CaMV35S promotor negative (Figure 7). The results of visual PAMMPs@DNA-LAMP were observed through color changes (color shifts from orange to green) (Figure 7A). The results of visual PAMMPs@DNA-LAMP were further confirmed by both PAMMPs@DNA-cPCR (Figure 7B) and PAMMPs@DNA-qPCR (Figure 7C). These results indicated that the visual PAMMPs@DNA-LAMP was reliable for practicable application.

4. DISCUSSION

In the molecular biological detections, DNA extraction is the first step. The difficulty of the extraction process and the quality of extraction are the factors that affect the subsequent detection. With the rapid development of GM crops, to protect consumer's right of knowing information about GMO products, the detection methods with simplicity, rapidity, and specificity are still needed to be developed. Transgene crop detection usually adopts molecular detection methods such as cPCR,[13,14] qPCR,[3,15] multi-qPCR,[16] and dPCR.[17,18] The premise of detection is to extract gDNA, while DNA extraction methods are usually purification with phenol/chloroform extraction and the extraction process needs harmful reagents and is time-consuming. Additionally, rapid nucleic acid extraction methods have been reported to simplify extraction process, such as those using paper,[19] alumina membrane,[20] silica,[21] cellulose,[22] and Chelex-100.[23] Although these extraction processes are simple and time-saving, complicated manufacturing or experimental processes are still required, which limits their wide application.

PAMMPs have biocompatibility, controllable chemistry, and physical properties and have been widely used in the biomedical field. Previous studies have reported that PAMMPs can bind primers and had been detected the amplified signals by the PCR method.[24] We found that PAMMPs can capture DNA and have high stability under various harsh conditions such as acid, alkali, and high temperature. We thus developed a simple and fast PAMMP-based DNA extraction method that can be used to extract DNA from various samples, including bacteria, mammalian cells, corn leaves, animal solid tissues, and human blood plasma.[12] This study found that PAMMPs can be used to rapidly and easily extract DNA from plant leaves and powder of GM crops, including the transgenic soybean, corn, rice, and rapeseed. This is important for the application of PAMMPs to GMO detection, especially for rapid GMO detection on the spot or in the field.

This study found that the DNA captured on PAMMPs (PAMMPs@DNA) can be directly detected by cPCR, qPCR, and LAMP. Especially, we found that PAMMPs@DNA of GMO can be rapidly and accurately detected by visual PAMMPs@DNA-LAMP. Because LAMP-based detections had more advantages than PCR in on-spot or field application, visual PAMMPs@DNA-LAMP is helpful for the on-spot or

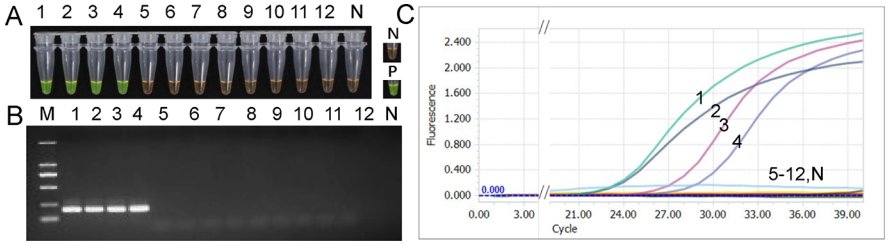

Figure 7. Detection of the CaMV35S promotor in multiple samples with PAMMPs@DNA-LAMP. (A−C) Detection of the CaMV35S promotor from the PAMMPs@DNA template with different assays. The CaMV35S promotor was detected from 12 samples by PAMMPs@DNA-LAMP (A), PAMMPs@DNA-cPCR (B), and PAMMPs@DNA-qPCR (C), respectively. 1−4, 1% GTS40-3-2, 1% MON863, soybean feed, and corn feed, respectively. 5−12, wheat flour, rice stick snack, tofu, soybean milk, maize starch, non-GM soybean, non-GM maize, and non-GM rice, respectively; N, ddH_2O.

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field detection of GMOs. This study demonstrates that visual PAMMPs@DNA-LAMP has high specificity, sensitivity, and reliability in the rapid detection of the CaMV35S promoter from various GMO products. Moreover, this study reveals that DNA captured on PAMMPs (PAMMPs@DNA) can be stably detected by visual PAMMPs@DNA-LAMP when stored at variant conditions (4, −20, and −80 °C) for a long time (tested to 30 days). Therefore, the combination of PAMMPs-based DNA extraction and visual PAMMPs@DNA-LAMP detection of PAMMPs@DNA provides a simple, rapid, and applicable tool for the GMO detection, especially for GMO detection on the spot or in the field.

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Author Contributions

Y.X. (the first author) prepared all PAMMPs and performed all DNA extraction and PCR/qPCR/LAMP detection with PAMMPs@DNA. Y.X. (the first author) wrote the manuscript. J.L. prepared all detected samples including powder and leaves to extract DNA using PAMMPs. F.D. evaluated the specificity and sensitivity of the PAMMPs@DNA-LAMP assay. J.W. analyzed the method's stability and applied it to detect the supermarket sample. J.S. gave a helpful discussion about GMO detection. J.X. (the corresponding author) gave a beneficial suggestion to analyze the result in this study and reviewed and edited the manuscript. J.W. (the corresponding author) conceptualized and supervised the study. J.W. (the corresponding author) also edited the manuscript.

Notes

The authors declare no competing financial interest.

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