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RT-RPA Assay Combined with a Lateral Flow Strip to Detect Soybean Mosaic Virus

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Soybean (*Glycine max* L.) is one of the most widely planted and used legumes in the world, being used for food, animal feed products, and industrial production. The soybean mosaic virus (SMV) is the most prevalent virus infecting soybean plants. This study developed a diagnostic method for the rapid and sensitive detection of SMV using a reverse transcription-recombinase polymerase amplification (RT-RPA) technique combined with a lateral flow strip (LFS). The RT-RPA and RT-RPA-LFS conditions to detect the SMV were optimized using the selected primer set that amplified part of the VPg protein gene. The optimized reaction temperature for the RT-RPA primer and RT-RPA-LFS primer used in this study was 38°C for both, and the minimum reaction time was 10 min and 5 min, respectively. The RT-RPA-LFS was as sensitive as RT-PCR to detect SMV with 10 pg/µl of total RNA. The reliability of the developed RT-RPA-LFS assay was evaluated using leaves collected from soybean fields. The RT-RPA-LFS diagnostic method developed in this study will be useful as a diagnostic method that can quickly and precisely detect SMV in the epidemiological investigation of SMV, in the selection process of SMV-resistant varieties, on local farms with limited resources.

Keywords **:** lateral flow strip (LFS), reverse transcription recombinase polymerase amplification (RT-RPA), soybean mosaic virus (SMV)

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Soybean (*Glycine max* L.), belonging to the order *Rosales* and the family *Fabaceae*, is an annual dicotyledonous plant native to East Asia - including Korea (Lee et al., 2015b). Soybean is one of the most widely planted and used legumes worldwide, being used for food, animal feed products, and industrial production (Lee et al., 2012). In 2021, soybean ranked third for per capita food grain consumption in Korea (Ministry of Agriculture, Food and Rural Affairs, 2022). At least 46 viruses belonging to 27 distinct taxonomic groups have been reported and found in soybeans worldwide (Tolin and Lacy, 2004). More than 14 of these viruses have been found in soybeans grown in Korea (Cho, 2007; Cho and Chung, 1976; Jang et al., 2018; Kil et al., 2017; Kim et al., 2006; Lee et al., 1980, 1985, 2015c; Lim et al., 2014; Nam et al., 2009, 2012; Shin et al., 2014; Yoon et al., 2018). Among them, soybean mosaic virus (SMV; of the genus *Potyvirus*) occurs most frequently in the soybean cultivation fields in Korea (Jo et al., 2020). SMV is not only one of the most prevalent viruses, it reduces productivity and quality in soybeans (Goodmand and Oard, 1980).

Cho and Chung (1976) reported on SMV in Korea, finding that it is a member of the genus *Potyvirus* in the family *Potyviridae* (Domier et al., 2011). The SMV genome is comprised of a single-stranded RNA encoding at least 11 proteins (Jayaram et al., 1992; Liu et al., 2016), with SMV being transmitted non-persistently by more than 35 species of aphids (Irwin and Goodman, 1981). The transmission level of SMV through seeds ranged from 0 to 43% depending on the soybean line and virus strain analyzed (Domier et al., 2007, 2011). Symptoms of SMV are comprised of leaf mottling, distortion of leaves, necrosis, and overall stunting of the plants, often exhibiting mottling on the seeds derived from the infected plants (Hajimorad et al., 2018). It is therefore important to develop rapid and sensitive methods by which the SMV virus can be detected directly and early in the field as a first step towards the effective management of the virus.

Diagnosis of SMV is achieved in an enzyme-linked immunosorbent assay, reverse transcription polymerase chain reaction (RT-PCR), and loop-mediated isothermal amplification (LAMP) (Botelho et al., 2016; Lee et al., 2015b; Maury et al., 1986). Due to its speed, specificity, and sensitivity, an RT-PCR or reverse transcription-quantitative polymerase chain reaction (RT-qPCR) is the most commonly used nucleic acid-based detection method for plant diseases to SMV (Lopez et al., 2009). These protocols require laboratory-based sample processing, take 1 to 2 h to obtain the results, utilize high price thermal cyclers, and require trained researchers to properly use.

Recombinase polymerase amplification (RPA), developed by Piepenburg, is remarkable due to its low cost, convenience, faster processing time, high specificity, and sensitivity (Piepenburg et al., 2006). The RPA reaction is based on the system containing three enzymes: recombinase, single-stranded DNA-binding protein, and strand-displacing polymerase (Supplementary Fig. 1A) (Li et al., 2019; Piepenburg et al., 2006; Yonesaki and Minagawa, 1985). The RPA assay requires constant low temperatures (30-42°C), and achieves amplification in a short time span of 15 min or less. The lateral flow strip (LFS) assay coupled with RPA can cause the extremely rapid visualization of RPA products (Crannell et al., 2014). The RPA-LFS assay is based on the Immuno-chromatographic with modified primer 5′ end with antigenic labels such as carboxyfluorescein (FAM), and Biotin and Digoxigenin (DIG) (Crannell et al., 2016; Lobato and O'Sullivan, 2018). The RPA amplicons containing FAM and biotin or FAM and DIG bind the nitrocellulose membrane, which visualizing the band on the strip with anti-Biotin, anti-DIG antibodies, and anti-FAM gold conjugates (Supplementary Fig. 1B). This study is aimed to establish a one-step RT-RPA-LFS assay to detect SMV, and to validate for on-site SMV detection in soybean samples from farms with equipment-free thermal source.

Materials and Methods

Plant materials and total RNA extraction. Soybean (Chamol, 01-0003-2013-2) leaves that showed mottle, mosaic, and yellow symptoms were obtained in 2022 from the National Institute of Crop Science of the Rural Development Administration, Korea. The plants were stored in a deep freezer at –70°C. The total RNA was extracted from the symptomatic leaves using an easy-BLUE total RNA extraction kit according to the manufacturer's instructions (iNtRON Biotechnology Inc., Seongnam, Korea). The total RNA from the SMV-infected soybean leaves were confirmed by RT-PCR with specific primer to SMV (Lee et al., 2012) (Supplementary Table 1). The total RNA was stored at –70°C for later use.

Design of the RT-RPA primer and modification. To design primers specific for RT-RPA assay, the viral protein genome-linked (VPg) and coat protein (CP) gene sequences of the SMV isolates (NC_002634.1, FJ640979.1, AY294044.1, FJ807700.1, MT603835.1, MT603826.1, FJ548849.1, MH919385.1, KT285170.1, and KP710868.1) were obtained from the GenBank database. The conserved regions of the SMV VPg gene were then found using multiple sequence alignments of MEGA 11 (Tamura et al., 2021). The RT-RPA primers were designed according to the TwistDx instruction manual and the PrimedRPA program (TwistDX, Ltd., Cambridge, UK) (Higgins et al., 2019). After the five primer sets targeting the SMV VPg and CP gene were designed, primer set was selected based on the RT-RPA results for the optimal. The FAM and biotin were attached to the 5′ end of the RT-RPA primers and were used for optimizing the RT-RPA-LFS assay.

RT-PCR assays. The RT-PCR was performed using the 2× SuPrimeScript RT-PCR Premix according to the manufacturer's protocol (GeNet Bio, Daejeon, Korea). The 20 µl of the total reaction volume was contained in 10 µl of 2× SuPrimeScript RT-PCR Premix, 7 µl of nuclease-free water, 1 µl of each of 10 pmoles of forward and reverse primers, and 1 µl of total RNA. The RT-PCR was conducted with the specific primer to SMV, soybean yellow mottle mosaic virus (SYMMV), tomato spotted wilt virus (TSWV), and cucumber mosaic virus (CMV) as described previously, and designed using the clover yellow vein vi-

rus (ClYVV) primer (Lee, 2022; Lee et al., 2012; Yoon et al., 2014) (Supplementary Table 1). The soybean *ACT* gene was used as the internal control for the RT-PCR assay (Libault et al., 2008) (Supplementary Table 1). The thermocycling conditions were as follows: reverse transcription at 50°C for 30 min; initial denaturation at 95°C for 10 min, followed by 35 cycles of denaturation at 95°C for 30 s; annealing at 55°C for 30 s and extension at 72°C for 40 s; and final extension at 72°C for 5 min in the All-in-One Cycler thermal cycler (Bioneer, Daejeon, Korea). The RT-PCR products were analyzed on 2% agarose gels stained with an ethidium bromide solution (Sigma-Aldrich, St. Louis, MO, USA).

RT-RPA-LFS assay. The RT-RPA assay was performed according to the manufacturer's instructions for the TwistAmp Basic kit (TwistDX, Ltd.). The RPA kit contains positive control primer and template to confirm the activity of kit components. The positive control containing RPA kit were conducted using 1 µl of the positive control DNA, 29.5 µl of rehydration buffer, 8 µl of the positive control primer mix, 2.5 µl of 280 mM magnesium acetate, and 9 µl of nuclease-free water (Qiagen, Hilden, Germany) with a total reaction volume of 50 µl. The components were incubated for 20 min at 38°C in an All-in-One Cycler thermal cycler (Bioneer). The RT-RPA assay to detect SMV was carried out using 1 µl of total RNA isolated SMV-infected soybean leaves, 29.5 µl of rehydration buffer, 2.4 µl of 4 µM forward primer (RPA_SMV_VPg: 5′-CTATATGGAGTTGAGCCAGAGAATTACA-3′), 2.4 µl of 4 µM reverse primer (RPA_SMV_VPg: 5′-TCTTTCCAATGAAATAAGCCTGTAAACC-3′), 1.0 µl of RevertAid reverse transcriptase (Thermo Fisher Scientific, Waltham, MA, USA), 2.5 µl of 280 mM magnesium acetate, and 11.2 µl of nuclease-free water, with a total reaction volume of 50 µl. The reaction was conducted for 15 min at 38°C in a thermal cycler. To combine the RT-RPA with a LFS assay, the RT-RPA amplified products were diluted at 1:99 with HybriDetect Assay Buffer in the Milenia GenLine HybriDectect 1 (Milenia Biotec, Giessen, Germany) in new reaction tube. The LFS was dipped into the mixture and incubated for 5 min at room temperature. The line with one purple band in the control line was considered negative, and the line with two visible bands in both control line and test line indicated a positive (Supplementary Fig. 1B). To detect the SMV without thermal equipment, some of the thermal sources were used to conduct RT-RPA-LFS assay such as heating block, thermos, and normothermia.

Results

Establishment of the RPA-LFS assay for the detection of SMV. The tests were initially carried out to ensure the RT-RPA primer could amplify the target site of 207 bp (RPA_SMV_VPg) in the SMV-infected leaf (Fig. 1A). The positive control to test the activity of the kit components was showed an amplification product of 141 bp (Fig. 1A). The RT-RPA combined with the LFS was successful in its detection of SMV against the SMV VPg gene and did not detect the no template control and healthy soybean plant similarly to the result on the agarose gel (Fig. 1B). These results suggested that the RT-RPA LFS could be used for detection of SMV in soybeans.

Specificity tests of RPA primers. The detection specificity of the primer sets was evaluated by RT-RPA and RT-

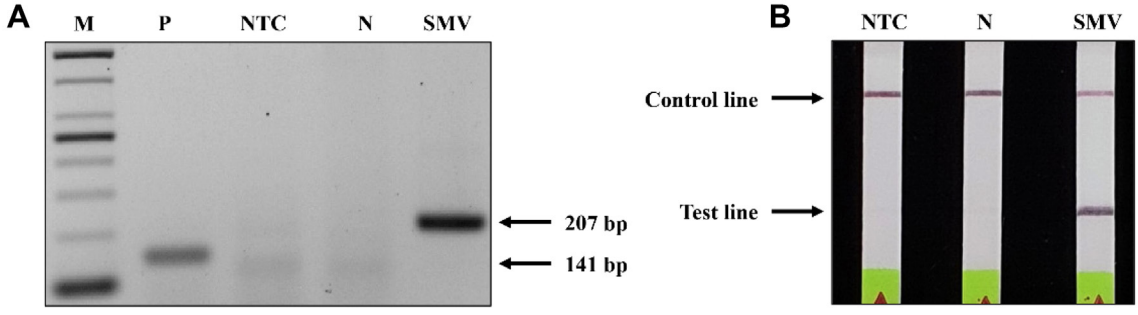

Fig. 1. Establishment of the reverse transcription-recombinase polymerase amplification (RT-RPA) experimental system for the detection of soybean mosaic virus (SMV). (A) The RT-RPA products were visualized on a 2% agarose gel. The amplicon size expected was 207 bp for the SMV primer. (B) The RT-RPA assay combined with lateral flow strip. The RT-RPA products were diluted at 1:99 with the HybriDetect Assay Buffer. Lane M, 100 bp DNA ladder marker (Cosmo Gentech, Seoul, Korea); lane P, the positive control provided by the manufacturer; lane NTC, no template control contained water; lane N, healthy soybean sample (negative control); lane SMV, SMV-infected template.

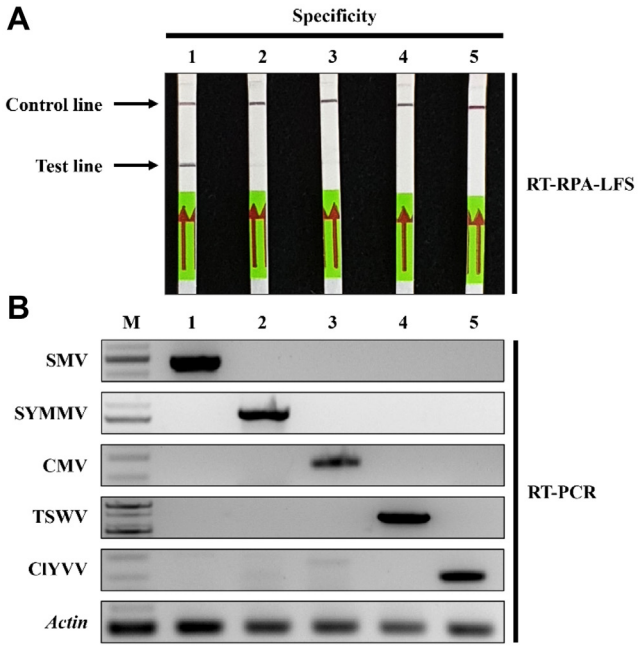

Fig. 2. The specificity of the reverse transcription-recombinase polymerase amplification (RT-RPA) assay and RT-RPA assay combined with the lateral flow strip (LFS) assay. The experiment used the soybean mosaic virus (SMV), soybean yellow mottle mosaic virus (SYMMV), cucumber mosaic virus (CMV), tomato spotted wilt virus (TSWV), and clover yellow vein virus (ClYVV) as they were the major soybean viruses. (A) The results of the RT-RPA-LFS assay to detect SMV. (B) Reverse transcription polymerase chain reaction (RT-PCR) products were visualized on 2% agarose gels. Lane 1, the SMV-infected sample; lane 2, the SYMMV-infected sample; lane 3, the CMV-infected sample; lane 4, the TSWV-infected sample; lane 5, the ClYVV-infected sample. Lane M is a 100 bp DNA marker. The *Actin* was used as an internal control.

RPA-LFS assay using the virus′ infected soybean. The RT-RPA-LFS results indicated that only the SMV-infected sample was shown as a distinct band without cross-reaction to SYMMV, TSWV, CMV, and ClYVV, which was not observed in the test line (Fig. 2A). The presence of the single SMV, SYMMV, TSWV, CMV, and ClYVV infected sample were verified by the RT-PCR using specific primers, respectively (Fig. 2B, Supplementary Table 1). Each sample was confirmed so that they were isolated from the soybean by an internal control primer that targeted the Actin gene.

Optimization of the RT-RPA-LFS assay. The optimal reaction conditions were determined through examination of temperature and reaction time. To validate the RT-RPA-LFS optimum reaction temperature, the reaction was carried out at serial different temperatures (30°C, 34°C, 38°C, 42°C, 46°C, and 50°C) for 15 min. The results of the different temperatures were visualized as a red band on the test line at 30 up to 46°C (Fig. 3A). The results showed that the red band faded on the strip at 46°C. To determine the RT-RPA-LFS minimum reaction time, the reaction was conducted at serial different reaction time (1 min, 5 min, 10 min, 15 min, 20 min) at 38°C (Fig. 3B). The red band on the test line was detectable after 5 min; however, the best reaction time was 15 min, which showed a thick band.

Sensitivity tests of the RPA primers. To evaluate the sensitivity of the RT-RPA LFS assay, a 10-fold serial dilution of the SMV total RNA 10 to 10^{-5} ng) was used to test the RT-RPA-LFS and RT-PCR. The sensitivity was established through a comparison of the RT-RPA-LFS with RT-

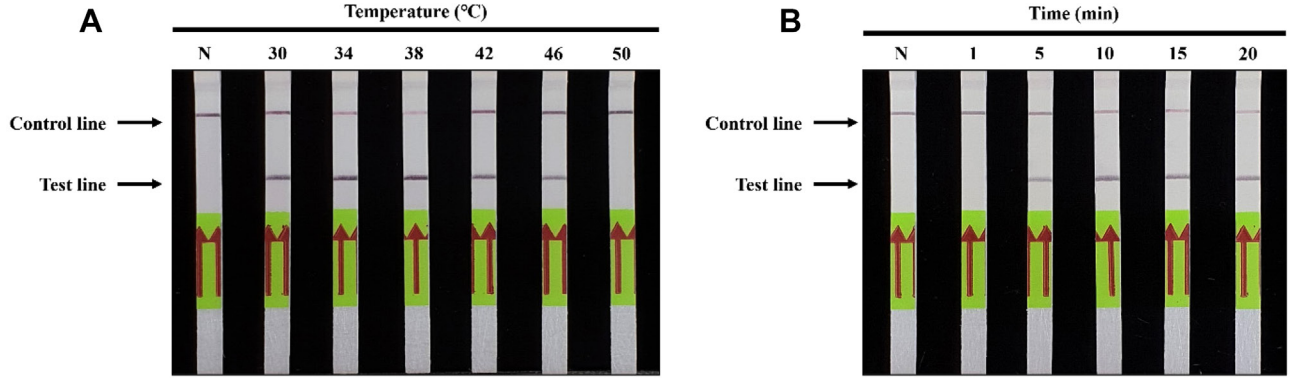

Fig. 3. The optimization of the reaction temperature and minimum time for the reverse transcription-recombinase polymerase amplification (RT-RPA) technique combined with a lateral flow strip (RT-RPA-LFS) assay. (A) The different conditions for each temperature were set up at 30°C, 34°C, 38°C, 42°C, 46°C, and 50°C. The determination of the optimal temperature of the RT-RPA-LFS reaction. The RT-RPA-LFS were shown as a red band from 30°C to 46°C on the test line for 15 min. (B) Assessment of the optimal reaction time of the RT-RPA assay at 38°C, from 1 to 20 min. The results were visualized with a distinct line at the test line from 5 to 20 min. The N was a healthy soybean plant sample.

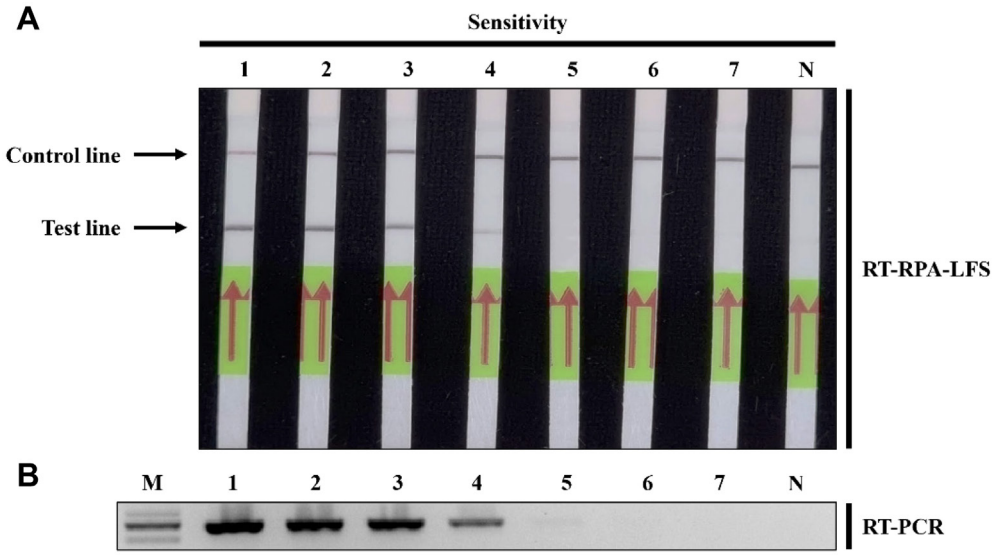

Fig. 4. The sensitivity of the reverse transcription-recombinase polymerase amplification technique combined with a lateral flow strip (RT-RPA-LFS) assay for detecting soybean mosaic virus (SMV). The RT-RPA-LFS and reverse transcription polymerase chain reaction (RT-PCR) were performed in a 10-fold serial dilution of total RNA from 10 to 10^{-5} ng. (A) In the RT-RPA-LFS assay, the detection limit was 1×10^{-2} ng (total RNA). (B) The RT-PCR were visualized on agarose gels. The RT-PCR assay was performed in a range of total RNA concentration from 10 to 10^{-2} ng. Lane M was a 100 bp DNA ladder maker. Lane N was a healthy soybean plant sample.

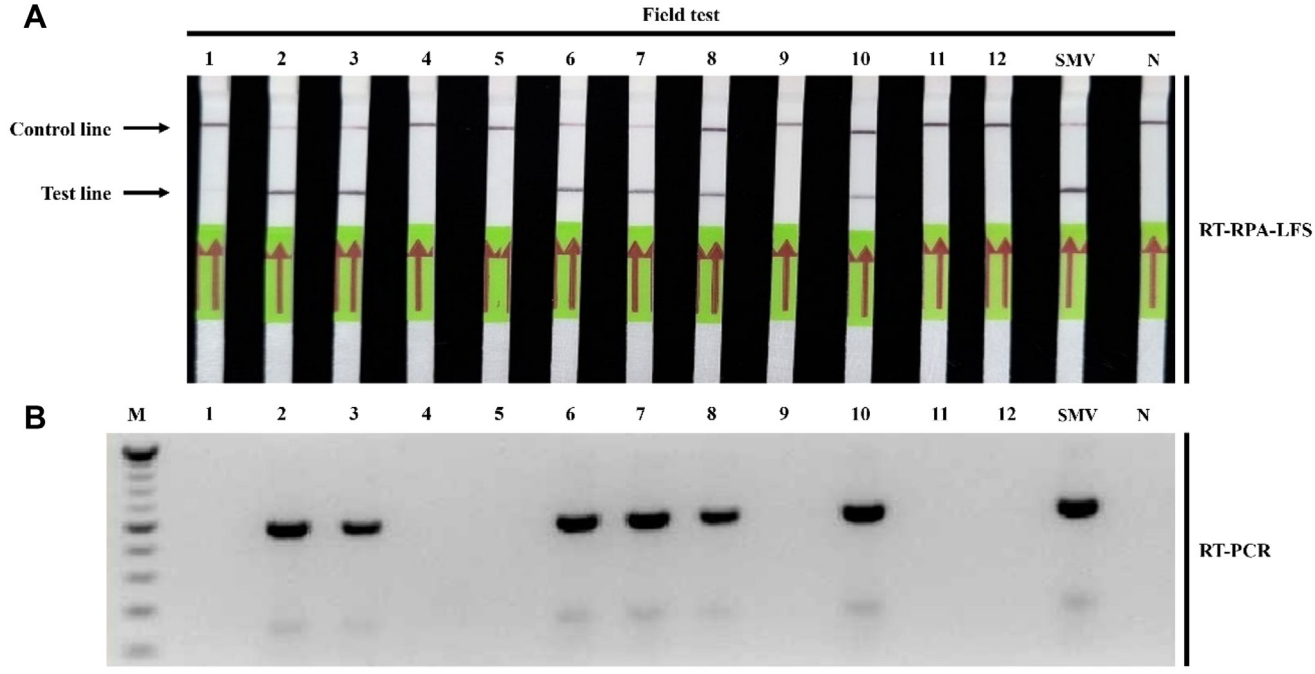

Fig. 5. The application of the reverse transcription-recombinase polymerase amplification technique combined with a lateral flow strip (RT-RPA-LFS) assay to the field samples. (A) The detection of soybean mosaic virus (SMV) in the field samples using the RT-RPA-LFS assay. (B) The reverse transcription polymerase chain reaction (RT-PCR) assay was compared using soybean samples collected from the field. Lane M, 100 bp DNA ladder maker; lanes 1-12, viral symptom-like soybean leaf samples; lane SMV, the SMV-infected sample (positive sample); lane N, the healthy soybean plant (negative sample).

PCR. The concentration of the total RNA isolated from the SMV-infected leaves was quantified using a BioTek Epoch microplate spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The RT-RPA-LFS results of the sensitivity showed a red band at least 10^{-2} ng (Fig. 4A). The results of the RT-PCR showed similar RT-RPA-LFS sensitivity, which could be observed by a band of at least 10^{-2} ng total RNA (Fig. 4B).

Application of the RT-RPA-LFS assay to detect SMV in field-collected samples. To confirm the reliability of the RT-RPA-LFS assay, 12 soybean samples with or without SMV and symptoms were used. The samples were collected in a soybean cultivation field in Gimje-si, Korea. The soybean samples were isolated from the total RNA and the test was conducted by RT-PCR and RT-RPA-LFS. Among the 12 soybean samples, six leaves were detected with SMV by the RT-RPA-LFS assay (Fig. 5A), which was consistent with the RT-PCR results (Fig. 5B). These results indicated that the RT-RPA-LFS assay could be used for SMV detection and screening in soybean fields.

RT-RPA-LFS assay using equipment-free thermal sources. To assess the equipment-free conditions, the RT-RPA-LFS was conducted on the heating block, thermos, and normothermia (Fig. 6A). The temperature of the thermal supplies was adjusted at 38°C in the heating block and thermos. After the RT-RPA-LFS reaction, the temperature was changed from 38 to 36.8°C in thermos conditions. The incipient temperature of the hand condition was 35°C. While the RT-RPA-LFS was conducted, the hand's temperature went back and forth between 34 and 35°C, measured by an electron thermometer. After the RT-RPA reaction was finished using the thermal supplies, the product was diluted with a HybriDetect Assay Buffer and used in an LFS. The red bands on the LFS were visualized by various conditions without non-specific amplification (Fig. 6B). This demonstrated that the RT-RPA-LFS assay could

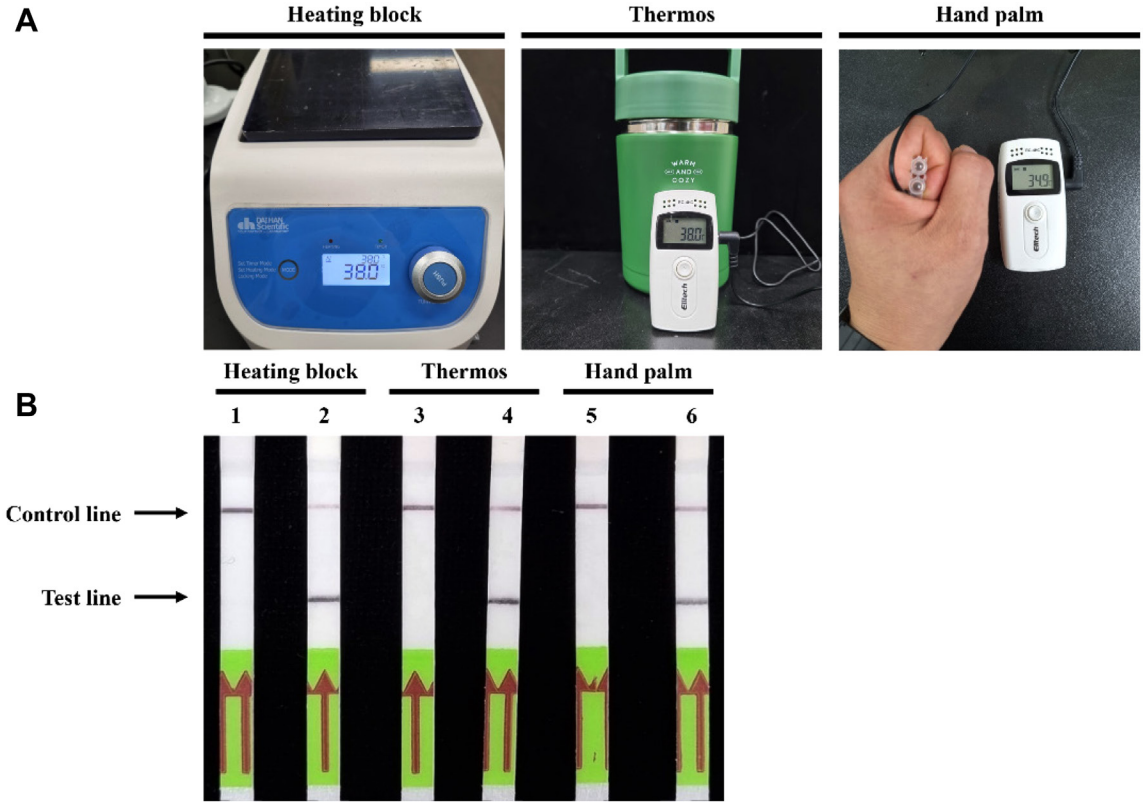

Fig. 6. The various equipment-free conditions for on-site diagnosis using the reverse transcription-recombinase polymerase amplification technique combined with a lateral flow strip (RT-RPA-LFS) assay. (A) The various substituted conditions used, such as a heating block (left), thermos (middle), and normothermia (right). The temperature at each condition was 38°C in a heating block, from 36.8°C to 38°C in a thermos, and from 34°C to 35°C in the palm of the hand. (B) Detection of the soybean mosaic virus (SMV)-infected sample using the RT-RPA-LFS assay in the thermal source without a thermocycler, such as the heating block (lanes 1-2), thermos (lanes 3-4), and hand palm (lanes 5-6). Lanes 1, 3, 5, healthy soybean plant; lanes 2, 4, 6, SMV-infected sample.

be utilized as an on-site diagnosis method.

Discussion

This study established that an RT-RPA-LFS assay could be used for the detection of the SMV. The specificity of RT-RPA-LFS assay for SMV detection did not show cross-reactivity with four soybean-infecting viruses (Fig. 2). Through the evaluation of reaction conditions, the RT-RPA-LFS assay can successfully amplify from flexible incubation temperature and within 5 min. Applicability of RT-RPA-LFS assay for on-site detection of SMV was determined by screening soybean leaves in a soybean cultivation field. In addition, the results of RT-RPA-LFS assay with simple thermal sources were showed this method was portable and user-friendly for on-site detection of SMV without an expensive thermocycler. The benefits of the RT-RPA-LFS assay were that it was rapid, user-friendly, portable, specific, and efficient. The results showed that the RT-RPA-LFS was likely to aid in the early and convenient detection of SMV in the laboratory and field.

In the past, the diagnosis systems were developed through PCR-based method to detect SMV such as RT-PCR, RT-qPCR, and RT-LAMP (Botelho et al., 2016; Kim et al., 1999; Lee et al., 2015b). These methods are limited in their on-site applicability, with RT-PCR and RT-qPCR requiring expensive instruments, especially RT-qPCR which demands a costly specific probe; while RT-LAMP has issues with cross-contamination among the four to six primers and with the detection of non-specific amplicons (Mehetre et al., 2021; Yan et al., 2014). In addition, extremely sensitive new methods, such as digital PCR (dPCR) and high throughput sequencing (HTS), are currently being developed. The dPCR and HTS can detect viruses in symptomless samples and identify an arbitrary viral disease (Lee et al., 2015a, 2023). Although dPCR and HTS have many advantages, there are limitations in that they are prone to representing false-positive results, requiring costly instruments and materials, and demanding particular software to analyze the data (Lee et al., 2015a, 2023). A comparison with other techniques indicated that the RT-RPA-LFS assay was appropriate for rapid and convenient diagnosis of SMV in soybean cultivation fields.

Many branches of bacteriology, mycology, and clinical medicine have researched the use of RPA assays (Jiang et al., 2022; Li et al., 2022; Liu et al., 2021). The RPA that is available is a combination of flocculation assay, electrochemical assay, chemiluminescent assay, silicon microring resonator-based photonic assay, surface-enhanced Raman scattering assay, and LFS assay (Del Rio et al., 2015; Kunze et al., 2016; Li et al., 2019; Qi et al., 2018; Tsaloglou et al., 2018; Wang et al., 2017). The RT-RPA combined with an LFS was first reported by Qi et al. (2018) and has becoming a promising method. Although RT-RPA-LFS has been actively researched as a diagnostic tool for plant diseases, duplex and multiplex RT-RPA-LFS have yet to be reported on. Most RT-RPA-LFS assays were developed using a probe-base kit that has since been discontinued by the manufacturer (Zhou et al., 2022; Zou et al., 2022a, 2022b).

This study showed that the RT-RPA-LFS procedure was capable of amplifying without a particular sophisticated thermocycler. The step for RNA extraction was able to be streamlined by finding the optimal extraction buffers (Yoon et al., 2014). Further research is needed to overcome a number of challenges, such as simplifying the series of processes, duplex and multiplex amplification, and ultimately commercializing the mobile package for use in agricultural fields.

Conflicts of Interest

No potential conflicts of interest relevant to this article was reported.

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Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (http://www.ppjonline.org/).

References

Botelho, S. R., Martins, T. P., Duarte, M. F., Barbosa, A. V., Lau, D., Fernandes, F. R. and Sanches, M. M. 2016. Development of methodologies for virus detection in soybean and wheat seeds. *MethodsX* 3:62-68.

Cho, E.-K. 2007. Severity of cowpea mosaic virus and putkong disease monitoring and purification of cowpea mosaic virus.

Res. Plant Dis. 13:30-33 (in Korean).

Cho, E. K. and Chung, B. J. 1976. Studies on identification and classification of soybean virus diseases in Korea. *Res. Plant Dis.* 15:61-68.

Crannell, Z., Castellanos-Gonzalez, A., Nair, G., Mejia, R., White, A. C. and Richards-Kortum, R. 2016. Multiplexed recombinase polymerase amplification assay to detect intestinal protozoa. *Anal. Chem.* 88:1610-1616.

Crannell, Z. A., Rohrman, B. and Richards-Kortum, R. 2014. Equipment-free incubation of recombinase polymerase amplification reactions using body heat. *PLoS ONE* 9:e112146.

Del Rio, J. S., Steylaerts, T., Henry, O. Y. F., Bienstman, P., Stakenborg, T., Van Roy, W. and O'Sullivan, C. K. 2015. Real-time and label-free ring-resonator monitoring of solid-phase recombinase polymerase amplification. *Biosens. Bioelectron.* 73:130-137.

Domier, L. L., Hobbs, H. A., McCoppin, N. K., Bowen, C. R., Steinlage, T. A., Chang, S., Wang, Y. and Hartman, G. L. 2011. Multiple loci condition seed transmission of soybean mosaic virus (SMV) and SMV-induced seed coat mottling in soybean. *Phytopathology* 101:750-756.

Domier, L. L., Steinlage, T. A., Hobbs, H. A., Wang, Y., Herrera-Rodriguez, G., Haudenshield, J. S., McCoppin, N. K. and Hartman, G. L. 2007. Similarities in seed and aphid transmission among soybean mosaic virus isolates. *Plant Dis.* 91:546-550.

Goodman, R. M. and Oard, J. H. 1980. Seed transmission and yield losses in tropical soybeans infected by soybean mosaic virus. *Plant Dis.* 64:913-914.

Hajimorad, M. R., Domier, L. L., Tolin, S. A., Whitham, S. A. and Saghai Maroof, M. A. 2018. Soybean mosaic virus: a successful potyvirus with a wide distribution but restricted natural host range. *Mol. Plant Pathol.* 19:1563-1579.

Higgins, M., Ravenhall, M., Ward, D., Phelan, J., Ibrahim, A., Forrest, M. S., Clark, T. G. and Campino, S. 2019. PrimedRPA: primer design for recombinase polymerase amplification assays. *Bioinformatics* 35:682-684.

Irwin, M. E. and Goodman, R. M. 1981. Ecology and control of soybean mosaic virus. In: *Plant diseases and vectors: ecology and epidemiology*, eds. by K. Maramorosch and K. F. Harris, pp. 181-229. Academic Press, Cambridge, MA, USA.

Jang, Y. W., Jo, Y., Cho, W. K., Choi, H., Yoon, Y. N., Lim, S. M., Lee, Y. H., Bae, J. Y. and Lee, B. C. 2018. First report of bean common mosaic necrosis virus infecting soybean in Korea. *Plant Dis.* 102:2051.

Jayaram, C., Hill, J. H. and Miller, W. A. 1992. Complete nucleotide sequences of two soybean mosaic virus strains differentiated by response of soybean containing the Rsv resistance gene. *J. Gen. Virol.* 73:2067-2077.

Jiang, T., Wang, Y., Jiao, W., Song, Y., Zhao, Q., Wang, T., Bi, J. and Shen, A. 2022. Recombinase polymerase amplification combined with real-time fluorescent probe for *Mycoplasma pneumoniae* detection. *J. Clin. Med.* 11:1780.

Jo, Y., Yoon, Y. N., Jang, Y.-W., Choi, H., Lee, Y.-H., Kim, S.-M., Choi, S. Y., Lee, B. C. and Cho, W. K. 2020. Soybean viromes in the Republic of Korea revealed by RT-PCR and next-generation sequencing. *Microorganisms* 8:1777.

Kil, E.-J., Park, J., Choi, H.-S., Kim, C.-S. and Lee, S. 2017. Seed transmission of tomato yellow leaf curl virus in white soybean (*Glycine max*). *Plant Pathol. J.* 33:424-428.

Kim, S.-M., Lee, J.-B., Lee, Y.-H., Choi, S.-H., Choi, H.-S., Park, J.-W., Lee, J.-S., Lee, G.-S., Moon, J.-K., Moon, J.-S., Lee, K.-W. and Lee, S.-H. 2006. First report of soybean dwarf virus on soybean (*Glycine max*) in Korea. *Res. Plant Dis.* 12:213-220 (in Korean).

Kim, Y.-H., Kim, O.-S., Lee, B.-C., Roh, J.-H., Kim, M.-K., Im, D.-J., Hur, I.-B. and Lee, S.-C. 1999. Detection of soybean mosaic virus using RT-PCR. Korean *J. Crop Sci.* 44:253-255.

Kunze, A., Dilcher, M., Abd El Wahed, A., Hufert, F., Niessner, R. and Seidel, M. 2016. On-chip isothermal nucleic acid amplification on flow-based chemiluminescence microarray analysis platform for the detection of viruses and bacteria. *Anal. Chem.* 88:898-905.

Lee, H.-J., Choi, S., Cho, I.-S., Yoon, J.-Y. and Jeong, R.-D. 2023. Development and application of a reverse transcription droplet digital PCR assay for detection and quantification of plantago asiatica mosaic virus. *Crop Prot.* 169:106255.

Lee, H. J., Kim, M.-K., Lee, S. G., Choi, C. S., Choi, H.-S., Kwak, H. R., Choi, G. S. and Chun, C. 2015a. Physiological characteristics of melon plants showing leaf yellowing symptoms caused by CABYV infection. *Hortic. Sci. Technol.* 33:210-218 (in Korean).

Lee, H. S., Lee, M. H. and Tochihara, H. 1980. An infectious virus isolated from soybeans. *Korean J. Plant Prot.* 9:175-179.

Lee, H. T. 2022. Development of lateral flow reverse transcription recombinase polymerase amplification (LF RT-RPA) for detection of Cucumber mosaic virus (CMV) on *Cnidium officinale* in field. M.S. thesis. Jeonbuk National University, Jeonju, Korea.

Lee, S. H., Choi, Y. M., Kim, J. S. and Chung, B. J. 1985. Identification of alfalfa mosaic virus from soybean. *Korean J. Plant Pathol.* 1:33-37 (in Korean).

Lee, Y.-H., Bae, D.-H., Kim, B.-S., Yoon, Y.-N., Bae, S.-D. Kim, H.-J. Mainali, B. P., Park, I.-H., Lee, S.-H. and Kang, H.-W. 2015b. Detection of soybean mosaic virus by reverse transcription loop-mediated isothermal amplification. *Res. Plant Dis.* 21:351-320 (in Korean).

Lee, Y. H., Lim, S. T., Yoon, Y. N., Jeon, M. G., Yun, H. T., Ko, J. M., Lee, S. H., Lee, K. W. and Baek, I. Y. 2012. Incidence of soybean viral diseases in Korea. *Korea Soybean Digest* 29:7-15.

Lee, Y.-H., Yoon, Y.-N., Yun, H.-T., Baek, I. Y., Lim, S., Moon, J. S. and Lee, S.-H. 2015c. First report of bean common mosaic virus infecting soybean in South Korea. *Plant Dis.* 99:1189.

Li, J., Macdonald, J. and von Stetten, F. 2019. Review: a comprehensive summary of a decade development of the recombinase polymerase amplification. *Analyst* 144:31-67.

Li, J., Zhong, Q., Shang, M.-Y., Li, M., Jiang, Y.-S., Zou, J.-J.,

Ma, S.-S., Huang, Q. and Lu, W.-P. 2022. Preliminary evaluation of rapid visual identification of *Burkholderia pseudomallei* using a newly developed lateral flow strip-based recombinase polymerase amplification (LF-RPA) system. *Front. Cell. Infect. Microbiol.* 11:804737.

Libault, M. A. R. C., Thibivilliers, S., Bilgin, D. D., Radwan, O., Benitez, M., Clough, S. J. and Stacey, G. 2008. Identification of four soybean reference genes for gene expression normalization. *Plant Genome* 1:44-54.

Lim, S., Lee, Y.-H., Igori, D., Zhao, F., Yoo, R. H., Lee, S.-H., Baek, I. Y. and Moon, J. S. 2014. First report of peanut mottle virus infecting soybean in South Korea. *Plant Dis.* 98:1285.

Liu, D., Shen, H., Zhang, Y., Shen, D., Zhu, M., Song, Y., Zhu, Z. and Yang, C. 2021. A microfluidic-integrated lateral flow recombinase polymerase amplification (MI-IF-RPA) assay for rapid COVID-19 detection. *Lab Chip* 21:2019-2026.

Liu, J.-Z., Fang, Y. and Pang, H. 2016. The current status of the soybean-soybean mosaic virus (SMV) pathosystem. *Front. Microbiol.* 7:1906.

Lobato, I. M. and O'Sullivan, C. K. 2018. Recombinase polymerase amplification: basics, applications and recent advances. *Trends Anal. Chem.* 98:19-35.

Lopez, M. M., Llop, P., Olmos, A., Marco-Noales, E., Cambra, M. and Bertolini, E. 2009. Are molecular tools solving the challenges posed by detection of plant pathogenic bacteria and viruses?. *Curr. Issues Mol. Biol.* 11:13-46.

Maury, Y., Duby, C., Bossennec, J.-M. and Boudazin, G. 1986. Group analysis using ELISA: determination of the level of transmission of soybean mosaic virus in soybean seed. *Agronomie* 5:405-415.

Mehetre, G. T., Leo, V. V., Singh, G., Sorokan, A., Maksimov, I., Yadav, M. K., Upadhyaya, K., Hashem, A., Alsaleh, A. N., Dawoud, T. M., Almaary, K. S. and Singh, B. P. 2021. Current developments and challenges in plant viral diagnostics: a systematic review. *Viruses* 13:412.

Ministry of Agriculture, Food and Rural Affairs. 2022. Cultivation status of flowering plants in Korea 2021. URL http://lib.mafra.go.kr/Search/Detail/49887/ [16 May 2023].

Nam, M., Kim, S. M., Domier, L. L., Koh, S., Moon, J. K., Choi, H. S., Kim, H. G., Moon, J. S. and Lee, S.-H. 2009. Nucleotide sequence and genomic organization of a newly identified member of the genus Carmovirus, soybean yellow mottle mosaic virus, from soybean. *Arch. Virol.* 154:1679-1684.

Nam, M., Park, S.-J., Kim, Y.-J., Kim, J.-S., Park, C. Y., Lee, J.-S., Choi, H.-S., Kim, J.-S., Kim, H. G. and Lee, S.-H. 2012. First report of peanut stunt virus on *Glycine max* in Korea. *Plant Pathol. J.* 28:330.

Piepenburg, O., Williams, C. H., Stemple, D. L. and Armes, N. A. 2006. DNA detection using recombination proteins. *PLoS Biol.* 4:e204.

Qi, Y., Yin, Q., Shao, Y., Li, S., Chen, H., Shen, W., Rao, J., Li, J., Li, X., Sun, Y., Lin, Y., Deng, Y., Zeng, W., Zheng, S., Liu, S. and Li, Y. 2018. Rapid and visual detection of *Coxiella burnetii* using recombinase polymerase amplification combined with lateral flow strips. *Biomed Res. Int.* 2018:6417354.

Shin, J.-C., Kim, M.-K., Kwak, H.-R., Choi, H.-S., Kim, J.-S., Park, C.-Y. and Cha, B. J. 2014. First report of clover yellow vein virus on *Glycine max* in Korea. *Plant Dis.* 98:1283.

Tamura, K., Stecher, G. and Kumar, S. 2021. MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* 38:3022-3027.

Tolin, S. A. and Lacy, G. H. 2004. Viral, bacterial, and phytoplasmal diseases of soybean. In: *Soybean: improvement, production, and uses*, eds. by H. R. Boerma and J. E. Specht, pp. 765-819. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI, USA.

Tsaloglou, M.-N., Nemiroski, A., Camci-Unal, G., Christodouleas, D. C., Murray, L. P., Connelly, J. T. and Whitesides, G. M. 2018. Handheld isothermal amplification and electrochemical detection of DNA in resource-limited settings. *Anal. Biochem.* 543:116-121.

Wang, J., Koo, K. M., Wee, E. J. H., Wang, Y. and Trau, M. 2017. A nanoplasmonic label-free surface-enhanced raman scattering strategy for non-invasive cancer genetic subtyping in patient samples. *Nanoscale* 9:3496-3503.

Yan, L., Zhou, J., Zheng, Y., Gamson, A. S., Roembke, B. T., Nakayama, S. and Sintim, H. O. 2014. Isothermal amplified detection of DNA and RNA. *Mol. Biosyst.* 10:970-1003.

Yonesaki, T. and Minagawa, T. 1985. T4 phage gene uvsX product catalyzes homologous DNA pairing. *EMBO J.* 4:3321-3327.

Yoon, J.-Y., Choi, G.-S., Cho, I.-S. and Choi, S.-K. 2014. Development of a novel sap extraction buffer for simultaneous detection of pepper viruses using VC/RT-PCR. *J. Korean Soc. Int. Agric.* 26:41-47 (in Korean).

Yoon, Y. N., Jo, Y., Cho, W. K., Choi, H., Jang, Y., Lee, Y. H., Bae, J. Y. and Lee, B. C. 2018. First report of tomato spotted wilt virus infecting soybean in Korea. *Plant Dis.* 102:461.

Zhou, Y., Zheng, H. Y., Jiang, D. M., Liu, M., Zhang, W. and Yan, J. Y. 2022. A rapid detection of tomato yellow leaf curl virus using recombinase polymerase amplification-lateral flow dipstick assay. *Lett. Appl. Microbiol.* 74:640-646.

Zou, X., Dong, C., Ni, Y., Yuan, S. and Gao, Q.-H. 2022a. Rapid detection of strawberry mottle virus using reverse transcription recombinase polymerase amplification with lateral flow strip. *J. Virol. Methods* 307:114566.

Zou, X., Yuan, S., Dong, C. and Gao, Q. 2022b. Development and evaluation of an isothermal recombinase polymerase amplification–lateral flow assay for rapid detection of strawberry vein banding virus in the field. *Crop Prot.* 158:105994.