Loop-mediated Isothermal Amplification Assay to Rapidly Detect Wheat Streak Mosaic Virus in Quarantined Plants

Siwon Lee1,†, Jin-Ho Kim2,†, Ji-Young Choi2,3 and Won-Cheoul Jang2*
1Water Supply and Sewerage Research Division, National Institute of Environmental Research, Incheon 404-708, Korea
2Department of Chemistry, College of Natural Science, Dankook University, Cheonan 330-714, Korea
3Institute of Tissue Regeneration Engineering (ITREN), Dankook University, Cheonan 330-714, Korea

(Received on June 12, 2015; Revised on August 9, 2015; Accepted on August 12, 2015)

We developed a loop-mediated isothermal amplification (LAMP) method to rapidly diagnose Wheat streak mosaic virus (WSMV) during quarantine inspections of imported wheat, corn, oats, and millet. The LAMP method was developed as a plant quarantine inspection method for the first time, and its simplicity, quickness, specificity and sensitivity were verified compared to current reverse transcription-polymerase chain reaction (RT-PCR) and nested PCR quarantine methods. We were able to quickly screen for WSMV at quarantine sites with many test samples; thus, this method is expected to contribute to plant quarantine inspections.

Keywords: LAMP, loop-mediated isothermal amplification, quarantine, wheat streak mosaic virus, WSMV

Wheat streak mosaic virus (WSMV) is an unreported virus in Korea (Animal, Plant and Fisheries Quarantine and Inspection Agency, 2013) and quarantine has raised the possibility that WSMV can cause serious food loss and economic damage (French and Robertson, 1994) by infecting wheat, corn, oats, and millet. Quarantine inspections have used reverse transcription-polymerase chain reaction (RT-PCR) and nested PCR quarantine methods. We were able to quickly screen for WSMV at quarantine sites with many test samples; thus, this method is expected to contribute to plant quarantine inspections.

We developed a LAMP method to detect WSMV in a one-step quick screen during quarantine inspection. Samples of WSMV and reference viruses [Cereal chlorotic mottle virus (CCMV) and Cucumber mosaic virus (CMV)] were collected through import approval of prohibited goods. Nucleic acids were extracted and cDNA was synthesized using a method reported previously. RNA of 170–200 ng/µl was extracted from the samples, and 100 µl cDNA was synthesized from the RNA.

The WSMA complete genome (NC_001886), 15 WSMV strains and their base sequences, and the base sequences of 20 reference virus strains that could infect the same host were collected from the National Center for Biotechnology Information. Two sets of WSMV-specific LAMP primers were designed based on the base sequences collected using the LAMP primer designing software PrimerExplorer (Table 1).

Three WSMV template cDNAs were chosen and reacted for 1 hr at three different temperatures (60, 62, and 65°C) to select the best LAMP conditions for detecting WSMV after 10 min at 95°C and 1 min at 4°C. As a result, condition 3 using an 11 µl total volume and no distilled water showed the most specific reaction. The analysis indicated that the specific reaction occurred at all temperatures, so the most stable conditions at 62°C were selected (Fig. 1). In addition, RNA of 170–200 ng/µl was extracted from the samples, and 100 µl cDNA was synthesized from the RNA.

The WSMA complete genome (NC_001886), 15 WSMV strains and their base sequences, and the base sequences of 20 reference virus strains that could infect the same host were collected from the National Center for Biotechnology Information. Two sets of WSMV-specific LAMP primers were designed based on the base sequences collected using the LAMP primer designing software PrimerExplorer (Table 1).

The LAMP method developed here can detect WSMV in imported samples within 1.5 hrs (30 min for cDNA synthesis and 60 min for LAMP) after RNA extraction. Thus,
Development of LAMP Assay for Detecting *Wheat streak mosaic virus*

Table 1. Loop-mediated isothermal amplification primer sequence of the amplified poly-protein coding gene to rapidly detect *Wheat streak mosaic virus*

| Set       | Primer | Sequence (5'→3')                                      | Length (nt) | G+C (%) | Tm (°C) |
|-----------|--------|------------------------------------------------------|-------------|---------|---------|
| WSMV_1    | F3     | GAGTTGGAGGAAGGAAA                                    | 18          | 44.4    | 49.0    |
|           | B3     | TTCGAAAAACGGTAAAG                                    | 18          | 44.4    | 49.3    |
|           | FIP    | GGACCAGAATATGAGTCTTTACACCGCAGAGT                     | 39          | -       | -       |
|           | BIP    | AGGAAGGTATGCTTTCATTTGCGGAGTATACTTTGCAAGAGC          | 41          | -       | -       |
| WSMV_2    | F3     | TACGACTCAGATATTGCTGT                                 | 20          | 40.0    | 50.2    |
|           | B3     | GCCTATGATATACTTGC A                                  | 20          | 40.0    | 49.4    |
|           | FIP    | TCGGTCTCGGAGATAGCGGAAAGAGGTGGAGA                     | 36          | -       | -       |
|           | BIP    | ACAAGGGAGGTCTTTGAGGATTTCGCCAAAGGTGAGTAA             | 41          | -       | -       |

This method reduces the time to detect WSMV from the previous 10 hrs using the RT-PCR, electrophoresis, nested PCR, and additional electrophoresis steps. Furthermore, it is much simpler and easier to use as the standard quarantine method. Additionally, specificity improved compared to that of the PCR method.

The WSMV cDNA template for LAMP was diluted up to $10^{-9}$. The RT-PCR primer combination [forward: 5'-TGG CGA TGA AGA TGT CAG-3', reverse: 5'-CCA TTT CTG TGA AGG CTT T-3' (834 bp)] was used previously to diagnose WSMV. The PCR template result was used for the nested PCR reaction to amplify the specific 299 bp gene (Lee et al., 2013a). The detection sensitivities of the two methods were compared. As a result, a specific band was formed at up to a $10^{-2}$ dilution in the nested PCR but the specific ladder from the LAMP assay was analyzed at up to a $10^{-3}$ dilution (Fig. 2).

After LAMP assay, the prepared 10 µl of LAMP products were digested with 10 U of restriction enzyme *BstI* (New England Biolabs, US) at 37°C for 2 hrs, then they were subjected to electrophoresis in a 1.5% agarose gel and visualized (digestion fragments [194 + 156 bp]) (Supplementary Fig. 2).

The LAMP assay was applied to quickly screen for WSMV during quarantine inspection of imported wheat, corn, oats, and millet. Instead of using Taq DNA polymerase, LAMP amplifies denatures, anneals, and extends at
the same temperature by using Bst polymerase, resulting in quicker detection than that of the existing PCR method. In addition, the 5'→3' exonuclease feature provides high detection sensitivity and a quick result (Ahn et al., 2010; Cho et al., 2013). The LAMP developed in this study is simple and more specific rather than the current two-step quarantine methods using RT-PCR and nested PCR and reduced processing time by approximately 8 hrs. Furthermore, the LAMP method had 10 fold higher detection sensitivity than the current two-step PCR method. We expect that this LAMP method will be applied for use during quarantine inspections in the future.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (grant number: 2009-0093829).

References

Ahn, Y. C., Nam, Y. H., Park, S. N., Cho, M. H., Sco, J. W., Yoon, I. K., Park, Y. H. and Jang, W. C. 2008. Detection of Mycobacterium tuberculosis by loop-mediated isothermal amplification assay. J. Korean Chem. Soc. 52:273–280.

Animal, Plant and Fisheries Quarantine and Inspection Agency. 2013. List of plant quarantine viruses in Korea newly revised in 2013. Res. Plant Dis. 19:67–75.

Cho, M. H., Jang, W. C. and Choi, J. G. 2013. Detection for methicillin resistant Staphylococcus aureus in using bio-chip based loop mediated isothermal amplification assay. J. Korean Chem. Soc. 57:81–87.

French, R. and Robertson, N. L. 1994. An RT-PCR method for the detection of the wheat streak mosaic virus. J. Virol. Methods 49:93–100.

Lee, S. 2013. A study of molecular biological detection methods for seed-transmitted viruses in quarantine. Ph.D. thesis. Dankook University, Cheonan, Chungcheongnam-do, Korea.

Lee, S., Cha, M., Kim, S. M., Heo, N. Y., Shin, Y. G. and Lee, S. H. 2014. Development of nucleotide primers for diagnostic RT-PCR and nested PCR detection of three seed-transmitted viruses (CRLV, SpLV and WCIMV) in quarantine. J. Agric. Life Sci. 48:75–83.

Lee, S., Kang, E. H., Chu, Y. M., Shin, Y. G. and Ahn, T. Y. 2013a. Development of PCR diagnosis system for plant quarantine seed-borne Wheat streak mosaic virus. Korean J. Microbiol. 49:112–117.

Lee, S., Kang, E. H., Heo, N. Y., Kim, S. M., Kim, Y. J. and Shin, Y. G. 2013b. Detection of Carnation necrotic fleck virus and Carnation ringspot virus using RT-PCR. Res. Plant Dis. 19:36–44.

Lee, S., Kang, E. H., Shin, Y. G. and Lee, S. H. 2013c. Development of RT-PCR and nested PCR for detection of four quarantine plant viruses belonging to Nepovirus. Res. Plant Dis. 19:220–225.

Lee, S., Lee, J. Y., Shin, Y. G., Lee, S. H. and Ahn, T. Y. 2015. Development and verification of nested PCR assay for detection of Tobacco rattle virus in plant quarantine. J. Bacteriol. Virol. 45:54–61.

Lee, S. and Shin, Y. G. 2014. Development and practical use of RT-PCR for seed-transmitted Prune dwarf virus in quarantine. Plant Pathol. J. 30:178–182.

Shin, Y. G. and Rho, J. Y. 2014. Development of a PCR diagnostic system for Iris yellow spot tospovirus in quarantine. Plant Pathol. J. 30:440–444.

Table 2. Optimal loop-mediated isothermal amplification conditions

| Components          | Condition (µl) |
|---------------------|----------------|
|                     | 1              | 2              | 3              |
| Template            | 1.5            | 1.5            | 1.5            |
| Bst. 10× reaction buffer | 2            | 2              | 2              |
| dNTP (10 mM)        | 2              | 2              | 2              |
| Primers             |                |                |                |
| F3 (10 pmol)        | 0.6            | 0.6            | 0.6            |
| B3 (10 pmol)        | 0.6            | 0.6            | 0.6            |
| FIP (10 pmol)       | 1.4            | 1.4            | 1.4            |
| BIP (10 pmol)       | 1.4            | 1.4            | 1.4            |
| Bst. polymerase (12 U) | 1.5           | 1.5            | 1.5            |
| Total (µl)          | 9              | 4              | 0              |

Table 2. Optimal loop-mediated isothermal amplification conditions

| Components          | Condition (µl) |
|---------------------|----------------|
|                     | 1              | 2              | 3              |
| Template            | 1.5            | 1.5            | 1.5            |
| Bst. 10× reaction buffer | 2            | 2              | 2              |
| dNTP (10 mM)        | 2              | 2              | 2              |
| Primers             |                |                |                |
| F3 (10 pmol)        | 0.6            | 0.6            | 0.6            |
| B3 (10 pmol)        | 0.6            | 0.6            | 0.6            |
| FIP (10 pmol)       | 1.4            | 1.4            | 1.4            |
| BIP (10 pmol)       | 1.4            | 1.4            | 1.4            |
| Bst. polymerase (12 U) | 1.5           | 1.5            | 1.5            |
| Total (µl)          | 9              | 4              | 0              |