Optimization and Application of Loop-mediated Isothermal Amplification Detection Method on Little Cherry Virus 1

Deya Wang (wangdeyasdny@163.com)
Zaozhuang University https://orcid.org/0000-0001-7865-6008
Zhili Li
Zaozhuang University
Weikang Yang
Zaozhuang University
Chenmeng Gao
Zaozhuang University
Xinran Cao
Qingdao Agricultural University
Yanmei Deng
Zaozhuang University

Short report

Keywords: Little cherry virus 1, loop-mediated isothermal amplification, detecting, Shandong

Posted Date: March 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-267415/v1

Abstract

Background: *Little cherry virus 1* (LChV-1) is one of the most pathogenic virus that frequently infect cherry and have caused serious economic damage to production and quality of cherry. But current detection methods of LChV-1 was less.

Objective: This study aims to optimization of loop-mediated isothermal amplification (LAMP) detection method on *Little cherry virus 1*, and lies the foundation for the application of LChV-1 fast diagnosis in the field in Shandong province.

Methods: We performed one-step RT-LAMP at temperature of 59℃ for 60 minutes, whereas the reaction system contained 5.2 mM Mg^{2+}, 8 U Bst 2.0 DNA polymerase, 1.4 mM deoxyribonucleotide and 0.2 and 1.6 μM of the outer and inner primers, respectively. And,we used the RT-LAMP method to detect 65 field samples from Shandong province, and discovered that 5 samples were infected by LChV-1, the detection results were consistent with the RT-PCR results.

Conclusions: The RT-LAMP method is highly specific, sensitive, practicable and efficient and suitable method for detection LChV-1 samples.

Synopsis

The RT-LAMP method for LChV-1 established and optimized in our study can be used for fast detection of samples infected by LChV-1, lying the foundation for fast diagnosis and comprehensive prevention of LChV-1 in the field.

Main Text

Virus diseases have becoming a key factor affecting yield and quality of cherry. At present, there are 14 virus diseases infecting sweet cherry in China [1–3], of which the Little cherry disease (LChD) have been found in most cherry growing areas. LChD is associated to two different viral species, Little cherry virus 1 (LChV-1) and Little cherry virus 2 (LChV-2), showing symptoms of size and sweetness reducing, ripening delaying of cherry fruits [4, 5]. LChV-1 belongs to the *Crinivirus* genus, family *Closteroviridae*, and was first discovered in Poland in 2004, mainly spread mainly through grafting and insects in the field. LChV-1 contains a positive single-strand RNA with 17,000 nts, with several open reading frames (ORFs). Current detection methods of LChV-1 mainly include ELISA, molecular hybridization, RT-PCR, immunoelectron microscopy [6]. However, ELSA, molecular hybridization and immunoelectron microscopy have complex experimental procedures and low sensitivity. At present, a diagnostic protocol of LChV-1 based on a one-step RT-LAMP was established, but the experimental results showed that the dependence on a mobile detection device [7]. This study aims to establish a specific, fast detection method of LChV-1, and lies the foundation for the application of LChV-1 fast diagnosis in the field.

Firstly, three sets of DNA primers (Tm/length/Loction, etc) were designed and synthesized based on the nucleotide sequence of the coat protein of LChV-1 for one step RT-LAMP detection system (Table 1), and the F3-3/B3-3 and FIP3-3/BIP3-3 primers were selected as the optimized primers. Analysis by gel electrophoresis showed that the product of RT-LAMP were positive bands (omitted).

Table 1
Primers for the RT-LAMP and RT-PCR detection of LChV-1

Primer combination	Primer Name	Sequence (5'-3')	Loction
I	F3-1	TTGTTAGAAGAACATTTCAGAGA	12740–12746
	B3-1	TGTCATTTTCTTTGTCACTGG	12938–12958
	FIP-1	CCAAAGCGGAAGTCCATTCAG-ATTGTCTTTA	12804–12824
		AGAAAAGTTCTGGTG	12764–12788
	BIP-1	AATCGACTACAACTAAATTAGCCGA-GCCATT	12852–12876
		CATATTTGATTTTTCCAAC	12905–12929
II	F3-2	GTTGACTGACGAATTCTTGAA	12718–12738
	B3-2	TGCCATTCATATTTGATTTTTCC	12908–12930
	FIP-2	AGGAGTCAATTGTACACCAGAAC-TTGTTAG	12780–12802,
		AAGAACATTTCAGAGA	12740–12762
	BIP-2	AATGGACTTCCGCTTTGGTATC-GCATCTTTG	12807–12828,
		CTATCGGCTAA	12869–12888
III	F3-3	GAAGAACATTTCAGAGATATTGTCT	12746–12770
	B3-3	TGTCATTTTCTTTGTCACTGG	12938–12958
	FIP-3	GAAGTAAAATACGATACCAAAGCGG-AAAAG	12816–12840,
		TTCTGGTGTACAATTGAC	12776–12798
	BIP-3	AAAAGTTCTGGTGTACAATTGAC-GCCATTCA	12776–12798,
		TATTTGATTTTTCCAAC	12905–12929

According to the reaction system of Warm Start LAMP Kit, we optimized the reaction temperature and time duration. After we amplified the product by setting the temperature gradient, we found that LChV-1 RT-LAMP results showed amplification at 56 ~ 63 ℃, whereas at 59 ℃, the bands showed an obvious ladder form, with clear bands of approximately 100–200 bp. After we set the time duration gradient, we found that there were amplified bands between 40 and 80 min, and after 60 min, the yield of the amplified

product stopped increasing. After we optimized the primers, the results showed successful amplification at different primer concentration ratios. Combining the instruction of Warm Start LAMP Kit that the internal/external primer concentration ratio was 8:1, we determined that the reaction temperature was 59°C, the time duration was 60 min, and the internal/external primer concentration ratio was 8:1 (Fig. 1). Then we separated the LAMP products of the LChV-1 infected samples using electrophoresis, we excised bands of approximately 200 bp for cloning. The sequencing results showed that the size of the cloned fragments was 206 bp. Sequence alignment using the NCBI database showed that the nucleotide homology between the LAMP product and the Tai'an LChV-1 isolate (GenBank: KR736335) was 96%, indicating that the obtained sequence is the genomic sequence of LChV-1 (omitted). Next, to verify method specificity, we detected the cherry samples infected by LChV-1, CVA and PDV using the RT-LAMP method. Only samples infected by LChV-1 were detected as positive, showing that this RT-LAMP system can be used for specific detection of LChV-1. Finally, we detected 5 samples infected by LChV-1 out of 65 candidates collected from Yantai, Tai'an, Linyi and Zaozhuang using RT-LAMP, with a detection rate of 7.7%. The RT-LAMP and RT-PCR results were consistent (Table 2).

Table 2

Detection of field samples

Region (city)	Variety	Tree old (year)	Number of samples	Positive number	
				RT-LAMP	RT-PCR
Yantai	Hongdeng,Sammy,Tieton	5–12	33	3	3
Tai'an	Tieton and Hongdeng	8–13	11	1	1
Linyi	Hongdeng	3–9	13	1	1
Zaozhuang	Hongdeng and Tieton	6–13	8	0	0

Discussion

LChV-1 is one of the most pathogenic virus that frequently infect cherry and have caused serious economic damage to production and quality of cherry [8, 9]. The present study aimed to establish a loop-mediated isothermal amplification (LAMP) method for the detection of LChV-1. The RT-LAMP method is fast, simple and sensitive, and is widely used in plant virus detection. In our study, we designed three groups of specific primers by sequence analysis of LChV-1. After primer screening and reaction system optimization, we established a specific RT-LAMP method for LChV-1 detection. This method can run one reaction within 60 min using water bath, whereas the reaction time of RT-PCR is 2h. Therefore, the RT-LAMP method established and optimized in our study can be used for fast detection of samples infected by LChV-1, lying the foundation for fast diagnosis and comprehensive prevention of LChV-1 in the field.

Conclusion

We establish a specific, fast detection RT-LAMP method of LChV-1, and the RT-LAMP method was used to detect 65 field samples from Shandong province, and discovered that 5 samples.

Abbreviations

RT-PCR: reverse transcription PCR; LChV-1: *Little cherry virus 1*; LChD: Little cherry disease; LAMP: loop-mediated isothermal amplification; CVA: *cherry virus A*; PDV: *Prune dwarf virus*

Declarations

Acknowledgements

We are grateful to Prof. Xuefeng Yuan, Shandong Agriculture University for valuable suggestions and critical modification on the manuscript.

Authors' contributions

DW conceived and designed the study. ZL, WY and LG performed the experiments. XC analyzed the epidemiological data. YD wrote the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Shandong Provincial Natural Science Foundation (ZR2019PC011), Scientific Research Foundation for Ph.D.Programs of Zaozhuang University (2018BS040), and Science and technology Program of Zaozhuang (2019NS03).

Availability of data and material

Healthy cherry samples and cherry samples infected with LChV-1, *Prune dwarf virus* (PDV) and *Cherry virus A* (CVA) virus were provided by Virus genetic engineering lab, Zaozhuang University.

Ethics approval and consent to participate

Not applicable.

Consent for publication

The authors declare that they agreed to publish this paper with the permission of the publishing houses

Competing interests

The authors declare that they have no competing interests.

References

1. Cui J C, Wang W H, Jia X H, Wang Z H, Tong W. Problems and development countermeasures of sweet cherry industry at home and abroad [J]. Journal of Fruit Science, 2017, 34(5): 620-631.
2. Zong X J, Wang W W, Wei H R, Wang J W, Chen X, Xu L, Liu Q Z. Development and application of a multiplex RT-PCR assay for detecting three sweet cherry virus species[J].Scientia Agricultura Sinica, 2014, 47(6): 1111-1118.
3. Wang W W, Zhong XJ, Wei H R, Wang J W, Zhu D Z, Tan Y, Liu Q Z. Symptom and detection of the virus causing mosaic disease in sweet cherry trees in Tai'an, Shandong[J]. Plant Protection, 2015, 41(2): 97-101, 129.
4. Rao W L, Li F, Zuo R J, Li R. First report of Little cherry virus 2 in flowering and sweet cherry trees in China[J]. Plant Disease, 2011, 95(11): 1484.
5. Lu M G, Gao R, Chen R R, Wu B, Zhang Z X, Li S F. First Report of Little cherry virus 1 in sweet cherry trees in China[J]. Plant Disease, 2015, 99(8): 1191.
6. Bruisson S, Lebel S, Walter B. Comparative detection of a large population of grapevine viruses by TaqMan® RT-qPCR and ELISA[J]. Journal of virological methods, 2017, 240: 73-77.
7. Tahzima R, Foucart Y, Peusens G. New sensitive and fast detection of Little cherry virus 1 using loop-mediated isothermal amplification (RT-LAMP)[J]. Journal of virological methods, 2019, 265: 91-98.
8. Dong W, Song Yakun, Wu Mingqin, Wang L, Wu Y Y . Advances in research of cherry virus diseases[J]. Chinese Agricultural Science Bulletin, 2005, 21(5): 332-336.
9. Wang J, Zhu D, Tan Y, et al. Complete nucleotide sequence of little cherry virus 1 (LChV-1) infecting sweet cherry in China[J]. Archives of virology, 2016, 161(3): 749-753.

Figures

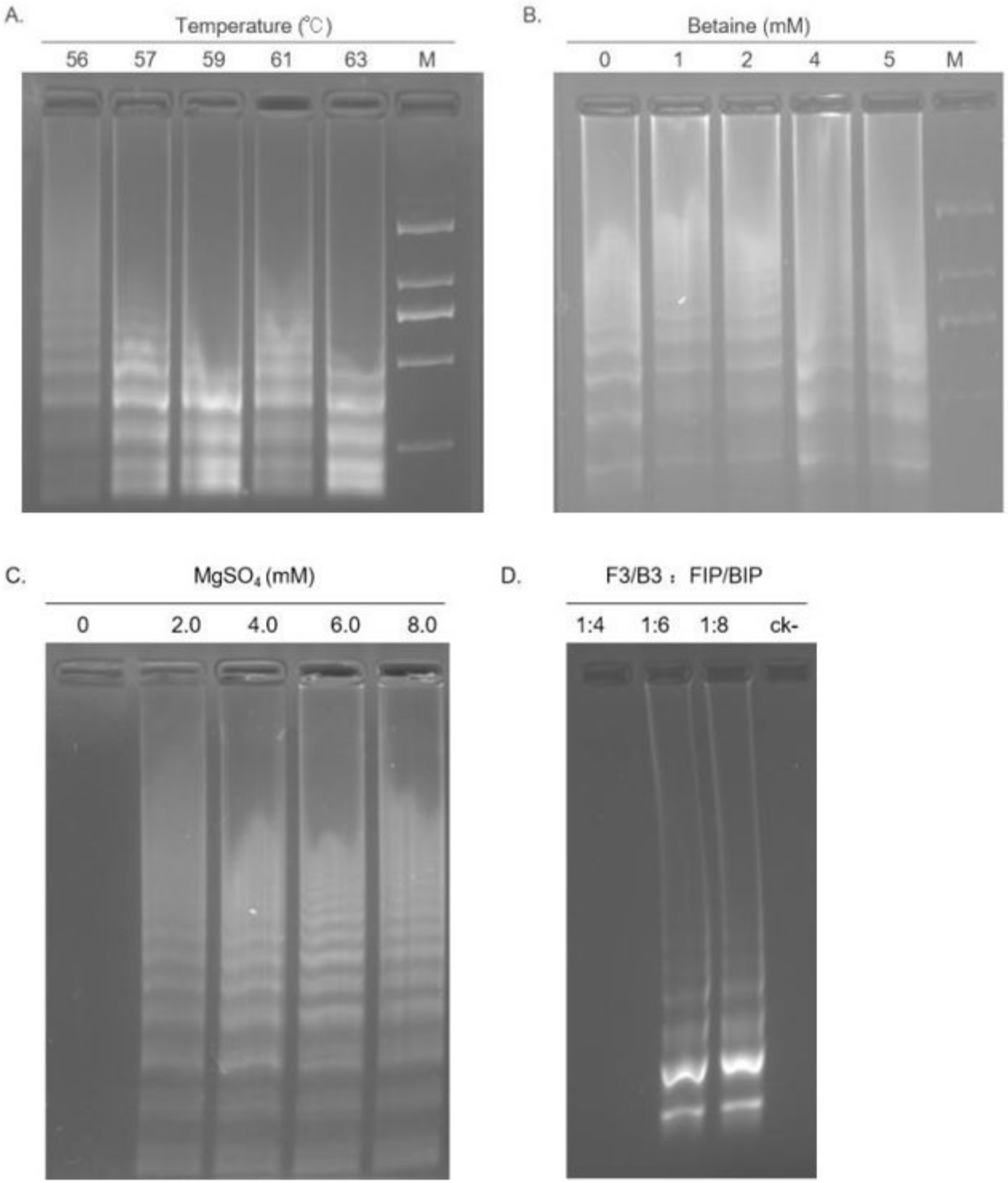

Figure 1

Screening of optimal temperature, time and internal/external primer ratio of the LChV-1 RT-LAMP reaction for RT-LAMP