DETECTION OF POTATO VIRUSES BY REVERSE TRANSCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION

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INTRODUCTION

Loop-mediated isothermal amplification assay (LAMP) facilitates fast and sensitive detection of DNA and RNA targets. The speed of detection of RNA can be improved by the addition of reverse transcriptase to form an RT-LAMP assay. RT-LAMP was reported as a favorable method of detection of many RNA viruses with a sensitivity similar to, or higher than, the real-time RT-PCR. The advantage of RT-LAMP is also its speed. When the assay is performed in the real-time fluorescent detection mode, the target virus can be detected in 5-30 minutes since the amplification commence. Furthermore, polymerases used in the LAMP reaction are less sensitive to amplification inhibitors than polymerases used in PCR reaction. Consequently, the RT-LAMP can be performed on crude tissue extracts or crude RNA preparations, saving the time and cost the assay. Availability of many color-changing approaches for detection of the LAMP amplicons facilitates the development of RT-LAMP variants with visual detection as a point-of-care test. Here, we present a development and optimization of RT-LAMP assays to detect the most important potato viruses including potato virus Y (PVY), potato virus M (PVM), potato leafroll virus (PLRV), potato virus S (PVS) and potato virus X (PVX).

MATERIAL AND METHODS

The juice from potato leaves (A) was squeezed out (B) and then diluted from 100 to 2 million times with the extraction buffer (C) (Fig. 1). RT-LAMP Isothermal AMPlification Kit. (e.g., Novazym Polska S.A., Cat. No.: RT-LAMP-02). Kit includes all enzymes and reagents necessary for fluorescent RT-LAMP so only target-specific primers and RNA templates have to be provided by the user. To increase the sensitivity of fluorescent detection, the kit contains pyrophosphatase, an enzyme degrading magnesium pyrophosphate. Thus, for the turbidimetric detection another kit should be chosen, e.g., Isothermal Mastermix - no dye, no pyrophosphatase (Cat. No.: ISO-001t,Novazym Polska S.A.). In such case, to detect RNA pathogens, the thermostable reverse transcriptase should be also purchased and supplemented into the reaction mix. The detection of DNA doesn't require this enzyme (Fig. 2). Using a CFX96 Touch™ Real-Time PCR Detection System (BioRad Ltd) the thermal profile to 60 cycles of 30 sec at 65°C. Follow the amplification by melting temperature analysis (65°C to 98°C, 0.5°C/sec) (Fig. 3).

Fig. 1 Procedure for obtaining dilutions from the juice.

Reactions number	1	96
Primer working mix[a]	2.63 µl	263 µl
H_2O nucleaze-free[b]	0.27 µl	27 µl
Reverse transcriptase[c]	0.10 µl	10 µl
Isothermal Mastermix	6.00 µl	600 µl

Fig. 2. Reaction mix
[a]The final concentration of primers in the reaction is 1.5 µM for FIP/BIP, 0.75 µM for LF/LB and 0.376 µM for F3/B3.
[b]For colorimetric detection instead of water add 0.2 µl of 6 mM stock of HNB dye and 0.07 µl of nuclease-free H_2O.
[c]For detecting DNA pathogens in place of reverse transcriptase add water. To save costs, choose kits w/o reverse transcriptase.

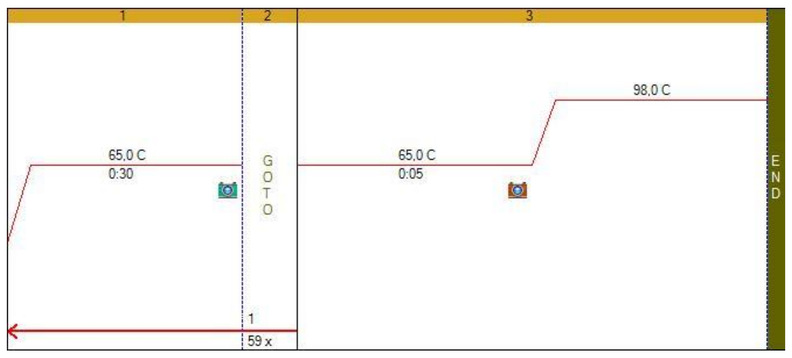

Fig. 3. Temperature profile on a CFX96 Touch™ Real-Time PCR Detection System (BioRad Ltd)

RESULTS AND DISCUSSION

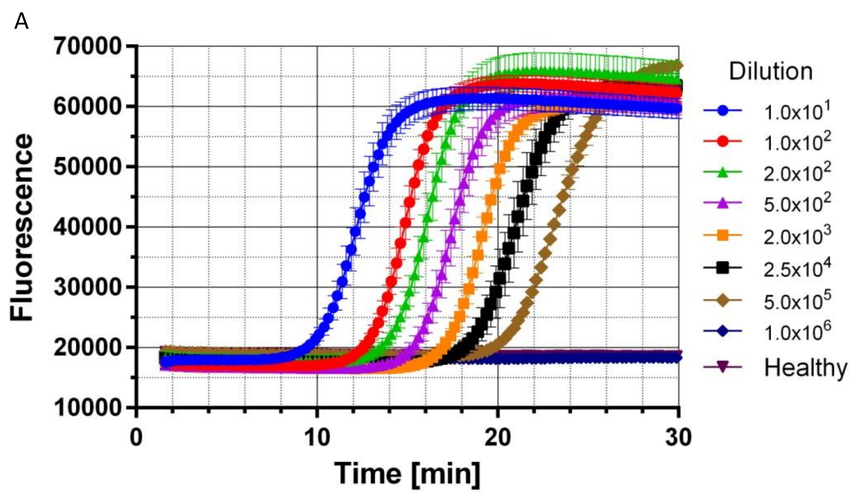

Fig. 4. Comparison of sensitivity RT-LAMP (A) and RT-PCR sensitivity (B). On panel B: 100 bp DNA marker (Novazym) - M, RNA dilution from 10 x to 0.5 million - lane 1 14, RNA from healthy plant - lane 15, water in RT reaction lane 16, PVY RNA - lane 17.

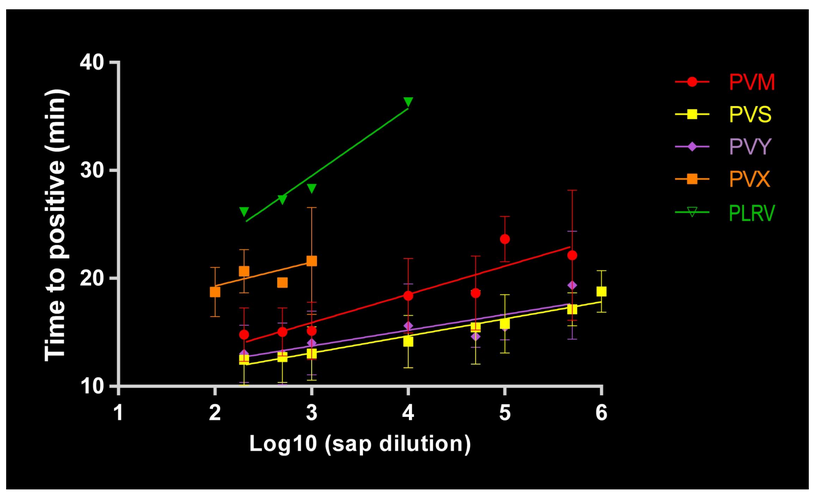

Fig. 5. Time after which a positive result was obtained for samples infected with five viruses: PVM, PVS, PVY, PVX, PLRV. Results obtained with the RT-LAMP test. The dilution of potato juice was used as a template in the test.

Amount of RNA								RT-LAMP with HNB
10 ng/µl	1 ng/µl	100 pg/µl	10 pg/µl	1 pg/µl	100 fg/µl	10 fg/µl	NTC (water)	
+	+	+	+	+	+	+	-	
+	+	+	+	-	-	-	-	
+	+	+	+	-	-	-	-	
+	+	+	+	-	-	-	-	
+	+	+	+	+	-	-	-	
+	+	+	-	-	-	-	-	
+	+	+	+	+	+	+	-	
+	+	+	+	+	+	+	nt	
+	+	+	+	-	-	-	nt	

Fig. 6. Sensitivity of RT-LAMP with hydroxy naphthol blue.The "+" sign means a positive result, the "-" sign is negative

CONCLUSION

1. The RT-LAMP test can be performed without RNA isolation.
2. Reduction of test time and cost.
3. It was also confirmed that the hydroxy naphthol blue may be useful in the development of a colorimetric version of the RT-LAMP test. However, this method requires further refinement.

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