台灣農業研究 (J. Taiwan Agric. Res.) 74(2):177–187 (2025)
https://doi.org/10.6156/JTAR.202506_74(2).0007

Research Article

Evaluation of the Use of One-Step Quantitative RT-PCR for Detection of Potato Virus X

Cheng-Ping Kuan[1,*], Ya-Ting Liu[2], Ssu-Yu Lin[3], Mei-Ju Lin[4], Shu Chen[5], and Yin-Hewey Cheng[6]

Abstract

Kuan, C. P., Y. T. Liu, S. Y. Lin, M. J. Lin, S. Chen, and Y. H. Cheng. 2025. Evaluation of the use of quantitative RT-PCR for the detection of potato virus X. J. Taiwan Agric. Res. 74(2):177–187.

This study developed a real-time reverse transcription-polymerase chain reaction (RT-PCR) detection method for potato virus X (PVX) by designing a specific primer pair based on the coat protein (CP) gene. The assay demonstrated a sensitivity approximately 10–100 times higher than conventional RT-PCR for PVX detection. Furthermore, it exhibited high specificity, accurately detecting PVX without cross-reacting with other solanaceous viruses or uninfected healthy plants. The reliability of this method was further confirmed through field testing on PVX-infected potato samples. Due to its high sensitivity and specificity, the real-time RT-PCR assay developed in this study has significant potential for the early detection of PVX infections in potatoes and tomatoes.

Key words: Potato virus X, Real-time RT-PCR, Detection.

INTRODUCTION

Potatoes (*Solanum tuberosum* L.) are susceptible to more than 40 virus species (Martelli *et al.* 2008; Kreuze *et al.* 2020). The principal viruses in potato crops causing significant economic damage in the world are potato virus X (PVX), potato virus Y (PVY), potato virus S (PVS), potato virus A (PVA), potato virus M (PVM), and potato leaf roll virus (PLRV) (Kumar *et al.* 2019). Potatoes are propagated vegetatively using seed tubers, facilitating the rapid and extensive spread of viruses. Therefore, the primary strategy for managing these viral diseases is to use healthy, virus-free seed potatoes and to test mother tubers and tissue culture planting materials to ensure free of virus infection (Kumar *et al.* 2019).

PVX is a member of the Potexvirus genus. Virus particles are filamentous with a single-stranded RNA genome, measuring approximately 515 × 13 nm (Jeevalatha *et al.* 2016). Symptoms caused by PVX infection include mild mottling or necrotic streaks, with highly susceptible potato varieties displaying pale or dark green spots on the leaves and irregular

Received: November 21, 2024; Accepted: February 26, 2025.
* Corresponding author, e-mail: pcr123@tari.gov.tw
[1] Associate Research Fellow, Crop Resource and Biotechnology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[2] Project Assistant, Crop Resources and Biotechnology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[3] Assistant Research Fellow, Crop Resource and Biotechnology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[4] Assistant Research Fellow, Plant Pathology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[5] Research Fellow and Division Director, Crop Resources and Biotechnology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[6] Research Fellow and Division Director, Department of Plant Protection and Utilization, Fengshan Tropical Horticultural Experiment Branch, Taiwan Agricultural Research Institute, Kaoshung, Taiwan, ROC.

small spots between the veins (Bendahmane *et al.* 1997; Tommiska *et al.* 1998). PVX can cause yield reductions ranging from 10% to 30% (Jeevalatha *et al.* 2016), and even 40% to 50% when it is in coinfection with PVA or PVY. PVX spreads primarily through agricultural tools or mechanical contact between healthy and infected plants (Jeevalatha *et al.* 2016; Kumar *et al.* 2019; Rashid *et al.* 2021). The main control measure for PVX is using virus-free seed potatoes (Kumar *et al.* 2019). Developing a straightforward and accurate diagnostic method is thus essential for the early detection of PVX infection.

Various diagnostic methods have been employed to identify PVX in potatoes, including visual inspection, mechanical inoculation onto indicator plants, electron microscopy, and enzyme-linked immunosorbent assay (ELISA) (Kumar *et al.* 2021). However, these techniques are often time-consuming, have low sensitivity, are non-specific, and are unsuitable for use on dormant tubers (Raigond *et al.* 2019). Recent advances have led to the use of more sensitive methods such as reverse transcription-polymerase chain reaction (RT-PCR) (Soliman *et al.* 2000; Yu *et al.* 2008; Jamal *et al.* 2012; Mandal *et al.* 2012), multiplex RT-PCR (Singh *et al.* 2004; Peiman & Xie 2006; Bostan & Peker 2009; Kumar *et al.* 2017). Although some methods can simultaneously detect multiple potato viruses (Agindotan *et al.* 2007; Jeevalatha *et al.* 2016), their sensitivity remains limited. In this study, the improved real-time RT-PCR method using a one-step approach is expected to detect PVX in potato leaves and tuber samples and compared to the RT-PCR method.

MATERIALS AND METHODS

Virus sources

Virus isolates, such as PVX, tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), cucumber mosaic virus (CMV), pepper mild mottle virus (PMMoV), PVY, pepper mosaic virus (PMV), chili vein mosaic virus (CVMV), and pepper vein mottle virus (PVMV), were obtained by Dr. Ting-Ching Deng (former virus specialist in Plant Pathology Division, Taiwan Agricultural Research Institute), and were maintained in tobacco (*Nicotiana benthamiana*) in the greenhouse (Cheng *et al.* 2013). Potatoes suspected of PVX infection were collected in a field in central Taiwan. PVX was inoculated and propagated in tobacco plants (*N. benthamiana*), tomato (*S. lycopersicum*), and potato (*S. tuberosum*).

RNA extraction

The purification method was mainly based on Agindotan (Agindotan *et al.* 2007), with slight modifications. 0.1 gram of tissue, collected from potato leaf/tuber, either PVX-infected or virus-free was ground using liquid nitrogen. The powdered tissue was then extracted with 0.5 mL of extraction buffer (diethyl pyrocarbonate (DEPC)-treated phosphate buffered saline (PBS) buffer (pH 7.4) containing 2% (w/v) polyvinylpyrrolidone-40 (PVP-40), 0.2% (w/v) egg albumin, and 1% (w/v) Na_2SO_3). To 36 µL of crude sap, 4 µL of RNA-secure reagent (20×) (Ambion) was added, mixed thoroughly, and incubated at 60℃ for 10 min. Throughout the experiment, crude sap was diluted using the extraction buffer.

Construction of the PVX-specific gene

The RNA from PVX-infected strains underwent a PCR reaction using the PVX-cp-751-F/R primer (as listed in Table 1). The amplified fragments were cloned into the pGEM-T Easy vector (Promega, Madison, WI, USA) and transformed into *Escherichia coli* (Protech, Taipei, Taiwan). Recombinant vector DNA was purified using the GeneSpin DNA extraction kit (Protech, Taipei, Taiwan). The plasmid containing the full-length PVX cp sequences was amplified by PCR with T7 + PVX-cp-112-F and PVX-cp-751-R primers. The resulting fragment was purified and used as a template for in vitro transcription with T7 RNA polymerase, following the MEGAscript® Kit protocol (Ambion™, Austin, TX, USA). The reaction products were dissolved in nuclease-free water, and the RNA concentration was calculated and serially diluted for sensitivity testing.

Table 1. The primers and probes used in this experiment.

Primers and probe	Sequence (5′–3′)[z]	Amplicon size (bp)
PVX-cp-751-F	TACTCGAAAGATGTCAGCACCAGCT	751
PVX-cp-751-R	GGGGTAGGCGTCGGTTATGTAGA	
PVX-cp-112-F	CCTATTCCAACGGCATCAG	112
PVX-cp-112-R	GTCAGCATCCAGTTCCATAC	
PVX-cp-112-Probe	TCGCTGCTGCCAGTCTGGC	
T7 + PVX-cp-112-F	FAM[y]-TAATACGACTCACTATAGGCCTATTC CAACGGCATCAG-BHQ1[y]	

[z] GeneBand Accession no. AF272736
[y] FAM: 6-carboxyfluorescein; BHQ1: black hole x quencher1

Design of PVX primers and probes

Primers and probes were designed using Primer Express™ software (Applied Biosystems, Foster City, CA, USA). The primers (PVX-cp-112-F/R) were specifically targeted to the cp gene sequence of PVX, with an expected product size of 112 bp. The RT-PCR amplified sequence included a binding site for the specific fluorescent probe PVX-probe (Table 1) to ensure that during nucleic acid amplification, the specific fluorescent probe generates fluorescence upon polymerase degradation, allowing the real-time RT-PCR machine to detect the fluorescent signal. The 5′ and 3′ ends were labeled with the fluorescent dyes FAM (6-carboxyfluorescein, excitation wavelength = 494 nm) and minor groove binder (MGB) (excitation wavelength = 520 nm), respectively.

RT-PCR and real-time RT-PCR methods

The RT-PCR reaction conditions were set to 50℃ for 15 min, and 95℃ for 3 min, followed by 40 cycles of 95℃ for 15 s, 60℃ for 30 s, and 72℃ for 30 s, with a final extension at 72℃ for 5 min and holding at 16℃. The PCR reagents included SuperScript™ III RT/Platinum™ Taq Mix (Invitrogen, San Diego, CA, USA) 0.5 μL, 2× Reaction Mix 12.5 μL, each 0.2 μM of PVX-cp-112 F/R primer 1 μL, probe 50 nM, Rox 50 nM, and plant RNA in a total reaction volume of 25 μL. The real-time RT-PCR conditions were 50℃ for 5 min, and 95℃ for 2 min, followed by 40 cycles of 95℃ for 3 s and 60℃ for 30 s. The real-time RT-PCR machine used was the ABI PRISM® 7500 Sequence Detection System (Applied Biosystems, Foster City, CA, USA).

ELISA

This experiment followed the method outlined by Clark & Adams (1977). A 0.2 g sample of diseased leaf tissue (tomato or potato) was ground in 2 mL of 15 mM sodium carbonate buffer (pH 9.6) and added to an ELISA plate (100 μL $well^{-1}$). Each sample was tested in duplicate, and the plates were incubated at 37℃ for 4 h. The plates were washed three times with 1× PBST (137 mM NaCl, 1.5 mM KH_2PO_4, 1 mM Na_2HPO_4, 0.05% Tween 20, pH 7.4), followed by the addition of PVX antibodies (DSMZ; Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) and a 2-hour incubation at 37℃. After washing three times with 1× PBST, goat anti-rabbit immunoglobulin secondary antibodies (Invitrogen, San Diego, CA, USA, 100 μL $well^{-1}$), dissolved in phosphate buffer (137 mM NaCl, 1.5 mM KH_2PO_4, 1 mM Na_2HPO_4, 0.05% Tween 20, pH 7.4), were added. The plates were incubated at 37℃ for 2 h. After washing four times with 1× PBST, a 150 μL $well^{-1}$ solution of 1 mg mL^{-1} alkaline phosphatase enzyme substrate (ρ-NPP, Amresco, Solon Ind., OH, USA) was added for color development. After 20 to 30 min of reaction, the absorbance at 405 nm was measured using an ELISA reader (PTI max microplate reader, Molecular Devices, Sunnyvale, CA, USA).

RESULTS

Specificity of RT-PCR and real-time RT-PCR for PVX detection

To develop primers and probes specific to PVX, this study utilized sequence alignment of the highly conserved region of the PVX coat protein (cp) gene. The designed primers, PVX-cp-112-F and PVX-cp-112-R, along with the probe PVX-cp-112-probe (listed in Table 1), were tested for specificity against a variety of other Solanaceae viruses, including PMMoV, PVX, TMV, ToMV, PVMV, CVMV, and PMV from tomato or pepper and PVY from potato. PMMoV, TMV, ToMV, PVY, PVMV, CVMV, and PMV were used by RT-PCR and real-time RT-PCR assays, respectively. The healthy plants of tomato were as healthy controls. A no-template control (NTC) using ddH_2O was also included to ensure no contamination. The agarose gel electrophoresis analysis revealed that the PVX-positive controls, which included cloned PVX plasmids and PVX-infected plants, produced a specific 112 bp nucleic acid fragment. No amplification was detected in the samples from other Solanaceae viruses or the healthy plants (Fig. 1). This result confirms the high specificity of the primers and probe for PVX.

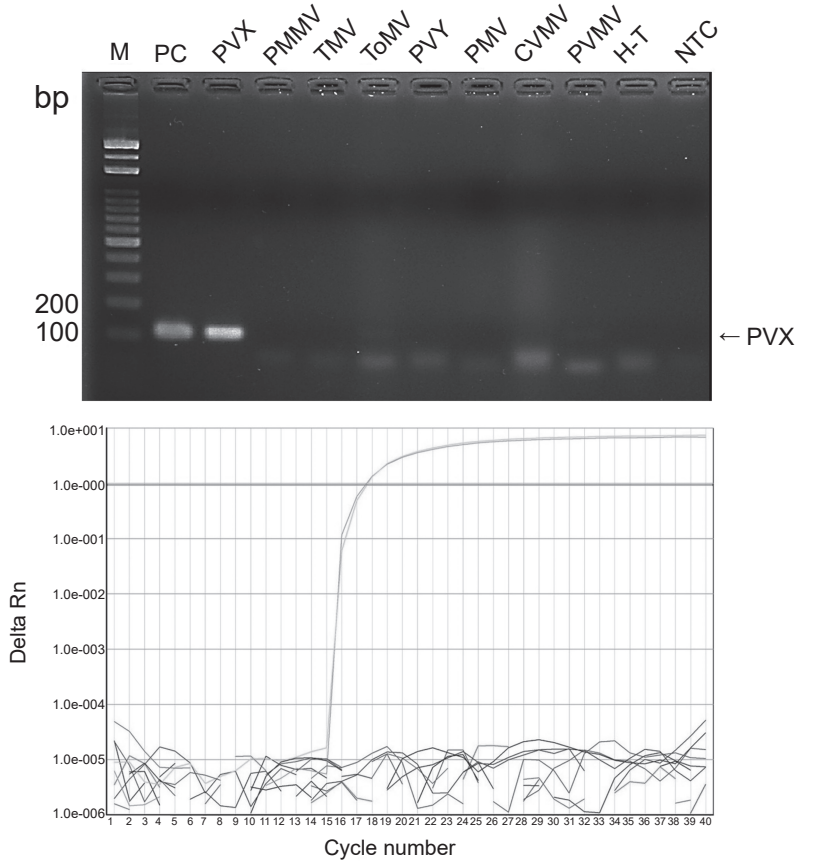

Fig. 1. Specificity evaluation of potato virus X (PVX) detection based on the reverse transcription-polymerase chain reaction (RT-PCR) (upper) and real-time RT-PCR (lower) assays. Amplification of PVX, pepper mild mottle virus (PMMV), tobacco mosaic virus (TMV), tomato mosaic virus (ToMV), potato virus Y (PVY), pepper mosaic virus (PMV), chili vein mosaic virus (CVMV), and pepper vein mottle virus (PVMV)-infected plants by RNA extracts. Health tomato samples as negative control (H-T). In RT-PCR, a 112-bp amplicon was generate only from positive control (PC) and PVX. Lane NTC: No Template Control and Lane M: 100 bp ladder.

Additionally, real-time RT-PCR results were consistent with those of RT-PCR, showing positive signals only for the PVX-positive controls, with Ct values of 12.89 and 15.1 for the PVX plasmid and PVX-infected plants, respectively. No reactions were observed for other viruses, healthy plants, or the negative controls. These findings indicate that the primers and probes are highly specific for PVX detection, without cross-reactivity with other Solanaceae viruses.

Sensitivity test of PVX

To understand the relationship between the detected viral load and the detection limit of PVX in tomatoes and potatoes after artificial infection, RT-PCR and ELISA were used for Evaluation. The detection limit of ELISA in tomatoes reached 10^6 dilution factor, while in potatoes, it reached 10^3. The results indicate that the detection limit in tomatoes is approximately 1,000 times greater than in potatoes. The detection limit of RT-PCR in tomatoes reached 10^8 dilution factor, while in potatoes, it reached 10^5. The results indicate that the detection limit in tomatoes is approximately 1,000 times greater than in potatoes (Table 2). For the same material (tomato/potato), the sensitivity of RT-PCR is approximately 100 times higher than that of ELISA.

The sensitivities of the RT-PCR and real-time RT-PCR methods for detecting PVX were evaluated using whole RNA extracted from PVX-infected plants and in vitro transcribed PVX coat protein RNA. The PVX coat protein (CP) RNA was detected at a concentration of 10^3 copies using RT-PCR and 10 copies using real-time RT-PCR (Fig. 2 and Fig. 3). These results indicate that the real-time RT-PCR method is approximately 100 times more sensitive than traditional RT-PCR in detecting low levels of PVX.

Detection of PVX in potato from field samples

The detection of PVX in potato samples was conducted by extracting total RNAs from the leaf/tuber tissue of suspected infected plants. Healthy potato leaves and a NTC were negative controls. The presence of PVX was assessed using RT-PCR, real-time RT-PCR, and ELISA. According to the results, both RT-PCR and real-time RT-PCR could successfully detect PVX from field samples (Table 3). Seven out of 20 field samples

Table 2. Comparison of reverse transcription-polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) for detecting potato virus X (PVX) using the detection limit of extracted infected leaf samples.

	Tomato[z]		Potato[z]	
Dilution factor	RT-PCR	ELISA $A_{405\ nm}$	RT-PCR	ELISA $A_{405\ nm}$
1	+	3.484+	+	3.064+
1 × 10	+	3.545+	+	2.116+
1×10^2	+	3.490+	+	1.134+
1×10^3	+	2.482+	+	0.266+
1×10^4	+	0.702+	+	0.087–
1×10^5	+	0.483+	+	0.076–
1×10^6	+	0.295+	–	0.070–
1×10^7	+	0.161–	–	0.090–
1×10^8	+	0.102–	–	0.085–
1×10^9	–	0.070–	–	0.084–
1×10^{10}	–	0.105–	–	0.101–
H-CK	–	0.105–	–	0.101–

[z] Plant tissue testing starting quantity: 0.1 g (RT-PCR); 0.2 g (ELISA).

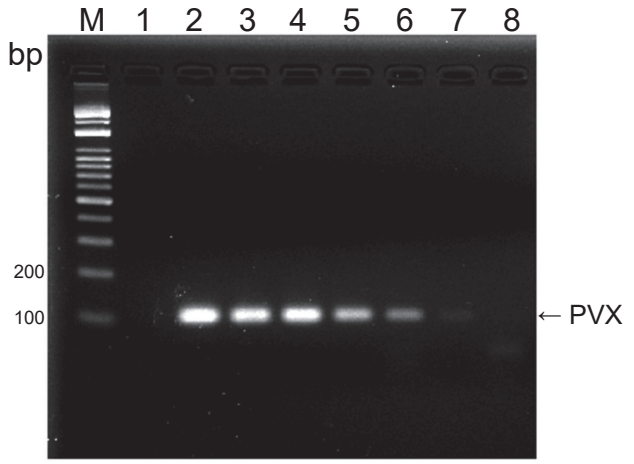

Fig. 2. The detection limit of reverse transcription-polymerase chain reaction (RT-PCR) assay based on cRNA copy number of potato virus X (PVX). PVX copy number: 1: NTC 2. Positive control, 3: 10^7; 4: 10^6; 5: 10^5; 6: 10^4; 7: 10^3; 8: 10^2 copies uL^{-1}.

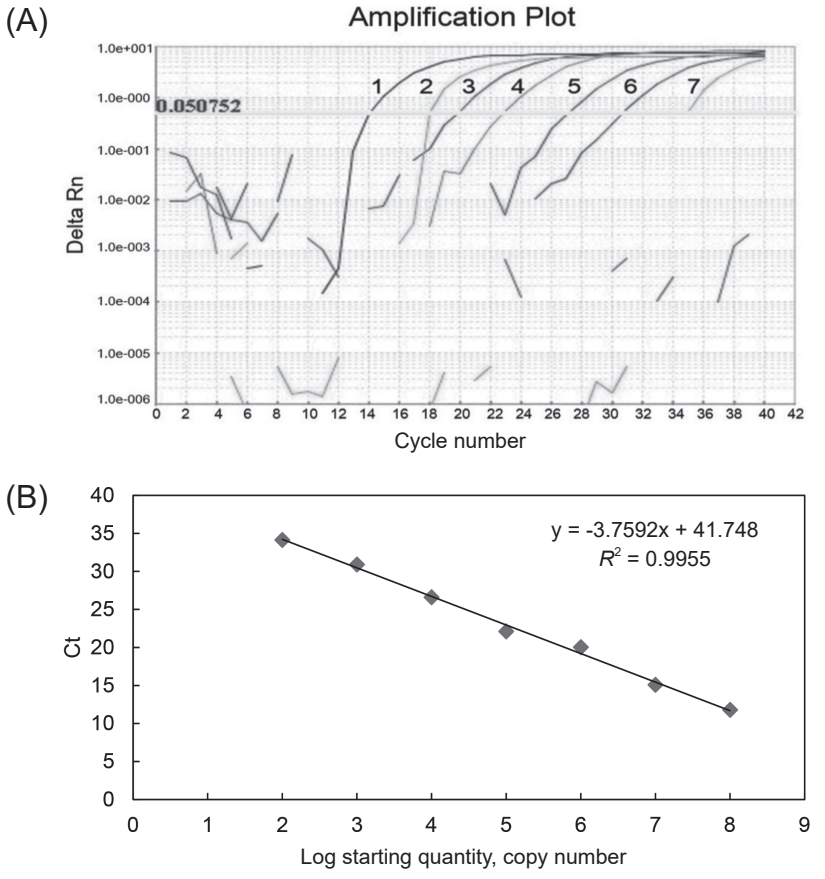

Fig. 3. Sensitivity detection of potato virus X (PVX) using real-time reverse transcription-polymerase chain reaction (RT-PCR) assay: (A) The results of real-time RT-PCR; and (B) The real-time RT-PCR was plotted as a standard curve. PVX copy number: 1: 10^7; 2: 10^6; 3: 10^5; 4: 10^4; 5: 10^3; 6: 10^2; 7: 10^1 copies uL^{-1}.

Table 3. Comparison of different methods for detecting potato virus X (PVX) infection in field-grown potatoes.

Tissues[z]	RT-PCR	Real-time RT-PCR	Ct	Enzyme-linked immuno-sorbent assay (ELISA)	$A_{405\ nm}$
S1	+[y]	+	30.41	+	0.452
S2	+	+	29.82	+	0.771
S3	–[y]	–	ND	–	0.109
S4	–	–	ND	–	0.142
S5	–	–	ND	–	0.195
S6	+	+	24.16	+	0.539
S7	–	–	ND	–	0.072
S8	–	–	ND	–	0.020
S9	–	–	ND	–	0.084
S10	+	+	29.92	+	0.761
S11	–	–	ND	–	0.122
S12	–	–	ND	–	0.141
S13	–	–	ND	–	0.084
S14	+	+	31.17	–	0.057
S15	+	+	21.87	+	0.947
S16	–	–	ND	–	0.139
S17	–	–	ND	–	0.172
S18	+	+	30.09	–	0.160
S19	–	–	ND	–	0.184
S20	–	–	ND	–	0.161
Positive control[x]	+	+	12.51	+	2.939
Negative control[x]	–	–	–	–	0.212
NTC[x]	–	–	–	–	0.162

[z] S1–S15: leave tissues; S16–S20: tuber tissues.
[y] "+" indicates positive reaction, whereas "–" indicates a negative reaction.
[x] Negative control: healthy tomato; Positive control: PVX-infected tomato; NTC: no template control.

(leaves or tubers) were positive for PVX using RT-PCR. In contrast, only five samples were positively tested using ELISA.

DISCUSSION

PVX is a highly destructive virus that causes a significant threat to potato crops due to its ease of transmission through mechanical contact. It has also been found in potato fields in Taiwan (Deng *et al.* 1992). Effective management of PVX requires early detection or using of virus-resistant cultivars. PVX is primarily transmitted through potato tubers, the application of this real-time RT-PCR assay for large-scale testing of potato tubers will add substantial value to the research. Currently, RT-PCR and ELISA are widely used as PVX detection methods. Internationally, multiplex detection methods for potato viruses have been developed, allowing the detection of multiple viruses (PVX, PVY, PVA, and PLRV). However, real-time RT-PCR thermocycler have limitations (limited by the number of instrument fluorescence channels). As a result, it is not possible to simultaneously detect all four viruses while incorporating an additional internal control for validation (Agindotain *et al.* 2007). The real-time RT-PCR method developed in this study amplifies a 112 bp fragment of the PVX viral gene. One key advantage of this method is its ability to prepare

samples in a single reaction tube, which eliminates the need for a separate cDNA synthesis step and enables one-step nucleic acid amplification. This simplifies the detection process by reducing the number of operational steps and enhances the efficiency of virus detection.

Detection from diluted PVX-infected leaf tissue showed that, regardless of whether RT-PCR or ELISA was used, the detectable dilution factor in infected tomato tissue could detect lower than that in potato tissue. This suggests that the PVX concentration in tomatoes is higher than in potatoes. This phenomenon requires more sampling and repeated experiments for validation, as the current results are only from a preliminary test. There is no related research mentioned, but PVX can infect both tomato and potato plants, causing varying symptoms. In tomatoes, PVX infection leads to mosaic patterns and slight stunting, while in potatoes, it typically results in mild mosaic symptoms or remains latent. The severity of PVX symptoms can be influenced by factors such as mixed infections with PVY (Verchot 2021). Sensitivity tests indicated that the real-time RT-PCR method developed in this study is comparable to previous findings by Agindotan *et al.* (2007), demonstrating at least a 10-fold increase in sensitivity. Notably, while Agindotan *et al.* (2007) utilized a two-step real-time RT-PCR method, this study's one-step approach streamlines the process, further improving detection efficiency. In other words, the sensitivity results showed that the real-time RT-PCR in this study is 100 times more sensitive than conventional RT-PCR and 10 times superior to the two-step method. Field tests on 20 potato samples (leaves or tubers) revealed that the real-time RT-PCR method was consistent with traditional RT-PCR in terms of positive virus detection. However, the real-time RT-PCR assay detected more positive samples than double-antibody sandwich ELISA (DAS-ELISA), RT-PCR identified 7 positive samples, whereas ELISA detected only 5 samples. In addition, the use of potato tubers showed that only one sample was detected positively by RT-PCR and real-time RT-PCR; however, ELISA could not detect the target virus. The inability of DAS-ELISA to detect PVX in some potato tuber samples might be due to the viral concentration being below the detection threshold or the uneven distribution of the virus in dormant tubers like PVY in potato (Whitworth *et al.* 2012). PVX tends to multiply during the sprouting process (Betti *et al.* 2012), leading to a higher viral load in sprouts, which makes it easier to be detected. Consequently, sprouting tuber samples are more suitable for DAS-ELISA detection. The one-step real-time RT-PCR assay demonstrated a 10-fold higher sensitivity compared to RT-PCR, confirming its superiority in detecting PVX, particularly in cases of low viral load. Moreover, the consistency between RT-PCR and real-time RT-PCR in detecting positive samples reinforces the reliability of the developed assay. Some samples that tested negative by ELISA were detected as positive by RT-PCR, highlighting the limitations of ELISA in detecting low-concentration or unevenly distributed viruses.

Previous studies have demonstrated that real-time RT-PCR is more sensitive than conventional RT-PCR for detecting plant RNA viruses (Babu *et al.* 2017; Kapoor *et al.* 2017; DeShields *et al.* 2019; Mohandas & Bhat 2020; Naveen & Bhat 2020; Wang *et al.* 2020). In addition, since RNA extraction in this study does not require the use of commercial kits and only utilizes simple isolation without complex procedures, it can reduce detection costs for large-scale sample screening. With minimal sample preparation requirements, the one-step real-time RT-PCR assay exhibits high resistance to inhibitors found in plant tissues, thereby improving specificity and sensitivity. The developed one-step real-time RT-PCR assay represents a significant advancement in PVX detection technology. Its rapid, sensitive, and specific detection capabilities, make it an ideal alternative to conventional RT-PCR for use in

tissue culture plants, virus-free certification programs, and screening of PVX-resistant cultivars. By facilitating early detection and reducing the spread of PVX, this assay contributes to more effective management of potato crops, ultimately supporting agricultural productivity and sustainability.

REFERENCES

Agindotan, B. O., P. J. Shiel, and P. H. Berger. 2007. Simultaneous detection of potato viruses, PLRV, PVA, PVX and PVY from dormant potato tubers by TaqMan® real-time RT-PCR. J. Virol. Methods 142:1–9. doi:10.1016/j.jviromet.2006.12.012

Babu, B., B. K. Washburn, T. S. Ertek, S. H. Miller, C. B. Riddle, G. W. Knox, … M. L. Paret. 2017. A field-based detection method for Rose rosette virus using isothermal probe-based Reverse transcription-recombinase polymerase amplification assay. J. Virol. Methods 247:81–90. doi:10.1016/j.jviromet.2017.05.019

Bendahmane, A., K. Kanyuka, and D. C. Baulcombe. 1997. High-resolution genetical and physical mapping of the Rx gene for extreme resistance to potato virus X in tetraploid potato. Theor. Appl. Genet. 95:153–162. doi:10.1007/s001220050543

Betti, C., C. Lico, D. Maffi, S. D'Ageli, M. M. Altamura, E. Benvenuto, ... S. Baschieri. 2012. Potato virus X movement in *Nicotiana benthamiana*: New detailsrevealed by chimeric coat protein variants. Mol. Plant Pathol. 13:198–203. doi:10.1111/J.1364-3703.2011.00739.X

Bostan, H. and P. Peker. 2009. The feasibility of tetraplex RT-PCR in the determination of PVS, PLRV, PVX and PVY from dormant potato tubers. Afr. J. Biotechnol. 8:4043–4047.

Cheng, Y. H., T. C. Deng, C. C. Chen, C. P. Kuan, and C. A. Chang. 2013. Identification and occurrence of Pepper mottle virus isolated from Peppers in Taiwan. J. Taiwan Agric. Res. 62:360–371. (in Chinese with English abstract) doi:10.6156/JTAR/2013.06204.06

Clark, M. F. and A. N. Adams. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475–483. doi:10.1099/0022-1317-34-3-475

Deng, T. C., S. J. Tsao, S. W. Tsai, C. H. Huang, and M. M. Tseng. 1992. Occurrence of potato viruses in Taiwan and resistance to virus Y of some promising clones as determined by ELISA. J. Agric. Res. China 41:361–370. (in Chinese with English abstract) doi:10.29951/JARC.199212.0007

DeShields, J. B., N. Moroz, L. E. Braley, G. A. Mora-Romero, and K. Tanaka. 2019. Recombinase polymerase amplification (RPA) for the rapid isothermal detection of *Spongospora subterranean* f. sp. *subterranea* and potato mop-top Virus. Am. J. Potato Res. 96:617–624. doi:10.1007/s12230-019-09750-7

Jamal, A., L. Korsten, and R. A. A. Van der Vlugt. 2012. Real-time quantitative RT-PCR for the detection of potato virus X in potato plants. J. Virol. Methods 185:1–7. doi:10.1007/BF02360415

Jeevalatha, A., P. Kaundal, R. Kumar, B. Raigond, M. Gupta, A. Kumar, ... B. P. Singh. 2016. Analysis of the coat protein gene of Indian *Potato virus X* isolates for identification of strain groups and determination of the complete genome sequence of two isolates. Eur. J. Plant Pathol. 145:447–458. doi:10.1007/s10658-016-0858-1

Kapoor, R., N. Srivastava, S. Kumar, R. K. Saritha, S. K. Sharma, R. K. Jain, and V. K. Baranwal. 2017. Development of a recombinase polymerase amplification assay for the diagnosis of banana bunchy top virus in different banana cultivars. Arch. Virol. 162:2791–2796. doi:10.1007/s00705-017-3399-9

Kreuze, J. F., J. A. C. Souza-Dias, A. Jeevalatha, A. R. Figueira, J. P. T. Valkonen, and R. A. C. Jones. 2020. Viral diseases in potato. p.389–430. *in*: The potato crop: Its agricultural, nutritional and social contribution to humankind. (Campos, H. and O. Ortiz. eds.) Springer. Cham, Switzerland. 518 pp. doi:10.1007/978-3-030-28683-5_11

Kumar, R., A. Jeevalatha, R. Baswaraj, S. Sharma, and M. Nagesh. 2017. A multiplex RT-PCR assay for simultaneous detection of five viruses in potato. J. Plant Pathol. 99:37–45.

Kumar, R., P. Kaundal, R. K. Tiwari, S. Siddappa, H. Kumari, K. C. Naga, and M. Kumar. 2021. Rapid and sensitive detection of potato virus X by one-step reverse transcription-recombinase polymerase amplification method in potato leaves and dormant tubers. Mol. Cell. Probes 58:101743. doi:10.1016/j.mcp.2021.101743

Kumar, R., R. K. Tiwari, A. Jeevalatha, P. Kaundal, S. Sharma, and S. K Chakrabarti. 2019. Potato viruses and their diagnostic techniques: An overview. J. Pharm. Phytochem. 8:1932–1944.

Mandal, B., A. Kumar, P. Rani, and R. K. Jain. 2012. Complete genome sequence, phylogenetic relationships and molecular diagnosis of an Indian isolate of potato virus X. J. Phytopathol. 160:1–5. doi:10.1111/

j.1439-0434.2011.01848.x

Martelli, G. P., M. J. Adams, J. F. Kreuze, and V. V. Dolja. 2008. Family Flexiviridae: A case study in virion and genome plasticity. Annu. Rev. Phytopathol. 45:73–100. doi:10.1146/annurev.phyto.45.062806.094401

Mohandas, A. and A. I. Bhat. 2020. Recombinase polymerase amplification assay for the detection of piper yellow mottle virus infecting black pepper. Virus Dis. 31:38–44. doi:10.1007/s13337-019-00566-x

Naveen, K. P. and A. I. Bhat. 2020. Reverse transcriptase loop-mediated isothermal amplification and reverse transcriptase recombinase amplification assays for rapid and sensitive detection of cardamom vein clearing virus. 3 Biotech. 10:250. doi:10.1007/s13205-020-02238-w

Peiman, M. and C. Xie. 2006. Development and evaluation of a multiplex RT-PCR for detecting main viruses and a viroid of potato. Acta Virol. 50:129–133. doi:10.4149/av_2017_403

Raigond, B., A. Verma, S. Roach, T. Kochhar, Shilpa, J. Arjunan, ... S. K. Chakrabarti. 2019. One-step reverse transcription loop-mediated isothermal amplification: A simple, sensitive and rapid assay for detection of potato virus X in potato leaves and tubers. Indian Phytopathol. 72:321–328. doi:10.1007/s42360-019-00147-4

Rashid, M. O., J. H. Li, Q. Liu, Y. Wang, and C. G. Han. 2021. Molecular detection and identification of eight potato viruses in Gansu province of China. Curr. Plant Biol. 25:100184. doi:10.1016/j.cpb.2020.100184

Singh, R. P., A. D. Dilworth, M. Singh, and D. L. McLaren. 2004. Evaluation of a simple membrane-based nucleic acid preparation protocol for RT-PCR detection of potato viruses from aphid and plant tissues. J. Virol. Methods 121:163–170. doi:10.1016/j.jviromet.2004.06.012

Soliman, A. M., A. A. Shalaby, B. N. Barsoum, G. G. Mohamed, M. K. Nakhla, H. M. Mazyad, and D. P. Maxwell. 2000. Molecular characterization and RT-PCR-ELISA detection of a potato virus X (PVX) isolate from Egypt. Ann. Agric. Sci. (Cairo) 4:1791–1804.

Tommiska, T. J., J. H. Hämäläinen, K. N. Watanabe, and J. P. T. Valkonen. 1998. Mapping of the gene Nxphu that controls hypersensitive resistance to potato virus X in *Solanum phureja* IvP35. Theor. Appl. Genet. 96:840–843. doi:10.1007/s001220050810

Verchot, J. 2021. Potato virus X: A global potato-infecting virus and type member of the Potexvirus genus. Mol. Plant Pathol. 23:315–320.

Wang, Y., R. Chen, X. Nie, Z. Zhong, C. Li, K. Li, and B. Nie. 2020. Rapid and sensitive detection of potato virus Y by isothermal reverse transcription-recombinase polymerase amplification assay in potato. Mol. Cell. Probes 50:101505. doi:10.1016/j.mcp.2019.101505

Whitworth, J. L., P. B. Hamm, and P. Nolte. 2012. Distribution of potato virus Y strains in tubers during the post-harvest period. Am. J. Pot. Res. 89:136–141. doi:10.1007/s12230-012-9235-6

Yu, X. Q., H. Y. Wang, Y. F. Lan, X. P. Zhu, X. D. Li, Z. F. Fan, ... Y. Y. Wang. 2008. Complete genome sequence of a Chinese isolate of Potato virus X and analysis of genetic diversity. J. phytopathology 156:346–351. doi:10.1111/j.1439-0434.2007.01365.x

使用單步驟定量 RT-PCR 法於馬鈴薯 X 病毒檢測之評估

關政平[1,*] 劉雅婷[2] 林思妤[3] 林玫珠[4] 陳述[5] 鄭櫻慧[6]

摘要

關政平、劉雅婷、林思妤、林玫珠、陳述、鄭櫻慧。2025。使用單步驟定量 RT-PCR 法於馬鈴薯 X 病毒檢測之評估。台灣農業研究 74(2):177–187。

本研究依據馬鈴薯 X 病毒 (potato virus X; PVX) 之鞘蛋白 (coat protein) 基因設計專一性引子對，利用即時反轉錄聚合酶連鎖反應 (real-time reverse transcription-polymerase chain reaction; real-time RT-PCR) 與 TaqMan 螢光探針，建立馬鈴薯 X 病毒的檢測技術。本研究所開發之 real-time RT-PCR 法的靈敏度，在檢測 PVX 上相較於 RT-PCR 法，提高約 10–100 倍，此外，此檢測具有高度特異性，可成功檢測 PVX，且不會與其他茄科病毒或未感染的健康植物發生交叉反應。透過檢測田間感染 PVX 的馬鈴薯，進一步確認此檢測方法的穩定性。有鑑於 real-time RT-PCR 的高特異性和敏感性，本研究所開發的 real-time RT-PCR 檢測在評估馬鈴薯或番茄受 PVX 感染的早期檢測具有高度應用效益。

關鍵詞：馬鈴薯 X 病毒、即時定量 RT-PCR、偵測。

投稿日期：2024 年 11 月 21 日；接受日期：2025 年 2 月 26 日。
* 通訊作者：pcr123@tari.gov.tw
[1] 農業部農業試驗所遺傳資源及生物技術組副研究員。臺灣 臺中市。
[2] 農業部農業試驗所遺傳資源及生物技術組計畫助理。臺灣 臺中市。
[3] 農業部農業試驗所遺傳資源及生物技術組助理研究員。臺灣 臺中市。
[4] 農業部農業試驗所植物病理組助理研究員。臺灣 臺中市。
[5] 農業部農業試驗所遺傳資源及生物技術組研究員兼組長。臺灣 臺中市。
[6] 農業部農業試驗所鳳山熱帶園藝試驗分所植物保護及園產品加工系研究員兼系主任。臺灣 高雄市。