Keywords: Colorimetric assay; DAS-ELISA assay; IC-RT-LAMP assay; IC-RT-PCR assay; Potato virus Y

Introduction

Potato (*Solanum tuberosum* L.), as the world's most important non-grain food crop [1], historically originated in the Andean mountains of South America about 8000 years ago, and gradually has undergone an immense spread around the world. It is mainly cultivated for its underground storage called tubers, and is central to global food security

0.2% polyvinyl pyrrolidone and 2% of egg albumin (Sigma A5253). e infected preparations were serially diluted (vefold dilution) at the same bu er. Aliquots of 195 l of prepared samples were added to each well, and the plates were incubated overnight at 4°C. Plates were then washed three times with washing bu er, incubated for 4 h at 37°C, with 190 l per well of alkaline phosphatase-conjugated PVY IgG diluted in sample bu er, washed again, and incubated lastly for 90 min, with p-nitrophenylphosphate (1 mg/ml), in 10% diethanolamine, pH 9.8. Data were expressed and recorded using Multiskan at A_{405} nm.

One-step IC–RT-PCR

e one-step IC–RT-PCR was developed using a PVY-speci c primer designed on the basis of virus coat protein (CP) gene (Table 1) [4]. e protocol, to generate one-step IC–RT-PCR products, was divided into two successive sections as below:

Section 1: e same as DAS-ELISA method, here, PCR tubes were rst coated with PVY speci c IgG diluted in coating bu er and incubated for 4 h in 37°C. Tubes, in the following, were washed with washing bu er (see "DAS-ELISA Assay" section). e extractions of

Section 1: Just the same as the section 1 of IC–RT-PCR procedure (see above).

Section 2: Each reaction was performed in a total volume of 50 l: 10 mM DTT, 5 U of RNase Ribonuclease Inhibitor (Fermentas Co., cat. no. EO0381), 20 mM Tris–HCl, pH 8.8, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 2 mM Betaine (Sigma-Aldrich, Oakville, ON, Canada), 1 mM $MgSO_4$, 10 mM each dNTP, 0.2 M each of F3 and B3, 0.8 M each of primer FIP and BIP, 1.25 U of AMV reverse transcriptase (Fermentas Co., cat. no.EP0641), and 8 U of Bst DNA polymerase (New England Biolabs Inc.). Tubes were then incubated at 60°C for 90 min in water bath. It is noticeable that the rst 45 min is allocated only to synthesize cDNA, while in the second round, LAMP amplicons are ampli ed. An agarose gel electrophoresis system (optional; 1.5%) under UV illumination could be also employed to visualize positive reactions: 5 l of each product is loaded on a 1.5% agarose gel. e details of colorimetric assay are described below:

Magnesium pyrophosphate: Like other metal indicators, magnesium pyrophosphate must be added before reaction. At the end of the ampli cation process, positive reactions were accompanied by a visible darker phase in the tubes in consequence of the formation of magnesium pyrophosphate [13,34], which can be easily visualized with the naked eye. It is noticeable that the turbidity of the positive samples is stable, but just for a short time, which should be consequently judged soon a er taking out of the samples either from the water bath or the thermal cycler.

SYBR® Premix Ex TaqTM II: To conquer time-dependent instability of magnesium pyrophosphate-based detection method, an alternative visual system using SYBR® Premix Ex TaqTM II was employed [20,25,35]. Hence, 2 l SYBR® Premix Ex TaqTM II (Perfect Real Time, Takara Bio Co., Ltd., RR081A) was added into each completely nished one-step IC–RT-LAMP reaction containing 25 l of products; all positive reactions were e ectively identi ed. Under UV illumination (302 nm), a green colour pattern is an identical characteristic of all positive reactions as the same was monitored in this study.

Hydroxynaphthol blue (HNB) dye: In this protocol, 1 l of the hydroxynaphthol blue dye (3 mM, Lemongreen, Shanghai, China) is mixed prior to ampli cation; all positive reactions can be easily identi ed using the naked eye, interestingly with no probable

even though presumptive diagnosis of PVY can be relatively simple when typical symptomatology is evident, symptoms in plants are not always specific, and can be confused with those caused by other biotic or abiotic agents. On the other hand, detection of deleterious viruses in symptomless plant material for preventive control is a compulsory

stronger than the others. Due to such remarkable features, the application of this colorimetric assay using visual observation systems, particularly, HNB, SYBR Green I and GeneFinder™ seems to be more e ective as a new viral diagnostic method for epidemiological studies of PVY, particularly in less well equipped laboratories, and might be helpful in clarifying virus–vector interaction. As the last point of view, the current diagnostic approach can be suitable not only for laboratory

worldwide.

References

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