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Shuchen (Elena) Yan

Department of Pathology and Microbiology

Supervisory Committee
Dr. Juan Carlos Rodriguez (Chair)
Dr. Fred Kibenge (Co-supervisor)
Dr. Huimin Xu (Co-supervisor)
Dr. Larry Hale
Dr. Yingwei Wang

Examination Committee
Dr. Chelsea Martin (Chair)
Dr. Yingwei Wang
Dr. Larry Hale
Dr. Angela Riveroll
Dr. Huimin Xu

Development and validation of multiplex polymerase chain reaction based diagnostic procedures for detecting eight viruses and one viroid in the Potato Nuclear Stock Certification Program

The Canadian Food Inspection Agency implements the Potato Nuclear Stock Certification Program to ensure the production of high-quality and disease-free seed potatoes. Currently, this program employs double and triple antibody sandwich enzyme-linked immunosorbent assays (DAS- or TAS-ELISA) and return-polyacrylamide gel electrophoresis (R-PAGE) for detecting the targeted viruses and PSTVd (Potato spindle tuber viroid), respectively. Pathogen-specific antibodies for ELISA are commercially available for detecting potato virus A, M, S, X, Y (all strain types), potato leafroll, latent, and mop-top viruses. However, the aforementioned methods are highly labour-intensive and time-consuming procedures with low sensitivity.

In this study, multiplex PCR-based procedures were developed and validated to simultaneously index the potato nuclear stock materials. Two multiplex RT-PCR and three multiplex RT-qPCR procedures were developed for primary screening and confirmatory testing of all targeted pathogens under this program, respectively. As little as 1.0 µl of tissue sap from a microplant or 1.0 pg of total RNA extract was sufficient for reliable RT-PCR detection. Additionally, virus-specific RNA could be detected in RNA extracts with almost no non-specific amplification in the presence of other viral RNAs. The use of potato genome-specific primer set as an internal control provided a reference for assessing the quality of the RNA extracts and the amplification of the targeted RNAs. Furthermore, the genomes of virus and viroid isolates or strains used in this research were verified using the next-generation sequencing (NGS) technology to ensure the accuracy of the RT-PCR primers employed. All tests conducted using the multiplex RT-PCR and RT-qPCR protocols were validated using another diagnostic assay and known/unknown microplants.

Overall, the developed and validated multiplex-PCR-based procedures in this study profoundly improved the sensitivity, specificity, efficiency, and feasibility with reductions in time and labour expenses for routine diagnostic testing and are ready for implementation into the Potato Nuclear Stock Certification Program.