Canadian Agri-Science Cluster for Horticulture 3

Update to Industry

2018-2019

Activity title: Investigating the occurrence and distribution of potato tuber necrosis-inducing viruses in Canada and studies on varietal responses to the viruses for minimizing economic losses cause by the pathogens
Name of Lead Researcher: Xianzhou Nie
Names of Collaborators and Institutions: Dr. Mathuresh Singh, Agricultural Certification Services, Fredericton, New Brunswick Mr. Jacques Lavoie, New Brunswick Department of Agriculture, Aquaculture and Fishery, Grand Falls, NB Dr. Vikram Bisht, Manitoba Agriculture Dr. Laixin Wang, McCain Foods Mr. Gary Hawkins, McCain Foods Dr. Huimin Xu, CFIA-Charlottetown Laboratory Dr. Sean Li, CFIA-Charlottetown Laboratory
Activity Objectives (as per approved workplan): The overall objectives of this activity are: 1) Revealing the occurrence and significance of potato tuber necrosis-inducing viruses in Canada; 2) Developing accurate and efficient procedures to detect the viruses in hosts and vectors and to unveil the spread and epidemiology of the viruses, especially the soil-borne PMTV; 3) Investigating varietal responses to the most prominent tuber necrosis-inducing virus(es), and identifying insensitive and/or resistant cultivars to the viruses. **For the fiscal year 2018-2019, the objectives are:** 1. Initiation of the development of a PCR protocol suitable for detection of PMTV and its fungal vector Spongospora subterranea f.sp. subterranean (Sss) from soil and plant tissues; 2. Investigating the incidences/occurrences of necrotic viruses (mainly PMTV and PVY^{NTN}) in potatoes in the participating provinces (mainly Manitoba and New Brunswick) in 2018; 3. Initiation of the studies on the sensitivity of up to 6 potato cultivars to Alfalfa mosaic virus-induced internal necrosis.

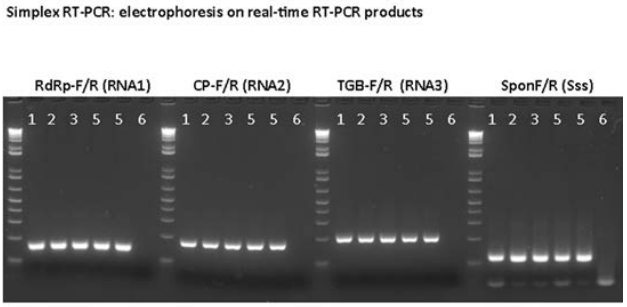
Research Progress to Date (use plain language):

We have met or exceeded all objectives for the 2018-2019 fiscal year despite the late start of the project (Dec 2018).

Objective 1. We have made an excellent progress on the development of a duplex real-time PCR/high resolution melting (HRM) of DNA for simultaneous detection of potato mop-top virus (PMTV) and its fungal vector Spongospora subterranea f. sp. subterranean (Sss) soil. As a part of the preparation for the project prior to its formal launch, we collected soil from several fields in New Brunswick that had been known to be infested with PMTV and Sss, and set up a platform in a contained growth room at the Fredericton Research and Development Centre (FRDC). Using the platform, we used common tobacco as the baiting plants and successfully infected approximate 50% tobacco plants with PMTV. The infection was confirmed by both ELISA test using PMTV-specific antibody (Agdia) and a conventional RT-PCR procedure reported previously (Hu et al. 2015). This platform plays a significant role in the project because PMTV is known for its self-elimination in potato plants and is thus impossible to be artificially inoculated and maintained in potato.

Although PCR-based diagnosis procedures for separate detection of PMTV and Sss have been reported (Qu et al 2011; Hu et al. 2016), no protocol for simultaneous detection of both pathogens is available. To facilitate an efficient detection of both PMTV and Sss, especially the detection of them in soil, we adapted the Sss primer pairs reported by Qu et al. (2011) and designed new primer pairs for the genomic RNA 1, RNA2 and RNA3 of PMTV, respectively. As anticipated, the primers led to the successful amplification of the target RNA at 138 bp (Sss), 149 bp (PMTV-RNA 1), 179 bp (PMTV-RNA2) and 207 bp (PMTV-RNA3) (Fig. 1. Top panel). A duplex RT-PCR for detection Sss and PMTV was also achieved (Fig. 1 bottom panel).

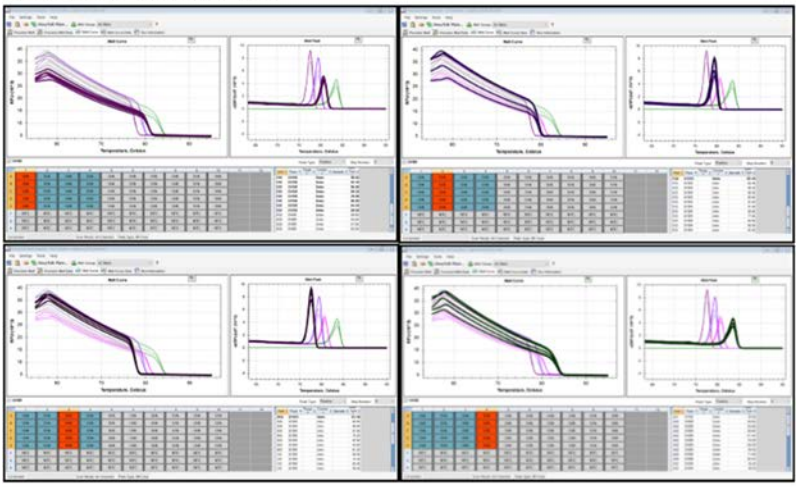
Fig. 1. Conventional RT-PCR for detection of Potato mop-top virus (PMTV) and its fungal vector Spongospora subterranea f. sp. subterranean (Sss). Top panel, simplex RT-PCR; Bottom panel, duplex RT-PCR.



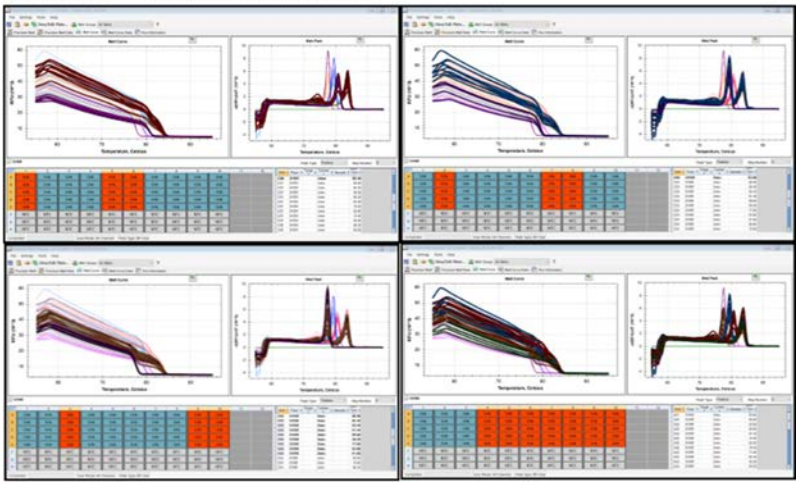
We then proceeded to the more efficient gel electrophoresis-free real-time duplex PCR/HRM for simultaneous detection of PMVT and Sss , and as shown in Fig. 2, virus and its fungal vector can be detected and differentiated effectively. The newly developed procedure is being optimized and is anticipated to be used for survey of PMTV infestation in soil.

Fig. 2. Real-time RT-PCR/high resolution melting (HRM) of DNA analysis for detection of Potato mop-top virus (PMTV) and its fungal vector Spongospora subterranea f. sp. subterranean (Sss). The dark line indicates the target molecules (RNA1, RNA2 and RNA3 of PMTV, and Sss)

Melting curve - simplex RT-PCR



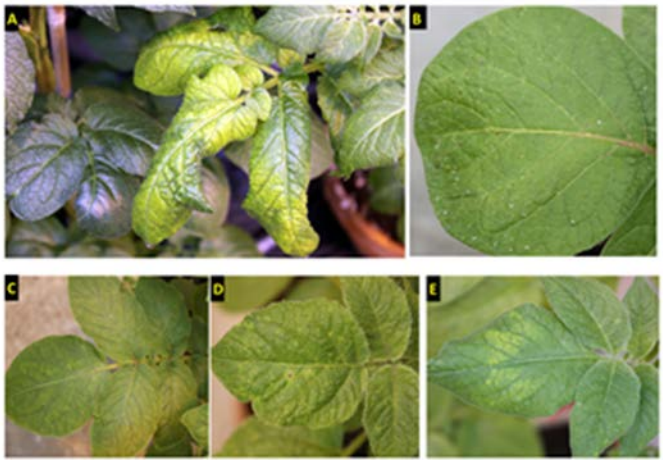
Melting curve - simplex and duplex RT-PCR



Objective 2. We received and tested 248 tubers from Manitoba and 20 from NB for all four targeted viruses, namely potato mop-top virus (PMTV), tobacco rattle virus (TRV), alfalfa mosaic virus (AMV) and potato virus Y strain NTN (PVY^{NTN}) by PCR. Among the samples, 6 were tested positive for PMTV. No other viruses were detected. Due to the late launch of the project, we are yet to verify the PCR results with other methods including ELISA.

Objective 3. In 2015, alfalfa mosaic virus (AMV) was found to be the causal agent of severe internal necrosis in tuber of cultivar Innovator (Nie et al. 2015), which prompted us to suspect that a necrotic strain of AMV similar to the one reported by Osward in 1950 was encountered. To further characterize the isolate (designated as isolate CaM), we inoculated it to six potato cultivars and one breeding clone. Upon mechanical inoculation, sporadic needle-sized necrotic spots occurred on the inoculated leaves of cultivars Innovator, Yukon Gold, Rochdale Gold-Dorée and Shepody. Two weeks after the inoculation, yellowish spots started to appear on the newly emerged leaves; which may be coupled with the needle-sized necrotic spots, depending on the cultivar (Fig. 3). As time progressed, the calico symptoms become more profound whereas the necrotic spots remained at the same level.

Fig 3. Example symptoms expressed on leaves of potato plants after primary infection with alfalfa mosaic virus (AMV) isolate CaM. A. Systemic symptoms on leaves of F87084. Left, leaf of a mock-inoculated plant; right, leaf of an AMV-inoculated plant at 21 days post-inoculation (dpi). B. Necrotic spots on an AMV-inoculated leaflet of Innovator at 7 dpi. C to E. Systemic symptoms (C, Chieftain; D, Rochdale Gold-Dorée; E. Yukon Gold) on leaves that emerged from AMV-inoculation at 14 dpi.



We sequenced the completed genome of the AMV isolate CaM and Ca175-1, an isolate that was deemed non-necrotic in a previous study by Xu and Nie (2006). Our results show that CaM and Ca175-1 belonged to different genetic group: IA-I-IB for CaM and IB-II-IA for Ca175-1. Despite the difference in genetics, CaM and Ca175-1 induced similar foliage symptoms in Innovator and its parental cultivar Shepody. We are in the process to evaluate the symptoms in tubers in all the tested cultivars.

References:

Qu XS et al. 2011. Journal of Applied Microbiology 110, 769–777. **Hu** X et al. 2016. Canadian Journal of Plant Pathology 38: 231-242. **Nie** X et al. 2015. Plant Disease 99: 1658-1658. **Xu** H and Nie J. 2006. Phytopathology 96:1237-1242. **Oswald** J W. 1950. Phytopathology 40:973-991.

Extension Activities (presentations to growers, articles, poster presentations, etc.):

Two oral presentations were given:

(1) Potato Tuber Necrotic Viruses. NB Seed Potato Day, February 28, 2019, Grand Falls, NB. Nie X, Singh M, Lavoie J, and Bisht V. "Canadian Agricultural Partnership (CAP) – Canadian Horticultural Council (CHC) cluster III - Activity 15 (2018 – 2023)". The meeting was attended by over 50 people including growers, extension staff and industry;

(2) Northeast Potato Technological Forum 2019. Charlottetown, PEI, 20-21 March 2019. Nie X, Dickison V, Singh M, De Koeyer D, Xu H, Bai Y, and Hawkins G. 2019. Potato tuber necrosis induced by alfalfa mosaic virus depends on potato cultivars rather than on virus strains. Approximately 50 research and development personnel from various organizations from NB, PEI, NS and Maine attended the meetings.

Early Outcomes (if any) or Challenges:

Despite the late launch of the project, we made significant progress on the project, and met or exceeded all objectives set for the fiscal year. In the fiscal year, we will conduct a pilot field trial to test 11 most common potato cultivars and 4 new AAFC release for their sensitivity to PMTV-caused tuber necrosis, aka spraing disease. We will ask our participating industrial partners for cultivars that they would like to be tested on the following years providing the piolet trial is proven successful.

Key Message(s):

1. We are progressing well in the development of a novel real time-PCR/high resolution melting of DNA analysis for simultaneous of potato mop-top virus (PMTV) and its fungal vector Spongospora subterranea f. sp. subterranean (Sss) in soil and plant tissues;
2. Based on the sequence analysis and pathological results obtained, the alfalfa mosaic virus (AMV) isolate previously deemed a “necrotic strain” was probably a regular strain.

This project is generously funded through the Canadian Agri-Science Cluster for Horticulture 3, in cooperation with Agriculture and Agri-Food Canada’s AgriScience Program, a Canadian Agricultural Partnership initiative, the Canadian Horticultural Council, and industry contributors.

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

CANADIAN AGRICULTURAL PARTNERSHIP
Innovate. Grow. Prosper.

Canadian Horticultural Council | Conseil canadien de l’horticulture
The voice of **Canadian fruit and vegetable growers**