Canadian Agri-Science Cluster for Horticulture 3

Update to Industry

Final Report – 2018 – 2023

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, AAFC

Names of Collaborators and Institutions:
Mathuresh Singh, potato virology/pathology/diagnostics, ACS, Fredericton, New Brunswick;
Jacques Lavoie, , potato specialist NBDAAF, Grand Falls, New Brunswick;
Vikram Bisht, potato pathologist, Manitoba Agriculture, Carman, Manitoba;
Laixin Wang, principal scientist-global science, potato processing quality, McCain Foods, Florenceville, New Brunswick;
Gary Hawkins, potato varieties, McCain Foods, Florenceville, New Brunswick;
Huimin Xu, virologist, CFIA-Charlottetown Laboratory, Charlottetown, Prince Edward Island;
Sean (Xiang) Li, soil pathologist, CFIA-Charlottetown Laboratory, Charlottetown, Prince Edward Island

Activity Objectives:
1) 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;
2) Revealing the occurrence and significance of potato tuber necrosis-inducing viruses in Canada;
3) Investigating varietal responses to the most prominent tuber necrosis-inducing virus(es), and identifying insensitive and/or resistant cultivars to the viruses.

Specifically, for each of the 5 years,
FY 2018-2019

1. Initiation of the development of a PCR protocol suitable for detection of PMTV and its protist vector *Spongospora subterranea* f.sp. *subterranea*n (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.

FY 2019-2020

1. Unveiling the incidences/occurrences of necrotic viruses (mainly PMTV and PVY^{NTN}) in potatoes in the participating provinces (mainly Manitoba and New Brunswick) in 2019;
2. Understanding the sensitivity of up to 6 potato cultivars to Alfalfa mosaic virus-induced internal necrosis and the sensitivity of up to 5 newly released potato clones/cultivars to PVY^{NTN}-induced potato tuber necrotic diseases;
3. Initiation of the studies on the sensitivity to PMTV-induced necrosis in up to 12 potato cultivars – first year field trial of group one cultivars.

FY 2020-2021

1. Unveiling the incidences/occurrences of necrotic viruses (mainly PMTV and PVY^{NTN}) in potatoes in the participating provinces (mainly Manitoba and New Brunswick) in 2020;
2. Understanding the sensitivity of up to 6 potato cultivars to Alfalfa mosaic virus-induced internal necrosis and the sensitivity of up to 5 newly released potato clones/cultivars to PVY^{NTN}-induced potato tuber necrotic diseases;
3. Unveiling the sensitivity to PMTV-induced necrosis in up to 12 potato cultivars – second year trial of group one cultivars.

FY 2021-2022

1. Unveiling the incidences/occurrences of necrotic viruses (mainly PMTV and PVY^{NTN}) in potatoes in the participating provinces (mainly Manitoba and New Brunswick) in 2021;
2. Understanding the sensitivity of up to 6 potato cultivars to Alfalfa mosaic virus-induced internal necrosis and the sensitivity of up to 5 newly released potato clones/cultivars to PVY^{NTN}-induced potato tuber necrotic diseases;
3. Unveiling the sensitivity to PMTV-induced necrosis in up to 13 potato cultivars – first year trial of group two cultivars.

FY 2022-2023

1. Unveiling the incidences/occurrences of necrotic viruses (mainly PMTV and PVY^{NTN}) in potatoes in the participating provinces (mainly Manitoba and New Brunswick) in 2022;
2. Understanding the sensitivity of up to 6 potato cultivars to Alfalfa mosaic virus-induced internal necrosis and the sensitivity of up to 5 newly released potato clones/cultivars to PVY^{NTN}-induced potato tuber necrotic diseases;
3. Unveiling the sensitivity to PMTV-induced necrosis in up to 13 potato cultivars – second year trial of group one cultivars.

Research Progress & Results*:*

1) Development of a novel PCR-based diagnostic method called high-resolution melting DNA (HRM) assay for detection of potato mop-top virus (PMTV) and its protist vector *Spongospora subterranea* f. sp. *subterranean* (Sss) in soil

Although various PCR-based methods for detection of the targeted viruses in plants had already been developed by various groups including us and were available when the project started, no molecular method was available for PMTV detection from soil directly. To bridge the gap, we developed a novel PCR-mediated high-resolution DNA melting (HRM) assay for simultaneous detection of PMTV and Sss in soil. To achieve this objective, we first established a tobacco-based PMTV-baiting system to identify soil that was infested with PMTV. We thereafter evaluated various nucleic acid extraction protocols for soil to find the most reliable procedure that could result in successful reverse transcription (RT)-PCR-gel electrophoresis detection of both PMTV and Sss. To facilitate more efficient detection, we designed new primer pairs for PMTV genomic RNA species (i.e., RNA-Rep, RNA-CP, and RNA-TGB; also knowns as RNA 1 to 3, respectively) and analyzed together with the existing Sss primers via real-time RT-PCR. As anticipated, the resulting PCR products (also known as amplicons) exhibited melting profiles that could be readily differentiated by HRM on the same PCR machine. Under duplex RT-PCR format, all PMTV and Sss primer combinations led to successful detection of respective PMTV RNA species and Sss in the soil samples by HRM analyses. To assess the efficacy and reliability of the newly developed HRM assay for PMTV and Sss, soil samples were collected from various spots of six fields at four different farms/sites in New Brunswick, Canada, and subjected to the HRM assay and the tobacco-baiting assay. By HRM assay, we found a positive detection of PMTV and Sss in 63-100% samples collected from fields in which PMTV-infected tubers had been observed.

In contrast, the samples from fields where neither PMTV- nor Sss-infected tubers had been observed resulted in negative (0%) detection by the assay. These results were consistent to the tobacco-based bioassay for PMTV and Sss: of the soil samples collected from PMTV-infested fields, 63-83% and 100% led to PMTV and Sss infections in the bait tobacco plants, respectively, whereas no (0%) PMTV- or Sss-infected plants were obtained from soil samples collected from PMTV- and Sss-free fields. This work has been published in the scientific journal Plant Disease (Nie et al. 2021. Plant Disease 105: 948-957. DOI: 10.1094/pdis-06-20-1321-re). HRM assay, which can be achieved in 2-3 days after a soil sample is collected, not only exhibited superiority in terms of time required for PMTV and Sss detection in comparison to the bait plant-based bioassay, which takes at least 2 weeks after the baiting plants being transplanted to the soil, but also showed great reliability and efficacy for large scale survey of fields for PMTV and Sss infestation. Indeed, using this method, we identified a hotspot of a PMTV-infested field for the field trial to assess cultivar sensitivity to PMTV-caused internal necrosis (i.e., spraing disease).

2) Revealing the occurrence and significance of potato tuber necrosis-inducing viruses in Canada

We tested a total of over 1600 tuber samples during the project period (2018-2022) for the four targeted viruses (i.e., alfalfa mosaic virus – AMV, potato mop-top virus – PMTV, tobacco rattle virus – TRV and potato virus Y strain NTN – PVY^{NTN}. Of the approximate 100 tubers exhibiting necrosis (either external or internal or both), 90% and 1% tested PMTV and PVY positive, respectively, and the remaining were found to be free of the tested viruses. Of the 1577 random tubers tested, 180 tested positive for PMTV, 4 for PVY, 2 for TRV and 0 for AMV. These results demonstrate that PMTV was the predominant necrotic virus in the participating provinces in Canada. It is necessary to point out that most PMTV-positive tubers in each year were found to occur in certain fields. For instance, for 2019 crop, 17 out of 379 (4.5%) random tubers tested positive for PMTV, most of them (11/17, or ~65%) came from one field. For 2021 crop, of the 16 fields in which a total of 480 were tested, 6 were free of PMTV infection, 3 had a infection rate of 3-7%, 4 at 20-40%,3 at >60%. We also found that approximately 50% of the tubers tested positive for PMTV exhibited several forms of interna necrosis (spraing disease).

3) Varietal sensitivity to AMV, PVY^{NTN} and PMTV

AMV: We first sequenced the complete genome of CaM, an AMV isolate deemed necrotic, and Ca175-1, an AMV isolate deemed non-necrotic, and analyzed their pathogenicity in potato tubers. We demonstrated 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, both isolates induced similar foliage symptoms and tuber necrosis in cvs. Innovator and Shepody, suggesting that all AMV isolates/strains may have the ability to cause tuber necrosis. We thereafter tested a total of 25 cultivars (Innovator, Yukon Gold, Rochdale Gold-Dorée, Chieftain, Shepody, Jemseg, F87084, Lamoka, Russet Burbank, Goldrush, Russet Norkotah, Atlantic, Kennebec, Snowden, Dark Red Norland, AC Chaleur, Ranger Russet, AAC Canada, Gold-Dorée, Green Mountain, AAC Valley Crisp, Exploits, CalWhite, Cherokee, Eramosa, Katahdin) for their sensitivity to the virus induced internal necrosis under both current-season (i.e., primary) and tuber-borne (i.e., secondary) infections in the greenhouse. While all tested cultivars showed a certain level of sensitivity to the virus-caused tuber necrosis, there are differences among them. For instance, cvs. Russert Burbank, Lamoka; Ranger Russet, Karahdin, Exploits, AC Chaleur and Eramosa showed low sensitivity to AMV-caused internal necrosis, whereas cv. Dark Red Norland, Innovator, Shepody, CalWhite, Cherokee and Green Mountain exhibited high sensitivity to the virus-induced internal tuber necrosis. One peer-reviewed article has been published (Nie et al. 2000. Plant Disease 104:340-347 https://doi.org/10.1094/PDIS-04-19-0827-RE).

PVY^{NTN}: We tested a total 99 advanced advanced clones created by AAFC for their susceptibility to PVY^{NTN} infection and sensitivity to PVY^{NTN} -caused tuber necrosis named potato tuber necrotic ringspot disease (PTNRD), along with PTNRD sensitive cv. Yukon Gold, in the greenhouse. Only one clone (F15062, a progeny of cv. AC Chaleur) exhibited sensitive to PVY^{NTN} -caused PTNRD, suggesting that overwhelming clones from AAFC breeding program are insensitive to PVY^{NTN} -caused tuber necrosis.

PMTV: We conducted standard trial (completely randomized design, 4 replicates) for cultivar sensitivity to PMTV-infection and the virus-caused tuber necrosis in a field that was identified/confirmed (see the progress of objective 1 above) to be infested with PMTV and Sss in 2019 (n = 15 cvs.), 2021 (n = 22 cv.) and 2022 (n = 19 cv.) on a total of 27 cultivars, each was tested for 2 years. Upon harvesting, tubers were divided into 4 groups and stored for 0, 3, 6 and 9 months at 4°C. Tubers at each time point were analyzed for spraing disease by cutting and for infection by PMTV by ELISA and RT-PCR testing. Our results demonstrated that the number of tubers with PMTV-related internal necrosis increased as the time of storage increased. For instance, for trial 2019, 15 out of 1200 tubers exhibited PMTV-associated spraing disease at 0 month; at 3-month, the number increased to 30; at 6-month, it increased to 35; and at 9-month,

the number increased further to 44. Of the 15 cultivars tested in 2019, Dark Red Norland showed the most susceptibility to PMTV-induced necrosis with an occurrence of ca. 7.8%, followed by Chieftain (6.5%), Kennebec (5.3%), Snowden (3.4%), Yukon Gold (2.5%), Atlantic (1.6%), Shepody (1.3%), Russet Norkotah (1.3%), Goldrush (0.9%), Lamoka (0.9%), and Russet Burbank (0%). For trial 2021 that was assessed at 3 month storage, of the 22 cultivars (9 for the second-year trial: Atlantic, Chieftain, Dark Red Norland, Goldrush, Kennebec, Russet Burbank, Shepody, Snowden, Yukon Gold; and 12 were for the first-year trial: AAC Canada Gold, AAC Valley Crisp, Caribou Russet, Hodag, Innovator, Ivory Russet, Manistee, Maritime Russet, Monica Russet, Mountain Gem Russet, Non Pareil Russet, and Reveille Russet) tested, Dark Red Norland was once again found to be the most susceptible (35.8%) to PMTV-induced spraing disease , followed by Chieftain (21.7%) and several smooth-skinned cultivars including Hodag (16%), Kennebec (15.8%), Atlantic (15%) and Snowden (13.3%). Russet cultivars generally exhibited lower sensitivity to the virus-induced tuber necrosis. Although lower infection was found in trial 2022, similar trend was observed: red skinned cultivars were the most sensitive to PMTV infection and the virus caused spraing disease, followed by smoothly-skinned; whereas russets were the least susceptible to both infection and the virus-induced tuber necrosis.

Key Message(s):

- A novel molecular diagnostic method that can be used for large scale survey for field infestation with potato mop-top virus (PMTV) and/or its protist vector *Spongospora subterranea* has been developed;
- PMTV has been determined to be the predominant necrotic virus in studied provinces; but its occurrence does not appear to have been spread to, or built up in, all fields;
- Russet cultivars were least sensitive to PMTV-infection and the virus caused tuber spraing disease;
- While AMV can cause internal necrosis in most cultivars, no occurrence was detected in the tested tubers.

Overall benefit to industry:

- The newly developed diagnostic method for detection of PMTV and its protist vector *Spongospora subterranea* (Sss, the powdery scab-causing pathogen) from soil samples directly can be used for large scale survey for fields for PMTV and/or Sss infestation, thus enabling the industry to develop mitigation strategies for manage the virus and its spread;
- While PMTV is an increasing concern to potato industry in Canada in general and the participating provinces in particular, the new knowledge and/or confirmation about its uneven occurrence in different field should give industry time to develop mitigation/management strategies on its spread and/or population built-up;
- The confirmation of russet cultivars' less insensitivities to PMTV infection and PMTV-caused spraing disease can be used as a part of cropping plan to minimize the economic losses caused by the virus on fields/farms that are heavily infested with the virus.

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 Fruit & Vegetable Growers of Canada and industry contributors.

CANADIAN AGRICULTURAL PARTNERSHIP
Innovate. Grow. Prosper.

Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

GROWERS OF CANADA | PRODUCTEURS DU CANADA