

Diagnostic optimization of viral detection and characterization for the Michigan seed potato certification program, 2021

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Potato virus Y (PVY) is a major concern throughout the US, including the North Central region, and is one of the primary diseases monitored and tested for in the seed certification process. Cost-effective and efficient detection of PVY in early generation potato seed lots will help prevent infected material from entering the production chain and will prevent unnecessary yield and profit loss. The MSU Potato and Sugar Beet Pathology (PSBP) program continues to work with the Michigan Department of Agriculture and Michigan Seed Potato Association seed inspectors to gather strain information from PVY-positive seed lots and also have been collaborating to increase handling capacity, efficiency and optimizing the viral detection and diagnostic protocols used in winter testing. Through this work we continue to: 1) investigate improved detection options to identify accurate, timely, and cost-effective methods for use in the Michigan seed potato certification and 2) monitor PVY strain prevalence in Michigan seed potatoes. The results of this work will help develop standard protocols for high-throughput, in-state tuber testing.

Materials & Methods:

Tuber testing methods, which do not require breaking tuber dormancy to sample sprouts or plantlets, were used. General (Mackenzie et al. 2015) and multiplex (Lorenzen et al. 2006, 2010; Chikh-Ali et al. 2013) reverse-transcriptase (RT) high-fidelity polymerase chain reaction (PCR) protocols will be compared to existing plantlet assays involving enzyme-linked immunosorbent assay (ELISA) to validate. In 2021, we selected six Snowden and Lamoka (variable or unreliable symptom expression) and Reveille Russet (reliable expression) seed lots for validation of dormant tuber methods. Samples of 200 tubers were taken from each seed lot. Each variety was sampled from two lots where visual PVY was either present or absent in summer field inspections (Table 1). Dormant tuber RT-PCR testing was conducted on all tubers in 10-tuber subsamples. After treatment with Rindite to break dormancy, subsamples were planted and grown out for standard leaflet ELISA. Subsets of positive samples (from research and commercial testing) will be subject to PVY strain confirmation by RT-PCR.

Results & Conclusions:

In 2021, PVY incidence was lower based on summer visual inspections, compared to 2020. In research lots, dormant tuber methods identified higher levels of PVY than estimated from the summer field inspections (Table 1). This could be due to in-field spread, varietal expression, strain differences, or variety by strain interactions, and strain typing is ongoing. Currently, we are conducting standard grow out methods to validate dormant tuber methods. We are also working to optimize a direct real-time RT-PCR assay (Mackenzie et al. 2015) to increase throughput and efficiency.

Table 1. RT-PCR results from seed lots assessed for PVY incidence based on summer field inspections. Results are based on positive PVY detections (%) using dormant tuber methods in 2021 (N=number of 10-tuber subsamples tested).

Variety	Typical Symptom Expression	N	Visual Summer (Jun-Jul)	Present (+) Absent (-) (Jun-Jul)	Dormant Tuber RT-PCR (Oct-Nov)	Leaflet ELISA Greenhouse (Jan)
Snowden	Unreliable	12	0.06	+	1.81	1.81
Snowden	Unreliable	13	0.00	-	2.59	1.66
Lamoka	Unreliable	11	0.03	+	0.95	0.95
Lamoka	Unreliable	12	0.00	-	0.00	0.00
Reveille	Reliable	20	0.40	+	6.70	N/A
Reveille	Reliable	20	0.00	-	2.21	N/A

Since 2019, we have developed the capacity to assess the strain types prevalent in Michigan seed growing regions. Our efforts have identified four major PVY strains, including N:Wi (most prevalent), N:O, NTN (tuber necrotic strain), and O. Due to flooding in Florida, the entire 2021-22 Michigan seed certification winter grow-out plots were lost. In place of the standard grow-out, MDARD will offer dormant tuber testing for seed lots intended for recertification and export and will further investigate the PVY strains present in positive samples (N = approx. 20,740 tubers will be tested in 2021-22). Preliminary observations from these samples suggest that PVY^{N:Wi} remains prevalent, however, PVY^{NTN}, PVY^{N:O} were also detected. A second tuber necrotic strain, PVY^E, has since been confirmed and indicates strain frequencies must be closely monitored to best inform the seed potato industry of potential risks.

We also conducting growth chamber assays using characterized PVY strains with elite potato germplasm using previously reported methods by Gundersen et al. (2019). Based on our assessment of PVY strain populations in Michigan, we have selected four characterized strains (N:Wi, NTN, N:O, O) and obtained them from collaborators at the University of Idaho. Six varieties were selected for preliminary experiments: Lamoka, Snowden, Mackinaw, Petoskey, Lady Liberty and MSZ242-13. These varieties represent current chip varieties used in Michigan and elite experimental varieties originating from the MSU Potato Breeding and Genetics program. We are currently optimizing protocols for maintenance of strains in virus-free *Nicotiana tabacum*. However, these preliminary strain by variety experiments will inform the seed potato industry of the yield and quality impacts that current PVY strains may have on prevalent chip varieties. We plan to increase the number of varieties and modify the strain panels used based on current growing practices and strain population assessments. This information will also confirm robust PVY resistance to multiple strains, further informing and directing future breeding efforts.

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