Recognition and Molecular Discrimination of Severe and Mild PVY^O Variants of *Potato virus Y* in Potato in New Brunswick, Canada

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Abstract

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A field isolate of *Potato virus Y* (PVY) was collected in New Brunswick, Canada in 2007 due to unusual symptoms observed on different potato cultivars. To unveil the PVY strain identity, tobacco and potato bioassays, PVY^O and PVY^N-specific antibody-based enzyme-linked immunosorbent assays, and reverse-transcription polymerase chain reaction (PCR)-based genotyping were carried out. All the assays demonstrated that the isolate, designated as PVY^O-FL in this study, belonged to the PVY^O strain group. Greenhouse tests with the potato cvs. FL 1533 and Jemseg confirmed the severe nature of infection by PVY^O-FL. The complete genome sequences of PVY^O-FL and PVY^O-RB, the latter a mild PVY^O isolate, were determined. BLAST analysis revealed that the two isolates shared 97 and 98% sequence identities at the nucleotide and polyprotein levels, respectively. Further BLAST analysis unveiled that PVY^O-FL shared 99.7% nucleotide sequence identity with PVY^O-Oz, an isolate reported in New York, United States,

whereas the PVY^O-RB isolate shared 99.2% sequence identity with PVY^O-139, a PVY^O isolate reported in New Brunswick, Canada. A phylogenetic tree of available, full-length sequences of PVY isolates demonstrated two subgroups within the PVY^O branch, one clustered with PVY^O-RB and the other with PVY^O-FL. Group-specific sense primers for differentiation of the two subgroups were developed and evaluated. A limited survey of potato tubers collected from a field plot at the Potato Research Centre, Agriculture and Agri-Food Canada, using the newly developed PCR primers, indicated that 65.3 and 2.4% of the PVY^O-positive tubers were infected with PVY^O isolates belonging to the PVY^O-FL and PVY^O-RB subgroups, respectively. Assessment of the pathogenicity of three representative isolates from each subgroup on the potato cv. Jemseg demonstrated that severe and mild symptoms were induced by the PVY^O-FL-like and PVY^O-RB-like isolates, respectively.

Potato virus Y (PVY, genus *Potyvirus*, family *Potyviridae*) is one of the most economically important viruses of the potato crop worldwide (29). The virus possesses a single-stranded, positive-sense RNA genome of approximately 9.7 kb, with one open reading frame encoding a single polyprotein (24). PVY induces various foliar symptoms in potato plants ranging from mosaic to leaf-drop streaks and stunting, depending on cultivars and virus strains, and can cause up to 80% yield reduction (8,22,34).

Potato growers across North America have noticed an increase in PVY incidence in their potato crops in the last 10 years (5,21,23,28). The increase in PVY levels and the emergence of different strains of PVY, including the tobacco vein necrosis strain (PVY^N), the recombinant N:O strain (PVY^{N:O}), and the po-

tato tuber ringspot necrosis strain (PVY^{NTN}), in many potato-growing areas in North America are of particular concern (5,10,11,23,28). In New Brunswick, Canada, the common or ordinary strain (PVY^O) is known to be present (26). Although various symptoms may be induced by PVY^O, typical symptoms are normally observed on plants infected with this strain. For instance, necrotic lesions and veinal necrosis are typical symptoms caused by current season infection of the cv. Jemseg with PVY^O (27), whereas mosaic is the most common symptom caused by PVY^O infection of cultivars such as Russet Burbank, Atlantic, and Shepody (15,31,33).

During the summer of 2007, seed potato inspectors from the Canadian Food Inspection Agency (CFIA) noticed some unusually severe PVY-like symptoms in growers' fields of different potato cultivars in the Grand Falls area of New Brunswick, Canada. The symptoms consisted of mild mosaic, chlorosis, and necrotic lesions on leaflets, followed by severe veinal necrosis and dying of entire infected branches of several potato cultivars, including FL 1533, FL 1579, Atlantic, and Goldrush. The atypical PVY-like symptoms in these cultivars led the inspectors to suspect they might be caused by a newly emerged PVY isolate in New Brunswick, thus prompting studies to unveil the identity and properties of the causal agent. In this report, we present the identification of an isolate of PVY^O from New Brunswick, Canada that causes severe symptoms on potato, and the pathological and molecular characterization of this isolate as well as a PVY^O isolate that causes mild symptoms on potato and that is known to occur in this province. A reverse-transcription polymerase chain reaction (RT-PCR)-based assay for discrimination of these PVY^O variant groups was also developed and evaluated. To our knowledge, this is the first report describing the

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relationship between the PVY^O genome sequence and symptom severity in potato plants, and differentiation of PVY^O variant groups.

Materials and Methods

Viral samples. Potato leaf samples with suspected symptoms of viral infection were collected in the 2007 growing season from fields in Grand Falls, New Brunswick, Canada. One field planted with each of the cvs. FL 1533, FL 1579, Goldrush, and Russet Burbank was sampled from commercial potato-seed-producing farms. The foliar symptoms on cvs. FL 1533, FL 1579, and Goldrush included chlorosis and necrotic lesions on leaflets, followed by severe veinal necrosis and early dying of entire symptomatic branches; on cv. Russet Burbank, symptoms included severe mosaic on the leaves. Four fully expanded, symptomatic leaves from a diseased plant of each cultivar and three stems from FL 1533 were brought to the Agricultural Certification Services (ACS), Fredericton, New Brunswick, Canada, and the Potato Research Centre, Agriculture and Agri-Food Canada (PRC-AAFC), Fredericton, New Brunswick, Canada, for further serological (ACS), biological (ACS and PRC-AAFC), and molecular identification (PRC-AAFC) of the causal agent. The three stems from FL 1533 were grafted onto healthy potato plantlets to maintain the original virus culture in the greenhouse. Following preliminary identification, one representative isolate (the isolate from the FL 1533, designated as PVY^O-FL) of the virus detected in these samples was selected for further biological and molecular characterization. In addition, greenhouse-maintained PVY isolates of PVY^N-Jg, PVY^{NTN}-SI, PVY^{N:O}-Mb58, and PVY^O-RB (17–20) were used for comparison with these New Brunswick field isolates.

Enzyme-linked immunosorbent assay. Samples of potato leaves and stems were first tested by enzyme-linked immunosorbent assay (ELISA) with virus-specific antibodies for common viruses of potato, including PVY, *Potato virus S* (PVS), *Potato virus A* (PVA), *Potato virus M* (PVM), *Potato virus X* (PVX), and *Potato leafroll virus* (PLRV) (Phyto Diagnostics, North Saanich, BC, Canada), as described previously (28). After determining that PVY was the only virus of the six tested that was present in all of the samples, the same samples were tested further using PVY antibodies for specific PVY serotypes. The PVY-Poly and 4C3 antibodies used are strain-nonspecific and capable of detecting all PVY strains, whereas MAb2 is PVY^O serotype specific and 1F5 is PVY^N serotype specific (6).

Symptom observation in potato and tobacco plants. The common tobacco (*Nicotiana tabacum* L.) cv. Samsun and potato (*Solanum tuberosum* L.) cvs. FL 1533, FL 1879, FL 1207, FL 1867, Jemseg, and Yukon Gold were used to characterize the plant pathological properties of the New Brunswick PVY isolates. Four tobacco seedlings and four virus-free potato plantlets of each cultivar were mechanically inoculated with PVY isolates, as described previously (28), and grown in a greenhouse with 12 h of light/day (90 $\mu\text{m}^2/\text{s}$) at 18 to 22°C. Tobacco seedlings and potato plantlets treated with buffer served as the healthy (negative) control treatments, and Russet Burbank infected with PVY^O (PVY^O-RB) (17) as the positive control treatment. The development of necrosis on inoculated leaves (local lesions) and systemic mosaic or necrosis of branches and whole plants was recorded weekly for 13 weeks after inoculation. After harvest of the potato tubers, the tubers were examined for symptoms of potato tuber necrotic ringspot (PTNR) (18), then stored at room temperature in the dark for 3 months and examined monthly for PTNR symptoms. The experiment was repeated two times in two subsequent years.

RT-PCR-based genotyping. RT-PCR-based PVY genotyping was carried out as described previously (9,20). Two steps were involved: (i) P1 gene-based PVY^O/PVY^{N:N:O/NTN} determination (17) and (ii) recombinant joint (RJ)-based PVY^N/PVY^{N:O}/Eu-PVY^{NTN} determination (19). Another format of multiplex RT-PCR developed by Lorenzen et al. (14) was used to verify results of the above genotyping assays. In addition, a new coat protein (CP)-gene-based method of PVY^O isolate group differentiation was developed in this study (see Results). The P1 gene-based RT-PCR

assay can differentiate PVY^O from PVY^{N:N:O/NTN} (17), and the RJ-based RT-PCR assay can determine the common recombinant events found in PVY^{N:O} and PVY^{NTN} (19). The PCR assay was performed on a PTC-200 Thermocycler (MJ Research, Watertown, MA). The amplification products (10 μl) were separated by electrophoresis on 1.2% agarose gels containing 1 $\times$ GelRed (Biotium Inc., Hayward, CA) and photographed under UV light with a FluorChem Imaging System (Alpha Innotech, San Leandro, CA).

Complementary DNA cloning and sequencing. For the purpose of cloning and sequencing the complete genome of PVY, total RNA was isolated from leaves of PVY^O-RB- or PVY^O-FL-infected potato or tobacco plants, as described above, using the LiCl procedure (16). RT-PCR-based cloning was performed using nine sets of primers that span the entire genome of PVY (for primer sequences, polarities and approximate locations, see 18). Each primer pair was projected to result in a DNA fragment of 1.0 to 1.3 kb, overlapping with adjacent fragments by approximately 100 bp at each end. For the PVY^O-RB isolate, all primer pairs successfully amplified the target fragments whereas, for the PVY^O-FL isolate, three primer pairs (nos. 3, 6, and 7) (18) failed to generate visible fragments. Upon cloning and sequencing the amplicons, three primer pairs (5'-ATTGCCAAGCAGGGTTATTG3'/5'-GCTCCATGCAGACAATCTCA3', 5'-TCGTGGACTGTGATGCATTT3'/5'-ATGCGACCA TCATTTTCAGTG3', and 5'-GAAATGCAAGCCTTGGGTAG3'/5'-CCCTATTGATGGCTTCCTCA3') were designed to amplify the missing fragments of PVY^O-FL. In addition, the 5' rapid amplification of cDNA ends system (version 2.0; Invitrogen, Carlsbad, CA) was employed to determine or verify the 5'-end nucleotides of both isolates according to the manufacturer's instructions.

The details of the RT-PCR assay conditions were described previously (20). The putative PVY fragments amplified by the various primers were separated on a 1.2% agarose gel and extracted from the gel using a DNA extraction kit (Qiagen, Valencia, CA). Purified fragments were cloned into a pDrive Cloning Vector (Qiagen) according to the manufacturer's instructions. Two cDNA clones of each fragment were sent to Robarts Research Institute (London, Ontario, Canada) for forward and reverse sequencing using the T7 promoter and SP6 promoter primers, respectively (for more information, see the manufacturer's instructions on pDrive Cloning Vector; Qiagen). Each isolate was fully sequenced twice, from RNA of infected tobacco leaves and from RNA of infected potato leaves of cv. Jemseg, with identical results. Sequence identities and alignments of these isolates with various potato virus species were analyzed using BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>) and ClustalW (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>). MEGA version 4.2 (32) was employed to analyze the phylogeny of PVY isolates at the complete genome sequence level.

Results

Serological-, molecular-, and biological-based assays of the field virus samples. Except for PVY, ELISAs gave negative readings for each potato virus evaluated (*data not shown*), indicating that PVY was the only virus of the six tested that was present in these New Brunswick field samples of potato foliage, and suggesting that the symptoms on all four potato cultivars were caused by PVY. As expected, positive ELISA readings were recorded for all potato foliar samples with the strain-nonspecific polyclonal (PVY-Poly) and monoclonal (4C3) antibodies (Table 1). For the strain-specific antibodies, all the samples gave positive ELISA readings to the PVY^O-specific antibody MAb2 but not to the PVY^N-specific antibody 1F5, except for a PVY^O + PVY^N-infected Russet Burbank leaf sample (Table 1), demonstrating that the PVY strains in these plant samples possessed a PVY^O-serotype.

It has been well established that PVY^{N:O}, PVY^{NTN-HN2}, and PVY^{NTN-NW} isolates possess a PVY^O-serotype but a PVY^N-pathotype in tobacco and a PVY^N-type P1 gene in the genome (4,9,20). The PCR assay results demonstrated that the New Brunswick field isolates of PVY possessed a PVY^O-type P1 gene and were free of known RJs (Supplementary Figure 1). The tobacco-based bioassay indicated that the New Brunswick PVY isolates as well as the

PVY^O-RB isolate induced mosaic symptoms on tobacco leaves, whereas PVY^N, PVY^{N:O}, and PVY^{NTN} isolates induced severe veinal, petiole, and stem necrosis, illustrating the PVY^O pathotype of the New Brunswick field isolates. No symptoms were observed on the negative control tobacco plants treated with buffer. Except for PVY^{NTN}, none of the isolates induced PTNR symptoms in tubers of cv. Yukon Gold, which is highly susceptible to PTNR (9,23). Based on these results, it was concluded that PVY^O was the causal agent of the unusually severe PVY-like symptoms observed on potato cultivars in New Brunswick, Canada. One representative

isolate recovered from cv. FL 1533 and designated as PVY^O-FL was selected for further characterization.

Sequence characterization of PVY^O-FL and PVY^O-RB. Prior to cloning and sequencing, pathogenicity of a representative isolate of each of PVY^O-FL and PVY^O-RB (17) was again confirmed on potato and tobacco plants in a greenhouse. PVY^O-FL induced more severe symptoms than PVY^O-RB on all the cultivars tested, including FL 1533, FL 1879, FL 1207, FL 1867, and Jemseg (Fig. 1; for cvs. FL 1879, FL 1207, and FL 1867, *data not shown*). For Jemseg, more local and systemic lesions were observed on the PVY^O-FL-

Table 1. Enzyme-linked-immunosorbent assay (ELISA) results for potato leaves and stems sampled from New Brunswick, Canada in 2007 and tested using various *Potato virus Y* (PVY) antibodies

Potato cultivar and tissue tested	ELISA (OD ₄₀₅) (mean $\pm$ standard deviation) ^a			
	PVY-Poly	4C3	MAb2	1F5
FL 1533 leaf	1.065 $\pm$ 0.055	0.770 $\pm$ 0.020	2.160 $\pm$ 0.050	0.004 $\pm$ 0.002
FL 1533 stem	0.580 $\pm$ 0.020	0.255 $\pm$ 0.025	0.605 $\pm$ 0.015	0.002 $\pm$ 0.002
FL 1579 leaf	2.293 $\pm$ 0.080	1.683 $\pm$ 0.024	2.820 $\pm$ 0.014	0.004 $\pm$ 0.002
Goldrush leaf	1.867 $\pm$ 0.009	0.890 $\pm$ 0.014	2.460 $\pm$ 0.057	0.004 $\pm$ 0.002
Russet Burbank leaf	1.323 $\pm$ 0.038	0.737 $\pm$ 0.019	1.643 $\pm$ 0.019	0.004 $\pm$ 0.002
PVY ^O + PVY ^N -infected Russet Burbank leaf	1.557 $\pm$ 0.023	0.573 $\pm$ 0.005	1.760 $\pm$ 0.028	1.230 $\pm$ 0.014
Virus-free Russet Burbank leaf	0.001 $\pm$ 0.001	0.001 $\pm$ 0.001	0.002 $\pm$ 0.002	0.001 $\pm$ 0.001

^a ELISAs were completed using various antibodies: PVY-Poly = polyclonal antibody that reacts with all known strains of PVY; and 4C3, MAb2, and 1F5 = monoclonal antibodies that react with all known strains, PVY^O strains, and PVY^N strains, respectively. OD₄₀₅ = optical density measured at 405 nm. Each value represents the mean $\pm$ standard deviation of four separate leaves or stems from the same plant assayed by ELISA (except three for FL 1533 stem). Numbers in bold represent ELISA-positive values for that assay. Samples were considered positive when the OD $\pm$ was $\geq$ 0.100 and four times greater than that of the negative (healthy) control samples (28).

Fig. 1. Symptoms induced by *Potato virus Y* (PVY) isolates PVY^O-FL and PVY^O-RB on potato cvs. FL 1533 and Jemseg were mechanically inoculated with PVY^O-FL or PVY^O-RB as described by Singh et al. (28). Symptoms were monitored for up to 3 months after inoculation. The experiments with FL 1533 and Jemseg were carried out in greenhouses at the Agricultural Certification Services and the Potato Research Centre, Agriculture and Agri-Food Canada, respectively, in New Brunswick, Canada. Photos of the FL 1533 plants were taken 35 days post inoculation (dpi). Photos of Jemseg plants were taken 21, 31, and 41 dpi; for inoculated leaves and noninoculated leaves located above the inoculated leaves, the photos were taken 8 and 14 dpi, respectively.

inoculated plants than on the PVY^O-RB-inoculated plants (Fig. 1, Jemseg), although this difference was not quantified statistically. Inoculation with PVY^O-FL led to plant death 31 days post inoculation (dpi), whereas PVY^O-RB did not kill the plants even by 41 dpi (Fig. 1, Jemseg). These results demonstrated that PVY^O-FL is a variant of PVY^O that causes severe symptoms, whereas PVY^O-RB is a variant of PVY^O that causes mild symptoms (hereafter referred to as severe and mild variants, respectively). Both isolates induced similar mosaic symptoms on tobacco leaves (data not shown).

The genome size was 9,698 nucleotides, excluding poly(A), for both the PVY^O-FL and PVY^O-RB New Brunswick isolates (Supplementary Table 1). The two isolates shared 97 and 98% sequence identities at nucleic acid and polypeptide levels, respectively. Comparison of the PVY^O-FL sequence with sequences of other PVY isolates retrieved from GenBank demonstrated that PVY^O-FL shared approximately 99.7% sequence identity with PVY^O-Oz (accession number EF026074), a PVY^O isolate reported in the northeastern United States (2), and 96% identity with PVY^O-139 (accession number U09509), a PVY^O isolate reported in New

Fig. 2. Phylogeny of isolates of the *Potato virus Y* common strain (PVY^O). Full-length sequences of PVY^O were used for phylogenetic analysis employing MEGA4 with the neighbor-joining method (32). The scale bar represents genetic distance. Numbers at the nodes indicate the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates). The PVY^O isolates and associated GenBank accession numbers are shown.

Fig. 3. Differentiation of isolates of the *Potato virus Y* common strain (PVY^O)-FL and PVY^O-RB groups using reverse transcription-polymerase chain reaction (RT-PCR) assay. **A**, Alignment of sequences of PVY^O isolates for designing PCR primers for differentiation of PVY^O-FL and PVY^O-RB isolates. Numbers in the alignment indicate the nucleotide locations in the input sequences reported by the GenBank accession number and PVY isolate name. The primers (5' to 3') were derived from the shaded nucleotides. FL-S8592 (targeting the PVY^O-FL/Oz-SCRI-O group, i.e., PVY^O-FL isolates), Yo139-S8658 (targeting the PVY^O-RB/139 group, i.e., PVY^O-RB isolates), and Yo-S8818 (targeting all PVY isolates) are sense primers; and Yo-9056 (targeting all PVY isolates) is an antisense primer. **B**, Schematic diagram of PCR amplification products for differentiation of PVY^O-FL/Oz-SCRI-O and PVY^O-RB/139 isolates. Target segments and resulting PCR products are illustrated. **C**, Multiplex RT-PCR assay results for differentiating PVY^O-FL, PVY^O-RB, and other PVY isolates. Lanes 1–4: PVY^O-FL; lanes 5–8: PVY^O-RB; lanes 9–12: PVY^N-Mb58; lanes 13–16: PVY^N-SI; lanes 17–20: PVY^N-Jg; lanes 21–24: healthy potato plant samples; M = DNA ladder (from top to bottom: 2,000, 1,200, 800, 400, 200, and 100 bp).

Brunswick (26). In contrast, PVY^O-RB shared 99.2% sequence identity with PVY^O-139 and 97% identity with PVY^O-Oz.

A phylogenetic tree constructed using publicly available full-length sequences of PVY isolates illustrated that several groups (namely, PVY^{C/non-potato}, PVY^O, nonrecombinant PVY^{N/NTN}, PVY^{N:O/Wilga}, and recombinant PVY^{NTN}) were present. This is consistent with the results of previous studies (9,10). Interestingly, two subgroups were observed in the PVY^O branch, one including PVY^O-139, -RB, and -SASA-110 and the other including PVY^O-Oz, -FL, and -SCRI-O. An additional tree generated using only the PVY^O full-length sequences showed consistent results with the above analysis, except for PVY^O-SASA-110 (Fig. 2). The latter, which loosely clustered with PVY^O-RB/-139 in the tree shown in Supplementary Figure 2 and which grouped with PVY^O-139 in previous studies (9,10), was placed between the PVY^O-RB/-139 and PVY^O-FL/-Oz/-SCRI-O groups (Fig. 2). This might represent a third PVY^O variant type.

Differentiation of PVY^O types. To differentiate PVY^O-FL and PVY^O-RB isolates, the aligned nucleotide sequences were examined for regions suitable for designing primers capable of discriminating these two types of isolates. The 5' proximal end of the CP gene demonstrated polymorphisms; therefore, primers were selected from this region. The sense primer FL-S8593 (5'-AAGCAG CAAGAAAGATGCAAG-3') matched with PVY^O-FL, -Oz, and -SCRI-O; RB-S8658 (5'-ATAAAGATGTGAATGCCGCA-3') matched with PVY^O-139 and -RB; and S8819 (5'-GCAACT CAATCACAGTTTGA-3') and the antisense primer A9056 (5'-AGAAATGTGCCATGATTGCCT-3') matched with all isolates (Fig. 3A). A 464-bp amplicon was predicted to be produced by FL-S8593/A9056 primers for PVY^O-FL, -Oz, and -SCRI-O isolates; a 399-bp fragment by RB-S8658/A9056 primers for PVY^O-RB and -139 isolates; and a 238-bp fragment by S8819/A9056 primers for all isolates, including PVY^O-SASA-110 (Fig. 3B). Indeed, multiplex RT-PCR assays with all the primers resulted in amplification of the projected fragments for the respective isolates (Fig. 3C); a fragment at each of 464 bp (PVY^O-FL isolate group specific) and 238 bp (common to all isolates) for PVY^O-FL (lanes 1 to 4); and at 399 bp (PVY^O-RB isolate group specific) and 238 bp (common to all isolates) for PVY^O-RB; and one fragment of 238 bp for all remaining PVY samples, including PVY^{N:O} (lanes 9 to 12), PVY^{NTN} (lanes 13 to 16), and PVY^N (lanes 17 to 20). The healthy potato samples did not produce any of these amplicons (Fig. 3C, lanes 21 to 24).

The newly developed RT-PCR primers were further evaluated using potato tubers collected from a field plot at the PRC-AAFC, New Brunswick, Canada, from cultivars infected with unknown strains of PVY. The tubers were first screened using the P1 gene-based RT-PCR assay for PVY^O and PVY^{N/N:O/NTN} (17), and the PVY^O-positive samples were then subjected to PVY^O-FL/-FL-like and PVY^O-RB/-RB-like determination. Of the 397 tubers tested from the four cvs. Atlantic (122 tubers), Yukon Gold (66 tubers), Kennebec (116 tubers), and Russet Norkotah (93 tubers), 124 (31.2%) and 26 (6.5%) were determined to be PVY^O and PVY^{N/N:O/NTN} positive, respectively. Among the PVY^O-positive samples, 3 (2.4%), all from Russet Norkotah, tested positive for the PVY^O-RB isolate group, and 81 (65.3%) (9 from Kennebec, 23 from Atlantic, 14 from Yukon Gold, and 35 from Russet Norkotah) for the PVY^O-FL isolate group. Interestingly, 40 (42.3%) (5 from Atlantic, 9 from Yukon Gold, and 26 from Russet Norkotah) of the samples failed to generate either PVY^O-FL- or PVY^O-RB-specific bands, although these samples produced the common PVY band at 238 bp, suggesting the presence of a PVY^O-SASA-110-like or an uncharacterized PVY^O isolate in these tubers.

To test the correlation between the RT-PCR assay-determined isolate type and the pathogenicity of the virus isolates on potato plants, three randomly selected tubers that tested positive for PVY^O-FL-like isolates were planted together with three tubers that had tested positive for PVY^O-RB-like isolates. Symptomatic leaves of the resulting plants were used to inoculate virus-free Jemseg plants. By 18 dpi, isolates A6, A97, and A45 that belonged to the

PVY^O-FL variant group based on the RT-PCR assay (Fig. 4) induced severe symptoms on Jemseg, whereas isolates R51, R9, and R70 that belonged to the PVY^O-RB variant group based on the RT-PCR assay induced much milder symptoms on Jemseg plants (Fig. 4A).

Discussion

Viral diseases pose a continuous challenge for potato production worldwide. Stringent phytosanitary measures and seed-potato certification programs have been integrated into the production systems in many countries, including Canada and the United States, to reduce virus problems in potato crops (30). However, despite the overall success of these programs, emergence of new viruses or new virus strains has occurred even in countries where strict certification programs and rules are implemented (4,5,13,29). Therefore, with unusually severe PVY-like symptoms observed on various potato cultivars in New Brunswick, Canada in 2007 by experienced CFIA field inspectors, and confirmed as part of this study as being caused by PVY, we speculated that a novel strain or infections by multiple viral strains might have occurred. This prompted urgent requests by the potato growers and inspectors to unveil the identities and properties of the causal agents. Upon completion of ELISA screening for viruses known to occur in New Brunswick, PVY was the only virus detected in the potato plants, indicating that the symptoms were probably due to PVY infection. These fields were planted with Elite 1 and 2 seed of the highest classes (1), in which current-year virus symptoms are rare; therefore, the observation of symptoms initially on foliage of one stem of each symptomatic plant, spreading subsequently to other stems and foliage, suggested that infection likely occurred and spread as a result of aphid vectors present during the growing season.

Recent progress on PVY research has depicted complicated molecular or biological diversities and evolutionary pathways of the virus. Among the known potato PVY isolates, PVY^O and PVY^N have been demonstrated to be parental strains (7,18,20). From these strains, various recombinant substrains or isolates, including PVY^{N:O} (PVY^{N-Wi}), Eu-PVY^{NTN}, PVY^{NTN-HN2}, PVY^{NTN-NW}, and many more have emerged (2,4,9,13,17,19,20,23). Although the pathogenicity on potato of many of these isolates has not been described, some isolates may incite atypical symptoms in potato plants, as evidenced by the PVY^{NTN} isolates discovered in the 1980s and 1990s (12,29). Therefore, when isolates of PVY causing atypical symptoms were encountered in several potato cultivars in New Brunswick, this necessitated further investigation to unveil the serological, pathological, and molecular properties of these isolates. PVY^O- and PVY^N-specific antibodies revealed the serotypes of the isolates. This was followed by P1 gene- and RJ-based RT-PCR genotyping. Surprisingly, all of the tests indicated that the field samples contained a PVY^O-like isolate exhibiting PVY^O serotype and PVY^O genotype. Results of a tobacco-based bioassay concurred with the assessments. Analysis of the genome sequences demonstrated that an isolate designated as PVY^O-FL possessed a PVY^O-like genome that shared 99.7% sequence identity with isolates of PVY^O-Oz and 97% identity with PVY^O-139. Like other PVY^O isolates such as PVY^O-Oz and PV-575 (2,10), PVY^O-FL did not induce PTNR symptoms in tubers of the sensitive potato cv. Yukon Gold.

Results of the PVY phylogenetic analysis in this study supported previous studies on phylogenetic relationships among isolates of PVY and pm PVY evolution (7,9,10,13,20,25). Moreover, the results demonstrated the existence of at least two groups of PVY^O, represented by PVY^O-RB/-139 and PVY^O-FL/-Oz/-SCRI-O. PVY^O-RB isolates, which induced typical, mild symptoms, and PVY^O-FL isolates, which incited atypical, severe symptoms on the potato cultivars evaluated, might represent two pathological groups, the former classified as a "mild" isolate and the latter as a "severe" isolate. If this hypothesis is correct, PVY^O-Oz and PVY^O-SCRI-O should induce severe symptoms on potato plants while PVY^O-139 should induce mild symptoms. Because limited pathological information is available on these isolates infecting potato

Fig. 4. Pathogenicity assays of representative *Potato virus Y* common strain (PVY^O-FL and PVY^O-RB isolates identified from field leaf samples of the potato cv. Jemseg. **A** minisurvey using the newly developed reverse transcription-polymerase chain reaction (RT-PCR) assay together with the P1 gene-based RT-PCR assay (17) on potato tubers collected from a field plot at the Potato Research Centre, Agriculture and Agri-Food Canada, New Brunswick, Canada, identified 81 and 3 tubers positive with PVY^O-FL- and PVY^O-RB-like isolates, respectively. Three randomly selected PVY^O-FL-like positive tubers (A6, A97, and A45) and all three PVY^O-RB-like positive tubers (R51, R9, and R70) were planted in a greenhouse. The resulting plants were used for mechanical inoculation of virus-free Jemseg plants, which were then subjected to RT-PCR assay for determination of PVY^O types. **A**, Symptoms on Jemseg 18 days post inoculation. **B**, RT-PCR assay results for differentiating PVY^O isolate types on inoculated Jemseg plants shown in **A**. Lane M = DNA ladder (from top to bottom: 2,000, 1,200, 800, 400, 200, and 100 bp); RB = known PVY^O-RB isolate, and FL = known PVY^O-FL isolate (positive control treatments).

plants (2,3,26), further studies with additional isolates are needed to test this hypothesis. Nevertheless, the sequence differences between the PVY^O-FL/-Oz/-SCRI-O and PVY^O-RB/-139 groups might be useful for differentiating the two groups. Using primers derived from the 5' proximal end of the CP gene, isolates of the two groups were readily identified and differentiated in this study. When this procedure was combined with P1 gene-based genotyping (17) to field tuber samples, three types of PVY^O were detected; namely, PVY^O-Oz/-FL/-SCRI-O, PVY^O-139/-RB, and PVY^O-SASA-110-like or an uncharacterized PVY^O type. The PVY^O-FL/-Oz/-SCRI-O type appeared to be the predominant group in potato tubers collected from four cultivars in New Brunswick. PVY isolates identified as the PVY^O-FL/-Oz/-SCRI-O type induced more severe symptoms than the PVY isolates identified as the PVY^O-139/-RB type when inoculated onto potato cv. Jemseg, which was consistent with the severity of symptoms exhibited by PVY^O-FL and PVY^O-RB isolates, respectively. These results demonstrate the potential of CP gene-based genotyping for rapid identification of PVY^O variants.

BLAST analysis using the CP gene sequence of PVY^O-RB/-139 and PVY^O-FL/-Oz to compare with nucleotide sequences available in genetic databases demonstrated that the majority (97%) of the sequences appeared to be PVY^O-FL/-Oz (*unpublished data*). However, due to the lack of pathological data and descriptions associated with these available sequences, it was not possible to ascertain pathogenicity of the isolates on potato. Clearly, further studies on the biological, serological, molecular, and pathological properties of PVY^O variants, including PVY^O-O5, which reacts to a PVY^N-specific antibody (11), are needed to better understand these PVY groups.

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