Identification and Molecular Characterization of Recombinant Potato Virus Y (PVY) in Potato from South Korea, PVY^{NTN} Strain

Mohamad Chikh-Ali, Mariana Rodriguez-Rodriguez, and Kelsie J. Green, Department of Entomology, Plant Pathology, and Nematology, University of Idaho, Moscow, 83844-2329; Dong-Jun Kim, Inno Seed Co., Dong-myeon Chuncheon-si Gangwon-do, 24210, South Korea; Sang-Min Chung, Life Science Department, Dongguk University–Seoul, Seoul, 04620, South Korea; Joseph C. Kuhl, Department of Plant Science, University of Idaho, Moscow, 83844-2333; and Alexander V. Karasev,[†] Department of Entomology, Plant Pathology, and Nematology, University of Idaho, Moscow, 83844-2329, and Bioinformatics and Computational Biology Program, University of Idaho, Moscow, 83844-3050

Abstract

Potato is an important source of food in South Korea, and viruses represent a significant threat to sustainable and profitable potato production. However, information about viruses affecting the potato crop in South Korea is limited. In 2017, potato plants of five cultivars exhibiting foliar mosaic, crinkling, and mottle were collected in two seed potato production areas, in Gangwon-do and Jeollabuk-do Provinces, and subjected to virus testing and characterization. Potato virus Y (PVY) was found associated with mosaic symptoms, and samples were characterized using reverse transcription polymerase chain reaction (RT-PCR) and whole genome sequencing. All analyzed PVY-positive samples were found to represent the same recombinant PVY strain: PVY^{NTN}. Three PVY

isolates were subjected to whole genome sequencing using overlapping RT-PCR fragments and Sanger methodology, and all three were confirmed to represent strain PVY^{NTNa} after a recombination analysis of the complete genomes. In phylogenetic analysis, the three South Korean isolates were placed most closely to several PVY^{NTNa} isolates reported from Japan and Vietnam, suggesting a common source of infection. This is the first report and complete molecular characterization of a PVY^{NTN} strain present in the country, and because this strain induces tuber necrotic ringspot disease in susceptible cultivars of potato, appropriate management tools need to be implemented to mitigate potential tuber quality losses.

Potato has been cultivated in South Korea since at least the early 1800s, first in the mountain areas during the summer, and later expanded to the lowlands to meet the increased demand for potato consumption (Horton et al. 1988). Today, potato is one of the main food crops in South Korea, and in 2015 a total area of 20,704 ha was planted with potatoes and about 546,000 tons of potato were produced (Soley and Choi 2016). Domestic production accounted for approximately 75% of potato consumption in South Korea, and the rest was supplied as imports, with most of the fresh potato being imported from the United States and Australia (Soley and Choi 2016). Potato production in South Korea is carried out in three planting seasons: spring (67% of total production), summer (highland, 24%), and autumn (9%) (Soley and Choi 2016). The major cultivar Sumi is used for table potatoes, whereas Atlantic is the main cultivar used for chipping potatoes. Daeji is the main cultivar grown in the South during the autumn season (Soley and Choi 2016). In the 1970s, an increase of virus disease pressure caused potato yields to drop dramatically, which led to the collapse of seed certification programs, mainly owing to the loose standards that were not able to control potato diseases

effectively (Horton et al. 1988). In the 1980s, the seed certification program was reorganized to meet the growers' demand for high-quality seed potatoes. This helped to increase potato yield from about 11 t/ha in the 1970s to 20 t/ha in the 1990s and to 26.3 t/ha in 2015 (Horton et al. 1988; Soley and Choi 2016). Despite the significant role potato viruses played in potato production in South Korea, information on potato viruses in the country was scarce up to now.

Potato virus Y (PVY) is a major virus affecting potato production everywhere potato is grown. PVY exists as a complex of multiple strains, and these strains can be defined based on their host range in solanaceous hosts, necrotic phenotypes in potato cultivars with PVY hypersensitivity genes and in tobacco, and molecularly based on whole genome sequences. Currently, five nonrecombinant and 36 recombinant types have been defined based on their genome structures (Green et al. 2017, 2018; Karasev and Gray 2013). Prevalence of PVY strains in different potato production areas differs (Chikh-Ali et al. 2016a, b; Elwan et al. 2017; Galvino-Costa et al. 2012b; Ogawa et al. 2012; Schubert et al. 2015) and may be determined by the predominant potato cultivars expressing strain-specific resistance (Funke et al. 2017).

Multiple recombinant strains of PVY cause various potato tuber defects (Benedict et al. 2015; Le Romancer et al. 1994), in particular potato tuber necrotic ringspot disease (PTNRD), which reduces the quality of affected tubers, rendering them unmarketable (Gray et al. 2010). One specific recombinant, PVY^{NTN}, has been reported from most potato producing areas, and in some areas PVY^{NTN} was found to be the dominant strain (Chikh-Ali et al. 2016b; MacKenzie et al. 2018). PVY^{NTN} exhibits two recombination patterns: PVY^{NTNa} has three recombination junctions in the P3, VPg, and CP regions, whereas PVY^{NTNb} has an additional recombination junction in the P1 coding region (Schubert et al. 2007). PVY^{NTNa} isolates are common and encountered worldwide, whereas PVY^{NTNb} isolates are uncommon and have been found only in Europe and the Middle East (Chikh Ali et al. 2008; Schubert et al. 2007).

The efficient control of PVY in a given potato production area requires the identification of PVY strains prevalent in that area, in order to deploy appropriate detection tools and potato cultivars with corresponding resistance genes. PVY population structure was studied extensively in many potato producing areas including Europe

[†]Corresponding author: Alexander V. Karasev;
E-mail: akarasev@uidaho.edu

Funding: This work was funded in part through grants from USDA-NIFA Hatch Projects (USDA-NIFA-Hatch, IDA01537 and IDA01560), USDA-NIFA-SCRI (2014-51181-22373), USDA-ARS (58-8042-6-049), Northwest Potato Research Consortium, and the Idaho Agricultural Experiment Station. This work was also supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry (IPET) through the Agri-Bio Industry Technology Development Program funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (317011-04-2-HD020).

*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary table is published online.

Accepted for publication 15 July 2018.

Table 1. Summary of Korean potato sample testing and potato virus Y (PVY) strain typing^a

Sample lab ID	Potato cultivar	PVY ImmunoStrip test	Multiplex PCR results (bp)	Identification
K1-1	Superior	+	441/633/1,307	NTNa
K1-2	Superior	+	441/633/1,307	NTNa
K1-3	Superior	+	441/633/1,307	NTNa
K1-4	Superior	+	441/633/1,307	NTNa
K1-5	Rose Valley	+	441/633/1,307	NTNa
K1-6	Rose Valley	+	441/633/1,307	NTNa
K1-7	Dubaek	+	441/633	NTN
K1-8	Dubaek	+	441/633	NTN
K1-9	Dubaek (control)	–	...	...
K1-10	Dubaek	+	441/633/1,307	NTNa
K2-1	Chubaek	+	441/633/1,307	NTNa
K2-2	Chubaek	+	441/633/1,307	NTNa
K2-3	Suyoung	+	441/633/1,307	NTNa

^a Foliar samples were collected based initially on visual symptoms of mosaic and crinkling; all collected symptomatic samples were tested for the presence of PVY by ImmunoStrip test, and applied to FTA cards. Nucleic acids extracted from the dried FTA cards were used in reverse transcription polymerase chain reaction (PCR) typing to strain (Chikh Ali et al. 2010).

(Bahrami Kamangar et al. 2014; Boonham et al. 2002; Kerlan et al. 1999; Schubert et al. 2007; Yin et al. 2012), the Middle East (Anfoka et al. 2016; Chikh-Ali et al. 2010, 2016a; Elwan et al. 2017), North America (Funke et al. 2017; Gray et al. 2010; MacKenzie et al. 2018; Nanayakkara et al. 2012), South America (Colavita et al. 2017; Galvino-Costa et al. 2012a, b), East Asia (Chikh Ali et al. 2013b; Ogawa et al. 2012), Southeast Asia (Chikh-Ali et al. 2016b; Schubert et al. 2015), and Oceania (Kehoe and Jones, 2016). In South Korea, wide spread of PVY was reported in the past (Choi et al. 2004), but no studies up until now have been conducted on PVY strain identification and prevalence in potato fields; thus, the aim of the current study was to identify PVY strains present in South Korea and to characterize PVY isolates collected there.

Materials and Methods

Sample collection. Potato leaf samples were collected from potato fields representing five different cultivars: one of North American origin (Superior) and four local South Korean cultivars (Rose Valley, Dubaek, Chubaek, and Suyoung) (Table 1). The first sample collection was from potato fields near Chuncheon-si, Gangwon-do province, in May 2017, followed by a second collection near Buan-gun, Jeollabuk-do province, in October 2017 (Fig. 1). In the first round of sampling (K1 series), 10 samples, named K1-1 through K1-10, were

Fig. 1. A map of Korea indicating the locations where all potato samples were collected. The black circle indicates the capital city, Seoul, and the red circles point to the locations of potato fields sampled.

collected from three potato fields during the late spring growing season (Table 1). In each field, two to four plants showing virus-like symptoms, mainly mottle (Fig. 2), were selected for testing. In the second sampling (K2 series), three plants showing mottle symptoms were collected from two fields during the autumn growing season (Table 1). In both cases, samples were first tested with a strain-nonspecific PVY ImmunoStrip test (Agdia, Elkhart, IN) (Fig. 2). All PVY-positive samples, plus one PVY-negative sample (Fig. 2), were printed on FTA Classic Cards (Whatman, U.K.) according to the manufacturer's instructions. The cards were dried at room temperature for 1 h, sealed in a plastic bag with silica gel, and transported to the University of Idaho Virology Laboratory for further analysis. These dried FTA cards were stored in their original sealed plastic bags at -20°C until further use for nucleic acid extraction.

RNA extraction, reverse transcription polymerase chain reaction (RT-PCR), and sequencing. RNA was extracted from the FTA cards according to the method of Ndunguru et al. (2005), essentially as described previously (Chikh-Ali et al. 2016a, b). RNA pellets were dissolved in 50 μl of nuclease-free water, heated at 65°C for 5 min, and then chilled on ice for 3 min. Samples were tested for the presence of potato mop-top virus (PMTV), tobacco rattle virus (TRV), alfalfa mosaic virus (AIMV), potato virus M (PVM), potato virus A (PVA), potato virus X (PVX), potato virus S (PVS), and potato leafroll virus (PLRV) using the RT-PCR with specific primers as described previously (Elwan et al. 2017). For PVY strain identification, the multiplex RT-PCR typing assays were done according to the method of Chikh-Ali et al. (2010). Control isolates representing nine strains of PVY and additional recombinant variants were obtained from the University of Idaho laboratory collection (Chikh Ali et al. 2013a).

PVY isolates K1-1, K1-3, and K2-3 (Table 1) were randomly selected for whole genome sequencing. Whole genome sequencing was performed directly on a series of overlapping PCR fragments amplified from cDNA synthesized from these samples as described in Green et al. (2017). Successfully amplified PCR products, as determined by presence of bands of appropriate size on a 1.2% agarose gel, were treated with Exosap-It (Affymetrix, Cleveland, OH) and submitted for Sanger sequencing to Elim Biopharmaceuticals, Inc. (Hayward, CA). Individual sequence reads were assembled using the SeqMan Pro program of the Lasergene 14 Suite (DNASTAR, Madison, WI, USA). The nearly complete genome sequences will appear in GenBank under the following accession numbers: MH603861 (K1-1), MH603862 (K1-3), and MH603863 (K2-3).

Sequence analysis. Sequence identity of the South Korean sequences was checked using the BLAST program provided by the National Center for Biotechnology Information. PVY isolates with whole genomes sequenced, available in the GenBank database and identified as belonging to the PVY^{NTNa} strain (a total of 75 isolates as of March 2018), were used for the multiple alignment and subsequent phylogenetic analysis along with the complete sequences of the three South Korean isolates that had been selected for whole genome sequencing (Supplementary Table S1). The PVY^{NTNa} isolate PVY-12 (AB185833) was used as an outgroup. For the multiple sequence alignment, MUSCLE (Edgar 2004) implemented in the program MEGA version 7 (Kumar et al. 2016) was used with the default parameters. Phylogeny inference was conducted based on the whole genomes using the maximum likelihood method implemented in MEGA version 7 with 100 bootstrap replicates, with parameters modified to reflect the best model chosen for the data using the model selection tool in MEGA version 7 (Kumar et al. 2016). Additionally, the sequence identities and mean distances between isolates were calculated in MEGA version 7.

Results

Symptomatic samples exhibiting various degrees of mosaic, crinkling, and mottle were confirmed as PVY-positive based on the ImmunoStrip assay (Agdia), producing two distinct bands on the test strip (Fig. 2). Nine samples from three cultivars were collected near Chuncheon-si, Gangwon-do province, with approximately 30% of the plants exhibiting visual symptoms of mosaic. Three samples of

two cultivars were collected near Buan-gun, Jeollabuk-do province, where about 60% of the plants had visual symptoms of mosaic. One asymptomatic sample collected near Chuncheon-si in May 2018 tested negative for PVY and was used as a PVY-healthy control for downstream assays (Fig. 2B).

Fig. 2. Potato plants with visual symptoms of mosaic (A) or asymptomatic (B) were sampled and tested for potato virus Y (PVY) presence using a commercial ImmunoStrip test (Agdia). The symptomatic plant (A) was positive for PVY, producing two purple lines, control and test lines, on the ImmunoStrip kit; the asymptomatic plant (B) produced a single purple control line. The sample in A corresponds to the fourth lane (reading left to right) in the K1 set and the sample in B to the ninth lane in the K1 set on the gel in Figure 3.

Fig. 3. Reverse transcription polymerase chain reaction (PCR) assay for the identification of potato virus Y (PVY) isolates collected from a potato field in Korea. Samples were subjected to molecular typing according to the method of Chikh-Ali et al. (2010). Positions and sizes of the PCR products are indicated; H = healthy potato control; M = 100-bp DNA markers; NTNa = PVY^{NTNa} positive control; O = PVY^O positive control; N = PVY^N positive control; and N-Wi = PVY^{N-Wi} positive control.

Fig. 4. Phylogenetic analysis of the complete genome of 78 isolates of potato virus Y^{NTNa} (PVY^{NTNa}) including the three isolate sequences in the current study. The PVY^{NTNb} isolate PVY-12 (AB185833) was used as an outgroup. Lineages containing one sequence from southern Japan, three sequences from northern Japan, and two from Vietnam are indicated by arrows. The evolutionary history was inferred by using the maximum likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (−24,729.3162) is shown. The percentage of trees (higher than 70%) in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach and then selecting the topology with superior log likelihood value. The analysis involved 79 nucleotide sequences. Codon positions included were first + second + third + noncoding. All positions containing gaps and missing data were eliminated. A total of 9,514 positions were in the final dataset. Evolutionary analyses were conducted in MEGA7.

RT-PCR assays. Although initially samples were selected visually owing to mosaic and crinkling, and using PVY-specific ImmunoStrips (Agdia), all extracted nucleic acid samples were also subjected to testing for other viruses, as well as PVY, using RT-PCR. Of the 12 PVY-positive samples and one PVY-negative sample, four samples (K1-5 to K1-8) from potato cultivars Rose Valley and Dubaek, two each, were found positive for PVS (not shown). All samples were found negative for all the other viruses tested (PVX, PVA, AIMV, PVM, PLRV, PMTV, and TRV). When subjected to RT-PCR typing, 10 PVY-positive samples produced three bands of sizes 1,307, 663, and 441 bp characteristic of PVY^{NTNa} (Fig. 3; Chikh Ali et al. 2010), and two samples produced two faint bands of sizes 663 and 441 bp (Table 1; Fig. 3). The serologically PVY-negative sample was found negative for all viruses, including PVY, in the multiplex RT-PCR as well (Figs. 2 and 3, lane 9).

Sequencing and sequence analysis. The nucleotide sequences of nearly whole genomes of three isolates (K1-1, K1-3, and K2-3) were determined to be 9,626, 9,572, and 9,586 nt, respectively, excluding the 5' and 3' termini. All three genomes coded for a single open reading frame of 3,061 amino acids, typical of PVY. The reciprocal identity levels between the three isolates were very high, reaching 99.7 to 99.8% with an overall mean distance of 0.003. When subjected to BLAST analysis against the nucleotide sequences available in GenBank, the South Korean PVY isolates shared the highest nucleotide identity of 99.6 to 99.7% with isolates of the PVY^{NTNa} strain, NTNHIR3 (AB711143, Japan), VNP411 (HG810949, Vietnam), and VNP413 (HG810950, Vietnam).

To determine the precise relationships between the three sequenced PVY^{NTN} isolates from South Korea, and other PVY^{NTN} isolates across the globe, all 75 PVY^{NTNa} sequences identified in the GenBank database, plus an PVY^{NTNb} outgroup sequence, were downloaded and used for the alignment with the K1-1, K1-3, and K2-3 whole genomes. A global PVY^{NTN} phylogenetic tree was generated in MEGA based on this alignment (Fig. 4). The PVY^{NTN} whole genomes available in the GenBank database were grouped into multiple lineages, without any apparent geographic affinity, possibly reflecting the natural evolution of this PVY recombinant and global movement of seed potatoes. However, some clades could be defined within these lineages, linked to specific geographical locations where the corresponding PVY isolates were collected. For instance, three sequences, isolates Eu-12Jp (AB702945), NTKGAM2 (AB711145), and NTNTK1 (AB711146), originating from northern Japan, were placed in a tight clade (Fig. 4), despite being collected and sequenced in two different labs (Chikh Ali et al. 2013b; Ogawa et al. 2012).

In phylogenetic analysis, the South Korean PVY isolates were placed in a well-defined clade together with three isolates: NTNHIR3 from Hiroshima, Japan, and VNP411 and VNP413 from Tian Lang, Vietnam, respectively (Fig. 4). The overall mean distance for this subgroup was 0.004, whereas it was 0.006 for all PVY^{NTNa} isolates analyzed (78 in total). Isolate NTNHIR3 was described by Ogawa et al. (2012), and isolates VNP411 and VNP413 were described by Schubert et al. (2015).

Discussion

PVY is a major virus pathogen threatening seed and ware potato production worldwide. The PVY problem worsened across the globe during the last three decades owing to the emergence and spread of recombinant PVY strains (Karasev and Gray 2013). One particular recombinant, PVY^{NTNa}, was found associated with external tuber damage, called PTNRD, in susceptible potato cultivars (Gray et al. 2010; Le Romancer et al. 1994). PVY^{NTNa} has been reported in most of East and Southeast Asia, including Japan (Chikh Ali et al. 2013b; Ogawa et al. 2012), China (Hu et al. 2009b), Indonesia (Chikh-Ali et al. 2016b), and Vietnam (Schubert et al. 2015).

Viruses, including PVY, present a significant biotic stress affecting potato production in South Korea (Choi et al. 2004; Horton et al. 1988; Soley and Choi 2016). Presence of PVY isolates in potato exhibiting N- and O-serotypes was reported in South Korea (Choi

et al. 2004), but, prior to the current study, PVY strains circulating in potato in South Korea were not identified (Choi et al. 2004), and no whole genome PVY sequences were available for the virus isolates from potato. Only a few partial capsid protein sequences were available in the GenBank database, but these were not sufficient to identify the strain of PVY (Green et al. 2017, 2018; Karasev and Gray 2013). This lack of accurate information on PVY strains circulating in potato fields placed breeding programs in South Korea at risk of breeding for resistance against off-target PVY strains. In the current study, all PVY isolates collected were identified as PVY^{NTNa} using multiplex PCR assay (Chikh Ali et al. 2010). Three PVY^{NTNa} isolates from South Korea described in this study were found closely related to isolates from southern Japan (Ogawa et al. 2012) and Northern Vietnam (Schubert et al. 2015), which may suggest a common infection source, likely seed potato sourced from the same or closely related suppliers. The fact that only one PVY isolate found in southern Japan (NTNHIR3) was phylogenetically close to the three South Korean sequences (Fig. 4) may suggest that at least two separate introductions of the PVY^{NTN} strain occurred in Japan, with PVY^{NTNa} isolates from northern Japan being distinct from PVY^{NTNa} found in Hiroshima (Fig. 4). In this case, the origin of the South Korean PVY^{NTN} isolates would be hypothesized to be common with PVY^{NTN} isolates from Vietnam (Schubert et al. 2015) and with the NTNHIR3 isolate from southern Japan (Ogawa et al. 2012).

The results presented revealed that the PTNRD-inducing strain PVY^{NTNa} occurs in South Korea, imposing a potential threat to PTNRD-susceptible potato cultivars. The question about other strains of PVY that may or may not be present in South Korean potato fields remains open. The current study should be followed by more comprehensive surveys to assess the prevalence of PVY^{NTNa} and possibly other PVY strains in South Korea.

Literature Cited

- Anfoka, G., Altaieb, M., and Abu-Obaida, M. 2016. First report of potato virus Y strain N-Wilga affecting potato in Jordan. *Plant Dis.* 100:2176.
- Bahrami Kamangar, S., De Jonghe, K., and Smaghe, G. 2014. Potato virus Y (PVY) strains in Belgian seed potatoes and first molecular detection of the N-Wi strain. *J. Plant Dis. Prot.* 121:10-19.
- Baldauf, P. M., Gray, S. M., and Perry, K. L. 2006. Biological and serological properties of potato virus Y isolates in northeastern United States potato. *Plant Dis.* 90:559-566.
- Benedict, C., McMoran, D., Inglis, D., and Karasev, A. V. 2015. Tuber symptoms associated with recombinant strains of potato virus Y in specialty potatoes under northwestern Washington growing conditions. *Am. J. Potato Res.* 92:593-602.
- Boonham, N., Walsh, K., Hims, M., North, J., and Barker, I. 2002. Biological and sequence comparisons of potato virus Y isolates associated with potato tuber necrotic ringspot disease. *Plant Pathol.* 51:117-126.
- Chikh-Ali, M., Alruwaili, H., Vander Pol, D., and Karasev, A. V. 2016a. Molecular characterization of recombinant strains of potato virus Y from Saudi Arabia. *Plant Dis.* 100:292-297.
- Chikh-Ali, M., Bosque-Perez, N., Vander Pol, D., Sembel, D., and Karasev, A. V. 2016b. Occurrence and molecular characterization of recombinant potato virus Y^{NTN} (PVY^{NTN}) isolates from Sulawesi, Indonesia. *Plant Dis.* 100:269-275.
- Chikh-Ali, M., Gray, S. M., and Karasev, A. V. 2013a. An improved multiplex IC-RT-PCR assay distinguishes nine strains of potato virus Y. *Plant Dis.* 97:1370-1374.
- Chikh Ali, M., Karasev, A. V., Furutani, N., Taniguchi, M., Kano, Y., Sato, M., Natsuaki, T., and Maoka, T. 2013b. Occurrence of potato virus Y strain PVY^{NTN} in foundation seed potatoes in Japan, and screening for symptoms in Japanese potato cultivars. *Plant Pathol.* 62:1157-1165.
- Chikh Ali, M., Maoka, T., and Natsuaki, K. T. 2008. Whole genome sequence and characterization of a novel isolate of PVY inducing tuber necrotic ringspot in potato and leaf mosaic in tobacco. *J. Phytopathol.* 156:413-418.
- Chikh Ali, M., Maoka, T., Natsuaki, K. T., and Natsuaki, T. 2010. The simultaneous differentiation of potato virus Y strains including the newly described strain PVY^{NTN-NW} by multiplex PCR assay. *J. Virol. Methods* 165:15-20.
- Choi, H.-S., Bhat, A.-I., Park, J.-W., Cheon, J.-U., Kim, J.-S., Pappu, H.-R., Choi, J.-K., and Takanami, Y. 2004. Studies on potato virus Y isolates infecting potato and tobacco in Korea. *Kyushu Univ. Inst. Repository* 49:253-262.
- Colavita, M. L., Lancia, M., Feingold, S. E., and Massa, G. A. 2017. Evidence of recombinant isolates of potato virus Y (PVY) in Argentina. *Am. J. Potato Res.* 94:275-282.
- Edgar, R. C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res.* 32:1792-1797.

- Elwan, E. A., Aleem, E. E. A., Fattouh, F. A., Green, K. J., Tran, L. T., and Karasev, A. V. 2017. Occurrence of diverse recombinant strains of potato virus Y circulating in potato fields in Egypt. *Plant Dis.* 101:1463-1469.
- Funke, C. N., Nikolaeva, O. V., Green, K. J., Tran, L. T., Chikh Ali, M., Quintero-Ferrer, A., Cating, R., Frost, K. E., Hamm, P. B., Olsen, N., Pavsek, M. J., Gray, S. M., Crosslin, J. M., and Karasev, A. V. 2017. Strain-specific resistance to potato virus Y (PVY) in potato and its effect on the relative abundance of PVY strains in commercial potato fields. *Plant Dis.* 101:20-28.
- Galvino-Costa, S. B. F., dos Reis Figueira, A., Camargos, V. V., Geraldino, P. S., Hu, X.-J., Nikolaeva, O. V., Kerlan, C., and Karasev, A. V. 2012a. A novel type of potato virus Y recombinant genome, determined for the genetic strain PVY^E. *Plant Pathol.* 61:388-398.
- Galvino-Costa, S. B. F., dos Reis Figueira, A., Rabelo-Filho, F. A. C., Moraes, F. H. R., Nikolaeva, O. V., and Karasev, A. V. 2012b. Molecular typing of potato virus Y isolates from Brazil reveals a diverse set of recombinant strains. *Plant Dis.* 96:1451-1458.
- Gray, S., De Boer, S., Lorenzen, J., Karasev, A., Whitworth, J., Nolte, P., Singh, R., Boucher, A., and Xu, H. M. 2010. Potato virus Y: An evolving concern for potato crops in the United States and Canada. *Plant Dis.* 94:1384-1397.
- Green, K. J., Brown, C. J., Gray, S. M., and Karasev, A. V. 2017. Phylogenetic study of recombinant strains of potato virus Y. *Virology* 507:40-52.
- Green, K. J., Brown, C. J., and Karasev, A. V. 2018. Genetic diversity of potato virus Y (PVY): Sequence analyses reveal ten novel PVY recombinant structures. *Arch. Virol.* 163:23-32.
- Horton, D., Kim, Y. C., Hahn, B. H., Kim, K. K., Mok, I. G., Lee, B. N., and Kim, D. M. 1988. Potato Research and Development in the Republic of Korea: Organization, Impact and Issues. International Potato Center, Lima, Peru.
- Hu, X., Meacham, T., Ewing, L., Gray, S. M., and Karasev, A. V. 2009a. A novel recombinant strain of potato virus Y suggests a new viral genetic determinant of vein necrosis in tobacco. *Virus Res.* 143:68-76.
- Hu, X. X., He, C. Z., Xiao, Y., Xiong, X. Y., and Nie, X. Z. 2009b. Molecular characterization and detection of recombinant isolates of potato virus Y from China. *Arch. Virol.* 154:1303-1312.
- Karasev, A. V., and Gray, S. M. 2013. Continuous and emerging challenges of potato virus Y in potato. *Annu. Rev. Phytopathol.* 51:571-586.
- Karasev, A. V., Hu, X., Brown, C. J., Kerlan, C., Nikolaeva, O. V., Crosslin, J. M., and Gray, S. M. 2011. Genetic diversity of the ordinary strain of potato virus Y (PVY) and origin of recombinant PVY strains. *Phytopathology* 101:778-785.
- Kehoe, M. A., and Jones, R. A. C. 2016. *Potato virus Y* strain nomenclature: Lessons from comparing isolates obtained over a 73-year period. *Plant Pathol.* 65:322-333.
- Kerlan, C., Tribodet, M., Glais, L., and Guillet, M. 1999. Variability of potato virus Y in potato crops in France. *J. Phytopathol.* 147:643-651.
- Kumar, S., Stecher, G., and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33:1870-1874.
- Kutnjak, D., Elena, S., and Ravnarik, M. 2017. Time-sampled population sequencing reveals the interplay of selection and genetic drift in experimental evolution of potato virus Y. *J. Virol.* 91:e00690-17.
- Kutnjak, D., Rupar, M., Gutierrez-Aguirre, I., Curk, T., Kreuze, J. F., and Ravnarik, M. 2015. Deep sequencing of virus-derived small interfering RNAs and RNA from viral particles shows highly similar mutational landscapes of a plant virus population. *J. Virol.* 89:4760-4769.
- Le Romancer, M., Kerlan, C., and Nedellec, M. 1994. Biological characterization of various geographical isolates of potato virus Y including superficial necrosis on potato tubers. *Plant Pathol.* 43:138-144.
- Lorenzen, J. H., Meacham, T., Berger, P. H., Shiel, P. J., Crosslin, J. M., Hamm, P. B., and Kopp, H. 2006. Whole genome characterization of potato virus Y isolates collected in the western USA and their comparison to isolates from Europe and Canada. *Arch. Virol.* 151:1055-1074.
- MacKenzie, T. D. B., Lavoie, J., Nie, X., and Singh, M. 2018. Differential spread of potato virus Y (PVY) strains O, N:O and NTN in the field: Implications for the rise of recombinant PVY strains in New Brunswick, Canada. *Am. J. Potato Res.* 95:301.
- Nanayakkara, U. N., Singh, M., Pelletier, Y., and Nie, X. 2012. Investigation of potato virus Y (PVY) strain status and variant population in potatoes in New Brunswick, Canada. *Am. J. Potato Res.* 89:232-239.
- Ndunguru, J., Taylor, N. J., Yadav, J., Aly, H., Legg, J. P., Avenling, T., Thompson, G., and Fauquet, C. M. 2005. Application of FTA technology for sampling, recovery and molecular characterization of viral pathogens and virus-derived transgenes from plant tissues. *Virol. J.* 2:45-56.
- Ogawa, T., Nakagawa, A., Hataya, T., and Ohshima, K. 2012. The genetic structure of populations of potato virus Y in Japan; based on the analysis of 20 full genomic sequences. *J. Phytopathol.* 160:661-673.
- Quintero-Ferrer, A., Robles-Hernandez, L., Gonzalez-Franco, A. C., Kerlan, C., and Karasev, A. V. 2014. Molecular and biological characterization of a recombinant isolate of potato virus Y from Mexico. *Arch. Virol.* 159:1781-1785.
- Schubert, J., Fomitcheva, V., and Sztangret-Wisniewska, J. 2007. Differentiation of potato virus Y strains using improved sets of diagnostic PCR-primers. *J. Virol. Methods* 140:66-74.
- Schubert, J., Thieme, T., Thieme, R., Ha, C. V., and Hoang, G. T. 2015. Molecular and biological characterization of potato virus Y isolates from Vietnam. *J. Phytopathol.* 163:620-631.
- Soley, G. T., and Choi, S. 2016. Potato and Potato Products. 2016 Potato Update, Republic of Korea. USDA-FAS, Report number KS1621. https://gain.fas.usda.gov/Recent%20GAIN%20Publications/2016%20Potato%20Update_Seoul_Korea%20-%20Republic%20of_7-22-2016.pdf.
- Thole, V., Dalmay, T., Burgyan, J., and Balazs, E. 1993. Cloning and sequencing of potato virus Y (Hungarian isolate) genomic RNA. *Gene* 123:149-156.
- Turco, S., Golyaev, V., Seguin, J., Gilli, C., Farinelli, L., Boller, T., Schumpp, O., and Pooggin, M. 2018. Small RNA-omics for virome reconstruction and antiviral defense characterization in mixed infections of cultivated *Solanum* plants. *Mol. Plant-Microbe Interact.* 31:707-723.
- Kimba, S. P. F., Ibaba, J. D., and Gubba, A. 2017. Potato virus Y strains infecting potatoes in the Msinga district in the province of KwaZulu-Natal, South Africa. *Crop Prot.* 96:188-194.
- Yin, Z., Chrzanowska, M., Michalak, K., Zagórska, H., and Zimnoch-Guzowska, E. 2012. Recombinants of PVY strains predominate among isolates from potato crop in Poland. *J. Plant Prot. Res.* 52:214-219.
- Yin, Z., Xie, F., Michalak, K., Pawelkowicz, M., Zhang, B., Murawska, Z., Lebecka, R., and Zimnoch-Guzowska, E. 2016. Potato cultivar Etola exhibits hypersensitive resistance to PVY^{NTN} and partial resistance to PVY^{Z-NTN} and PVY^{N-Wi} strains and strain-specific alterations of certain host miRNAs might correlate with symptom severity. *Plant Pathol.* 66:539-550.