Genetic Diversity of the Ordinary Strain of *Potato virus Y* (PVY) and Origin of Recombinant PVY Strains

Alexander V. Karasev, Xiaojun Hu, Celeste J. Brown, Camille Kerlan, Olga V. Nikolaeva, James M. Crosslin, and Stewart M. Gray

First, second, fourth, and fifth authors: Department of PSES, University of Idaho, Moscow, ID 83843; first, second, and third authors: Bioinformatics and Computational Biology Program, University of Idaho, Moscow, ID; sixth author: United States Department of Agriculture–Agricultural Research Service (USDA-ARS), Prosser, WA 99350; and seventh author: USDA-ARS, Cornell University, Ithaca, NY 14853.

Accepted for publication 7 February 2011.

ABSTRACT

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.

The ordinary strain of *Potato virus Y* (PVY), PVY^O, causes mild mosaic in tobacco and induces necrosis and severe stunting in potato cultivars carrying the *Ny* gene. A novel substrain of PVY^O was recently reported, PVY^O-O5, which is spreading in the United States and is distinguished from other PVY^O isolates serologically (i.e., reacting to the otherwise PVY^N-specific monoclonal antibody 1F5). To characterize this new PVY^O-O5 subgroup and address possible reasons for its continued spread, we conducted a molecular study of PVY^O and PVY^O-O5 isolates from a North American collection of PVY through whole-genome sequencing and phylogenetic analysis. In all, 44 PVY^O isolates were sequenced, including 31 from the previously defined PVY^O-O5 group,

and subjected to whole-genome analysis. PVY^O-O5 isolates formed a separate lineage within the PVY^O genome cluster in the whole-genome phylogenetic tree and represented a novel evolutionary lineage of PVY from potato. On the other hand, the PVY^O sequences separated into at least two distinct lineages on the whole-genome phylogenetic tree. To shed light on the origin of the three most common PVY recombinants, a more detailed phylogenetic analysis of a sequence fragment, nucleotides 2,406 to 5,821, that is present in all recombinant and nonrecombinant PVY^O genomes was conducted. The analysis revealed that PVY^{N:O} and PVY^{N-Wi} recombinants acquired their PVY^O segments from two separate PVY^O lineages, whereas the PVY^{NTN} recombinant acquired its PVY^O segment from the same lineage as PVY^{N:O}. These data suggest that PVY^{N:O} and PVY^{N-Wi} recombinants originated from two separate recombination events involving two different PVY^O parental genomes, whereas the PVY^{NTN} recombinants likely originated from the PVY^{N:O} genome via additional recombination events.

Potato virus Y (PVY) is an emerging pathogen in potato, seriously affecting yield (23) and quality of tubers in susceptible cultivars infected with PVY strains inducing potato tuber necrotic ringspot disease (PTNRD). PVY exists as a complex of strains which can be distinguished by their reactions toward a series of resistance genes in potato. A strain group eliciting hypersensitive response (HR) in potato genotypes carrying the *Ny* gene, such as ‘Desiree’ and ‘Maris Bard’, was named PVY^O, and those eliciting HR in potato genotypes carrying the *Nc* gene, such as ‘King Edward’, were named PVY^C strains (5,6). Strains that could overcome both *Ny* and *Nc* genes and did not elicit HR toward these two genes were named PVY^N strains (13,30,32). Existence of an additional resistance gene, *Nz*, was postulated based on HR reactions of a PVY strain group that did not elicit HR in either *Ny* or *Nc* genetic backgrounds (13,30).

Complete genome sequences are known for several PVY strains from the PVY^O and PVY^N groups, which are ≈8% different along the entire 9.7-kb genome. In addition to these main, parental genomes, multiple recombinants have been discovered, with segments of PVY^O and PVY^N sequences spliced in their genomes. The three best-studied recombinants are PVY^{NTN}, which contain three to four recombination junctions, and PVY^{N-Wi} and PVY^{N:O},

which have two or one recombinant junctions, respectively (9,18,22,30). All three of these recombinant strains induce a characteristic veinal necrosis in tobacco, like the PVY^N strain group, and hence are called “necrotic” strains. The PVY^{NTN} strain has attracted the most attention recently because of its association with PTNRD (2). However, recently, other PVY strains were reported to induce PTNRD in certain sensitive potato cultivars (10). This new information suggests that other strain groups, perhaps even the seemingly benign PVY^O group, should be subjected to further genetic and biological scrutiny. The origins of these widespread recombinants and interrelations between different recombinant types have not been clear up to now (11,18,19); however, at least two recombinant types, PVY^{N-Wi} and PVY^{N:O}, have been considered to be almost identical, both genetically and biologically (9,18,19,22,27).

In 2004 to 2006, a North American survey of seed potato crops focused on identifying and typing PVY-positive samples (10). All PVY isolates collected during this survey were typed using serological profiling with PVY-specific monoclonal antibodies (MAbs) and reverse-transcription polymerase chain reaction (RT-PCR) profiling using a multiplex assay identifying the two most prominent recombinant junctions in the PVY genome (18), and all were tested for their reaction on tobacco. In the course of this survey, an unusual group of isolates was revealed that represented a subgroup of the PVY^O strain that was distinguished by a positive reaction toward one PVY^N-specific MAb, 1F5 (15). Although this variant, named PVY^O-O5, was clearly identified as belonging to the PVY^O strain by a combination of molecular and biological data, the survey data suggested that it was a stable or

Corresponding author: A. V. Karasev; E-mail address: akarasev@uidaho.edu

*The e-Xtra logo stands for “electronic extra” and indicates that the online version contains two supplemental tables and Figure 2 appears in color online.

doi:10.1094/PHYTO-10-10-0284

© 2011 The American Phytopathological Society

perhaps expanding component of the PVY^O strain (10); thus, we hypothesize that it may have certain evolutionary advantages over the ordinary PVY^O isolates (10,15).

In order to characterize molecular and biological features of this new PVY^O-O5 variant that may be responsible for its spread in the United States, 44 isolates from the survey collection were subjected to whole-genome sequencing and subsequent phylogenetic analysis. A majority of these isolates were PVY^O-O5 variants; however, 13 additional PVY^O isolates were included to provide better reference between the two PVY^O subgroups. In this work, we determined that PVY^O-O5 represents a phylogenetically distinct lineage of isolates within a broader PVY^O clade, which demonstrated considerable diversity. Phylogenetic analyses of the PVY^O sequences common between nonrecombinant PVY^O and recombinant PVY^{NTN}, PVY^{N-Wi}, and PVY^{N-O} genomes allowed an insight into the origin of recombinant PVY strains.

MATERIALS AND METHODS

Virus strains and antibodies. PVY isolates L26 (12), Mont, Oz, and 423-3 (18) were from our laboratory collection. PVY isolate CW was obtained from a tuber of 'Cal White' showing PTNRD and was subsequently identified as a PVY^O isolate, based on serological and RT-PCR profiles and tobacco symptoms (J. Crosslin, *unpublished*). Isolates ME56, ME173, and ID269 were sequenced and described previously (15). Additional isolates from the PVY^O and PVY^O-O5 subgroups were from a U.S. PVY strain collection (10). These isolates were characterized on tobacco and by RT-PCR and serology as described previously (15), and are listed in Supplemental Table 1. All virus isolates were maintained as freeze-dried infected tobacco leaf material or as purified virus preparations at -20°C. Polyclonal antisera UID8 (from rabbit) and G500 (from goat) used for general detection of all PVY strains, along with four MABs (MAB 1F5, MAB 2, MAB SASA-N, and MAB SASA-O), were described previously (15). MAB 1F5 (Agdia, Elkhart, IN) reacts with isolates belonging to the PVY^N, PVY^{NTN}, PVY^{NA-N}, and PVY^O-O5 strains; MAB2 (Agdia) reacts with PVY^O, PVY^O-O5, PVY^{N-Wi}, and PVY^C; MAB SASA-N (Scottish Agricultural Science Agency, Edinburgh) reacts with PVY^N, PVY^{NTN}, and PVY^{NA-N}, and MAB SASA-O (Scottish Agricultural Science Agency) reacts with PVY^O, PVY^O-O5, PVY^{N-Wi}, and PVY^C.

Biological characterization. To study plant responses, each isolate was mechanically inoculated as described previously (12) onto tobacco seedlings (*Nicotiana tabacum* 'Burley') or onto in vitro potato plantlets at the 6- to 10-leaf stage of cultivars King Edward, Desiree, and Maris Bard. Three plants of each potato cultivar were used per each PVY isolate; three independent experiments were conducted with isolates Oz, Tb60, CW, ME173, and ID269. The symptom expression of the PVY strains in King Edward (expressing the *Nc* gene), Desiree (expressing the *Ny* gene), and Maris Bard (expressing the *Nc*, *Ny*, and *Nz* genes) has been recently reviewed by Singh et al. (30). Young *Chenopodium amaranticolor* plants were inoculated at the six- to eight-leaf stage. Inoculations were carried out using fresh leaf tissue from infected tobacco source plants kept in an insect-free growth chamber as described (12), after equalizing PVY concentration in the samples based on enzyme-linked immunosorbent assay (ELISA) tests. Infected leaf tissue was homogenized with mortar and pestle in a neutral phosphate buffer, and *C. amaranticolor* leaves were rub inoculated using carborundum as an abrasive. Two independent experiments were conducted. Inoculated plants were grown in an insect-free greenhouse maintained at ≈20°C with natural light. Symptom observation commenced 1 week after inoculation and continued daily for 5 to 7 weeks. Infection status of the inoculated potato plants was verified in triple-antibody sandwich (TAS)-ELISA tests conducted on noninoculated, mid- and upper-level leaves collected 2 to 3 weeks postinoculation.

Plant tissue was homogenized in a phosphate buffer (1:20, wt/wt) as described (15) prior to loading onto ELISA plates. Additional ELISA tests were performed on the uppermost potato leaf samples collected at the end of the experiments in order to check whether infection was fully systemic even in plants exhibiting HR. ELISA tests were also performed on inoculated and upper, uninoculated leaves of *C. amaranticolor*, in order to confirm PVY infection in plants exhibiting local lesions. TAS-ELISA was performed as previously described (15).

ELISA format, primers, RT-PCR, and sequencing. TAS-ELISA tests were performed following the previously described protocol (15). PVY-positive and PVY-negative potato samples were included in each ELISA experiment as controls. Samples were defined as positive if the absorbance value was 3× the average of healthy controls. For multiplex RT-PCR analyses, a PVY-Multi 12-primer set described by Lorenzen et al. (20) was used. All steps, including nucleic acid extraction, RT, and subsequent PCR followed the protocol of Lorenzen et al. (20). PCR products were separated in agarose gels and visualized with a fluorescent imager after staining with GelStar (Cambrex, Rockland, ME) or ethidium bromide. All PVY-infected plants were maintained in an insect-proof growth room at the University of Idaho. Prior to sequencing, all PVY isolates were typed by RT-PCR and by TAS-ELISA with strain-specific MABs to confirm initial strain assignments as described previously (15). The whole-genome sequencing for the 44 isolates listed above was performed on two large, overlapping RT-PCR-amplified fragments generated essentially as described previously (12). These overlapping fragments were amplified directly on RNAs extracted from tobacco plants infected with different PVY isolates (12,20). PCR products were purified using the Qiaquick purification kit (Qiagen, Valencia, CA) and sequenced directly using the Applied Biosystems Automated 3730 DNA analyzer with Big Dye Terminator chemistry and AmpliTaq-FS DNA Polymerase. For multiple alignments, CLUSTAL X was used with the default parameters (31). Sequence analyses and phylogenetic reconstructions were performed essentially as described previously (11,12).

RESULTS

Serology, RT-PCR typing, and tobacco reactions. The 44 PVY isolates all reacted with MAB2 but not with SASA-N, indicating that they belonged to the PVY^O or PVY^C serotype. Of the 44 isolates, 31 also reacted with MAB 1F5 and were assigned to the PVY^O-O5 subgroup. All 44 isolates were determined to belong to the PVY^O strain by the multiplex RT-PCR assay (20), and none of the isolates induced veinal necrosis in tobacco. Three additional PVY^O-O5 isolates (ME56, ME173, and ID269), described previously (15), were included in this comparison as controls.

Whole-genome sequence analysis. The entire genome of 31 PVY^O-O5 isolates and 13 typical PVY^O isolates was sequenced and compared with the genomes of the three previously sequenced PVY^O-O5 isolates. The respective accession numbers are given in Supplemental Table 1. Each whole-genome sequence was analyzed to verify whether it belonged to the PVY^O strain group and to identify any possible recombinants. Based on our sequence analysis, no recombinant genomes were found, and all 47 isolates were clearly identified as belonging to the PVY^O strain group. The characteristic, single-amino-acid substitution in the capsid protein, Q98R, previously suggested to be responsible for the reactivity with the 1F5 MAB (15), was identified in each of the PVY^O-O5 (1F5-positive) genomes. Each of the typical PVY^O (1F5-negative) genomes contained a glutamine (Q) residue at position 98 (Fig. 1).

The 47 PVY^O-O5 and PVY^O genomes were subjected to phylogenetic analysis using the neighbor-joining algorithm to determine whether the 1F5 serological marker may be linked to a

more general heterogeneity within the PVY^O strain group. Indeed, all but one PVY^O-O5 genome clustered in a separate lineage, distinct from other PVY^O isolates (Fig. 2). Only ME173 clustered outside of the common PVY^O-O5 clade (Fig. 2). Interestingly, an isolate reported from Canada in 1996 as Canadian PVY^O (or PVY^O-139), under the GenBank accession number U09509 (29), also grouped with the PVY^O-O5 lineage (Fig. 2). In this case, however, position 98 in the capsid protein is occupied by R (Fig. 1), suggesting a typical PVY^O (i.e., 1F5-negative serology would be expected for this isolate).

Biological characterization of the PVY^O-O5 variants. The PVY^O strain but not the PVY^N or PVY^{NTN} strain is known to induce local lesions of a distinct pinkish color on *C. amaranticolor* (Fig. 3A and B). Two PVY^O-O5 isolates, ID269 and ME173, along with three characterized PVY^O isolates did induce typical local lesions; however, the appearance and time course of development for these local lesions differed for the PVY^O-O5 and PVY^O isolates. Data for six isolates is summarized in Table 1; isolate PB-Oz (1), which belongs to the PVY^O subgroup, was used as a control. All isolates from both subgroups

Fig. 1. Amino acid alignment of the *Potato virus Y* (PVY) capsid protein (CP) fragment between amino acids 61 and 120, spanning position number 98. All 47 PVY^O and PVY^O-O5 isolates used in this work are presented, plus U09509 (O-139), PB-Oz, Mont, L26, NE-11, and RRA-1.

induced multiple, pinkish local lesions in inoculated leaves of *C. amaranticolor* (Table 1), consistent with our expectations. However, appearance of lesions was delayed for ID269 isolate from the PVY^O-O5 subgroup compared with PB-Oz, Tb60, CW, and ME-173 isolates (Fig. 3A and B). Initial symptoms (pinpoints for most isolates and diffuse chlorotic spots for CW) appeared only 11 days postinoculation (dpi) for ID269 versus 7 to 9 dpi for other PVY^O isolates. Typical chlorotic local lesions appeared 9 dpi for Tb60, 10 to 12 days for CW and ME-173, and at 15 dpi for ID269. Chlorotic lesions then turned into pink lesions within 1 day for Tb60 (10 dpi), and not before 20 dpi for ID269 (Table 1). PVY^O and PVY^O-O5 isolates were easily detected by TAS-ELISA in inoculated but not in upper, uninoculated leaves (Table 1).

Two PVY^O-O5 isolates (ID269 and ME173) and three PVY^O isolates (CW, PB-Oz, and Tb60 controls) were tested on a set of potato cultivars, including Desiree, King Edward, and Maris Bard, that carry different PVY resistance genes used to distinguish PVY strains (5,29) (Table 2). All PVY^O and PVY^O-O5 isolates induced an HR in both Desiree and Maris Bard and induced only mosaic and leaf crinkling in King Edward, in full agreement with our expectations for PVY^O group strains (Table 2). Importantly, lack of HR in King Edward clearly indicates that PVY^O-O5 isolates do not belong to the PVY^C strain group. The time course of the HR development in both Desiree and Maris Bard was somewhat different for the ID269 isolate as opposed to other tested isolates. Specifically, the HR appeared earlier for ID269 and the necrotic reaction was more severe in ID269-infected plants, leading to a full collapse of plantlets 5 to 6 weeks after inoculation. In all cultivar-isolate combinations where HR occurred, symptoms were typical of HR reactions (i.e., necrotic patterns on inoculated and noninoculated leaves, with necrotic lesions and vein necrosis, and leaf-drop symptom on lower leaves and, frequently, on intermediate leaves, including noninoculated leaves) (Fig. 3C). Mosaic and crinkle symptoms were observed in most cases on top leaves. ELISA tests performed on top leaves 3 weeks after inoculation confirmed systemic infection in all cases.

Phylogenies of the PVY^O sequences from PVY recombinants with PVY^O genome segment. Because of the diversity of the PVY^O sequences revealed in our study, all PVY^O sequences determined here as well as those present in GenBank were subjected to further scrutiny. As noted above, the recombinant strains of PVY, PVY^{N-Wi}, PVY^{N-O}, and PVY^{NTN} all contain the genome segments of PVY^O from nucleotides 2,406 to 5,821 (11). Phylogenetic trees were generated using this PVY genome segment from the 47 PVY^O genomes discussed above and an additional three PVY^{N-Wi}, five PVY^{N-O}, one PVY-NE11, and one PVY^{NTN} genomes sequenced in this work, plus several available in GenBank (Supplemental Table 2). Aside from the separate PVY^O-O5 lineage, three distinct clades were identified (Fig. 4): clade A contained two sequences represented by PVY^O isolates ME120 and ME236-4 (Fig. 4, designated as PVY^O-1), clade B included two groups of ordinary PVY^O isolates (designated PVY^O-2 and PVY^O-3) plus PVY^{N-Wi} recombinant isolates, and clade C included PVY^{N-O} and PVY^{NTN} isolates. Clade B can be subdivided into at least three subclades; specifically, PVY^O-2 and PVY^O-3 lineages containing all PVY^O sequences similar to a group of more diverse PVY^O isolates (subclade PVY^O-2) and another group very similar to PVY^O-Oz (PVY^O-3), and PVY^{N-Wi} recombinant isolates. Clade C, on the other hand, did not contain any isolates from the nonrecombinant, ordinary PVY^O group or from the PVY^O-O5 variant; the clade was formed exclusively by isolates from two recombinant strains, PVY^{N-O} and PVY^{NTN}.

DISCUSSION

The genetic diversity characteristic of positive RNA plant viruses is driven by three main factors: mutation, recombination,

Fig. 2. Phylogenetic relationships among 47 *Potato virus Y* (PVY^O) and PVY^O-O5 isolates used in this work plus select others based upon their entire genome sequences; two non-potato PVY isolates are presented as outgroups. One thousand bootstrap replicates were analyzed by the neighbor-joining algorithm, and the figure shows the 70% consensus tree. Bootstrap support percentage for each node is shown.

and reassortment (25,26,28). All three are hypothesized to produce a vast pool of virus genomes required for virus adaptation to new evolutionary niches (25,26). PVY is a perfect example of an RNA virus using high mutation rate and numerous recombinants to generate huge genetic diversity and, thus, survive and succeed in multiple hosts and in various environments (3,11,16,17).

In the past 20 years, much attention has been given to studies of multiple recombinants of PVY that spread across the globe, affecting potato crops (2,4,9,11,12,18,19). In Europe, the non-

recombinant PVY strains PVY^O and PVY^N have all but disappeared, replaced by recombinant strains PVY^{NTN} and PVY^{N-Wi} (3,17). However, in the United States and Canada, PVY^O remains the predominant strain infecting potato, and PVY^N is rare, although recombinant strains are increasing in incidence and distribution (1,10,14,24). In New Zealand, contrary to the situation in Europe and even in North America, recombinant PVY strains have not been identified and only the PVY^O and PVY^N strains were found in potato (8). The relative success of nonrecombinant PVY strains in North America and, especially, in New Zealand,

Fig. 3. Symptoms induced by *Potato virus Y* (PVY) isolates: **A**, ID269 and **B**, ME173 in inoculated leaves of *Chenopodium amaranticolor* 15 days postinoculation; **C**, ID269 and Oz in ‘Desiree’ potato 39 days postinoculation.

TABLE 1. Reaction of *Chenopodium amaranticolor* to infection by select *Potato virus Y*^O (PVY^O) and PVY^O-O5 isolates

Strain	1F5 ^a	Phylogenetic subgroup	Type of lesions at given days postinoculation ^b			ELISA test on leaves ^c	
			Initial pinpoint	Chlorotic local	Pink local	Inoculated	Uninoculated
PB-Oz	–	O	7	12	12	+++	–
Tb60	–	O	N/T	9	10	+++	–
CW	–	O	9	10	15	+++	–
ME173	+	O	8	11	15	+++	–
ID269	+	O5	11	15	20	+++	–
L26	+	NTN	None	None	None	–	–

^a 1F5 reactivity as described by Karasev et al. (15).
^b Three plants were used for each inoculation; N/T = not tested.
^c Triple-antibody sandwich enzyme-linked immunosorbent assay (ELISA) test with anti-PVY capturing and detecting PAb (optical density at 405 nm); +++ indicates an ELISA signal >3.0 and – indicates an ELISA signal equal to the noninfected background (<0.2) 12 h after adding the developing solution into the wells.

can be partly explained by their closed potato seed production systems and the lack of substantial importation of seed potato and new recombinant PVY strains with it (8,10). Nevertheless, other factors may also be involved (i.e., evolution of those non-recombinant strains through mutations that may make them more competitive).

From a technical standpoint, it is intrinsically more difficult and time consuming to study genetic diversity in nonrecombinant PVY strains, because such studies would require whole-genome

sequencing as a prerequisite. However, the PVY^O-O5 subgroup has provided such an opportunity to study genetic diversity and evolution within the PVY^O strain group. The PVY^O-O5 variant was first described by Ellis et al. (7), and distinguished based on simple serological reactivity to a particular, PVY^N-specific MAb, 1F5. Recently, in the course of a large-scale potato PVY survey, this variant was found in numerous locations within the United States and Canada (15). The reasons for the PVY^O-O5 survival and selection were not immediately understood because, in all

TABLE 2. Reaction of potato indicators to select *Potato virus Y*^O (PVY^O) and PVY^O-O5 isolates^a

Cultivar	ID269	ME173	Oz	Tb60	CW
King Edward	Mosaic	Mosaic	Mosaic	Mosaic	Mosaic
Desiree	HR	HR	HR	HR	HR
Maris Bard	HR	HR	HR	HR	HR

^a HR = hypersensitive response.

Fig. 4. Phylogenetic tree generated from a *Potato virus Y* (PVY) genome fragment, nucleotides 2,406 to 5,821, using the neighbor-joining algorithm for the set of PVY isolates presented in Supplemental Tables 1 and 2. Designations of the main identified clades (letters) and subclades (letters and numbers) are also shown.

respects other than serology, PVY^O-O5 isolates were found to be nonrecombinant, ordinary PVY^O isolates (15). Whole-genome sequencing performed here on 31 PVY^O-O5 isolates collected during the survey, and identified by their reactivity to the 1F5 MAb, provided us a first glimpse into the genetic make-up and diversity of the PVY^O-O5 subgroup.

Indeed, PVY^O-O5 isolates represent a phylogenetically distinct lineage that can be distinguished from ordinary PVY^O isolates (Fig. 2). Thus, the apparent spread of the PVY^O-O5 isolates in North America in recent years (15) may reflect an internal evolution within the PVY^O strain group and emergence of a more successful and better fit subgroup, PVY^O-O5. Interestingly, this ongoing evolution occurred largely unnoticed, and could be revealed only by the presence of the 1F5 serological marker in the virus capsid protein.

However, this 1F5 serological marker is certainly a good but not perfect tag for the uncovered evolutionary lineage. Our whole-genome phylogenetic analysis revealed one PVY isolate, ME-173, clearly placed in the ordinary PVY^O clade (Fig. 2), while this same isolate displayed typical PVY^O-O5 reactivity with the 1F5 MAb and had a characteristic, previously identified Q residue in the capsid protein position 98 (Fig. 1). On the other hand, a previously determined sequence of Canadian isolate PVY^O-139 (U09509) (27) clearly belonged to our newly identified PVY^O-O5 phylogenetic lineage (Fig. 2) but did not have the Q residue in position 98 of the capsid protein (Fig. 1). Reactivity of this PVY^O-139 isolate with the 1F5 MAb was not tested in the original work (27) but PVY^O-139 was recently reported to be 1F5 negative (21).

Our initial tests on *C. amaranticolor* and potato indicators demonstrated that this phylogenetic distinction between the PVY^O-O5 subgroup and other, ordinary PVY^O isolates may have some biological foundations. Specifically, an O5 isolate, ID269, induced delayed local lesions on *C. amaranticolor* relative to isolates from the PVY^O lineage, including isolate ME173 (Fig. 3A and B; Table 1). It may be interpreted that the PVY^O-O5 isolate was “milder” on *C. amaranticolor* than ordinary PVY^O isolates. On the other hand, in potato indicators carrying the *Ny* gene, such as Desiree and Maris Bard, the same ID269 isolate induced a much more severe HR than ordinary PVY^O isolates (Fig. 3C; Table 2). This systemic HR started earlier than for ordinary PVY^O isolates, developed faster, and exhibited much more severe necrosis than the one characteristic of PVY^O isolates. Our finding is consistent with recent data on the Canadian PVY isolate O-139, belonging to the PVY^O-O5 lineage (U09509) (Fig. 2), that can be distinguished biologically from other, ordinary PVY^O isolates on ‘Jemseg’ potato (21). A potato cultivar carrying the *Nc* gene, King Edward, was susceptible to all PVY^O isolates tested, including PVY^O-O5 isolates. This indicated that the PVY^O-O5 subgroup was distinct from PVY^C isolates.

This is the first study of the recently described subgroup of PVY^O strains, named PVY^O-O5, demonstrating phylogenetic and biological distinction between PVY^O-O5 and other isolates from the PVY^O strain group. In addition to this, based on the whole-genome phylogenies of ordinary PVY^O and serologically distinct PVY^O-O5 isolates, at least two separate lineages can be identified in the ordinary PVY^O clade. Initial biological characterization suggested that PVY^O-O5 isolates differed from ordinary PVY^O in their reactivity in indicator plants and in potato cultivars carrying the *Ny* gene. It remains to be determined whether these biological differences may be related to the recent emergence and spread of the PVY^O-O5 isolates observed in the United States (10).

With a substantial set of PVY^O isolates sequenced during this work, it became possible to address more fundamental questions related to the origin of recombinant strains of PVY containing large fragments of PVY^O-derived sequences (e.g., PVY^{N:O}, PVY^{N:Wi}, and PVY^{NTN}). Specifically, these questions are (i) do all three types of recombinants originate from the same PVY^O parent

sequence? (ii) do some recombinants represent recombination intermediates between other recombinant types? and (iii) how often did these recombination events happen? Examination of the phylogenetic tree generated from the PVY^O- and PVY^O-derived segment represented by nucleotides 2,406 to 5,821 for recombinant and nonrecombinant PVY isolates (Fig. 4) gives some insight into the origin of recombinants and preliminary answers to these questions. It appears that the origin of these PVY recombinants is likely polyphyletic, with PVY^{N:Wi} acquiring its PVY^O sequence from a parent distinct from the one that provided the same segment to PVY^{N:O} and PVY^{NTN}. PVY^{N:O} and PVY^{NTN} may have acquired their PVY^O sequences from the same or very close parental genomes. Consequently, the PVY^{N:O} recombinant may be viewed as an intermediate or precursor type that evolved into PVY^{NTN} through additional recombination events. Interestingly, the two simpler recombinants—PVY^{N:Wi}, with two recombination junctions (9), and PVY^{N:O}, with one recombination junction (22)—which have been considered all but identical biologically, are now placed in distinct phylogenetic clades and, thus, likely have independent origins. The branching of the tree (Fig. 4) may even suggest a more recent origin of the PVY^{N:Wi} recombinant type which is still close enough to its parent sequence of a nonrecombinant PVY^O. The structure of the tree presented in Figure 4 suggests that recombination between PVY genomes may be quite rare—only three clades could be identified, corresponding to three recombinant PVY types. Alternatively, selective pressure on the newly generated recombinant genome may be so high that only a few recombinant types could survive and succeed in genome propagation (11).

ACKNOWLEDGMENTS

This work was funded, in part, through grants from the United States Department of Agriculture (USDA)-NIFA-NRI (number 2009-35600-05025), USDA-NIFA-SCRI (number 2009-51181-05894), the USDA-ARS Cooperative Agreements 58-5354-7-540 and 58-1907-8-870, and the Idaho Potato Commission. Bioinformatics facilities at University of Idaho were supported by grants P20RR16448 and P20RR016454 from the National Institutes of Health, National Center for Research Resources. We thank L. Ewing for providing potato plantlets, T. Meacham for help with RT-PCR typing, and H. Fingerson for help in immunoassays.

LITERATURE CITED

- 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.
- Beckner, L., Horvath, H., Romhanyi, L., and Forster, H. 1984. Etiology of tuber ringspot disease in potato. *Potato Res.* 27:339-351.
- Blanchard, A., Rolland, M., Lacroix, C., Kerlan, C., and Jacquot, E. 2008. *Potato virus Y*: A century of evolution. *Curr. Topics Virol.* 7:21-32.
- Chrzanowska, M. 1991. New isolates of the necrotic strain of potato virus Y (PVY^N) found recently in Poland. *Potato Res.* 34:179-182.
- Cockerham, G. 1970. Genetical studies on resistance to potato viruses X and Y. *Heredity* 25:309-348.
- de Bokx, J. A., and Huttinga, H. 1981. Potato Virus Y. Descriptions of Plant Viruses, No. 242. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, England. Online. www.dpvweb.net/dpv/showdpv.php?dpvno=242.
- Ellis, P., Stace-Smith, R., Bowler, G., and Mackenzie, D. J. 1996. Production of monoclonal antibodies for detection and identification of strains of potato virus Y. *Can. J. Plant Pathol.* 18:64-70.
- Fomicheva, V. W., Fletcher, J. D., and Schubert, J. 2009. *Potato virus Y* strain spectrum in New Zealand—absence of recombinant N:O Strains. *J. Phytopathol.* 157:507-510.
- Glais, L., Tribodet, M., and Kerlan, C. 2002. Genomic variability in *Potato potyvirus Y* (PVY): Evidence that PVY^{N:W} and PVY^{NTN} variants are single to multiple recombinants between PVY^O and PVY^N isolates. *Arch. Virol.* 147:363-378.
- Gray, S. M., DeBoer, S. H., Lorenzen, J., Karasev, A. V., Whitworth, J., Nolte, P., Singh, R. P., Boucher, A., and Xu, H. 2010. *Potato virus Y*: A significant and evolving threat to potato crops in the United States and Canada. *Plant Dis.* 94:1384-1397.
- Hu, X., Karasev, A. V., Brown, C. J., and Lorenzen, J. H. 2009. Sequence

- characteristics of *Potato virus Y* recombinants. J. Gen. Virol. 90:3033-3041.
12. Hu, X., Meacham, T., Ewing, L., Gray, S. M., and Karasev, A. V. 2009. A novel recombinant strain of *Potato virus Y* suggests a new viral genetic determinant of vein necrosis in tobacco. Virus Res. 143:68-76.
 13. Jones, R. A. C. 1990. Strain group specific and virus hypersensitive reactions to infection with potyviruses in potato cultivars. Ann. Appl. Biol. 117:93-105.
 14. Karasev, A. V., Meacham, T., Hu, X., Whitworth, J., Gray, S. M., Olsen, N., and Nolte, P. 2008. Identification of PVY strains associated with tuber damage during an outbreak of PVY in potato in Idaho. Plant Dis. 92:1371-1371.
 15. Karasev, A. V., Nikolaeva, O. V., Hu, X., Sielaff, Z., Whitworth, J., Lorenzen, J. H., and Gray, S. M. 2010. Serological properties of ordinary and necrotic isolates of *Potato virus Y*: A case study of PVY^N misidentification. Am. J. Potato Res. 87:1-9.
 16. Kerlan, C. 2006. *Potato virus Y*. Descriptions of Plant Viruses no. 414. Association of Applied Biologists, UK. Online. www.dpvweb.net/dpv/showdpv.php?dpvno=414.
 17. Kerlan, C., and Moury, B. 2008. *Potato virus Y*. Pages 287-296 in: Encyclopedia of Virology, Third Edition, vol. 4. B. W. J. Mahy and M. H. V. Van Regenmortel, eds. Elsevier, Oxford.
 18. 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.
 19. Lorenzen, J. H., Nolte, P., Martin, D., Pasche, J. S., and Gudmestad, N. C. 2008. NE-11 represents a new strain variant class of *Potato virus Y*. Arch. Virol. 153:517-525.
 20. Lorenzen, J. H., Piche, L. M., Gudmestad, N. C., Meacham, T., and Shiel, P. 2006. A multiplex PCR assay to characterize *Potato virus Y* isolates and identify strain mixtures. Plant Dis. 90:935-940.
 21. Nie, B., Singh, M., Sullivan, A., Singh, R. P., Xie, C., and Nie, X. 2011. Recognition and molecular discrimination of severe and mild PVY^O variants of *Potato virus Y* in potato in New Brunswick, Canada. Plant Dis. 95:113-119.
 22. Nie, X., Singh, R. P., and Singh, M. 2004. Molecular and pathological characterization of N:O isolates of the *Potato virus Y* from Manitoba, Canada. Can. J. Plant Pathol. 26:573-583.
 23. Nolte, P., Whitworth, J. L., Thornton, M. K., and McIntosh, C. S. 2004. Effect of seedborne *Potato virus Y* on performance of Russet Burbank, Russet Norkotah, and Shepody potato. Plant Dis. 88:248-252.
 24. Piche, L. M., Singh, R. P., Nie, X., and Gudmestad, N. C. 2004. Diversity among *Potato virus Y* isolates obtained from potatoes grown in the United States. Phytopathology 94:1368-1375.
 25. Roossinck, M. J. 1997. Mechanisms of plant virus evolution. Annu. Rev. Phytopathol. 35:191-209.
 26. Roossinck, M. J. 2003. Plant RNA virus evolution. Curr. Opin. Microbiol. 6:406-409.
 27. Schubert, J., Fomitcheva, V., and Sztangret-Wisniewski, J. 2007. Differentiation of *Potato virus Y* using improved sets of diagnostic PCR-primers. J. Virol. Methods 140:66-74.
 28. Simon, A. E., and Bujarski, J. J. 1994. RNA-RNA recombination and evolution in virus-infected plants. Annu. Rev. Phytopathol. 32:337-362.
 29. Singh, M., and Singh, R. P. 1996. Nucleotide sequence and genome organization of a Canadian isolate of the common strain of potato virus Y (PVY^O). Can. J. Plant Pathol. 18:209-214.
 30. Singh, R. P., Valkonen, J. P. T., Gray, S. M., Boonham, N., Jones, R. A. C., Kerlan, C., and Schubert, J. 2008. Discussion paper: The naming of *Potato virus Y* strains infecting potato. Arch. Virol. 153:1-13.
 31. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. 1997. The CLUSTAL_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 25:4876-4882.
 32. Valkonen, J. P. T. 1997. Novel resistances to potyviruses in tuber-bearing potato species, and temperature-sensitive expression of hypersensitive resistance to potato virus Y. Ann. Appl. Biol. 130:91-104.