Identification of the Molecular Make-Up of the *Potato virus Y* Strain PVY^Z: Genetic Typing of PVY^Z-NTN

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ABSTRACT

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Potato virus Y (PVY) strains were originally defined by interactions with different resistance genes in standard potato cultivars. Five distinct strain groups are defined that cause local or systemic hypersensitive responses (HRs) in genetic background with a corresponding *N* gene: PVY^O, PVY^N, PVY^C, PVY^Z, and PVY^E. The nucleotide sequences of multiple isolates of PVY^O and PVY^N differ from each other by ≈8% along their genomes. Additionally, complete genome sequences of multiple recombinant isolates are composed of segments of parental PVY^O and PVY^N sequences. Here, we report that recombinant isolate PVY-L26 induces an HR in potato ‘Maris Bard’ carrying the putative *Nz* gene, and is not recognized by two other resistance genes, *Nc* and *Ny_{ibr}*. These

genetic responses in potato, combined with the inability of PVY-L26 to induce vein necrosis in tobacco, clearly define it as an isolate from the PVY^Z strain group and provide the first information on genome structure and sequence of PVY^Z. The genome of PVY-L26 displays typical features of European NTN-type isolates with three recombinant junctions (PVY^{EU-NTN}), and the PVY-L26 is named PVY^Z-NTN. Three typical PVY^{NTN} isolates and two PVY^N isolates, all inducing vein necrosis in tobacco, were compared with PVY-L26. One PVY^{NTN} isolate elicited HR reactions in Maris Bard, similar to PVY-L26, while two induced a severe systemic HR-like reaction quite different from the quasi-symptomless reaction induced by two PVY^N isolates. ‘Yukon Gold’ potato from North America produced HR against several PVY^{NTN} isolates, including PVY-L26, but only late and limited systemic necrosis against one PVY^N isolate. Consequently, according to symptoms in potato indicators, both PVY^Z and PVY^{NTN} isolates appeared biologically very close and clearly distinct from PVY^O and PVY^N strain groups.

Potato virus Y (PVY) is one of the most studied plant viruses (38,42) and one of the most economically important plant pathogens, often listed in the top five viruses affecting both yield and quality in field-grown vegetables (46). It is currently recognized as the most economically important virus affecting cultivated potato, including seed, fresh, and processed potato (64). PVY is also considered the most damaging virus in tobacco crops, and is the causal agent of major diseases and losses in pepper and tomato crops (39). PVY is distributed worldwide in these four crops, and is also reported to infect numerous weeds in the family *Solanaceae*. It is spread naturally by vegetatively propagated material, notably potato tubers, and by aphids in a nonpersistent manner, with over 50 aphid species recognized as potential vectors (53). PVY is the type member of the genus *Potyvirus* in the family *Potyviridae* (27,58).

Given its broad host range, which includes four widely cultivated plants, each having a large set of cultivars with different resistance backgrounds, PVY normally displays a high genetic variability (59). PVY history is highlighted by frequent emergence of atypical populations differing in virulence or aggressiveness, transmissibility, phenotype, serotype, or another trait (6,29). In the 1960s, potato isolates of PVY were divided into three strains: PVY^C, PVY^O, and PVY^N (5). PVY^C was identified by hypersensitive reactions (HRs) in potato cultivars expressing gene *Nc* (15,16). PVY^O infected these same cultivars without inducing HR (15,16) but induced HR in other cultivars bearing a tentatively named gene *Ny* (22,23), later designated *Ny_{ibr}* (9,35,

65). A third, historical strain, PVY^N (59), was distinguished from PVY^C and PVY^O by its ability to induce vein necrosis in tobacco (*Nicotiana tabacum*) but caused milder symptoms in most potato cultivars and faster translocation from foliage to progeny tubers (5). Moreover, PVY^N overcame both *Nc* and *Ny_{ibr}* (32) and did not elicit any HR in standard potato cultivars (61). In the 1980s, a new strain group, named PVY^Z, was characterized which overcame both *Ny_{ibr}* and *Nc* while eliciting HR in certain cultivars such as ‘Maris Bard’ and ‘Pentland Ivory’ bearing the hypothetical gene *Nz* (35). Later, a strain group, first designated as PVY^{ZE} and later as PVY^E, was characterized which overcame resistance in Maris Bard (40). The incidence, geographical distribution, and significance of both of these strain groups has never been reported; nevertheless, both PVY^Z and PVY^E were recently designated as PVY strains along with PVY^O, PVY^C, and PVY^N (59). However, if we exclude some review papers (39,59), much of the recent PVY literature ignores PVY^C, PVY^Z, and PVY^E and takes into account only PVY^N and PVY^O and recombinants between them.

Rapid developments in PVY molecular biology since the late 1980s made it possible to characterize and classify PVY strains based on molecular characteristics, which may or may not correspond to genetic PVY strain groups. In addition to the five PVY strains defined genetically on potato indicators, two main variants within the PVY^N group (PVY^{NTN} and PVY^{N-Wi}) were defined. These emerged during the last three decades and are now distributed worldwide (2,7,10,12,19–21,25,36,41,43,45,48,51,52, 56,67). PVY^{NTN} isolates were first characterized as being associated with a potato disease referred as potato tuber necrotic ring-spot disease (PTNRD) (4). PVY^{N-Wi} isolates, also called PVY^{N:O} in Canada and the United States, were first reported as differing from PVY^N in pathogenicity (13) and later in serotype (14). Both

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PVY^{NTN} and PVY^{N-wi} induce vein necrosis in tobacco and, consequently, fit within the PVY^N group. However, molecular characterization revealed that the majority are recombinants between PVY^O and PVY^N, and display genomes with segments of PVY^O-type and PVY^N-type sequences in various combinations. Most PVY^{NTN} isolates have three or, rarely, four recombinant junctions (57), whereas PVY^{N-wi} isolates usually have one or two recombinant junctions (30,43,44,49).

Other recombinant isolates were reported recently, such as U.S. isolate PVY-L26 which induced PTNRD, like PVY^{NTN}, although it induced only mosaic in tobacco, which implies that induction of the PTNRD is not linked to the induction of tobacco vein necrosis (32). Molecular characterization revealed that the genome of L26 contains three recombination junctions typical of European PVY^{NTN} isolates (32). However, L26 still had the amino acids (K-400 and E-419) in helper component proteinase (HC-Pro) reported as determinants of the tobacco necrotic response (61), indicating involvement of other determinants in this phenotype. A single amino acid substitution in the central region of the PVY-L26 HC-Pro was correlated with loss of induction of necrotic symptoms in tobacco (32).

All the genetic PVY strain reactions described were studied with European potato cultivars, and the great majority of PVY strains typed were collected in Europe (35,40). Relatively limited information exists on the presence of *N* genes in North American potato cultivars, and no comprehensive genetic typing of North American PVY strains has been completed. Our primary objective was to obtain a comprehensive biological characterization of isolate L26 using criteria defined by Singh et al. (59) (i.e., through reactions on potato cultivars bearing HR genes). L26 was tested under different temperature and light conditions along with standard isolates belonging to PVY^O, PVY^N, and PVY^{NTN}, mostly isolated from the North American field samples.

MATERIALS AND METHODS

Viral isolates and plant infection studies. Seven PVY isolates collected from potato in the United States during 2001 to 2008 were described previously (2,32,43) and isolate Tb60 (J. H. Lorenzen, *personal communications*) was also included; their known biological, serological, and molecular properties are summarized in Table 1. Isolate PVY^N-NZ was provided by Dr. Emmanuel Jacquot (INRA, Rennes France). It was collected in New Zealand (28) and displayed a nonrecombinant N-type sequence (57). This isolate was never tested on 'Yukon Gold' but it induced some tuber necrosis on many cultivars under experimental conditions (8). All isolates were maintained as lyophilized or frozen tissue from mechanically inoculated tobacco plantlets and by mechanical plant-to-plant transfer using potato and tobacco plants maintained in an insect-free, isolated growth chamber.

To study plant responses, each isolate was mechanically inoculated as described previously (32) onto tobacco seedlings ('Samsun' or 'Xanthi') or onto in vitro potato plantlets at the 6- to 10-leaf stage of 'King Edward', 'Desiree', Maris Bard, 'Russet Burbank', and Yukon Gold. Symptom expression in King Edward (expressing the *Nc* gene), Desiree (expressing the *Ny_{ibr}* gene), and Maris Bard (expressing the *Nc*, *Ny_{ibr}*, and *Nz* genes) was reviewed by Singh et al. (59). Russet Burbank was included as a susceptible control. In addition, Yukon Gold was included because it was previously used for characterization of isolate L26 (32). Inoculated plants were grown under three different conditions: an insect-free greenhouse maintained at ≈20°C with natural light and in growth chambers under artificial light provided by fluorescent and incandescent lamps with a day-and-night cycle of 18 and 6 h, respectively, and maintained at either 16 to 18 or 23 to 26°C. Three separate experiments were conducted between March and August 2009 and two additional experiments between March and June 2010. In the first, plants were grown only at 23 to 26°C in a growth chamber; in the second, plants were grown in the greenhouse and growth chambers maintained at both temperatures; and, in the last three experiments, plants were grown in the 23 to 26°C growth chamber and in the greenhouse.

Symptom observation commenced 1 week after inoculation and continued daily for 5 to 7 weeks. Infection status of the inoculated plants was verified in triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) tests conducted as previously described (37,50) on noninoculated, mid- and upper-level leaves collected 2 to 3 weeks postinoculation. Additional ELISA tests were performed on the uppermost leaf samples collected at the end of the experiments to check whether infection was fully systemic even in plants exhibiting HR. Additional ELISA tests were also performed to confirm infection in some plants exhibiting late necrosis on low, noninoculated leaves.

To verify that no cross-contamination occurred between different PVY strains, both TAS-ELISA and reverse-transcription polymerase chain reaction (RT-PCR) typing were used. Monoclonal antibodies (MAbs) MAb2 (Agdia, Elkhart, IN) and SASA-O (Scottish Agriculture Science Agency, Antibody Unit at SASA, Edinburgh) were used to identify PVY^O reactivity, while MAbs 1F5 (Agdia) and SASA-N (SASA) were used to identify PVY^N and PVY^{NTN} reactivity; the protocol followed a previously described procedure (37). RT-PCR typing was performed according to the protocol of Lorenzen et al. (44).

RESULTS

Results were consistent among all five experiments and all three environmental conditions. Symptoms were first observed 10 to 12 days postinoculation (dpi) on potato and tobacco plants grown in the chamber maintained at 23 to 26°C, and a few days later in the growth chamber maintained at 16 to 18°C. Under the

TABLE 1. Phenotypic traits of the *Potato virus Y* (PVY) isolates used in this study

Isolates	Strain	Tobacco bioassay	Specific traits		
			Serotype	Genome sequence ^a	PTNRD ^b
Oz	PVY ^O	Mosaic	PVY ^O	O-type sequence EF026074	No
Tb60	PVY ^O	Mosaic	PVY ^O	O-type sequence	No
Mont	PVY ^N	Vein necrosis	PVY ^N	N-type sequence AY884983	No
NZ	PVY ^N	Vein necrosis	PVY ^N	N-type sequence AM268435	Possible
423.3	PVY ^{NTN}	Vein necrosis	PVY ^N	Recombinant AY884982	Yes
N4	PVY ^{NTN}	Vein necrosis	PVY ^N	Recombinant FJ204165 ^c	Yes
HR1	PVY ^{NTN}	Vein necrosis	PVY ^N	Recombinant FJ204166 ^c	Yes
L26 ^d	PVY ^{NTN}	Mosaic	PVY ^N	Recombinant FJ204164 ^c	Yes

^a Sequences deposited in GenBank. Recombinant: genome displays three PVY^O/PVY^N recombination junctions.

^b Potato tuber necrotic ringspot disease (PTNRD) induced or not induced on 'Yukon Gold' progeny tubers after mechanical inoculation on foliage of young plantlets.

^c Genome displays three PVY^O/PVY^N recombinant junctions.

^d Special PVY^{NTN} with a mutation in helper component proteinase cistron.

greenhouse conditions, symptoms appeared at approximately the same time as under high-temperature conditions in one experiment (late May to June) but were delayed in subsequent experiments. ELISA tests confirmed that virus inoculation resulted in systemic infection in all cases, except for a few potato plantlets in which the leaflets were very small at the time of inoculation. ELISA tests also revealed rather frequent symptomless infections, including in Russet Burbank. Of the >300 inoculations performed during the entire set of experiments, only one mismatch or cross-contamination was identified with strain-specific ELISA and RT-PCR tests; this plant, in the first round of experiments, was excluded from the analysis.

Tobacco plantlets Samsun and Xanthi expressed typical PVY^O-like symptoms when inoculated by isolates Oz and Tb60 (i.e.,

mottling or vein-clearing on flat and well-expanded leaves). They expressed PVY^N-like symptoms when inoculated with isolates Mont, NZ, HR1, 423.3, and N4, notably vein necrosis and leaf curling. Mild vein necrosis was observed in most plantlets inoculated by isolates Mont and NZ, though leaf curling was very obvious, whereas severe vein necrosis, leaf distortion, and stunting occurred in plantlets inoculated by PVY^{NTN} isolates. Isolate PVY-L26 induced vein clearing that turned into very faint mosaic later, and, contrary to symptoms induced by isolates HR1, 423.3, and N4, displayed a total absence of both plant stunting and leaf curling.

Several types of PVY-induced symptoms were observed on potato. Mosaic and vein clearing symptoms developed on King Edward and Russet Burbank. Very small necrotic lesions were observed, infrequently and inconsistently, on their inoculated or noninoculated leaves. HR reactions developed in Desiree, Maris Bard, and Yukon Gold, usually manifested on the inoculated leaves initially as vein clearing that quickly turned into vein necrosis (Fig. 1A). Interveneal necrotic spots and halos rapidly expanded into large necrotic lesions that eventually expanded to cover the entire leaflets and petioles. These necrotic leaves usually withered or abscised within 1 day. Systemic necrosis, leaf-drop, and mosaic symptoms developed on the systemically infected leaves of plants exhibiting HR on inoculated leaves. Necrotic streaks or irregular necrotic blotches were often observed on stems in plants displaying leaf-drop symptoms. Leaves at an intermediate position on the stem developed expanding necrotic lesions and, sometimes, vein necrosis. These leaves could become entirely necrotic and withered but remained attached to the stem, hanging down (Fig. 1C), sometimes until the end of the experiment. Such characteristic leaf-drop streak symptoms were clearly visible in numerous isolate-cultivar combinations at 23 to 26°C as well as under greenhouse conditions and, to a lesser extent, at 16 to 18°C. Leaf-drop usually affected intermediate leaves but sometimes upper leaves as well. When infection turned into a quasi-collapse of the plant, only the top of it still retained some small leaves. Systemic necrosis was often rather severe, notably under greenhouse conditions (Fig. 1B). Uppermost leaves either showed mosaic symptoms (Fig. 1C) with sometimes severe crinkle and necrotic points or were symptomless. In these plants showing either local necroses or local and systemic necroses, systemic infection was confirmed by ELISA, in most cases with high virus titer. Symptoms observed on each of the potato cultivars are summarized in Table 2.

PVY^O symptoms. Isolates PVY^O-Oz and PVY^O-Tb60 induced local and systemic HR on Desiree, Maris Bard, and Yukon Gold. Local and systemic HR was clearly visible, especially the leaf-drop symptom which affected numerous leaves. Local lesions and vein necrosis were first observed at 10 to 11 dpi on inoculated leaves of plants grown in the 23 to 26°C growth chamber and greenhouse (Fig. 1A), and 1 day later on plants grown in the 16 to 18°C growth chamber. The leaf-drop symptom was less frequently observed in the 16 to 18°C growth chamber but readily developed under greenhouse conditions and in the 23 to 26°C growth chamber (Fig. 1B). Systemic mosaic with a more or less severe crinkling was clearly visible at 11-12 dpi under all three conditions and developed into severe systemic necrotic lesions and vein necrosis at 23 to 26°C and under greenhouse conditions (Fig. 1C). Systemic necrosis was less obvious and less frequent at 16 to 18°C. Uppermost leaves sometimes displayed severe mosaic, necrotic points, and some vein necrosis. No HR and only chlorotic halos on inoculated leaves and systemic mosaic and vein clearing were observed on King Edward and Russet Burbank plants infected with PVY^O-Oz or PVY^O-Tb60.

PVY^N symptoms. PVY^N-Mont infection was often symptomless (e.g., on Russet Burbank or Desiree) and only ELISA tests established inoculation success. Mosaic symptoms developed on Maris Bard and King Edward plants at 23 to 26°C and some crinkling

Fig. 1. Different types of symptoms that developed on potato cultivars after mechanical inoculation with *Potato virus Y* isolate PVY-Oz. **A**, Necrotic lesions on inoculated leaves: 'Desiree', 11 days postinoculation (dpi), greenhouse. **B**, Leaf-drop on intermediate leaves: 'Yukon Gold', 16 dpi, greenhouse. **C**, Severe systemic necrosis on an upper leaflet; mosaic with some necrotic points on apical leaves, rings on lower leaflets: Yukon Gold, 19 dpi, greenhouse.

on several King Edward plants. Yukon Gold plants displayed symptomless infection in greenhouse and lower-temperature conditions but mottle turning into yellowing at 15 to 20 dpi at higher temperatures.

Isolate PVY^N-NZ induced essentially symptomless infections in Desiree, Maris Bard, and Russet Burbank but faint mosaic and crinkling without any necrosis on King Edward. Most plants of these four cultivars remained standing tall, with large, healthy-

looking leaves (Fig. 2A to D). No symptoms on Yukon Gold plantlets inoculated by isolate PVY^N-NZ developed during the first 4 weeks of the experiment. However, mild and limited HR-like reactions occurred $\approx$ 7 weeks postinoculation, consisting of some yellowing turning into necrosis and typical leaf-drop on several lower noninoculated leaves. These HR-like symptoms did not extend to upper leaves and no symptoms (either necrosis, mosaic, or crinkle) were visible on the top of the plant. However,

TABLE 2. Reactions expressed on five potato cultivars inoculated by eight *Potato virus Y* (PVY) isolates^a

Isolates	'King Edward'	'Desiree'	'Maris Bard'	'Russet Burbank'	'Yukon Gold'
Oz	mo, crk	syst. HR	syst. HR	mo, crk	syst. HR
Tb60	mo, crk	syst. HR	syst. HR	mo, crk	syst. HR
Mont	mo, crk	no symptoms	no symptoms or faint mo, crk	no symptoms	no symptoms or faint mo
NZ	mo, crk	no symptoms	faint mo, crk	no symptoms	no symptoms, late syst. nec
L26	severe mo, crk	no symptoms or mild mo	syst. HR	mo, crk	syst. HR
423.3, N4	severe mo, crk	mild mo, no crk	some nec, ld on il, syst. vn, severe mo, crk	mild mo, faint crk	local HR, syst. vn, no ld on top
HR1	mo, crk	no symptoms or mild mo	syst. HR	mo, crk	syst. HR

^a Systemic (syst.) hypersensitive responses (HR): necrotic points, spots, and vein necrosis (nec) on inoculated leaflets (il); whole-plant necrosis, quick leaf-drop (ld), then premature fall down of these inoculated leaflets; leaf-drop symptoms on intermediate leaves; necrotic points, spots and vein necrosis on noninoculated leaves, sometimes on uppermost leaves associated or not with mosaic (mo) and crinkle (crk). Local HR: necrotic lesions, including some vein necrosis and leaf-drop symptom on inoculated leaves (sometimes leaf-drop can extend to some lower noninoculated leaves).

Fig. 2. Symptoms that developed on potato cultivars after mechanical inoculation with *Potato virus Y*-NZ isolate PVY^N. **A**, Symptomless infection: 'Desiree', 16 days postinoculation (dpi), greenhouse. **B**, Mosaic: 'King Edward', 16 dpi, greenhouse. **C**, Symptomless infection: 'Maris Bard', 16 dpi, greenhouse. **D**, Symptomless infection: 'Russet Burbank', 16 dpi, greenhouse.

systemic infection in both lower and upper leaves was confirmed by additional ELISA tests performed 6 weeks postinoculation.

PVY-L26 symptoms. Isolate PVY-L26 induced typical HR, manifested as vein necrosis and necrotic spots on the inoculated leaves of Maris Bard and Yukon Gold that were first observed at 9 dpi (higher temperature and greenhouse) or 10 dpi (lower temperature). This was followed by leaf-drop 1 day later (Fig. 3A) and leaf abscission $\approx$ 14 to 16 dpi. The first systemic HR symptoms were observed on Yukon Gold at 14 dpi and 1 day later on Maris Bard plants maintained in the higher-temperature growth chamber. Symptoms began as necrotic points and developed into various necrotic patterns: periveinal or necrotic halos, necrotic patches, on intermediate and upper leaves (Fig. 3B). The leaf-drop symptom which affected middle leaves was still visible 5 weeks after inoculation under greenhouse conditions (Fig. 3C).

Apical leaves usually displayed mottling and crinkling but also numerous scattered necrotic points. Taken as a whole, HR symptoms induced by PVY-L26 on Maris Bard and Yukon Gold were more or less similar to those induced by both PVY^O isolates. In contrast, only chlorotic symptoms were observed on systemically infected leaves of Desiree, King Edward, and Russet Burbank plants starting at 11 dpi. Infected Desiree plants were symptomless in the greenhouse (Fig. 3D) but showed faint mosaic symptoms in both growth chambers. King Edward also expressed consistent mosaic and vein clearing symptoms, with moderate to severe crinkling of leaflets. Only mild mosaic was seen on Russet Burbank. No necrosis, leaf-drop, or premature falling of leaves were observed on these three cultivars.

PVY^{NTN} symptoms. Isolates PVY^{NTN}-423.3 and PVY^{NTN}-N4 induced only mosaic symptoms on Desiree, King Edward, and

Fig. 3. Symptoms that developed on potato cultivars after mechanical inoculation with *Potato virus Y* isolate PVY-L26. **A**, Leaf-drop on lower leaves: 'Maris Bard', 12 days postinoculation (dpi), 23 to 26°C. **B**, Systemic necrotic lesions on an intermediate leaf: 'Yukon Gold', 16 dpi, greenhouse. **C**, Leaf-drop symptom at the end of experiment: Yukon Gold, L26, 34 dpi, greenhouse. **D**, Lack of any visible symptom: 'Desiree', 16 dpi, greenhouse.

Russet Burbank; that is, mild mosaic without crinkling on Desiree (Fig. 4A), severe mosaic and crinkling on King Edward, and faint mosaic with very moderate crinkling on Russet Burbank (Fig. 4B). These isolates, however, induced a very severe systemic reaction on Maris Bard, consisting of severe mosaic, sometimes associated with numerous clearly visible distinct chlorotic spots and severe crinkling on the whole foliage (Fig. 4C). Systemic necrosis, notably vein necrosis or necrotic points, also occurred even on upper leaves (Fig. 4C) but typical leaf-drop symptom developed on lower leaves (Fig. 4D and E). Symptoms on inoculated leaves were inconsistent necrosis or yellow to dark-green halo spots. Moreover, plantlets were significantly stunted, with leaf size greatly reduced. On the whole, these isolates did not induce typical HR on Maris Bard characteristic of PVY-L26. However, in all conditions, symptoms were different from those induced by isolates PVY^N-Mont and PVY^N-NZ, which produced only mild mosaic without crinkling and size reduction (Fig. 2C). On Yukon Gold, both PVY^{NTN}-423.3 and PVY^{NTN}-N4 induced vein necrosis, necrotic spots, and typical leaf-drop on inoculated leaves. Systemic HR-like symptoms, notably leaf-drop, were only seen later, 15 to 20 dpi, and extended only to low and intermediate leaves, with apical leaves being symptomless. These HR-like symptoms were visibly milder than those induced by both PVY^O isolates on this cultivar, although necroses ap-

peared much earlier and were more severe than those induced by PVY^N-NZ.

PVY^{NTN}-HR1 induced mosaic symptoms with or without crinkling on Desiree, King Edward, and Russet Burbank. Symptoms on Maris Bard were typical HR reactions (i.e., local necrotic lesions and vein necrosis, followed by leaf-drop). Yukon Gold was not tested under lower temperature and greenhouse conditions but it was found to display a severe leaf-drop and systemic HR at higher temperatures.

DISCUSSION

A disconnect between the genetic and molecular schemes for classifying PVY strains generates a lot of confusion and makes PVY strain descriptions cumbersome and difficult to understand, especially for plant breeders and virologists not directly linked with the PVY problem (59). Similar disconnects between phylogenetic placements and strain groupings defined according to HR phenotypes were recently reported for two other common potato viruses, *Potato virus S* (11,17) and *Potato virus S* (PVX) (18). Such a disconnect also adversely affects development of reliable diagnostic tools which need to target disease symptoms caused by specific strains, rather than particular molecular features of the PVY strains. With PVY, part of the problem is that most of the

Fig. 4. Symptoms that developed on potato cultivars after mechanical inoculation with *Potato virus Y* isolates PVY^{NTN}-423.3 and PVY^{NTN}-N4. **A**, Mild systemic mosaic without any crinkle or necrosis: 423.3, 'Desiree', 34 days postinoculation (dpi), 23 to 26°C. **B**, Faint systemic mosaic and crinkle: 423.3, 'Russet Burbank', 34 dpi, greenhouse. **C**, Severe mottle and crinkle with necrotic points on apical leaves: 423.3, 'Maris Bard', 25 dpi, 23 to 26°C. **D**, Leaf-drop on basal leaves still visible at the end of experiment: 423.3, Maris Bard, 34 dpi, 23 to 26°C. **E**, Severe mosaic, crinkle, and leaf-size reduction on top leaves, leaf-drop on low leaves: 423.3, Maris Bard, 25 dpi, 23 to 26°C.

recombinants of PVY (e.g., the PVY^{NTN} and PVY^{N-WI} strains) have not been tested on the standard set of potato indicator cultivars carrying known HR genes and, thus, were not typed genetically. Once such testing is completed, classifications based on molecular properties of PVY strains and genetic reactions of potato cultivars will merge, and a unified picture of PVY molecular genetics can be devised. Here, we made a first step to provide such a unified picture by characterizing genetic reactions of a standard set of potato cultivars toward newly described molecular recombinant isolate PVY-L26, classified previously as PVY^{NTN} (32).

This characterization was conducted in comparison with the reactions of nonrecombinant PVY^O and PVY^N isolates and other PVY^{NTN} recombinants. Isolates representing the PVY^O group (Oz and Tb60) and the PVY^N group (Mont and NZ) reacted as expected on tobacco as well as on Desiree, King Edward, and Maris Bard potato according to the latest convention (59). Indeed, both PVY^O isolates induced HR on Desiree and Maris Bard, whereas both PVY^N isolates overcame the resistance genes present in these cultivars. Moreover, none of them induced any HR reaction in King Edward, which only a PVY^C isolate would be able to do. On the other hand, PVY^{NTN} isolates from our collection reacted quite differently from PVY^N isolates. Indeed, isolates L26 and HR1 induced a typical HR reaction on Maris Bard but not on Desiree and King Edward. Although isolates 423.3 and N4 did not induce a typical systemic HR reaction on Maris Bard, they did induce severe symptoms quite different from the quasi-symptomless infection induced by both PVY^N isolates on this cultivar. Based on the potato indicator cultivar reactions, isolates L26 and HR1 would be classified as belonging to the PVY^Z strain group; but the reaction in tobacco (i.e., vein necrosis for HR1) is inconsistent with PVY^Z (35,40). Only L26 can currently be described as a PVY^Z isolate based on the historical genetic classification (35, 59). Therefore, we designate this isolate as PVY^Z-NTN, thus taking into consideration its genetic and molecular background and biological properties (Table 3). Nevertheless, if the tobacco vein necrosis was assumed subordinate to the potato HR genetic classification, both L26 and HR1 would be considered PVY^Z-NTN isolates.

PVY^Z was recently assigned a PVY strain status together with the three historical strains PVY^C, PVY^O, and PVY^N (59). The first PVY^Z isolates were described in England (35). They were characterized as representatives of a virus strain group able to overcome *Nc* and *Ny_{thr}* (35) which confer hypersensitive resistance to PVY^C and PVY^O, respectively. They were not isolates from the PVY^N strain group, given the reaction induced in tobacco ('White Burley' or Xanthi). Moreover, they elicited an HR in Maris Bard potato which was considered to contain a hypothetical HR gene named *Nz* (35). In addition to the four PVY^Z isolates described by Jones (35), one additional PVY^Z isolate was described from a field survey in France (40). No further characterization of these isolates was conducted because the original material collected in England had lost its infectivity (40) (J. P. T. Valkonen, *personal communication*), while the French isolate was not saved. As far as we know, no other PVY^Z isolate has been reported.

TABLE 3. Summary of observed reactions on indicator potato cultivars and tobacco selected according to Singh et al. (59)^a

Host	Genetic background ^b	PVY ^O	PVY ^N	PVY ^Z -NTN
'King Edward'	<i>Nc</i> : <i>ny</i> : <i>nz</i>	s	s	s
'Desiree'	<i>nc</i> : <i>Ny</i> : <i>nz</i>	HR	s	s
'Maris Bard'	<i>Nc</i> : <i>Ny</i> : <i>Nz</i>	HR	s	HR
<i>Nicotiana tabacum</i>	...	s	vn	s

^a PVY = *Potato virus Y*, s = susceptible (i.e., systemic infection without any necrosis), HR = hypersensitive resistance response (systemic or localized), and vn = local and systemic vein necrosis.

^b According to Singh et al. (59).

PVY-L26 is the first PVY^Z isolate to be thoroughly characterized. Indeed, it was reported to induce typical PTNRD symptoms in tubers (32), a trait not mentioned by Jones (35) or Kerlan et al. (40). From a molecular point of view, PVY-L26 appears as a variant within the PVY^{NTN} strain. The complete sequence of its genome is available (32) and it has a recombinant structure with three PVY^N/PVY^O recombination junctions at the same locations as seen in typical PVY^{EU-NTN} isolates (30,43,57). Other PVY^Z isolates should now be characterized in the same manner in order to check whether L26 is unique or represents a larger PVY^Z strain group; a recently reported isolate from Mexico, PVY-M3, may be a good second candidate for this characterization (54). Some heterogeneity among PVY^Z isolates occurs because PVY-L26 reacts with anti-PVY^N antibodies (32; this study), consistent with previously determined PVY^Z serological reactivity (R. Singh, *personal communication*), unlike the PVY^Z isolate found in France, which was recognized by anti-PVY^{OC} antibodies but not by anti-PVY^N antibodies (40).

The PVY^{NTN} isolates studied here induced systemic mottling in Desiree and King Edward, and reactions varying from typical systemic HR (isolate HR1) to severe crinkling with HR-like, systemic vein necrosis in Maris Bard (isolates 423.3 and N4). A marked difference in symptoms induced in Maris Bard was found under all environmental conditions tested for PVY^N (quasi-symptomless infection) and PVY^{NTN} (severe HR-like, systemic symptoms). Up until recently, such biological characterization for PVY^{NTN} isolates was lacking (59). PVY^{NTN}-Slo was reported to produce necrotic local lesions and systemic infection associated with severe necrosis in Maris Bard, whereas systemic mottling developed in all other cultivars tested, including Desiree and King Edward (3). Another PVY^{NTN} isolate (PVY^{NTN}-Frøril) collected in France from a naturally affected PTNRD tuber and used as a standard in many experiments (30,31,33,55) induced similar HR-like reaction in Maris Bard and mosaic in Desiree (C. Kerlan, *unpublished*).

HR reactions against viruses in potato were described by Cockerham (15), Jones (35), and Valkonen (62). Examples of temperature-dependent expression of HR to virus infection in potato were shown for PVX (1) and PVA (47). HR to PVY^O in potato and to PVY^N in two wild potato species was efficiently expressed at 16 to 18°C, as indicated by development of local necroses, whereas the HR was rendered less effective at higher temperatures (19 to 24°C) which was indicated by the systemic infection with leaf-drop and mosaic symptoms (63). Szajko et al. (60) also reported a temperature dependence of HR in potato 'Rywal'. We did not see such drastic differences in our experiments. Indeed, in combinations where HR was scored, local vein necrosis or necrotic lesions were readily observed at 16 to 26°C in growth rooms as well as under greenhouse. However, the leaf-drop symptom and some necrosis on top leaves were seen less frequently at 16 to 18°C and were milder than at higher temperatures.

The "leaf-drop streak" symptom, known since the 1930s (26), was then described as a PVY^O strain-specific trait (24). We have shown here that this symptom was also elicited by a PVY^Z-NTN isolate and by typical PVY^{NTN} isolates.

Yukon Gold was previously reported to confer HR with PVY^O (63), which was confirmed here with two PVY^O isolates (Oz and Tb60). It also reacted hypersensitively to PVY^Z-NTN, PVY^{NTN}, and even, to some extent, to PVY^N in our experiments. Its genome could contain HR genes specific to these strains (i.e., at least the gene *Ny_{thr}* and the putative gene *Nz*). Yukon Gold was registered in Canada in 1980 and is widely cultivated in North America. One parent of this cultivar was derived from a cross between the potato 'Katahdin' and the wild species *Solanum phureja* (34). Katahdin was shown to react hypersensitively to PVY^O and PVY^C but not to PVY^N (35). On the other hand, *S. phureja* populations have also been found resistant to PVY^O (66), notably by

developing leaf necrosis after graft inoculation by an undefined common strain of PVY (68).

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