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Screening Three Potato Cultivars for Resistance to Potato Virus Y Strains: Broad and Strain-Specific Sources of Resistance

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Abstract
Three potato cultivars, Payette Russet, Dark Red Norland, and Chieftain were challenged with four strains of potato virus Y (PVY), PVY^{O}, PVY^{Eu-N}, PVY^{N-Wi}, and PVY^{NTN}. Cultivars Dark Red Norland and Chieftain exhibited strain-specific, hypersensitive resistance to PVY^{O} and PVY^{NTN} strains.. These same two cultivars, Dark Red Norland and Chieftain, appeared to have an additional resistance source in their genomes providing partial resistance against PVY^{N-Wi} but were found fully susceptible to the non-recombinant PVY^{Eu-N} strain. Payette Russet was found immune to the same four strains of PVY; PVY^{O}, PVY^{Eu-N}, PVY^{N-Wi}, and PVY^{NTN}, and was additionally challenged with the total of 18 isolates of PVY representing 12 genetic variants of the virus from potato and non-potato solanaceous hosts. None of the 18 isolates of the virus was found able to replicate in the inoculated or upper non-inoculated leaves of Payette Russet, confirming the broad specificity of the Ry_{sto} gene present in the Payette Russet genome.

Keywords Potato virus Y · Resistance · *R* genes · *N* genes

Introduction

Two types of genes confer resistance to potato virus Y (PVY) in potato (Gebhardt and Valkonen 2001; Karasev and Gray 2013). *R* genes confer an extreme resistance (ER) or immunity which is very durable and is effective against a broad range of virus strains. Phenotypically, ER manifests itself as lack of any symptoms in an inoculated leaf and no detectable virus infection. The origin of *R* genes is in a pool of wild relatives of potato (*Solanum tuberosum*) (Cai et al. 2011), and it takes many years to introgress these genes into commercially acceptable cultivars. *N* genes confer a hypersensitive resistance (HR) response where a small group of plant cells infected with the virus dies forming a necrotic lesion which often restricts further movement of the virus outside of this lesion. Occasionally, when the virus spread is not completely restricted, the infection may spread through the entire plant, and in this case the HR reaction becomes systemic, visible as various types of systemic necrosis, such as vein necrosis, leaf drop syndrome, and stem streaking. Unlike ER, HR is strain specific, and very sensitive to environmental factors, especially temperature – it can be broken due to changes in the temperature (Cockerham 1970; de Bokx and Huttinga 1981; Jones 1990; Valkonen 1997; Kerlan et al. 2011). And, unlike *R* genes, *N* genes are present in many commercial cultivars, and in theory, could be used to manage resistance against PVY in potato. Both ER and HR can be used to control spread of PVY in potato, however, HR is more prone to breaking down, since it is strain-specific and sensitive to environmental conditions, most importantly to temperature (Karasev and Gray 2013).

PVY exists as a complex of strains and genetic variants which can be defined molecularly (Green et al. 2017a, b, 2018, 2020a, b) and, sometimes, biologically (Cockerham 1970; deBokx and Huttinga 1981; Jones 1990; Singh et al. 2008; Chikh-Ali et al. 2014). The HR response in a set of standard cultivars of *S. tuberosum* harboring three strain-specific *N* resistance genes, *Ny*, *Nc*, and *Nz*, was used in the past to define four strains genetically; PVY^{O} (triggering Ny_{tbr}), PVY^{C} (Nc_{tbr}), PVY^{Z} (Nz_{tbr}), and PVY^{N} (overcoming all three *N* genes without the HR response) (Cockerham 1970; Jones 1990; Singh et al. 2008; Chikh-Ali et al. 2014). Molecular characterization of PVY strains revealed that PVY^{O}, PVY^{N}, and PVY^{C} had non-recombinant genomes that

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formed three separate phylogenetic clades (Glais et al., 2002; Lorenzen et al. 2006a, b; Singh et al. 2008; Moury 2010; Karasev and Gray 2013; Green et al. 2017a, b). PVY^{Z}, on the other hand, was classified as either PVY^{NTN} or PVY^{NTN-NW} recombinant based on molecular characteristics (Hu et al. 2009; Kerlan et al. 2011; Chikh-Ali et al. 2014). There are multiple other recombinants, at least 35, most often built of PVY^{O} and PVY^{N} parental sequences, named PVY^{N-Wi}, $PVY^{N:O}$, PVY-NE11 and others (Green et al. 2018, 2021), but these were not defined genetically and were classified only based on molecular properties.

At the moment, the information on *N* genes available in potato cultivars grown in the U.S. is limited. A North American cultivar Yukon Gold was demonstrated to carry the Ny_{tbr} and Nz_{tbr} genes eliciting HR against PVY^{O} and PVY^{NTN}, respectively (Kerlan et al. 2011; Chikh-Ali et al. 2014). Potato cultivar Umatilla Russet was studied in both greenhouse and screen-house settings and found eliciting HR reaction to PVY^{O} and to PVY^{NTN} (Funke et al. 2017). Additionally, eight potato cultivars grown in the U.S. were challenged with five PVY strains, and the presence of Ny_{tbr} gene was demonstrated in cultivars Ranger Russet, Alturas, Western Russet, Yukon Gem, and Rio Grande Russet (Rowley et al. 2015). The Nz_{tbr} gene was found in two cultivars, Yukon Gem and Rio Grande Russet (Rowley et al. 2015). In addition to these two *N* genes, the existence of several others was postulated in Yukon Gem, eliciting HR against multiple strains of PVY (PVY^{N}, PVY^{NA-N}, PVY^{N-Wi}, $PVY^{N:O}$, PVY-NE11) (Rowley et al. 2015).

At least three single dominant *R* genes conferring ER to PVY have been identified over the years, these are Ry_{adg} from *S. tuberosum* ssp. *andigena* (Munoz et al. 1975), Ry_{sto} from *S. stoloniferum* Schlechtd. et Bché. (Cockerham 1970), and Ry_{chc} from *S. chacoense* Bitt. (Hosaka et al. 2001). These *R* genes have been used by breeding programs to introduce PVY resistance to new potato varieties, although progress has been slow so far. A few years ago, Payette Russet, a dual-use commercial cultivar, was released harboring Ry_{sto} resistance gene (Novy et al. 2017). The presence and inheritance of this Ry_{sto} gene was inferred based on molecular markers linked to this resistance genes, and although in field experiments Payette Russet was found to be PVY-resistant, the strain of the challenging virus was not disclosed, and hence additional testing of the susceptibility of this cultivar to a multitude of PVY strains and genetic variants was desirable.

Here, a study was conducted to expand our screening to three additional North American cultivars for various resistance sources to PVY strains, including *N* and *R* resistance genes. Specifically, three potato cultivars were studied under greenhouse conditions for their ability to elicit a resistance response against four of the most common strains of PVY. The cultivars Dark Red Norland, Chieftain, and Payette Russet were tested against strains PVY^{O}, PVY^{NTN}, PVY^{N-Wi}, and PVY^{Eu-N} in search of the HR reaction or immunity to a virus challenge. Payette Russet, known to have an extreme resistance gene Ry_{sto} in its genetic background (Novy et al. 2017), was challenged with additional five strains and three genetic variants of PVY to evaluate robustness of its broad PVY resistance due to the presence and efficiency of the Ry_{sto} gene.

Materials and Methods

Potato Cultivar Sources and Plant Maintenance

The cultivar Maris Bard was originally received from the National Potato Germplasm Collection in Sturgeon Bay, WI, as tissue culture plantlets. Cultivars Desiree, Dark Red Norland, Chieftain, and Payette Russet were obtained from the University of Idaho Nuclear Seed Potato Program (provided by Lorie Ewing and Jenny Durrin). Plantlets were cut and transferred to new media every 8 weeks and after transfer, plantlets were transplanted in soil in 2–8 weeks. While in vitro, the plantlets were periodically subjected to RT-PCR tests for main potato viruses to confirm their virus-free status.

Maris Bard and Desiree were used as control cultivars in each experiment, with three plants inoculated per strain. This was done to help determine correct infection response with each PVY strain as well as confirm the infectivity of the inoculum in each experiment. Symptoms elicited in these two cultivars and HR reactions triggered by strains PVY^{O}, PVY^{NTN}, PVY^{N-Wi}, and PVY^{Eu-N} have been well documented (Kerlan et al. 2011; Chikh-Ali et al. 2014; Rowley et al. 2015; Funke et al. 2017).

Reference Isolates of PVY, Inoculations, Phenotype Screening, and Laboratory Testing

All isolates of PVY, used in this work as references for PVY strains, were from the laboratory collection at the University of Idaho and almost all of them were previously subjected to whole genome sequencing (Table 1). Their serological and genetic assignments and origin are listed in Table 1 along with corresponding references. PVY isolates were maintained in tobacco cv. Burley in an insect-free, climate-controlled growth room. This PVY isolate collection was subjected to periodic screening using ELISA and RT-PCR testing to verify and control the identity of each PVY isolate as described earlier (Karasev et al. 2010; Nikolaeva et al. 2012; Chikh-Ali and Karasev 2015; Funke et al. 2017). Infected tobacco tissue was used as an inoculum source for the potato plants. Tobacco leaves were homogenized in a phosphate inoculation buffer (pH 7.0) at a dilution rate of 1:10 (w:v) with a mortar and pestle on ice. For all cultivars, potato plants were mechanically inoculated at the six- to ten-leaf stage using carborundum (silicon carbide). Three

Table 1 Molecular and phenotypic traits of the PVY isolates used in this study

Isolates	Strain[a)]	Genotype	Tobacco bioassay[b)]	Serotype	Genome sequence[c)]	Reference
Tb60	PVY^{O}	PVY^{O}	Mos	O	NA	Lorenzen et al. 2006a
Oz	PVY^{O}	PVY^{O}	Mos	O	EF026074	Baldauf et al. 2006
ID269	PVY^{O}	PVY^{O}-O5	Mos	O5	FJ643477	Karasev et al. 2010
N1	PVY^{N-Wi}	PVY^{N-Wi}	VN	O	HQ912863	Karasev et al. 2011
Alt	$PVY^{N:O}$	$PVY^{N:O}$	VN	O	AY884985	Lorenzen et al. 2006a
Pondo4	261–4	261–4	VN	O	KY848023	Green et al. 2017a
Mont	PVY^{N}	PVY^{N}	VN	N	AY884983	Lorenzen et al. 2006a
HR1	PVY^{Z}	PVY^{NTN} (*syn.* PVY^{Z}-NTN)	VN	N	FJ204166	Hu et al. 2009
L26	PVY^{Z}	PVY^{NTN} (*syn.* PVY^{Z}-NTN)	Mos	N	FJ204165	Hu et al. 2009
NE-11	NE-11	NE-11 (long)	VN	N	DQ157180	Piche et al. 2008; Green et al. 2017a
ID20	NE-11	NE-11 (short)	VN	N	HQ912867	Karasev et al. 2011; Green et al. 2017a
PVY-AGA	E	E	VN	N/AST	JF928459	Galvino-Costa et al. 2012a, b
HI-14	C	C1	Mos	O	KX580384	Chikh-Ali et al. 2016
Poha2	C	C-Poha	Mos	O	MF134862	Green et al. 2017b
Poha6	C	C-Poha	Mos	-	MF134866	Green et al. 2017b
Tam13	SA-N	Tamarillo	VN	N	MT380736	Green et al. 2020a
Tam15	SA-N	Tamarillo	VN	-	MT380738	Green et al. 2020a
Tam17	SA-N	Tamarillo	NS	N	MT380740	Green et al. 2020a

[a]Strains listed according to Karasev and Gray (2013), Green et al. (2017a), Green et al. (2017b),Green et al. (2020a)

[b]Tobacco symptoms: VN, vein necrosis; Mos, mosaic and vein clearing; NS, no symptoms, asymptomatic infection

[c]Sequences deposited in GenBank

terminal leaflets on three leaves per plant were inoculated. Each inoculated leaflet was punched to mark it and allow for symptom tracking and subsequent testing of the inoculated leaves. After inoculation the plants were rinsed to remove excess inoculum and grown in climate-controlled growth chambers, with a 16 h light/8 h dark cycle and maintained at 20–22 °C (Kerlan et al. 2011; Chikh-Ali et al. 2014; Rowley et al. 2015; Funke et al. 2017). Three plants of each cultivar were inoculated with each PVY isolate per experiment, and three plants of each cultivar were left as healthy controls; each experiment was repeated at least two times. A control *Nicotiana benthamiana* plant was inoculated with each PVY isolate in each experiment, to ensure the viability of the inoculum. The symptom assessment started 4–5 days after inoculation and was carried out for 6–8 weeks.

Serological Analysis, RT-PCR, and Differentiating Primers

PVY presence in inoculated and non-inoculated leaves, and serological reactivity of the PVY isolates was tested in a TAS-ELISA format, as described by Nikolaeva et al. (2012). All tests included control PVY isolates from the laboratory collection, with distinct serological patterns characteristic of PVY^{O} and PVY^{N} strains. In addition to a polyclonal antiserum, Asc5 (Funke et al. 2017; Karasev et al. 2010), three strain-specific monoclonal antibodies were used: SASA-O (Scottish Agriculture Science Agency [SASA], Edinburgh, Scotland) which recognizes PVY^{O}, PVY^{O}-O5, $PVY^{N-Wi/N:O}$ and PVY^{C}; 1F5 (Agdia, Elkhart, IN) which reacts with PVY^{Eu-N}, PVY^{O}-O5, and PVY^{NTN}; and SASA-N (Scottish Agriculture Science Agency, Edinburgh, Scotland) which identifies PVY^{Eu-N}, PVY^{NA-N}, and PVY^{NTN}.

Two different multiplex RT-PCR assays were performed on PVY-positive samples identified by TAS-ELISA, following the methods of Lorenzen et al. (2006a, b) and Chikh Ali et al. (2013a). Immuno-capture reverse transcription (IC-RT-PCR), and PCR reactions were performed essentially as described previously (Chikh Ali et al. 2013b, 2016). Control strains of PVY and healthy controls were from the University of Idaho laboratory collection (see Table 1), and assays were conducted at the same time on all samples collected during a particular experiment.

Results

The collection of PVY strains and genetic variants maintained in the Virology Laboratory of the University of Idaho, included virus isolates from the Pacific Northwest of the

U.S., but also some PVY isolates collected in other states and obtained from collaborators in other countries. These genetic variants of PVY represented a large set of PVY isolates from potato and non-potato hosts exhibiting various pathotypes in potato, tobacco, pepper, cape gooseberry, and tamarillo; their biological and molecular characteristics are summarized in Table 1 which also contains references to more detailed descriptions of individual PVY strains and genotypes. During the screening experiments, special attention was placed on the PVY strains most commonly found in the U.S., such as PVY^{O}, PVY^{NTN}, PVY^{N-Wi}, and PVY^{Eu-N}, and these were used for the study of symptoms elicited by PVY strains in cultivars Dark Red Norland and Chieftain.

Dark Red Norland and Chieftain

When cultivars Dark Red Norland and Chieftain were tested against the isolate Tb60, representing the PVY^{O} strain, it induced local lesions on inoculated leaves of both cultivars, which appeared at 6–11 days post-inoculation (dpi) in Dark Red Norland, and at 18 dpi in Chieftain (Fig. 1). These lesions expanded and resulted in pronounced vein necrosis of the inoculated leaves at 18 dpi for Dark Red Norland, and at 11–13 dpi for Chieftain. At 27–31 dpi both cultivars developed systemic vein necrosis and at 44 dpi they developed necrotic lesions on upper, non-inoculated leaves (Table 2). Leaf drop was observed by 37 dpi for Dark Red Norland and 44 dpi for Chieftain, which also developed mottling at 13–24 dpi (Table 2). Three of the six Dark Red Norland plants inoculated with PVY^{O} in two separate experiments were dead after 50 dpi. Mont (PVY^{Eu-N}) infection stayed largely asymptomatic in Dark Red Norland and induced only mosaic in Chieftain by 35 dpi (Table 2). L26 (PVY^{NTN}) induced vein necrosis on inoculated leaves in Dark Red Norland at 18 dpi, with symptoms of mosaic in upper non-inoculated leaves (13 dpi), crinkling (13 dpi), and systemic necrosis and leaf drop (44 dpi) developing over the course of the testing period (Table 2). Following PVY^{NTN} inoculation, Chieftain developed mosaic in inoculated leaves at 13 dpi and vein necrosis by 18 dpi. Systemic mosaic symptoms in upper, non-inoculated leaves developed into systemic mottle at 27–31 dpi, and at the same time vein necrosis was appearing systemically, with leaf drop ensuing at 44 dpi (Table 2). PVY^{N-Wi} induced mosaic and necrotic lesions on inoculated leaves of either Dark Red Norland or Chieftain. Chieftain developed systemic mosaic in upper, non-inoculated leaves at 13 dpi which turned into mottle at 27 dpi (Table 2). Dark Red Norland showed systemic mosaic in upper, non-inoculated leaves at 13 dpi, and very mild systemic vein necrosis was observed at 31 dpi on one out of six infected plants (Table 2). Symptoms observed in

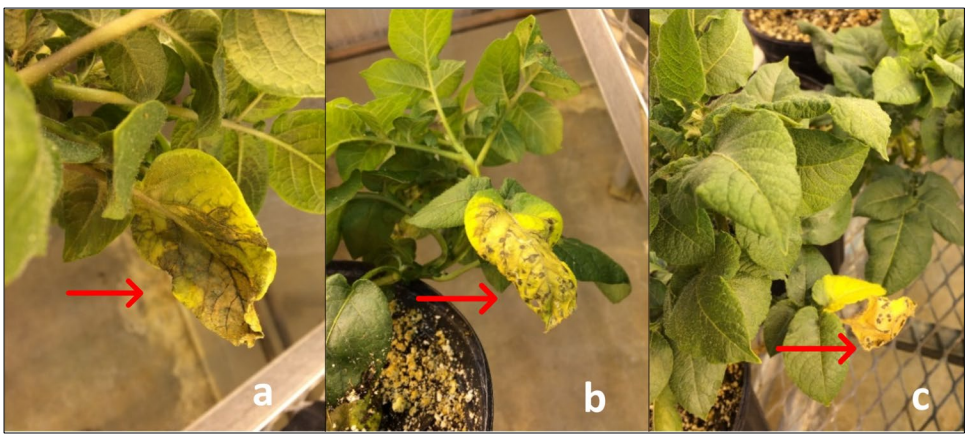

Fig. 1 Symptoms of local lesions and vein necrosis expressed on inoculated leaves, 18 days post-inoculation with the PVY isolate Tb60 (PVY^{O}): **a** cv. Chieftain; **b** cv. Dark Red Norland; and (**c**) cv. Maris Bard (control). Red arrows show inoculated leaves with necrotic lesions

Table 2 Summary of symptoms expressed by different potato cultivars upon mechanical inoculation with four isolates of PVY representing four PVY strains

Cultivar	Isolate (PVY strain)			
	N1 (PVY^{N-Wi})	L26 (PVY^{NTN})	Tb60 (PVY^{O})	Mont (PVY^{N})
Maris Bard	M, Cr, St [a]	M, Cr, LL, SN	M, Cr, LL, SN	M, Cr
Desiree	MM	M	M, Cr, LL, SN	MM
Chieftain	M, LL	M, SN, LD	M, LL, SN, LD	M
Dark Red Norland	M, LL, SN	M, Cr, SN, LD	LL, SN, LD	NS

[a]Symptom abbreviations: M, Mosaic; Cr, crinkling; St, stunting; LL, local lesions; SN, systemic necrosis; LD, leaf drop; MM, mild mosaic. "NS" designates no symptoms, however systemic virus infection was confirmed by ELISA and RT-PCR

Chieftain were similar to the ones described by Gundersen et al. (2019).

Our control cultivars Desiree and Maris Bard were tested in each experiment along with Dark Red Norland and Chieftain to confirm the presence and activation of known *N* genes. Both test cultivars showed a necrotic HR response to PVY^{O} in inoculated leaves, exhibiting vein necrosis and necrotic lesions (Fig. 1; Table 2). Over time, systemic necrosis developed in plants of Desiree and Maris Bard inoculated with the PVY^{O} isolate. Leaf drop, vein necrosis and necrotic lesions on upper, non-inoculated leaves were observed along with symptoms of mosaic and crinkling (Table 2). Incidentally, Desiree exhibited the HR reaction to PVY^{O} (Tb60) but not to PVY^{NTN} (L26), while Maris Bard exhibited HR to both PVY^{O} (Tb60) and PVY^{NTN} (L26) (Table 2).

All the inoculated plants were tested at 5 weeks post inoculation and all Chieftain and Dark Red Norland plants were found systemically infected with PVY^{O}, PVY^{Eu-N}, PVY^{NTN}, and PVY^{N-Wi} (Fig. 2) despite the observed HR response, visible locally or systemically. Some of the control plants, however, cultivars Desiree and Maris Bard, did not show PVY presence in upper, non-inoculated leaves (Fig. 2) which likely reflected the restricted systemic movement of PVY^{O} in these two cultivars due to the presence of the Ny_{tbr} gene.

Payette Russet

Strains PVYO and $PVY.^{O5}$

Payette Russet plants were mechanically inoculated as described in Materials and Methods and checked for symptoms weekly starting at 6 dpi and continuing through 8 weeks post-inoculation, and all plants tested at the end of the experiment were found negative for systemic infection (Fig. 3). PVY^{O} and PVY^{O5} elicited no symptoms in Payette Russet for the duration of the observation and testing period (Table 3). The control Desiree plants began showing symptoms for PVY^{O} and PVY^{O5} strains 14–16 dpi with vein necrosis and necrotic lesions forming on inoculated leaves. PVY^{O5} induced water-soaked rings on inoculated leaves of Desiree that appeared at 16–21 dpi, and then progressed into necrotic lesions. The symptoms of local lesions and vein necrosis subsequently spread and became systemic 21 dpi. Mosaic symptoms developed between 21–40 dpi and leaf drop was observed at 28–43 dpi. Another control cultivar Maris Bard began developing symptoms at 9 dpi showing necrotic lesions on inoculated leaves (Table 2 and 3). Vein necrosis developed in inoculated leaves of Maris Bard 9–16 dpi and spread systemically at 14–21 dpi. A severe mosaic appeared by 14 dpi and at 21 dpi leaf drop developed. All

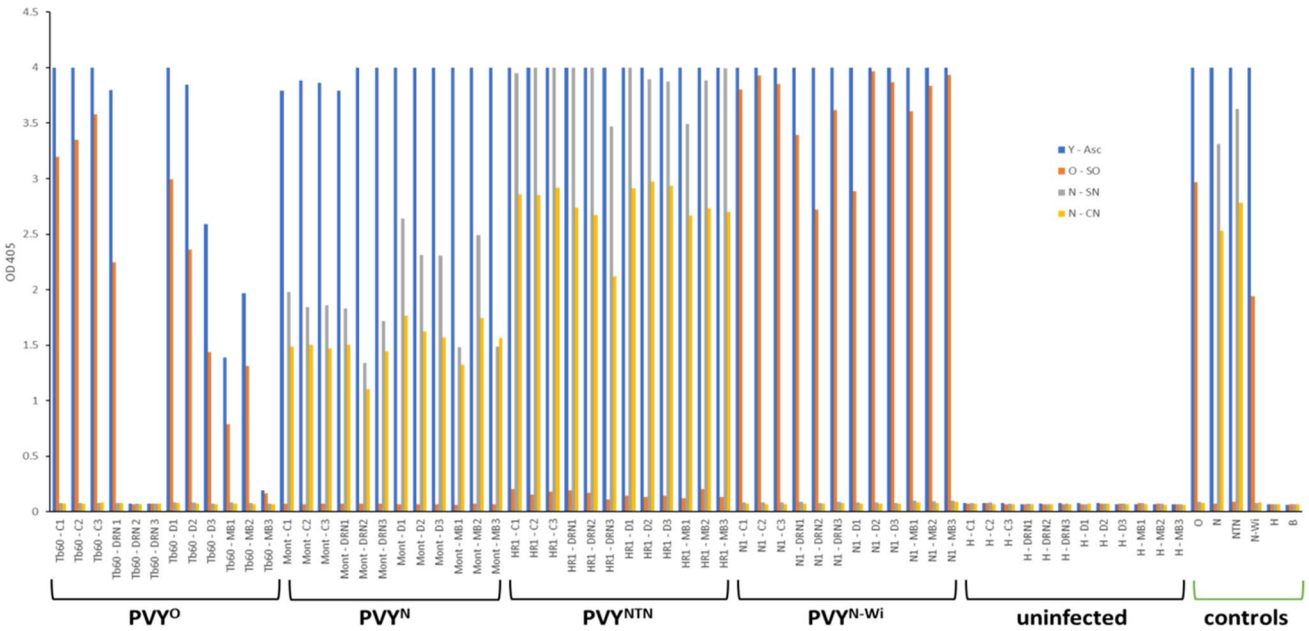

Fig. 2 TAS-ELISA detection of PVY infection in potato cultivars Chieftain (C), Dark Red Norland (DRN), Desiree (D), and Maris Bard (MB), 4 weeks post-inoculation. Three individual plants of each cultivar were inoculated with the isolates, Tb60 (PVY^{O}), Mont (PVY^{Eu-N}), HR1 (PVY^{NTN}), and N1 (PVY^{N-Wi}). Three plants were left uninfected as healthy controls. Controls came from the laboratory collection, and match isolates used to perform inoculations. OD_{405} signal generally reflects the concentration of the PVY strain in each individual plant. Different colored bars represent signals for a polyclonal antibody (PVY-specific, blue) and three different monoclonals (SASA-O, orange; SASA-N, gray; Agdia-N, yellow). Samples were considered positive if the signal for infected plants was three times higher than for an uninfected plant

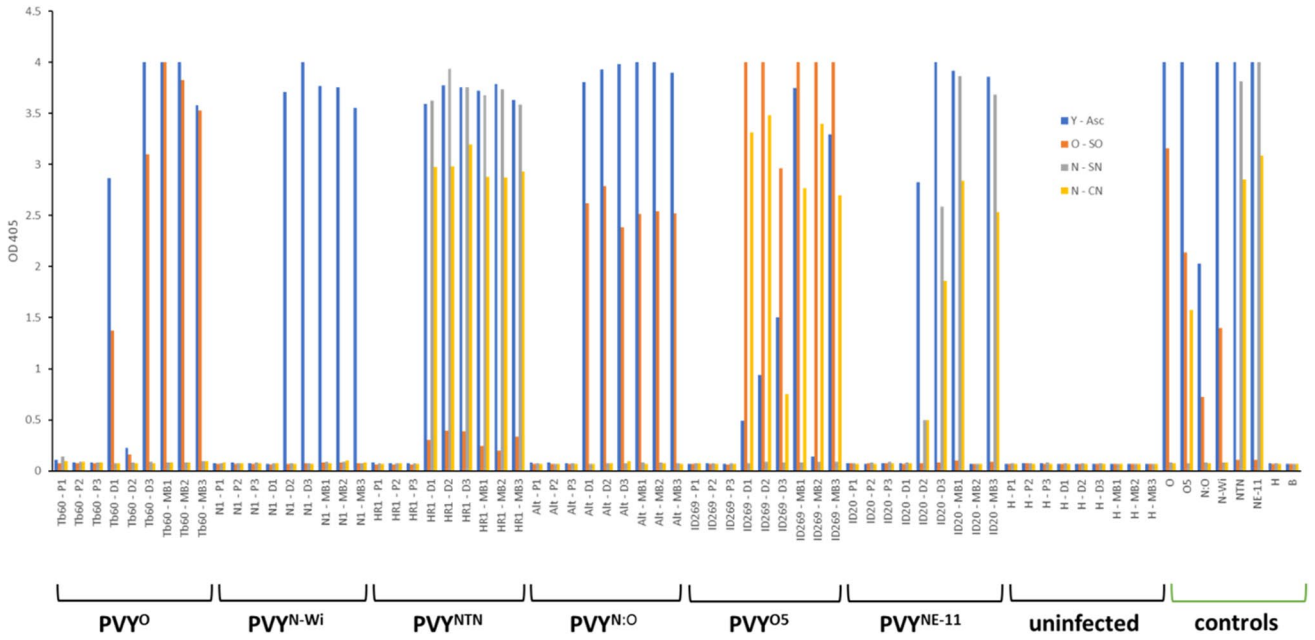

Fig. 3 TAS-ELISA detection of PVY infection in potato cultivars Payette Russet (P), Desiree (D), and Maris Bard (MB), 4 weeks post inoculation. Three plants per cultivar were inoculated with each isolate; Tb60 (PVY^{O}), N1 (PVY^{N-Wi}), HR1 (PVY^{NTN}), Alt ($PVY^{N:O}$), ID269 (PVY^{O5}), ID20 (PVY-NE11), and three plants left as uninoculated controls. Controls came from the laboratory collection, and match isolates used to perform inoculations. OD_{405} signal generally reflects the concentration of the PVY strain in each individual plant. Different colored bars represent signals for a polyclonal antibody (PVY-specific, blue) and three different monoclonals (SASA-O, orange; SASA-N, gray; Agdia-N, yellow). Samples were considered positive if the signal for infected plants was three times higher than for an uninfected plant

plants were tested at 28 dpi (Fig. 4) and 3/6 plants for both strains in controls Maris Bard and Desiree were found systemically infected. This 50% infection rate was expected due to the HR response from both cultivars, restricting virus systemic spread in both cultivars carrying the Ny_{tbr} gene.

Strains PVY.^{NTN} and PVY-NE11

Payette Russet was inoculated with PVY^{NTN} and PVY-NE11 isolates and displayed no symptoms in inoculated or upper, non-inoculated leaves (Table 2 and 3) during the entire observation period, and all plants tested at the end of the experiment were found negative for systemic infection (Fig. 3). In our controls, PVY^{NTN} and PVY-NE11 produced vein necrosis 9–16 dpi on inoculated leaves in Maris Bard but not in Desiree. Necrotic lesions began forming by 9–16 dpi on Maris Bard for both PVY^{NTN} and PVY-NE11, but none on Desiree. However, both cultivars showed systemic symptoms after 21 dpi. Desiree showed vein necrosis at 21–28 dpi, and necrotic lesions and leaf drop with PVY-NE11 28 dpi. Maris Bard began showing systemic vein necrosis and necrotic lesions 21–28 dpi, and leaf drop at 28–35 dpi. Both cultivars showed systemic mosaic (Table 3) for both PVY-NE11 and PVY^{NTN} at 14–21 dpi. When tested at 28 dpi, all six out of 6 plants inoculated with PVY^{NTN} were infected, but 5/6 Desiree and 4/6 Maris Bard were infected with PVY-NE11.

Strains PVY^{N-Wi} and $PVY^{N:O}$

Payette Russet was also challenged with PVY^{N-Wi} and $PVY^{N:O}$ but again showed no symptoms, and all plants tested at 35 dpi were negative by ELISA (Fig. 3; Table 3). Potato plants of cultivars Maris Bard and Desiree inoculated with PVY^{N-Wi} did not show any local lesions on inoculated leaves, and systemic symptoms began developing around 14 dpi for Maris Bard and 21 dpi for Desiree when mosaic/mottling began to show. These symptoms continued to be expressed on any new leaves formed for the rest of the testing period (Fig. 4), and gradually became more pronounced. The plants of Maris Bard and Desiree inoculated with $PVY^{N:O}$ showed only systemic mosaic symptoms (Table 3). Maris Bard plants showed mosaic around 14 dpi which eventually became more severe and turned into mottling. Desiree plants were slower to show symptoms, with the earliest sign of mosaic at 21 dpi. Five out of six inoculated Desiree plants were found infected with PVY^{N-Wi} when tested at 28 dpi (Fig. 3) and 6/6 plants infected with $PVY^{N:O}$. All Maris Bard plants inoculated with either PVY^{N-Wi} or $PVY^{N:O}$ were found infected (Fig. 3).

Table 3 Summary of symptoms expressed by Payette Russet and control cultivars Desiree and Maris Bard when tested against different strains of PVY mechanically inoculated using the inoculum from PVY isolate collection (see Table 1)

Isolate (PVY strain)	Payette Russet		Desiree		Maris Bard	
	Local	Systemic	Local	Systemic	Local	Systemic
Tb60 (PVY^{O})	NI [a)]	NI	VN, LL	M, LL, SN, Cr, LD	VN, LL	M, Cr, LD
Oz (PVY^{O})	NI	NI	VN, LL	M, LL, SN, Cr, LD	VN, LL	M, Cr, LD
ID269 (PVY^{O5})	NI	NI	VN, LL	M, SN, LL, LD, WSR	VN, LL	M, SN, LD
N1 (PVY^{N-Wi})	NI	NI	NS	M, Cr	NS	M, Cr, St
Pondo4 (PVY-261–4)	NI	NI	NS	M, Cr	NS	M, Cr, St
Alt ($PVY^{N:O}$)	NI	NI	NS	M	NS	M
L26 (PVY^{NTN})	NI	NI	NS	M, St	VN, LL	M, SN, LL, LD
HR1 (PVY^{NTN})	NI	NI	NS	M, St	VN, LL	M, SN, LL, LD
ID20 (PVY-NE11, short)	NI	NI	VN	M, SN, LL	VN, LL	M, Cr, SN, LL, LD
NE-11 (PVY-NE11, long)	NI	NI	NS	M, SN, LL	VN, LL	M, Cr, SN, LL, LD
AGA (PVY^{E})	NI	NI	NS	M	NS	M
Mont (PVY^{Eu-N})	NI	NI	NS	NS	NS	NS
H-14 (PVY^{C1})	NI	NI	NI	NI	LL	NI
Poha2 (PVY^{C}, Poha)	NI	NI	NS	NI	LL	NI
Poha6 (PVY^{C}, Poha)	NI	NI	NS	NI	NS	NI
Tam13 (PVY^{SA-N})	NI	NI	NI	NI	NI	NI
Tam15 (PVY^{SA-N})	NI	NI	NI	NI	NI	NI
Tam17 (PVY^{SA-N})	NI	NI	NI	NI	NI	NI

[a]Symptom abbreviations: M, Mosaic; Cr, crinkling; St, stunting; LL, local lesions; SN, systemic necrosis; LD, leaf drop; MM, mild mosaic; VN, vein necrosis; WSR, water soaked rings. "NS" designates no symptoms, but the systemic virus infection was confirmed by ELISA and RT-PCR. "NI" designates no symptoms and no infection confirmed by ELISA and RT-PCR in any of the plants tested

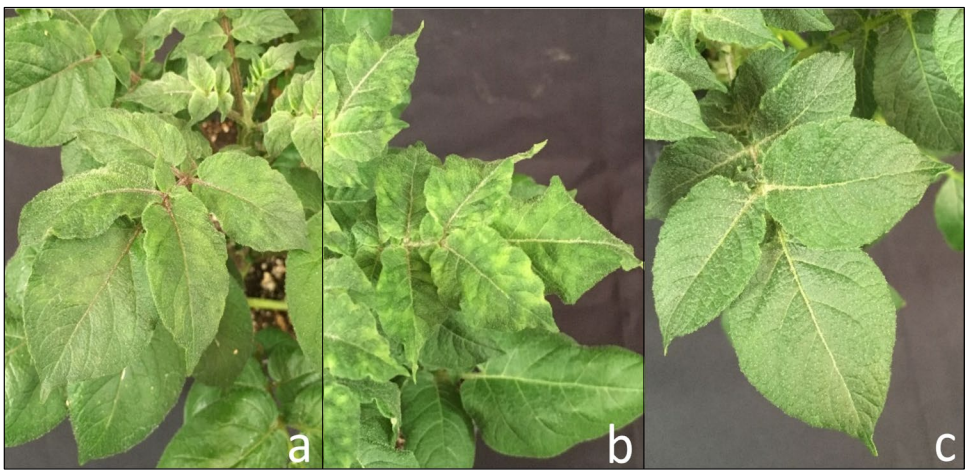

Fig. 4 Symptoms in upper, non-inoculated leaves of three cultivars inoculated with PVY isolate N1 (PVY^{N-Wi}), 29 days post-inoculation: **a** cv. Desiree and (**b**) cv. Maris Bard showing mosaic and mottling; and (**c**) asymptomatic leaves of cv. Payette Russet

Other PVY strains and genetic variants

To check if any other PVY strains and variants can infect cv. Payette Russet, it was challenged with additional eight isolates from the UI collection representing strains PVY^{E} and PVY^{C}, a genetic variant of PVY named 261–4, and also a newly discovered genetic variant of the PVY^{N} lineage, called PVY^{SA-N} (see Table 1). None of the eight isolates tested was found replicating in inoculated or upper, non-inoculated leaves of cv. Payette Russet for the duration of the experiment (Table 3). In control cultivars Desiree and Maris Bard, PVY^{E} was asymptomatic in inoculated leaves and induced mosaic in upper, non-inoculated leaves indicating susceptibility of Desiree and Maris Bard to systemic infection with this strain (Table 3). Isolate Pondo4 (variant 261–4) was asymptomatic on inoculated leaves of Desiree and Maris Bard, and produced mosaic and crinkling on upper, non-inoculated leaves; both Desiree and Maris Barded appeared

to be susceptible to the infection with the genetic variant 261–4 (Table 3). A tomato isolate HI-14 (PVY^{C1}) was found unable to infect Desiree either locally or systemically, while able to replicate in inoculated leaves of Maris Bard only, inducing local necrotic lesions and unable to spread systemically (Table 3). The two PVY isolates from cape gooseberry, Poha2 and Poha6 (PVY^{C}), were found replicating in inoculated leaves of both Desiree and Maris Bard, but unable to spread systemically in both cultivars; Poha2 induced local lesions in inoculated leaves of Maris Bard (Table 3). The three tamarillo isolates of PVY, Tam13, Tam15, and Tam17, were unable to replicate in either inoculated or upper, non-inoculated leaves of Desiree and Maris Bard (Table 3).

Discussion

While the HR reaction conferred by *N* genes specific to individual strains of PVY is considered a form of a host defense response in potato (Cockerham 1970; de Bokx and Huttinga 1981; Jones 1990; Singh et al. 2008; Chikh-Ali et al. 2014), it often provides only partial protection against the virus infection. The strain-specific genes Ny_{tbr} and Nc_{tbr} conferring resistance to PVY^{O} and PVY^{C}, were found to be triggered by genetic determinants of the virus located in the HC-Pro cistron (Moury et al., 2011; Tian and Valkonen, 2013, 2015), which may explain the gradual field selection of the PVY recombinants, such as PVY^{N-Wi} and PVY^{NTN}, carrying the HC-Pro cistron common with the PVY^{N} parent and unable to trigger HR conferred by these *N* genes (Glais et al., 2002; Lorenzen et al. 2006a; Singh et al. 2008; Hu et al. 2009; Karasev and Gray 2013; Funke et al. 2017; Tran et al. 2022). Recently, an additional Nz_{tbr} gene was identified in potato conferring resistance to the PVY^{NTN} recombinant, defining the PVY^{Z}-NTN strain of PVY (Jones 1990; Barker et al. 2009; Kerlan et al. 2011; Chikh-Ali et al. 2014). Ny_{tbr}, Nz_{tbr}, and possibly other strain-specific resistance genes were identified in multiple commercial potato cultivars grown in the U.S. (Kerlan et al. 2011; Rowley et al. 2015), including Alturas and Ranger Russet, commonly grown in the Columbia Basin (Rowley et al. 2015), and also in Dark Red Norland and Chieftain in this work (Table 2). This Ny_{tbr} gene-driven strain-specific selection was found very efficient in screen-house experiments (Funke et al. 2017) leading to rapid changes in the PVY strain composition during a single growing season which mimicked the changes observed in the commercial potato fields (Funke et al. 2017; MacKenzie et al. 2019; Tran et al. 2022). Consequently, introgression of the strain-specific *Ny* genes conferring resistance to only specific strains of PVY in newly released potato cultivars, such as PVY^{O} and PVY^{C}, does not actually solve the PVY problem and only shifts the strain composition in the field towards other, recombinant strains, such as PVY^{N-Wi} that now dominates the population of PVY isolates in potato production areas in the U.S. (Funke et al. 2017; MacKenzie et al. 2019; Tran et al. 2022). Pyramiding the strain-specific *Ny* genes in a single potato cultivar, e.g., Ny_{tbr} and Nz_{tbr} in Yukon Gold (Kerlan et al. 2011) or in Dark Red Norland and Chieftain (Table 2), may present a better, albeit only a short-term temporary solution restricting spread of PVY^{NTN} and PVY^{N-Wi} strains in the field, but still leaving room for possible emergence of new strains of the virus able to overcome these resistance genes. Of interest are the HR reactions exhibited by two PVY isolates, ID20 and NE11, representing strain NE11: both elicited systemic necrotic reactions in control cultivars Desiree and Maris Bard (Table 3) suggesting presence in their genetic background of an additional, hypothetical gene *Nne* postulated some time ago (Rowley et al. 2015). This may mean that these old European cultivars harbor additional resistance sources in their genome, besides *Ny* and *Nc* (Desiree) and *Ny, Nc,* and *Nz* (Maris Bard) (Cockerham 1970; de Bokx and Huttinga 1981; Jones 1990; Singh et al. 2008), conferring strain specific resistance against not only PVY^{C}, PVY^{O}, and PVY^{NTN}, but also against PVY-NE11 (see Table 3).

Deployment of *Ry* genes conferring broad, strain non-specific resistance to PVY seems a more acceptable strategy over the long run, provided such genes have reliable, stable, and predictive genetic markers and are confirmed to withstand all currently known strains of PVY. In our experiments, no symptoms were observed in Payette Russet following inoculation of all nine tested PVY strains and three additional genetic variants of the virus (Table 3), indicating that neither of these strains and variants were able to infect Payette Russet, not systemically and not even in an inoculated leaf. This suggested that the Ry_{sto} resistance gene present in the genome of Payette Russet (Novy et al. 2017) indeed conferred an ER against nine strains and additional three genetic variants of PVY maintained in our PVY collection. These tested isolates of PVY represented all genetic diversity of PVY found so far in the U.S. (Funke et al. 2017; Green et al. 2018; Tran et al. 2022), and thus Payette Russet may be deemed fully PVY-resistant or completely immune to PVY within the boundaries of the U.S. or even in North America (Table 3). Two isolates, Pondo4 (261–4) and AGA (PVY^{E}), represented non-US strains of the virus found in Brazil, Europe, Middle East, and China, and Payette Russet exhibited complete immunity to them too (Table 3). Six non-potato genetic variants of PVY from tomato, cape gooseberry, and tamarillo, representing PVY^{C} strain and a sub-lineage of the PVY^{N} strain were unable to replicate in Payette Russet as well (Table 3). While additional genetic variants of PVY, not available in the U.S. and in North America (see Green et al. 2018), will still need to be tested as a virus challenge in Payette Russet, we can conclude that this cultivar harbors a very robust, broad resistance source

against PVY, presented by the Ry_{sto} gene. Payette Russet may be confidently recommended as a parent for further breeding efforts introgressing this valuable source of resistance into commercial potato cultivars.

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Declarations

Conflict of Interest The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

Baldauf, P.M., S.M. Gray, and K.L. Perry. 2006. Biological and serological properties of *Potato virus Y* isolates in northeastern United States potato. *Plant Disease* 90: 559–566.

Chikh Ali, M., S. Gray, and A.V. Karasev. 2013. An improved multiplex IC-RT-PCR assay distinguishes nine strains of *Potato virus Y*. *Plant Disease* 97: 1370–1374.

Chikh Ali, M., A.V. Karasev, N. Furutani, M. Taniguchi, Y. Kano, M. Sato, T. Natsuaki, and T. Maoka. 2013. Occurrence of *Potato virus Y* strain PVY^{NTN} in foundation seed potatoes in Japan, and screening for symptoms in Japanese potato cultivars. *Plant Pathology* 62: 1157–1165.

Chikh-Ali, M., J.S. Rowley, J. Kuhl, S.M. Gray, and A.V. Karasev. 2014. Evidence of a monogenic nature of the *Nz* gene conferring resistance against *Potato virus Y* strain Z (PVY^{Z}) in Potato. *American Journal of Potato Research* 91: 649–654.

Chikh-Ali, M., D. Vander Pol, O.V. Nikolaeva, M.J. Melzer, and A.V. Karasev. 2016. Biological and molecular characterization of a tomato isolate of *Potato virus Y* (PVY) of the PVY^{C} lineage. *Archives of Virology* 161: 3561–3566.

Chikh-Ali, M. and Karasev, A.V. 2015. Immunocapture-multiplex RT-PCR for the simultaneous detection and identification of plant viruses and their strains: study case, potato virus Y (PVY). In: C. Lacomme (ed.), Plant Pathology: Techniques and Protocols, Methods in Molecular Biology, vol. 1302: 177–186 https://doi.org/10.1007/978-1-4939-2620-6_14.

Cockerham, G. 1970. Genetic 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.

Funke, C.N., O.V. Nikolaeva, K.J. Green, L.T. Tran, M. Chikh-Ali, A. Quintero-Ferrer, R.A. Cating, K.E. Frost, P.B. Hamm, N. Olsen, M.J. Pavek, S.M. Gray, J.M. Crosslin, and A.V. Karasev. 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 Disease* 101: 20–28.

Galvino-Costa, S.B.F., A. Figueira, V.V. Camargos, P.S. Geraldino, X. Hu, O.V. Nikolaeva, C. Kerlan, and A.V. Karasev. 2012. A novel type of *Potato virus Y* recombinant genome, determined for the genetic strain PVY^{E}. *Plant Pathology* 61: 388–398.

Galvino-Costa, S.B.F., A. dos Reis Figueira, F.A.C. Rabelo-Filho, F.H.R. Moraes, O.V. Nikolaeva, and A.V. Karasev. 2012. Molecular typing of *Potato virus Y* isolates from Brazil reveals a diverse set of recombinant strains. *Plant Disease* 96: 1451–1458.

Gebhardt, C., and J.P.T. Valkonen. 2001. Organization of genes controlling disease resistance in the potato genome. *Annual Review of Phytopathology* 39: 79–102.

Green, K.J., C.J. Brown, S.M. Gray, and A.V. Karasev. 2017. Phylogenetic study of recombinant strains of *Potato virus Y*. *Virology* 507: 40–52.

Green, K.J., M. Chikh-Ali, M.J. Melzer, and A.V. Karasev. 2017. *Potato virus Y* (PVY) isolates from *Physalis peruviana* are unable to systemically infect potato or pepper and form a distinct new lineage within the PVY^{C} strain group. *Phytopathology* 107: 1433–1439.

Green, K.J., C.J. Brown, and A.V. Karasev. 2018. Genetic diversity of potato virus Y (PVY): Sequence analyses reveal ten novel PVY recombinant structures. *Archives of Virology* 163: 23–32.

Green, K.J., C.N. Funke, J. Chojnacky, R.A. Alvarez-Quinto, J.B. Ochoa, D.F. Quito-Avila, and A.V. Karasev. 2020. *Potato virus Y* (PVY) isolates from *Solanum betaceum* represent three novel recombinants within the PVY^{N} strain group and are unable to systemically spread in potato. *Phytopathology* 110: 1588–1596.

Green, K.J., A. Quintero-Ferrer, M. Chikh-Ali, R.A.C. Jones, and A.V. Karasev. 2020. Genetic diversity of nine non-recombinant *Potato virus Y* (PVY) isolates from three biological strain groups: Historical and geographical insights. *Plant Disease* 104: 2317–2323.

Gundersen, B., D.A. Inglis, M.J. Pavek, and A.V. Karasev. 2019. Foliar and tuber reactions of three strains of *Potato virus Y* on five fresh market potato cultivars through three successive potato generations. *American Journal of Potato Research* 96: 519–531. https://doi.org/10.1007/s12230-019-09738-3.

Hosaka, K., Y. Hosaka, M. Mori, T. Maida, and H. Matsunaga. 2001. Detection of a simplex RAPD marker linked to resistance to potato virus Y in a tetraploid potato. *American Journal of Potato Research* 78: 191–196.

Hu, X., T. Meacham, L. Ewing, S.M. Gray, and A.V. Karasev. 2009. A novel recombinant strain of *Potato virus Y* suggests a new viral genetic determinant of vein necrosis in tobacco. *Virus Research* 143: 68–76.

Jones, R.A.C. 1990. Strain group specific and virus specific hypersensitive reactions to infection with potyviruses in potato cultivars. *The Annals of Applied Biology* 117: 93–105.

Karasev, A.V., and S.M. Gray. 2013. Continuous and emerging challenges of *Potato virus Y* in potato. *Annual Review of Phytopathology* 51: 571–586.

Karasev, A.V., O.V. Nikolaeva, X. Hu, Z. Sielaff, J. Whitworth, J.H. Lorenzen, and S.M. Gray. 2010. Serological properties of ordinary and necrotic isolates of Potato virus Y: A case study of PVY^{N} misidentification. *American Journal of Potato Research* 87: 1–9.

Karasev, A.V., X. Hu, C.J. Brown, C. Kerlan, O.V. Nikolaeva, J.M. Crosslin, and S.M. Gray. 2011. Genetic diversity of the ordinary strain of *Potato virus Y* (PVY) and origin of recombinant PVY strains. *Phytopathology* 101: 778–785.

Kerlan, C., O.V. Nikolaeva, X. Hu, T. Meacham, S.M. Gray, and A.V. Karasev. 2011. Identification of the molecular make-up of the *Potato virus Y* strain PVY^{Z}: Genetic typing of PVY^{Z}-NTN. *Phytopathology* 101: 1052–1060.

Lorenzen, J.H., T. Meacham, P.H. Berger, P.J. Shiel, J.M. Crosslin, P.B. Hamm, and H. Kopp. 2006. Whole genome characterization of *Potato virus Y* isolates collected in the western USA and their comparison to isolates from Europe and Canada. *Archives of Virology* 151: 1055–1074.

Lorenzen, J.H., L.M. Piche, N.C. Gudmestad, T. Meacham, and P. Shiel. 2006. A multiplex PCR assay to characterize *Potato virus Y* isolates and identify strain mixtures. *Plant Disease* 90: 935–940.

MacKenzie, T.D.B., X. Nie, V. Bisht, and M. Singh. 2019. Proliferation of recombinant PVY strains in two potato-producing regions of Canada, and symptom expression in 30 important potato varieties with different PVY strains. *Plant Disease* 103: 2221–2230.

Moury, B. 2010. A new lineage sheds light on the evolutionary history of *Potato virus Y*. *Molecular Plant Pathology* 11: 161–168.

Munoz, F.J., R.L. Plaisted, and H.D. Thurston. 1975. Resistance to potato virus Y in Solanum tuberosum ssp. andigena. *American Potato Journal* 52: 107–115.

Nikolaeva, O.V., D. Roop, S.F.B. Galvino-Costa, A.R. Figueira, S.M. Gray, and A.V. Karasev. 2012. Epitope mapping for monoclonal antibodies recognizing tuber necrotic strains of *Potato virus Y*. *American Journal of Potato Research* 89: 121–128.

Novy, R.G., J.L. Whitworth, J.C. Stark, B.L. Schneider, N.R. Knowles, M.J. Pavek, L.O. Knowles, B.A. Charlton, V. Sathuvalli, S. Yilma, C.R. Brown, M. Thornton, T.L. Brandt, and N. Olsen. 2017. Payette Russet: A dual-purpose potato cultivar with cold-sweetening resistance, low acrylamide formation, and resistance to late blight and *Potato virus Y*. *American Journal of Potato Research.* 94: 38–53.

Rowley, J.S., S.M. Gray, and A.V. Karasev. 2015. Screening potato cultivars for new sources of resistance to *Potato virus Y*. *American Journal of Potato Research* 92: 38–48.

Singh, R.P., J.P.T. Valkonen, S.M. Gray, N. Boonham, R.A.C. Jones, C. Kerlan, and J. Schubert. 2008. Discussion Paper: The naming of *Potato virus Y* strains infecting potato. *Archives of Virology* 153: 1–13.

Tran, L.T., K.J. Green, M. Rodriguez-Rodriguez, G.E. Orellana, C.N. Funke, O.V. Nikolaeva, A. Quintero-Ferrer, M. Chikh-Ali, L. Woodell, N. Olsen, and A.V. Karasev. 2022. Prevalence of recombinant strains of potato virus Y in seed potato planted in Idaho and Washington states between 2011 and 2021. *Plant Disease* 106: 810–817. https://doi.org/10.1094/PDIS-08-21-1852-SR.

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. *The Annals of Applied Biology* 130: 91–104. https://doi.org/10.1111/j.1744-7348.1997.tb05785.x.