Potato Virus Y Strains and Host Reactions in Potato

A Thesis

Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science

with a

Major in Plant Science

in the

College of Graduate Studies

University of Idaho

by

Hayam Alruwaili

Major Professor: Alexander V. Karasev, Ph.D.

Committee members: Jack Brown, Ph.D.; Nora Olsen, Ph.D.

Department Administrator: Paul McDaniel, Ph. D.

May 2016

Authorization to Submit Thesis

This thesis of Hayam Alruwaili, submitted for the degree of Master of Science with Major in Plant Science and “Potato Virus Y Strains and Host Reactions in Potato," has been reviewed in final form. Permission, as indicated by the signatures and dates given below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: _____ Date: _____

Alexander V. Karasev, Ph.D.

Committee Members: _____ Date: _____

Jack Brown, Ph.D.

_____ Date: _____

Nora Olsen, Ph.D.

Department Administrator: _____ Date: _____

Paul McDaniel, Ph.D.

Abstract

Potato virus (PVY) is the most destructive potato virus which currently causes economic losses in potato production worldwide, including in Saudi Arabia. The first objective of this study was to identify the PVY strains circulating in Saudi Arabia. Nine samples were collected and confirmed PVY-positive, and determined by RT-PCR to represent recombinant PVY strains NE-11 and SYR-III. Whole genome sequences were determined for two isolates, S2 (SYR-III) and S9 (NE-11). This is the first report of the occurrence of recombinant strains of PVY in Saudi Arabia.

Two types of resistance induced by two set of genes have been identified in potato against PVY. Our second objective was to characterize new *N* resistance genes in potato against PVY recombinants by phenotyping the progeny of F1 crosses between cultivars Yukon Gem and Russet Norkotah. It was determined that resistance to different recombinants of PVY is controlled by distinct N genes.

Acknowledgements

I first wish to thank the members of my committee, Dr. Alexander V. Karasev, Dr. Jack Brown, and Dr. Nora Olsen for their knowledge and encouragement. In particular, I am very grateful to Dr. Alexander V. Karasev, my adviser, supporter, critic, and friend. I am very fortunate for having the opportunity to work with him. I would like to acknowledge Dr. Mohamad Chikh-Ali who helped me all the time and kept me sane during the development of my thesis.

My family also deserves many thanks for being supportive and made everything possible. I would like to acknowledge my brother Sultan Alruwaili who helped me all the time. I would like to acknowledge my husband. He was always there when I needed support.

I would also like to thank the King Abdullah Scholarship Program, Saudi Arabia; they allowed me to study at the University of Idaho. I would like to thank Agricultural Development Company Farms in Al-Jouf, Saudi Arabia; they allowed me to collect the samples I used in my thesis.

Table of Contents

List of Figures

List of Tables

Chapter 1 Potato Virus Y strains in Saudi Arabia

Abstract

The practical importance of Potato virus Y (PVY) recombinant strains has increased due to the wide distribution and ability of some of the recombinants to induce potato tuber necrotic ring spot disease (PTNRD) that seriously affects the quality of tubers. In Saudi Arabia, potato production has increased five-fold during the last three decades reaching 450,000 tons per year. PVY has been reported as one of the main viruses infecting potatoes in the country, but no information was available on PVY strains circulating in the country. In August 2014, a small survey was conducted in a seed potato field in Al-Jouf, Saudi Arabia. Nine PVY-positive samples selected based on visual symptoms and serological reactivity were subjected to two multiplex RT-PCR assays and all nine determined to represent recombinant PVY strains, NE-11 and SYR-III. Whole genome sequences were determined for two isolates, S2 (SYR-III) and S9 (NE-11). Both recombinants, PVY-NE11 and SYR-III, were previously found associated with PTNRD but thought to be rare. However, the current identification of PVY-NE11 and SYR-III in a new geographic region suggests that these strains may not be as rare as believed previously. This is the first report on the occurrence of recombinant strains of PVY in Saudi Arabia.

Introduction

Potato virus Y (PVY) is the type species of the genus *Potyvirus*, family *Potyviridae*, the second largest family of plant viruses. (Adams *et al*., 2012). PVY is the most economically important and devastating virus infecting potato (*Solanum tuberosum L*.) crops worldwide, affecting both tuber yield and quality all over the world (Singh et al., 2008; Gray et al., 2010; Scholthof et al., 2011; Karasev and Gray, 2013). It is also associated with major diseases in other crops, like pepper and tomato (Kerlan and Moury, 2008). PVY exists as a

complexity of strains and variants that produce a range of symptoms in different hosts (Singh et al., 2008; Karasev and Gray, 2013). Potato tuber necrotic ringspot disease (PTNRD) was found to be associated predominantly with recombinant PVY strains (Beczner et al., 1984; LeRomancer et al. 1994; Lorenzen et al., 2008), although later demonstrated to be caused by other, non-recombinant strains as well (Gray et al., 2010). Because of PTNRD and its effect on tuber quality, recombinant strains of PVY attracted significant attention, and their incidence and geographical distribution in potato producing areas have been of concern in the last few years (Lorenzen et al., 2006a; Gray et al., 2010; Djilani-Khouadja *et al*., 2010; Bukhris-Bouhachem *et al*., 2010; Chikh Ali et al., 2010a; Galvino-Costa et al., 2012a,b; Anfoka et al., 2014).

Traditionally, PVY strains have been divided into PVY^{O}, PVY^{C}, PVY^{N}, PVY^{Z} and PVY^{E}, based on reactions of potato cultivars carrying the resistance genes Ny_{tbr}, Nc_{tbr} and Nz_{tbr}, and tobacco (*Nicotiana tabacum* L.; Jones, 1990; Kerlan *et al*., 1999; Singh *et al*., 2008; Karasev and Gray, 2013; Chikh-Ali et al., 2014). In the most recent classification, PVY isolates were divided into nine strains, PVY^{O}, PVY^{C}, PVY^{N}, PVY^{Z}, PVY^{E}, $PVY^{N\text{-}Wi}$, $PVY^{N:O}$, $PVY^{NA\text{-}N}$, and PVY-NE11 (Karasev and Gray, 2013). PVY^{Z}, PVY^{E}, $PVY^{N\text{-}Wi}$, $PVY^{N:O}$ and PVY-NE11 are recombinant strains with different recombinant structures with parental sequences representing most often PVY^{O} and PVY^{N}, but sometimes unknown parents, like PVY-NE11 (reviewed in Karasev and Gray, 2013). Most of the recombinants of PVY, including PVY^{Z}-NTN, PVY^{E}, $PVY^{NTN\text{-}NW}$, and PVY-NE11, caused PTNRD.

In Saudi Arabia, potato production is carried out under irrigation in two growing seasons, the spring and the fall seasons. Potato is produced for fresh market and for

processing into chips. Seed potatoes for the spring growing season ("Elite Class") are imported from several European suppliers located in the Netherlands, the UK, and France. Potato production of the spring growing season that is certified as seed potato is planted in the following fall season to produce "A Class" seed potatoes. In 2013, main potato producing areas in Saudi Arabia were Al-Jouf, Hail, Tabuk, Qasim, Riyadh, Hofuf and Najran (Fig. 1), with a total production area of 17,500 ha and a total production of 460,000 tons per year of which 45,500 tons were seed potatoes (FAOSTAT database, 2013). Surveys reported PVY as one of the main viruses infecting potatoes (Al-Shahwan et al., 1997; Sabir, 2012; Al-Saikhan et al., 2014). For instance, Al-Shahwan et al. (1997) detected PVY in 20-63% of potato samples collected from two main potato production areas in the North of Saudi Arabia, Tabuk and Hail, from both spring and fall growing seasons. However, PVY strain composition in potato production areas has not been systematically studied in Saudi Arabia. In neighboring countries, with similar potato production cycles, PVY population structure was studied in Syria and Jordan, where recombinants such as PVY^{NTN-NW} (SYR-I, -II and –III), PVY^{NTN}, and PVY^{N-Wi} were reported as dominant strains (Chikh Ali et al., 2010a; Anfoka et al., 2014). In Tunisia, a nearby Mediterranean country, PVY population was reported to be dominated by PVY^{NTN} with few exceptions belonging to PVY^{N} and PVY^{O} (Djilani-Khouadja *et al*., 2010; Bukhris-Bouhachem *et al*., 2010). Here, we report on the identification and characterization of nine recombinant PVY isolates from Saudi Arabia, and, for the first time, on the occurrence of significant recombinant strains of PVY, namely PVY-NE11 and SYR-III.

Material and Methods

Sample collection

A 25-ha seed potato field planted with the potato cultivar Hermes was sampled in August 2014, in the beginning of the fall growing season. The field was located near Al Jouf, Saudi Arabia, and belonged to the Al Jouf Agricultural Development Co. In the field 15 plants' leaves showing virus-like symptoms of mosaic and crinkling were initially tested with the general PVY immuno-strip test (Agdia, Elkhart, IN, USA) and PVY-positive samples were printed on FTA™ Classic Cards (Whatman™, UK) according to manufacturer's instructions (Kerlan, 2006). The cards were dried at room temperature for one hour, sealed in paper envelopes with silica gel and shipped to the University of Idaho laboratory for further analysis.

RNA extraction, RT-PCR, and sequencing

RNA was extracted from FTA cards according to the method of Ndunguru et al. (2005). RNA pellets were dissolved in 30 µl nuclease-free water, heated at 65ºC for 5 min, and then chilled on ice for 3 min. Reverse transcription (RT) was done according to the method of Chikh-Ali et al. (2013). For strain identification, two different multiplex RT-PCR assays reported by Lorenzen et al. (2006b) and Chikh Ali et al. (2010b) were performed with a slight modification. The multiplex RT-PCR (Chikh Ali et al., 2010b) was modified by replacing the primer n7577 with a new primer (n7892: 5'-CTCAACTCCAGATGGAACAATTGTC-3'). This resulted in two shorter PCR products, 992 bp and 761 bp, replacing the 1,307 bp and 1,076 bp, in the original protocol (Chikh Ali et al., 2010a), respectively. Control isolates representing nine strains of PVY and additional recombinant variants were from the laboratory collection (Chikh-Ali et al., 2013).

Two of the nine samples, S2 and S9, were selected for whole genome sequencing. The whole genome sequencing was performed directly on a series of overlapping PCR

fragments amplified on cDNA synthesized from the S2 and S9 samples using SYR-III-L4 and NE-11 specific primers, respectively, as described in Karasev et al. (2011). Successfully amplified PCR products were treated with Exosap-It (Affymetrix, Cleveland, OH) and submitted for Sanger sequencing to Genewiz, Inc. (South Plainfield, NJ). Individual sequence reads were assembled using the SeqMan program of the Lasergene 9 Suite (DNASTAR). The whole genome sequences for isolates S2 and S9 have been deposited in the GenBank database under the accession numbers KP793715 and KP793716, respectively.

Sequence analysis

Sequence analysis was carried out using the SeqMan Pro software (DNA Star; DNASTAR, Madison, WI, USA). For the multiple alignment, the program CLUSTAL X ver. 1.81 (Thompson et al., 1997) was used with the default parameters. Sequence identity was checked using the BLAST program provided by the National Center for Biotechnology Information (NCBI). Alignments were conducted based on the whole genome of representative isolates of PVY strains: PVY^{N}, $PVY^{NA\text{-}N}$, $PVY^{N\text{-}Wi}$, $PVY^{N:O}$, PVY^{NTN}, $PVY^{NTN\text{-}NW}$ (SYR-I, -II and -III), PVY^{O} and PVY^{C}. The aligned sequences were checked for recombination, using RDP (Martin and Rybicki, 2000), GENECONV (Sawyer, 1999), BOOTSCAN (Salminen et al., 1995), MAXCHI (Maynard-Smith, 1992), CHIMAERA (Posada and Crandall, 2001), 3Seq (Boni et al., 2007), and SiScan (Gibbs et al., 2000) methods in RDP4 Beta3 software (Martin et al., 2005). The analyses in RDP4 Beta3 package were done using default settings and a Bonferroni-corrected *P-value* cut off of 0.05. Only recombination points detected by more than 4 methods in the RDP4 Beta3 program were taken into consideration. Phylogeny inference was conducted using the neighbor joining (NJ)

method implemented in the program MEGA version 4 (Tamura *et al*., 2007) with 1,000 bootstrap replicates. Branches with bootstrap values <70% were collapsed.

Results

A seed field of potato cv Hermes was visually assessed for mosaic symptoms, and estimated to have ca. 3-5% of symptomatic plants. Fifteen leaf samples were collected exhibiting various degrees of mosaic and crinkling, and subjected to the general PVY testing using the PVY-specific immunostrips (Agdia). Nine samples were found PVY-positive, producing two distinct bands on the test strip, and these samples were separated and applied to the FTA™ Classic Cards (Whatman™, UK) for further characterization to strain.

RT-PCR assay

When all nine PVY-positive samples were subjected to the multiplex RT-PCR assay developed by Lorenzen et al. (2006b), six samples produced two bands of 181 and 452 bp, one sample produced a single band of 328 bp, and two samples produced a single band of 181 bp, suggesting PVY^{NTN} isolates in six samples and $PVY^{NA\text{-}N}$ in one (summarized in Table 1). In the multiplex PCR assay of Chikh Ali et al. (2010b), all samples produced PVYspecific bands (Fig. 2): three samples produced a band of 441 bp, two samples produced three bands of 278, 441 and 1,076 bp, and three samples produced two bands of 278 and 441 bp. A single 633-bp band was amplified from one sample (summarized in Table 1). Hence, all nine samples were determined to contain recombinant isolates of PVY – five were identified as SYR-III type, one as a NE-11 type, and three as possible SYR, N-Wi, or NTN type based on an inconclusive band pattern revealed in the Chikh-Ali et al. (2013) assay. All the various types exhibited similar mosaic and crinkling foliar symptoms. Such extensive

diversity in just one field suggests that PVY strain diversity in Saudi Arabia overall may be great.

Sequencing and Sequence analysis

Two samples, S2 (typed as SYR-III) and S9 (NE-11) were selected for whole genome sequencing as representative isolates. The sequences of their complete genomes were determined by direct sequencing of overlapping RT-PCR fragments, and were found 9,511 and 9,536-nt long, respectively, excluding poly(A) and 5'-terminal sequences. Both sequences coded for an open reading frame of 3,061 amino acids. S2 shared the highest identity (99.4%) with the isolate SYR-III-L4 (AB461454) reported from Syria, and 98.4% with SYR-II-DrH (AB461453) also reported from Syria (Chikh Ali et al., 2010a). Isolate S9 shared the highest identity (99.8%) with the isolate NE-11 reported from the United States (DQ157180; Lorenzen et al., 2008) and with the isolate ME-162 reported from China (JQ971975; Wang et al., 2012) followed by 98.4% identity to the isolate ID20 reported from the United States (HQ912867; Karasev et al., 2011).

In the recombination analysis, S2 and S9 had identical recombination structures to SYR-III-L4 and NE-11, respectively (Chikh Ali et al., 2010a; Lorenzen et al., 2008; see Fig. 3). The first recombination event in S2 (686-2415, Fig. 3) was detected by *P*-values using RDP (1.679×10^{-120}), GENECONV (2.876×10^{-113}), BootScan (1.195×10^{-119}), MaxChi (M; 1.026×10^{-27}), Chimaera (1.274×10^{-24}), 3Seq (1.264×10^{-215}) and SiScan (5.361×10^{-49}). The second recombination event in S2 (5833-8426; Fig. 3) was detected by *P*-values using RDP (2.938×10^{-129}), GENECONV (4.907×10^{-115}), BootScan (6.852×10^{-126}), MaxChi (9.682×10^{48}), Chimaera (7.517×10^{-40}), 3Seq (3.346×10^{-227}) and SiScan (1.218×10^{-54}). S9 had a single recombination event (2009-2489; Fig. 3) which was detected by *P*-values using RDP

(1.232×10^{-10}), GENECONV (7.649×10^{-08}), MaxChi (6.003×10^{-06}), 3Seq (8.426×10^{-12}), and SiScan (1.029×10^{-03}). The genome of S2 had O and N sequences identified as parents, while S9 had N and NA-N as parents for the first 2,489 nt of the genome and the rest of the genome originated from an unknown parent. When subjected to phylogenetic analysis together with representatives from major recombinant and non-recombinant lineages of PVY strains, S2 grouped closely with SYR-III-L4 while S9 was grouped closely to NE-11 and placed in a "NE-11" sub-cluster at an intermediate position between N and NA-N (Fig. 4).

Discussion

Potato production in Saudi Arabia has increased from about 90,000 tons in 1990 (AlShahwan et al., 1997) to about 460,000 tons in 2013 (FAOSTAT, 2013). With the continuous import of seed potatoes and the dramatic increase of potato production, the importance of potato virus diseases is also expected to increase. Several surveys reported PVY as the main virus infecting potatoes in Saudi Arabia (Al-Shahwan et al., 1997; Sabir, 2012; Al-Saikhan et al., 2014), yet no identification or characterization of PVY strains in the country have been reported. Several partial sequences of the coat protein (CP) area were reported for a few PVY isolates (Sabir, 2012; Al-Saikhan et al., 2014), but these were not sufficient to determine the strain type due to the complex recombination structure of PVY strains (Karasev and Gray, 2013). In the current study, two PVY recombinant types, SYR-III and PVY-NE11, were reported for the first time from Saudi Arabia. SYR-III is a recombinant of PVY with four RJs and shared properties with PVY^{NTN} and $PVY^{N\text{-}Wi}$ (Chikh Ali et al., 2010; Fig. 3).

Specifically, SYR-III was able to induce PTNRD in susceptible potato cultivars (like PVY^{NTN}), but unlike PVY^{NTN} had O-type serology and thus could have been erroneously

typed as PVY^{O}. SYR-III is one of the most common PVY strains in Syria (Chikh Ali et al., 2010a) and was recently found in Jordan (Anfoka et al., 2014). When S2 sequence was subjected to a BLASTN search through the GenBank database, an additional closely related sequence KC903166 was found (98%), representing a partial, 1.7-kb sequence from a tomato PVY isolate collected in 2009 in Iraq. The current report is adding Saudi Arabia to the geographic regions where SYR-III is found.

The origin of this S2 (SYR-III strain) isolate in Saudi Arabia is unclear. Al Jouf is separated from Syria by a desert, and no potato trade existed between the two counties for at least the last 10 years. There is also no potato seed trade with Jordan or Iraq, and, thus, neither Jordan nor Iraq would be likely sources of the SYR-III recombinant of PVY. Considering the fact that all these countries are importing seed potatoes from other regions, SYR-III might have been introduced via imported seed tubers which would imply a much wider distribution than had been reported so far.

The second Saudi isolate sequenced, S9 was identified as a PVY-NE11 recombinant using multiplex RT-PCR and sequence analysis of the whole genome. Since it was first reported in the United States (Lorenzen et al, 2008), the incidence of PVY-NE11 had been increasing (Gray et al., 2010; Karasev et al., 2011; unpublished data). Outside of the United States, an isolate found in China with sequence sharing high identity with the isolate NE-11 was submitted to the Genbank, though it was not originally identified as PVY-NE11 (JQ971975; Wang et al., 2012). Thus, S9 represents the only isolate of PVY-NE11 strain found outside of U.S. or China. The current finding of an NE-11 strain in Saudi Arabia also suggests that PVY-NE11 may have a wider distribution than was reported so far. PVY-NE11 has two RJs resulting in a recombinant genome with the first 2,000 nt belonging to the PVY^{N}

strain followed by a stretch of about 696 nt of the PVY^{NA-N} strain, and the rest of the genome coming from an unknown parent of an intermediate evolutionary position between N and NA-N (Lorenzen *et al*., 2008; Figs. 2, A, and 2, B). PVY-NE11 is also able to induce PTNRD (Piche et al., 2004; Lorenzen *et al*., 2008; Gray et al., 2010; our unpublished data). Although dry samples on the FTA cards would not allow probing the serological properties of Saudi isolates S9 (PVY-NE11) and S2 (SYR-III), the sequence analysis of the S2 and S9 genomes enabled us to predict the serotypes of these two isolates - S2 would have had Oserotype and S9 would have had N-serotype (Chikh-Ali et al., 2010a; Nikolaeva et al., 2012).

In conclusion, this is the first report on the identification of PVY strains in Saudi Arabia which revealed the existence of two significant recombinants, SYR-III and PVYNE11. The finding of PVY-NE11 and SYR-III in a new geographic area of the Middle East suggests a broader distribution of these PVY strains than thought before (Lorenzen et al., 2008; Chikh Ali et al., 2010a). It indicates that potato producers in Saudi Arabia need to be aware of the presence of these two strains in their potato production system, since both of these strains of PVY have been previously implicated in induction of PTNRD in susceptible cultivars.

Chapter 2

Studies on the reactions of potato *N* resistance genes to PVY strains

Abstract

Two types of resistance induced by two set of genes have been identified in potatoes against *Potato virus Y* (PVY). Extreme resistance, conferred by *R* genes, provides a broad, strain non-specific resistance against PVY. *R* genes are found in wild potato, and at least three *R* genes, Ry_{adg}, Ry_{sto}, and $Ry_{chc,}$ have been identified and mapped. The other resistance type is the hypersensitive resistance (HR) conferred by the *N* resistance genes in a strain specific manner. This type of resistance is common in cultivated potatoes and used to classify PVY strains. The resistance genes Ny_{tbr} and Nc_{tbr} have been effective to control PVY^{O} and PVY^{C} strains, respectively. PVY^{NTN} triggers the Nz_{tbr} resistance gene causing HR in potato cultivars carrying this gene. Various *N* resistance genes have been identified recently in commercial potato cultivars in North America, including the cv. Yukon Gem. These putative resistance genes were named as *Nw* (resistance against $PVY^{N\text{-}Wi}$ and $PVY^{N:O}$), *Ne* (resistance against PVY^{E}), *Nne* (resistance against PVY^{NE11}), *Nn* (resistance against PVY^{N}), and *Nna* (resistance against $PVY^{NA\text{-}N}$). In the current study the nature of these resistance genes were tested using the F1 population from the crossing Yukon Gem with the susceptible cultivar Russet Norkotah. As expected, the cultivar Yukon Gem reacted with HR against PVY strains $PVY^{N\text{-}Wi}$, PVY^{NE11}, PVY^{N}, $PVY^{NA\text{-}N}$, and PVY^{NTN}. Conversely, the parental cultivar Russet Norkotah did not show any necrotic reaction to any of PVY strains tested and all inoculated

plants were systemically infected which indicate the absence of any resistance to these strains. HR response was detected in the progeny of Yukon Gem x Russet Norkotah against all these PVY strains tested with variable segregation rates depending on the strain type which support the conclusion that different *N* resistance genes triggered by different PVY strains. The segregation rate of the progeny of Yukon Gem x Russet Norkotah was close to 1:1 when inoculated with $PVY^{N\text{-}Wi}$ which is the rate expected for a monogenic dominant nature of the *Nw* gene. The segregation rates need to be validated using larger numbers of progeny plants and more replicates. The current study was a preliminary study that proved the existence of different *N* resistance genes in Yukon Gem triggered by PVY strains tested and the nature of these genes need to be studied further.

Introduction

Potato virus Y (PVY) is the type species of the genus *Potyvirus*, family *Potyviridae* (Adams *et al*., 2012). PVY is a significant potato virus worldwide that reduces potato yield and quality (Singh *et al*., 2008; Karasev & Gray, 2013). Aphid vectors transmit PVY from infected to healthy plants in a non-persistent manner which requires a brief probe for the aphid on the infected plants to acquire the virus and transmit it to a new host. PVY exists in nature as a complex of strains and variants with variable properties and geographic distribution (Singh *et al*., 2008; Karasev & Gray, 2013). PVY strains have been classified into PVY^{O}, PVY^{C}, PVY^{N}, PVY^{Z} and PVY^{E}, based on hypersensitive reaction they induce in potato cultivars bearing the resistance genes Ny_{tbr}, Nc_{tbr} and Nz_{tbr}, and occurs in tobacco as well (*Nicotiana tabacum* L.; Jones, 1990; Kerlan *et al*., 1999; Singh *et al*., 2008; Karasev & Gray, 2013; Chikh-Ali *et al*., 2014). In the last three decades recombinant PVY strains have

emerged and expanded at the expense of traditional PVY strains in most potato production areas including PVY^{NTN}, $PVY^{N\text{-}Wi}$, $PVY^{N:O}$ and PVY^{NE11} (Karasev & Gray, 2013). Most of PVY recombinant strains, particularly PVY^{NTN}, cause potato tuber necrotic ringspot disease (PTNRD) which dramatically reduces the marketability of potato tubers (Beczner *et al*., 1984; Kerlan *et al*., 2011).

Two types of resistance induced by two set of genes have been identified in potatoes against PVY. Extreme resistance, conferred by *R* genes, provides a broad, strain non-specific resistance against PVY. *R* genes are found in wild potatoes, and at least three *R* genes, Ry_{adg}, Ry_{sto}, and $Ry_{chc,}$ have been identified and mapped (Sato *et al*., 2006). The other resistance type is the hypersensitive resistance (HR) conferred by the *N* resistance genes in a strain specific mode. This type of resistance is common in cultivated potatoes and used to classify PVY strains. Until recently, three *N* genes inducing HR were known, Ny_{tbr}, Nc_{tbr} and Nz_{tbr} conferring resistance to PVY^{O}, PVY^{C} and PVY^{Z} strains, respectively (Jones, 1990; Kerlan *et al*., 1999; Sigh *et al*., 2008; Chikh Ali *et al*., 2014). These resistance genes play a significant role in controlling these PVY strains. In a screening study of main potato cultivars in the United States for the existence of *N* resistance genes, Rowley *et al*. (2015) found that strains other than PVY^{O}, PVY^{C}, and PVY^{Z} could induce HR in commercial potato cultivars such as Yukon Gem. These putative resistance genes were named as *Nw* (resistance against $PVY^{N\text{-}Wi}$ and $PVY^{N:O}$), *Ne* (resistance against PVY^{E}), *Nne* (resistance against PVY^{NE11}), *Nn* (resistance against PVY^{N}), and *Nna* (resistance against $PVY^{NA\text{-}N}$; Rowley *et al*., 2015).

In the current study, the nature and characteristics of these genes were studied by inoculating representative isolates of PVY strains $PVY^{N\text{-}Wi}$, PVY^{NE11}, PVY^{N}, $PVY^{NA\text{-}N}$ and

PVY^{NTN} on progeny plants resulted from the crossing between Yukon Gem that harbors the hypothetical resistance genes and Russet Norkotah which is susceptible to these PVY strains.

Materials and Methods

Sources of potato cultivars

Cultivars Yukon Gem and Russet Norkotah were obtained from the University of Idaho Potato Tissue Culture Laboratory, Moscow, ID. These cultivars were maintained in tissue culture and periodically subjected to ELISA and RT-PCR tests against main potato viruses to confirm their virus-free status.

Crosses and plant maintenance

Virus-free tissue culture potato plantlets of Yukon Gem and Russet Norkotah, 6 of each, were planted in 1-gallon (3.78 liter) containers in a greenhouse bay at ~21-25^{o}C with Sunshine potting soil mix #4 a (Sungro, Seba Beach, AB Canada) and slow release fertilizer (Osmocote™). A 45% shade cloth was used to keep the plants cool. Lighting was 16 hour days and 8 hour nights. Water soluble fertilizer (Miracle Gro™) was added 2-3 times per week throughout the growing period. Plants were watered daily. At flowering, pollen was collected by emasculation of the buds and stored in gelatin capsules on ice and subsequently stored in a -20^{o}C freezer. Newly collected pollen was added to the same gelatin capsules each time pollinations were made. Just before natural flowers opening, flowers were pollinated after emasculation, and each flower was labeled with the date and cross made. Cheesecloth was used to cover the seeds while they matured. Fruits were removed from the plants at maturation (3 weeks) and then seeds were extracted from the fruits. Seeds were removed by cutting the fruits in half and scooping them out with a scalpel. Using cheesecloth, the seeds

were washed under plain water. They were then allowed to dry in the cheesecloth for one week before storing them in paper seed packets. Seeds were sowed, in both Spring (03/04/14) and Fall (09/03/14), directly to potting mix in 2 inch (5-cm) pots and then covered in medium grade vermiculite, and moisture was retained by covering the pots with transparent plastic covers. These plants were allowed to grow in growth chambers under artificial light provided by fluorescent and incandescent lamps with 15h day/9h night cycle and maintained at 2022°C. At 2-3 true-leaf stage, 3 to 4 weeks after planting, plantlets were transplanted into a 4 in (10-cm) pots containing soil mixed with slow release fertilizer (Osmocote™) and moved to a greenhouse.

Reference isolates of PVY, inoculations, phenotype screening, and laboratory testing

The isolate reference isolates shown in Table 1 from the laboratory collection at the University of Idaho were used for inoculation. All PVY isolates were maintained in tobacco (*Nicotiana tabacum* cv. White Burley) in an insect-free, climate-controlled growth chamber. Infected tobacco tissue homogenized in phosphate buffer (50 mM sodium phosphate, pH 7.0 plus 50 mM Na DIECA) at dilution rate 1:10 (w:v) with a mortar and pestle on ice was used as an inoculum.

All 188 potato plants (42 controls and 146 for other PVY isolates: ID20, RRA-1, Mont, HR1, and N1) were mechanically inoculated with carborundum, Phosphate buffer at a 7:1 dilution, and a cotton swab, at the six- to ten-leaf stage , and grown in the same growth chambers mentioned above with 15h day/9h night cycle and maintained at 20-22°C. The symptom observations started 4-5 days after inoculation and were carried out for 6-8 weeks. The symptoms were local and systemic, and included necrotic rings, HR response, mosaic, crinkling, leaf drop, and vein necrosis (Kerlan, 2006, Karasev and Gray, 2013).

All inoculated plants were tested for the development of the systemic PVY infection. Inoculated plants were tested 3 weeks post-inoculation using a triple antibody sandwich (TAS) ELISA (Karasev et al., 2010), and immunocapture (IC)-RT-PCR (Chikh-Ali et al., 2013). Three strain-specific monoclonal antibodies were used in addition to a polyclonal antiserum, MAb2 recognizing PVY^{O}, $PVY^{N\text{-}Wi}$, $PVY^{N:O}$, and PVY^{C} strains (McDonald and Kristjansson, 1993, Agdia, Elkhart, IN), 1F5 recognizing PVY^{N}, PVY^{O}-O5, and PVY^{NTN} (Ellis et al., 1996; Karasev et al., 2010, Agdia, Elkhart, IN), and SASA-N recognizing PVY^{N}, and PVY^{NTN} (Scottish Agriculture Science Agency, Edinburgh, Scotland). IC-RT-PCR typing was performed from the same ELISA plant extracts according to the protocol of Chikh-Ali et al. (2013).

Results

Crosses and seed production

Pollination was successful for 'Yukon Gem' x 'Russet Norkotah' and produced a total of six fruits, with a total of 1,028 seeds (Table 2-1). No fruits were obtained from the pollination 'Russet Norkotah x Yukon Gem'.

HR phenotype

In parental cv. Russet Norkotah, all isolates tested did not induce any local symptoms and all plants inoculated were positive to a challenging strain (Mont, HR1, N1, ID20, or RRA-1; see Table 2-1). Conversely, all PVY strains except $PVY^{NA\text{-}N}$ induced local HR of various types mainly necrotic rings and lesions, water soaked rings with yellowing in the parental cultivar Yukon Gem between 7-14 days post-inoculation (dpi) (Table 2-1; Fig. 2-1).

Inoculated leaves of progeny of the crosses were either symptomless or showed local HR presented as water-soaked rings developing into necrotic rings, and necrotic rings and lesions of various sizes (Fig. 2-1).

Segregation of the HR phenotypes in crosses

The segregation rates of local HR and the systemic infection varied depending on the strain used (Tables 2-2 and 2-3). For example, N1 had a ratio of 1:1 but Mont had a ratio of 1:30. No correlation was found between the local HR and systemic infection (Tables 2-2 and 2-3). The segregation the progeny of crosses.

Discussion

The potato *N* resistance genes confer HR in a strain specific manner restricting the virus movement in infected plants and the response of these genes is dependent on the environmental conditions (Jones, 1990; Valkonen, 1997; Chikh Ali *et al*., 2014; Rowley *et al*., 2015). The resistance genes Ny_{tbr} and Nc_{tbr} have been effective to control PVY^{O} and PVY^{C} strains, respectively. PVY^{NTN} triggers the Nz_{tbr} resistance gene causing HR in potato cultivars carrying this gene (Jones, 1990; Kerlan *et al*., 2011, Chikh Ali *et al*., 2013; Chikh Ali *et al*., 2014). Various *N* resistance genes were identified in commercial potato cultivars in North America, including Yukon Gem (Rowley et al., 2015). In the current study the nature of these resistance genes were tested using the F1 population resulted by crossing Yukon Gem with the susceptible cultivar Russet Norkotah. As expected, the cultivar Yukon Gem reacted with HR against PVY strains $PVY^{N\text{-}Wi}$, PVY^{NE11}, PVY^{N}, $PVY^{NA\text{-}N}$, and PVY^{NTN}. Conversely, the parental cultivar Russet Norkotah did not show any necrotic reaction to any of PVY strains tested and all inoculated plants were systemically infected which indicates the

absence of any resistance to these strains. These results were in agreement with those obtained earlier by Rowley et al., (2015). HR response was detected in the progeny of Yukon Gem x Russet Norkotah against all PVY strains tested with variable segregation rates depending on the strain type which might be an indicator of different N resistance genes triggered by different PVY strains. The segregation rate of the progeny of Yukon Gem x Russet Norkotah was close to 1:1 when inoculated with $PVY^{N\text{-}Wi}$ which is the rate expected for the monogenic dominant nature of the *Nw* gene. The segregation rates need to be validated using larger numbers of progeny plants and more replicates. The current study was a preliminary study that proved the existence of different *N* resistance genes in Yukon Gem triggered by PVY strains tested, and the nature of these genes need to be studied further.

Chapter 3

General Conclusions

Abstract

Potato virus (PVY) is the type member of the genus *Potyvirus*. PVY is the most destructive potato (*Solanum tuberosum L.*) virus which currently causes economic losses in potato production worldwide. Thus, PVY attracts global attention and intensive studies have been conducted to control PVY. In Saudi Arabia, PVY is one of the most important pathogens of potato, and typically detected in this crop by RT-PCR and ELISA. The first objective was to identify the PVY strains circulating in Saudi Arabia. To avoid a lengthy permitting process, and inspection by plant quarantine service in the U.S., a special sampling process utilizing FTA cards was used. Potato leaves displaying virus symptoms were collected at the location of Al-Jouf Agricultural Development Company Farms in Al-Jouf in the north of Saudi Arabia. Nine samples, all cv Hermes, were collected and initially confirmed PVY-positive using virus-specific immuno-strips (Agdia). These PVY-positive samples selected based on visual symptoms and serological reactivity were subjected to two multiplex RT-PCR typing assays and all nine determined to represent recombinant PVY strains, NE-11 and SYR-III. Whole genome sequences were determined for two isolates, S2 (SYR-III) and S9 (NE-11). Both recombinants, PVY-NE11 and SYR-III, were previously found associated with potato tuber necrotic ringspot disease (PTNRD) but thought to be rare. This is the first report on the occurrence of recombinant strains of PVY in Saudi Arabia.

Two types of resistance induced by two set of genes have been identified in potato against PVY. Extreme resistance, conferred by R genes, provides a broad, strain non-specific resistance against PVY. The other resistance type is the hypersensitive resistance (HR) conferred by the N resistance genes in a strain specific mode. This type of resistance is common in cultivated potato and used to classify PVY strains. These resistance genes are play a significant role in controlling these PVY strains. The second objective of this project was to initially characterize new *N* resistance genes in potato against PVY recombinants. We have studied the nature of these resistance genes through phenotyping the progeny of F1 crosses between cultivars Yukon Gem and Russet Norkotah following inoculation with recombinant PVY strains En-N, N-Wi, NTN, NE-11, and a non-recombinant PVY^{N}. Preliminary conclusion is that resistance to different recombinants of PVY is controlled by distinct N genes.

General Conclusions

PVY is one of the most important pathogens of potato. The first objective of this study was to identify the PVY strains circulating in Saudi Arabia**.** Several surveys reported PVY as the main virus infecting potatoes in Saudi Arabia (Al-Shahwan et al., 1997; Sabir, 2012; Al-Saikhan et al., 2014), yet no identification or characterization of PVY strains in the country have been reported. Nine field isolates were collected and typed. Two recombinant PVY strains were identified in these Saudi samples: PVY-NE11 and SYR-III. This is the first time NE-11 has been found outside of the U. States or China. The finding of SYR-III in Saudi Arabia indicates a wide distribution of SYR-III in the eastern Mediterranean. In the future, a larger study is needed to conclude more about the relative distribution of these recombinant PVY strains in Saudi Arabia, and the presence of any other strains.

The potato N resistance genes confer HR in a strain specific manner that restricts the virus movement in infected plants. Various N resistance genes have been identified recently in commercial potato cultivars in North America, including the cv. Yukon Gem. The second objective of this study was to study the nature of these resistance genes. They were tested using the F1 population from the crossing Yukon Gem with the susceptible cultivar Russet Norkotah. As expected, the cultivar Yukon Gem reacted with HR against PVY strains PVY^{N-Wi}, PVY-NE11, PVY^{N}, PVY^{NA-N}, and PVY^{NTN}. Conversely, the parental cultivar Russet Norkotah did not show any necrotic reaction to any of PVY strains tested and all inoculated plants were systemically infected which indicate the absence of any resistance to these strains. HR response was detected in the progeny of Yukon Gem x Russet Norkotah against all these PVY strains tested with variable segregation rates depending on the strain type which support the conclusion that different N resistance genes are triggered by different PVY strains. However, the current study was only a preliminary study that proved the existence of different *N* resistance genes in Yukon Gem triggered by PVY strains tested, and the nature of these genes still needs to be studied further, such as the exact nature of the interaction between the genes and virus, and how they are triggered.

This study represents two important next steps in advancing of our understanding of the most important and destructive potato virus in the world. More is now known about the presence of PVY in Saudi Arabia, and more is understood about the N genes in potato.

References

1. Adams, M. J., Zerbini, F. M., French, R., Rabenstein, F., Stenger, D. C., Valkonen, J. P. T.

   2012. Potyviridae. Pages 1069-1089 in: Ninth Report of the International Committee on Taxonomy of Viruses. A. M. Q. King, M. J. Adams, E. B. Carstens and E. J. Lefkowitz, eds. Elsevier Academic Press, San Diego, California.

2. Al-Saikhan, M. S., Alhudaib, K. A., Soliman, A. M. 2014. Detection of Three Potato Viruses Isolated from Saudi Arabia. Inter. J. Virol. 10:224-234.

3. Al-Shahwan, I. M., Abdalla, O. A., Al-Saleh, M. A. 1997. Viruses in the northern potatoproducing regions of Saudi Arabia. Plant Pathol. 46:91-94.

4. Anfoka, G., Haj-Ahmad, F., Altaleb, M., Abadi, M., Abubaker, S., Levy, D., Rosner, A., Czosnek, H. 2014. First report of recombinant *Potato virus Y* strains infecting potato in Jordan. Plant Dis. 98:1017.

5. Boni, M. F., Posada, D., Feldman, M. W. 2007. An exact nonparametric method for inferring mosaic structure in sequence triplets. Genetics 176:1035-1047.

6. Chikh Ali, M., Maoka, T., Natsuaki, T., Natsuaki, K. T. 2010a. PVYNTN^{-NW}, a novel recombinant strain of Potato virus Y predominating in potato fields in Syria. Plant Pathol. 59:31-41.

7. Chikh Ali, M., Maoka, T., Natsuaki, K. T., Natsuaki, T. 2010b. The simultaneous differentiation of *Potato virus Y* strains including the newly described strain PVY^{NTN-NW} by multiplex PCR assay. J. Virol. Methods 165:15-20.

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

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

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

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

12. Gibbs, M. J., Armstrong, J. S., Gibbs, A. J. 2000. Sister-scanning: a Monte Carlo procedure for assessing signals in recombinant sequences. Bioinformatics 16:573-582.

13. Gray, S., De Boer, S., Lorenzen, J., Karasev, A., Whitworth, J., Nolte, P., Singh, R., Boucher, A., and Xu, H. 2010. Potato virus Y: an evolving concern for potato crops in the United States and Canada. Plant Dis. 94:1384-1397.

14. Jones, R. A. C. 1990. Strain group specific and virus specific hypersensitive reactions to infection with potyviruses in potato cultivars. Ann. App. Biol. 117:93-105.

15. Karasev, A. V., and Gray, S. M. 2013. Continuous and emerging challenges of *Potato virus*

Y in potato. Ann. Rev. Phytopath. 51:571-586.

16. 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. Phytopath. 101:778-785.

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

18. Kerlan, C., and Moury, B. 2008. Potato virus Y. Pages 287-296 in: Encyclopedia of Virology, 3rd ed. vol. 4. B. W. J. Mahy and M. H. V. Van Regenmortel, eds. Elsevier, Oxford.

19. Kerlan, C., Tribodet, M., Glais, L., and Guillet, M. 1999. Variability of Potato virus Y in potato crops in France. J. Phytopath. 147:643-651.

20. Lorenzen, J. H., Meacham, T., Berger, P. H., Shiel, P. J., Crosslin, J. M., Hamm, P. B., and Kopp, H. 2006a. 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.

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

22. Lorenzen, J., Nolte, P., Martin, D., Pasche, J., and Gudmestad, N. 2008. NE-11 represents a new strain variant class of *Potato virus Y*. Arch. Virol. 153:517-525.

23. Martin, D., and Rybicki, E. 2000. RDP: detection of recombination amongst aligned sequences. Bioinformatics. 16:562-563.

24. Martin, D., Williamson, C., and Posada, D. 2005. RDP2: recombination detection and analysis from sequence alignment. Bioinformatics 21:260-262.

25. Maynard-Smith, J.1992. Analyzing the mosaic structure of genes. J. Mol. Evol. 34:126-129.

26. Nikolaeva, O. V., Roop, D. J., Galvino-Costa, S. B. F., dos Reis Figueira, A., Gray, S. M., and Karasev, A. V. 2012. Epitope mapping for monoclonal antibodies recognizing tuber necrotic isolates of *Potato virus Y*. Am. J. Potato Res. 89:121-128.

27. Ndunguru, J., Taylor, N. J., Yadav, J., Aly, H., Legg, J. P., Avenling, T., Thompson, G., and Fauquet, C. M. 2005. Application of FTA technology for sampling, recovery and molecular characterization of viral pathogens and virus-derived transgenes from plant tissues. Virol. J. 2:45.

28. 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. Phytopath. 94:13681375.

29. Posada, D., and Crandall, K. A. 2001. Evaluation of methods for detecting recombination from DNA sequences: computer simulations. PNAS USA 98:13757-13762.

30. Sabir, J. S. M. 2012. Identification of six *Potato virus Y* isolates from Saudi Arabia. African

J Biotech. 11:9709-9715.

31. Salminen, M. O., Carr, J. K., Burke, D. S., and McCutchan, F. E. 1995. Identification of breakpoints in intergenotypic recombinants of HIV type 1 by Bootscanning. AIDS Res. Human Retroviruses 11:1423-1425.

32. Sawyer, S. A. 1999. GENECONV: a computer package for the statistical detection of gene conversion. Distributed by the Author. Department of Mathematics. Washington University, St. Louis available online http://www.math.wustl.edu/~sawyer

33. Scholthof, K. G., Adkins, S., Czosnek, H., Palukaitis, P., Jaquot, E., Hohn, T., Hohn, B., Saunders, K., Candresse, T., Ahlquist, P., Hemenway, C., and Foster, G. 2011. Top 10 plant viruses in molecular plant pathology. Mol. Plant Pathol. 12:938-954.

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

35. Tamura, K., Dudley, J., Nei, M., and Kumar, S. 2007. MEGA: Molecular Evolutionary Genetics Analysis Software Version 4. Molecular Biology and Evolution 24:1596-1599.

36. 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 Research 22:4673-4680.

37. Wang, F., Wu, Y. H., Gao, Z. L., Zhou, B. G. 2012. First report of *Potato virus Y* in *Kalimeris indica* in China. Plant Dis. 96:1827.

Table 1.1. Strain identification of Potato virus Y (PVY) isolates collected from a potato field in Saudi Arabia, 2014 using multiplex RT-PCR assays.

Sample	PCR [1] amplicons	IDLorenzen [1)]	PCR [2] amplicons	IDChikh-Ali [2)]	Final ID	Recombination sites (nucleotide position)
S1	181/452	NTNfzdb	278/441	SYR-III?	SYR-III	
S2	181/452	NTN	278/441/761	SYR-III	SYR-III	P1(686), P3 (2415), VPg (5833), NIb (8426)
S3	181/452	NTN	441	?	NTN/ SYR	
S4	181/452	NTN	278/441	SYR-III?	SYR-III	
S5	181/452	NTN	278/441/761	SYR-III	SYR-III	
S6	181	-	441	?	?	
S7	181/452	NTN	278/441	SYR-III?	SYR-III	
S8	181	?	441	?	?	
S9	328	NA-N	633	NE11	NE11	HC-Pro (2009), P3 (2489)
NE-11	328	NA-N	633	NE11	NE11	HC-Pro (2009), P3 (2489)
SYR-III-1	181/452	NTN	278/441/761	SYR-III	SYR-III	P1(686), P3 (2415), VPg (5833), NIb (8426)

1): According to Lorenzen et al., (2006); 2): According to Chikh Ali et al. (2010 and 2013); PTNRD: potato tuber necrotic ringspot disease in potato cv. Yukon Gold; NE-11 and SYRIII-1 are reference isolates of PVY strains PVY-NE11 and SYR-III, respectively, which are maintained with PVY isolate collection of the University of Idaho.

Fig. 1.1 Map of Saudi Arabia showing the location of Al-Jouf (marked with a rectangle) where PVY isolates were collected. Red circles with corresponding names refer to main potato production areas in Saudi Arabia.

Fig. 1.2. RT-PCR assays for the identification of PVY isolates collected from a potato field from Saudi Arabia. Nine individual PVY-positive samples selected based on immuno-strip reactivity are labeled S1 through S9.

Upper panel (A), results of multiplex RT-PCR assay according to Lorenzen et al. (2006b); lower panel (B), results of multiplex RT-PCR assay according to Chikh Ali et al. (2010b). Positions and sizes of the characteristic PCR products are indicated; M indicates the lane for size markers.

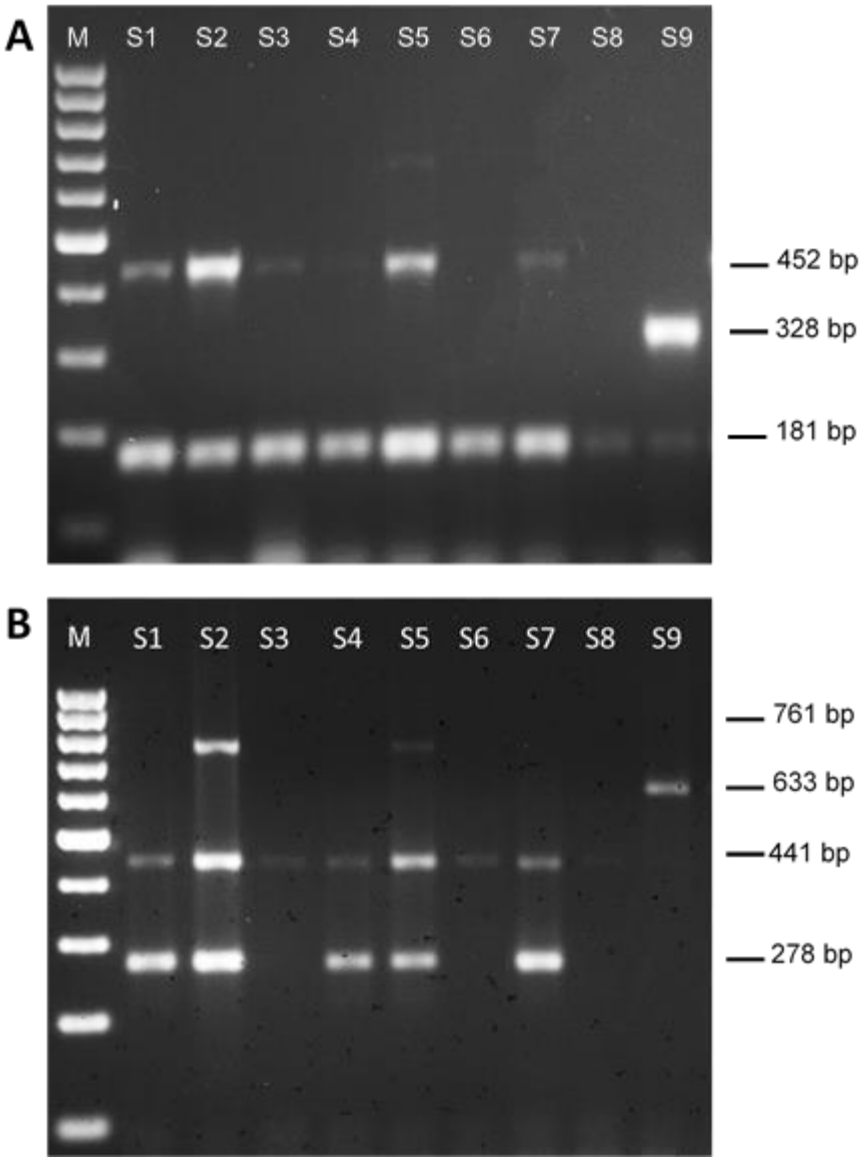

Fig. 1.3. Recombinant structure of S2 and S9 isolates of PVY as determined based on nucleotide sequence analysis. Rectangles designate virus genomes, and shading corresponds to the relatedness to the parental sequences, PVY^{O}, PVY^{N}, PVY^{NA-N}, and "unknown", indicated below the diagram. The PVY genome organization is presented at the top, individual cistrons are listed, with vertical lines corresponding to polyprotein cleavage sites. The two sequenced isolates, S2 and S9, are highlighted in bold; the two reference recombinant structures, SYR-III and NE-11, are italicized.

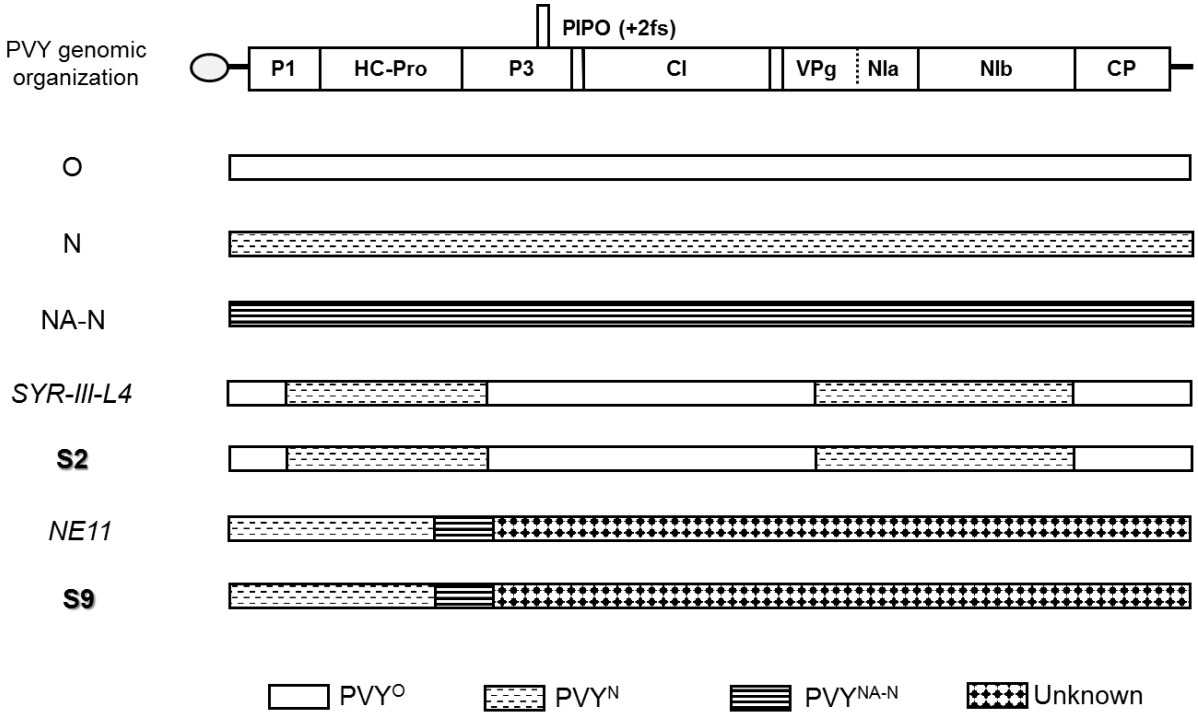

Fig. 1.4. Phylogenetic analysis of nucleotide sequences of the whole genomes for representative PVY isolates conducted with the neighbor joining (NJ) algorithm. Only nodes with bootstrap values higher 70% were retained. Brackets designate individual strains and variants of PVY according to Karasev and Gray (2013); red brackets indicate clades where S2 and S9 sequences are placed. Red colored font designates the S2 and S9 sequences paired with the closest reference sequences. Isolate name was followed by a corresponding accession number in the GenBank database.

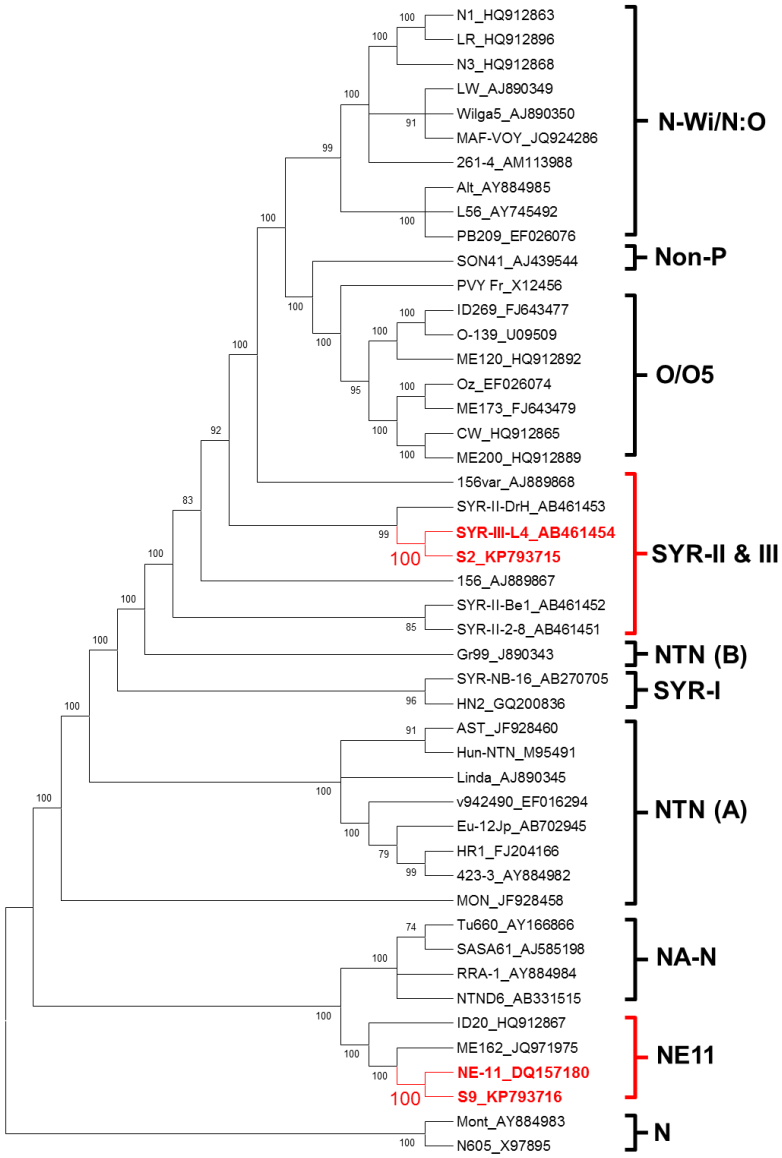

Table 2.1. Local reaction is symptoms observed (visually, and confirmed by ELISA) on inoculated leaves. The ratio is the number of plants with observed symptoms out of the total number of plants. Systemic reaction is the observation (visually, and confirmed by ELISA) of symptoms on upper (uninoculated) leaves. Numbers are given as number observed (+) / number with no symptoms observed (-). Reaction of the parental potato cultivars Yukon Gem and Russet Norkotah to PVY strains is given.

Isolate (Strain)	No. of plants	Local reaction		Systemic infection	
		Yukon Gem (Ratio)	Russet Norkotah (Ratio)	Yukon Gem (+/-)	Russet Norkotah (+/-)
N1 (PVY^{N-Wi})	5	HR (5/5)	No local symptoms (5/5)	(1/4)	(5/0)
HR1 (PVY^{NTN})	5	HR (5/5)	No local symptoms (5/5)	(5/0)	(5/0)
RRA1 (PVY^{NA-N})	3	Asymptomatic (3/3)	No local symptoms (3/3)	(0/3)	(3/0)
Mont (PVY^{N})	5	HR (4/5)	No local symptoms (5/5)	(0/5)	(5/0)
ID20 (PVYNE11)	3	HR (2/3)	Local HR (3/3)	(0/3)	(3/0)

Table 2.2. Segregation of the progeny of crosses between cvs Yukon Gem and Russet Norkotah according to their local HR (hypersensitive reaction) to PVY strains.

Isolate (Strain)	# of plants	# of displaying local HR	# of not displaying local HR	Ratio (+/-)
N1 (PVY^{N-Wi})	32	17	15	1:1
HR1 (PVY^{NTN})	31	4	27	1:7
RRA1 (PVY^{NA-N})	29	8	21	1:3
Mont (PVY^{N})	31	1	31	1:30

ID20 (PVY-NE11)	26	2	24	1:12

Table 2.3. Segregation of the progeny of crosses between cvs Yukon Gem and Russet Norkotah according to systemic infection with PVY strains. The plants tested for infection by ELISA and PCR.

Isolate (Strain)	# of plants	# of infected plants	# of negative plants	Ratio (+/-)
1. N1 (PVY^{N-Wi})	32	32	0	1:0
2. HR1 (PVY^{NTN})	31	31	0	1:0
3. RRA1 (PVY^{NA-N})	29	4	25	1:8
4. Mont (PVY^{N})	31	21	10	2:1
5. ID20 (PVY-NE11)	26	22	4	5.5:1

Figure 2.1. Hypersensitive reaction (HR) caused by PVY strains in Yukon Gem X Russet Norkotah on inoculated leaves. a, necrotic rings; b, water-soaked rings; c, lower leaf drop; d, necrotic lesions.