

Potato Virus Detection and Epidemiology of Recombinant PVY Strains in Seed Plots through Multiplex RT PCR

Medhanie Mehari Arefe

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Supervisors: Dr. Denis Griffin (Teagasc)
Dr. Dan Milbourne (Teagasc)
Mrs. Jeanne Moore (Teagasc)
Assoc. Prof. Carl Ng (UCD)



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ABSTRACT

Potato Virus Y(PVY) is the most important virus disease of potato (*Solanum tuberosum* L.) and ranked fifth in the world's top ten most important plant viruses. PVY has five common strains and more than ten recombinant strains, derived mostly from a parental lineage of PVY^N and PVY^O. In Ireland, the recombinant strains PVY^{NTN}, PVY^{N:O}, and PVY^{NWi} are more prevalent, which accounts for more than 90% of the PVY infections.

The Teagasc potato breeding program produces seed lots in yearly trials and advanced clones are exported for further trials abroad. These clones must meet seed potato certification standards, and as many of the new recombinant strains of PVY induces mild symptoms, visual inspection is not always adequate and laboratory diagnosis is used to determine PVY infection. This study was conducted to identify, and symptom characterize the old and recombinant strains of PVY in the Teagasc breeding programs of potato in Oak Park. It was conducted through multiplex RT PCR and compared with the captured and categorized pictures of the symptoms. Moreover, the methodologies used in the Center for extraction of crude samples and the results of ELISA and RT PCR were compared.

The results that were obtained from RT PCR on detection of PVY, PVS, and PVA, were found to be more sensitive than the ELISA technique. The recombinant strains PVY^{NTNa}, PVY^{NTNb}, and SYR III were found to be the more prevalent in the field. Moderate mosaic symptoms were observed to be common in most of the plants. PVY^{NTNa} was found associated with mild to moderate mosaic; whereas SYR III was linked with moderate to severe mosaic and crinkling of the leaves.

The identified recombinant strains further confirm the previous studies on their prevalence in Ireland. This study is the first to report the prevalence of SYR III

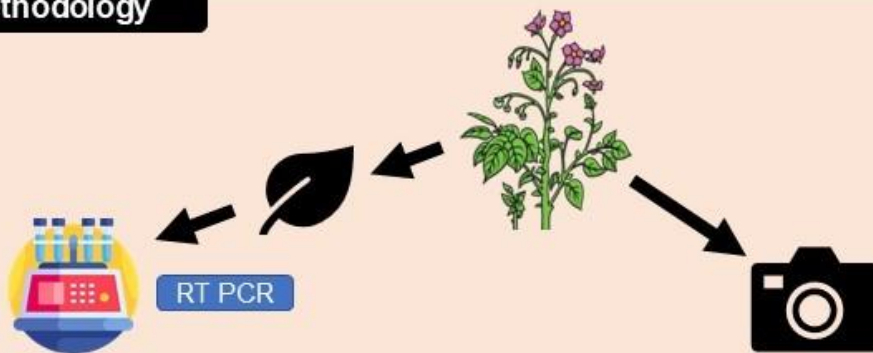
recombinant strain in Ireland. Though the study was conducted on a small scale, the symptoms observed, and their associated links with the strains give a preliminary result for better management in the field. Moreover, the results give a strong starting point for understanding the symptomology associated with different PVY strains to support further in-depth study of the relationship between the strains, varieties and the symptoms. Finally, a genotyping of the SYR III recombinant strain of PVY reported in this study and wider study on its distribution in Ireland is recommended.

GRAPHICAL ABSTRACT

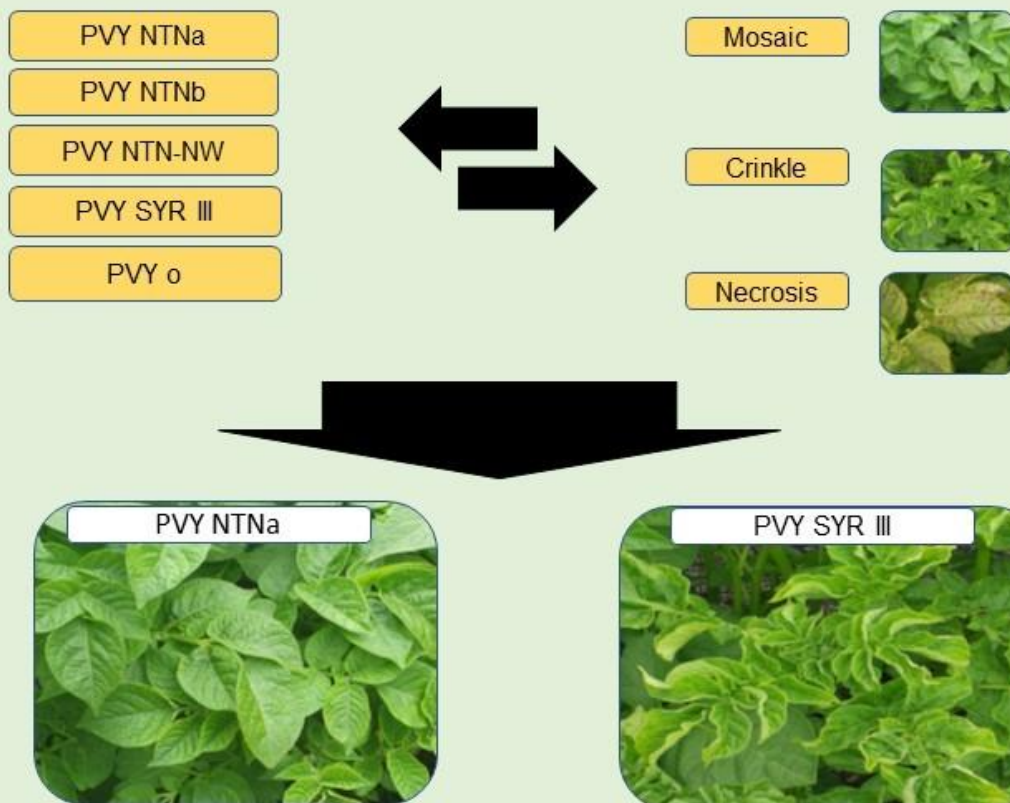
Research Question

- What are the *Potato Virus* YPVY recombinant strains present in Teagasc potato seed plots?
- Are the recombinant strains of PVY related with a specific symptoms?

Methodology



Result



1. INTRODUCTION

Potato (*Solanum tuberosum* L.), which belongs to the Solanaceae family ranks fourth as the most important crop in the world (Davie *et al.*, 2017). In 2019, global cultivated area and productivity were 17.3 million hectares and 21 tons/ha, respectively (FAOSTAT, 2020). Potato production is being seriously challenged due to different pathogens since it is clonally propagated the viruses persist in the crop. Generally, it is susceptible to more than fifty viruses and viroids (Jones, 2014), where potato leafroll virus (PLRV), potato virus Y (PVY), potato virus X (PVX), potato virus S (PVS), potato virus A (PVA), and potato virus M (PVM) are the more economically important ones negatively impacting crop yields (Singh, 1999).

Globally, PVY is the most important virus disease adversely affecting the production of solanaceous crops, mainly potato, pepper, tomato, tobacco, and other agriculturally important plants (Glais *et al.*, 2002; Galvino-Costa *et al.*, 2012; Karasev & Gray, 2013; Davie *et al.*, 2017; Green *et al.*, 2018). It is ranked fifth in the world's top ten most important plant viruses (Scholthof *et al.*, 2011). In potato, it causes substantial yield reduction and loss of tuber quality. In some cases, this is due to induction of potato tuber necrotic ringspot disease (PTNRD), typically associated with recombinant strains (Galvino-Costa *et al.*, 2012; Chikh-Ali *et al.*, 2016; Davie *et al.*, 2017; Funke *et al.*, 2017; MacKenzie *et al.*, 2019). The yield reduction varies between strains, and PVY^O have been shown to diminish yield by up to 80% (Kopp *et al.*, 2015). Yield losses of between 12 to 78% and 29 to 59% were observed when potato was infected by PVY^{N-Wi} and PVY^N, respectively (Onditi *et al.*, 2021). The variations in the yield reduction can be due to the fact that the poor growth of PVY infected plants may be compensated for by neighbouring healthy plants that gain from the additional space at low incidence (Valkonen, 2007).

PVY is a member of the genus *Potyvirus*, the largest group of plant pathogens (Glais *et al.*, 2002; Galvino-Costa *et al.*, 2012), comprising 128 approved and 89 tentative species (Fauquet *et al.*, (2005) referenced in Valkonen, 2007). PVY virions have a filamentous, flexuous form, having a length of 730-740nm and a diameter of 11-12 nm (Karasev & Gray, 2013 and references there in). They contain a single-stranded, messenger-sense RNA genome of approximately 9.7 kb in length excluding the poly(A) tail, covalently linked to a viral-encoded two proteins (VPg and coat protein) at the 5' end and to a 3' polyadenylated tail (Boonham *et al.*, 2002; Kehoe & Jones, 2016; Green *et al.*, 2018).

The RNA-dependent RNA polymerase of most RNA viruses lacks proofreading activity which results in a high error rate during virus replication (Choi, 2012). Due to these errors in replication, an RNA virus is a dynamic population termed quasi-species, corresponding to a swarm of sequences (Glais *et al.*, 2002) and it enables the rapid generation of genetic variability and novel variants (Gibbs & Ohshima, 2010). In nature, PVY exists as a complex of strains and are well known for their propensity to evolve through recombination, with different genotypes of the virus exchanging segments of their genome and are distinguished according to their pathogenicity, serology, and genomic analysis (Galvino-Costa *et al.*, 2012; Chikh-Ali *et al.*, 2016; Davie *et al.*, 2017; Della Bartola *et al.*, 2020).

As with most of the potato viruses, PVY originated from the Andes mountains (Valkonen, 2007) and spread all over the world. Multiple strains are found simultaneously within regions, fields and within individual plants across the world (Blanchard *et al.*, 2008; Dupuis *et al.*, 2019).

PVY was first identified by Smith in 1931 (De Bokx and Huttinga, (1981) referenced in Hutton *et al.*, 2015) and potato virus C (PVC) were first recognized in 1936 (Kehoe & Jones, 2016). In 1944, PVC was included within PVY, becoming strain group PVY^C, the original PVY was designated PVY^O, and additional strain group PVY^N (a veinal necrosis strain) was recognized later (Boonham *et al.*, 2002; Visser, 2012; Dupuis *et al.*, 2019). Furthermore, two additional strain groups were reported in the 1990s: PVY^Z and PVY^{ZE}, later renamed PVY^E (Kamangar *et al.*, 2014; Kehoe & Jones, 2016).

The PVY virus has undergone different genetic changes and additional strains were discovered later, mainly recombinant strains such as PVY^{NTN} in 1978, PVY^{N-Wi} and PVY^{N:O} in 1984 (MacKenzie *et al.*, 2019). The fast-rising population of the recombinant strains particularly PVY^{NTN} and PVY^{N:O} has then overcome the old strain PVY^O globally (MacKenzie *et al.*, 2018). So far the PVY complex includes five non-recombinant strains (PVY^O, PVY^{Eu-N}, PVY^{NA-N}, PVY^C, and PVY^{O-O5}) (Green *et al.*, 2018) and over 10 new recombinant strain variants composed of genome segments that came from the two “parental” genomes, PVY^O and PVY^N (also called PVY^{Eu-N}) (Visser *et al.*, 2012; Green *et al.*, 2018; Onditi *et al.*, 2021). The recombinant strains PVY^{NTN} (PVY-N Tuber Necrosis) and PVY^{N-Wi} (PVY-N Wilga) are the most widespread and causes decreased productivity of potato production in the world (Onditi *et al.*, 2021). A novel recombinant strain which shared the properties of PVY^{NTN} and PVY^{N-Wi} was reported by Chikh-Ali *et al.*, (2007) in Syria and designated as PVY^{SYR}. Based on recombination analysis, PVY^{SYR} isolates were then grouped into three recombination patterns, SYR-I, SYR-II and SYR-III (Chikh-Ali *et al.*, 2010a). Due to the characteristic symptoms that is similar to the PVY^{NTN} and the shared properties with PVY^{NTN} and PVY^{N-Wi}, SYR-I and SYR-II were then considered a new recombinant strain of the PVY^N strain group and designated as PVY^{NTN-NW} (Chikh-Ali *et al.*, (2007). The other recombinant genotype,

which is similar to recombinant genotype to SYR-I and SYR-II was designated as SYR-III (Chikh *et al.*, 2010a). Furthermore, the recombinant SYR III was reported to be prevalent in Saudi Arabia, Jordan, Israel and Egypt (Anfoka *et al.*, 2014; Chikh-Ali *et al.*, 2016; Elwan *et al.*, 2017 Avrahami *et al.*, 2019). In European potato production, PVY is considered co-introduced with potato importation in the 16th century (Blanchard *et al.*, 2008). As in the other parts of the world, the recombinant strains, PVY^{NTN} and PVY^{N-Wi}, are widespread and have replaced the historical strains and are showing a progressive increase in many countries in Europe (Glais *et al.*, 2002, Davie *et al.*, 2017, Dupuis *et al.*, 2019).

PVY, is mainly an aphid-borne virus transmitted in a non-persistent manner by more than 40 aphid species (Quenouille *et al.*, 2013; Kamangar *et al.*, 2014; Hutton *et al.*, 2015; Kehoe & Jones, 2016; MacKenzie *et al.*, 2018). The main sources of PVY inoculum in healthy plants are infected seed tubers. Aphids feeding on potato plants grown from infected tubers acquire PVY within a short period of time and transmit the virus to healthy plants within seconds (Wale *et al.*, 2008; Gadhave *et al.*, 2020). In a field experiment on the rate of transmission of three molecular groups of PVY isolates (PVY^O, PVY^{EU-NTN}, PVY^{NA-NTN}), Davie *et al.* (2017) showed that PVY^{EU-NTN} has the highest transmission rate to progeny tubers. The eight aphid species that are commonly considered as vectors of PVY are *Acyrtosiphon pisum*, *Aphis fabae*, *Aphis* spp., *Brachycaudus helichrysi*, *Macrosiphum euphorbiae*, *Myzus persicae*, *Phorodon humuli* and *Rhopalosiphum padi* (Dupuis *et al.*, 2019) where the peach potato aphid (*Myzus persicae*) is the most important in the transmission and spread of PVY to healthy plants (Wale *et al.*, 2008; Karasev & Gray, 2013; Gadhave *et al.*, 2020).

The symptoms and severity of potato virus diseases depends on different factors such as the time of initial infection, variety of potato, strain of virus and growing conditions (Karasev & Gray, 2013). PVY disease in potato can induce different symptoms, where the most common is a leaf mosaic associated with a characteristic patchy discoloration of the leaves (MacKenzie *et al.*, 2019). The main symptoms of the ordinary strain (PVY^O) in many potato cultivars are a severe mosaic, yellowing of leaflets and leaf drops (Boonham *et al.*, 2002; Della Bartola *et al.*, 2020). Secondary symptoms result in a dwarf plant with mottled or crinkled foliage (Wale *et al.*, 2008; Hutton *et al.*, 2015). PVY^C on the other hand can cause hypersensitive responses such as stipple streak (Hutton *et al.*, 2015). The N strain of PVY can be differentiated from PVY^C and PVY^O by its ability to induce severe veinal necrosis when infecting tobacco (*Nicotiana tabacum*) but causes milder mosaic symptoms, occasional leaf necrosis, and plant mortality in most potato cultivars (Boonham *et al.*, 2002; Glais *et al.*, 2002; Visser, 2012; Kamangar *et al.*, 2014; Hutton *et al.*, 2015; Dupuis *et al.*, 2019). These different symptoms of PVY strains disturbs the physiology of the crop and thereby affects the productivity.

Many of the emerging recombinant PVY strains, such as PVY^{NTN}, PVY^{N-Wi}, and PVY^{N:O} often induce mild or transient foliar symptoms, making them more difficult to detect during visual inspection in the field (Kamangar *et al.*, 2014; Lindner *et al.*, 2015; Della Bartola *et al.*, 2020; Onditi *et al.*, 2021). The NTN strain, PVY^{NTN} and PVY^{N-Wi} are particularly associated with two different tuber syndromes in susceptible potato cultivars, a potato tuber necrotic ringspot disease (PTNRD) and canoe-shaped cracks (Boonham *et al.*, 2002; Gray *et al.*, 2010; Visser, 2012; Kehoe & Jones, 2016; Green *et al.*, 2018). Moreover, the Syrian recombinant strain is also reported to be associated with PTNRD on susceptible potato cultivars (Chikh-Ali *et al.*, 2010a; Anfoka *et al.*,

2014; Elwan *et al.*, 2017). These tuber symptoms are often a major concern for potato production as they decrease tuber quality.

Control of plant viruses is more difficult than those caused by other pathogens because of the complex disease cycle, efficient transmission, and lack of viricides. The effective and sustainable management of a viral disease requires the integration of management practices such as avoidance of source of infection, control or avoidance of vectors, modification of cultural practices, and development of resistance in host plants (Varma, 1993; Basu & Srikanta, 2003). In order to limit the impact of existing strains on their hosts and avoid creating the conditions that drive further escalation of pathogenicity, it is essential to understand the circumstances under which pathogenic recombinant virus strains evolve (Visser *et al.*, 2012). Conducting regular virus surveys can help identify the regions and cultivars infected with the virus and permit replacing susceptible cultivars with resistant ones (Onditi *et al.*, 2021).

In the Irish potato sector, PVX, PVS, PVA, PVY, and PLRV are known to occur, where PVY predominantly accounts for 9.9% of the population (Hutton *et al.*, 2015). In Ireland, the recombinant strains PVY^{NTN}, PVY^{N:O} and the associated tuber necrotic disease (PTNRD) were reported in 2012 and 2013 (Hutton *et al.*, 2013; Hutton *et al.*, 2015). An RT PCR analysis of PVY strains conducted by Della Bartola *et al.* (2020) found that within the PVY^N and PVY^O serotypes, the recombinant genotypes accounted for more than 90% of the PVY infections and from the non-recombinant PVY genotypes, which represents a minority of the PVY isolates, are PVY^O, PVY^{Na-N}, and PVY^{Eu-N}.

The Teagasc potato breeding program produces over 400 seed lots of new advanced clones each year, with approximately 100 of these for export for further trials abroad

(personal communication with Dr. Denis Griffin). These clones must meet seed potato certification standards (Delaney, 2020). Regular field inspection and tests are required to identify healthy plants to comply with the standards for export. The rapid evolution of PVY mainly by viral recombination, has complicated strain characterization based upon host symptom expression and the traditional serological tests (MacKenzie *et al.*, 2019) which makes them more difficult to detect during visual inspection in the field. Furthermore, the traditional ELISA testing method cannot differentiate the recombinant strains such as between N and NTN, and the PVY^{N-Wi} is identified as an O strain (Kamangar *et al.*, 2014). This makes it important to identify the recombinant strains circulating in the breeding lines of the Teagasc potato breeding program using molecular methods. Due to its specificity and sensitivity, the reverse transcription polymerase chain reaction (RT-PCR) has been adopted for detection of many viruses and viroids (Singh, 1999). Moreover, as viruses cannot be directly controlled by crop protection products in the field, early, accurate and reliable detection and characterization of the PVY isolates prevailing in potato crops is therefore essential for better management (Hutton *et al.*, 2015; Della Bartola *et al.*, 2020). Understanding the prevalence and spread of the recombinant genotypes and their symptom expression will help in the early identification and management of the disease.

In this project, it was hypothesized that the type of potato virus strains previously reported in the Irish potato sector by Hutton *et al.*, (2015) and Della Bartola *et al.*, (2020) are present in the varieties under development and they would induce different symptoms and varying degrees of severity. Therefore, this project was aimed at the identification of recombinant strains of PVY in Teagasc breeding lines and to describe their symptoms in the potato crops, based on the observed symptoms in the field.

In order to address the objective, one hundred and fifty-five samples were collected randomly from symptomatic and asymptomatic crops planted in the Centre. An ELISA assay was conducted to assess the prevalence of the common viruses in the Centre PVY, PVS and PVA. This test was then compared with the results of a multiplex reverse transcriptase polymerase chain reaction (RT PCR) conducted for five viruses (PVY, PVX, PVA, PVS and PLRV). The samples were then subjected to multiplex RT PCR for recombinant strains using primers described by Chikh-Ali *et al.*, (2010) for simultaneous detection PVY isolates and modified by Rodriguez-Rodriguez *et al.*, (2020). Finally, the results were compared with the recorded and categorized symptoms according to type and degree severity. The symptoms were categorized into three groups as “mosaic”, “crinkling” and “necrosis”, with a rating of one to three, based on the level of expression. So, in this document for example the phrase “crinkling symptoms of the leaf” is used to express all the symptomatic characteristics of leaf deformations, mottling and curling. Furthermore, a comparison was conducted between the crude leaf sample extraction methods employed in the centre.

2. MATERIALS AND METHODS

This research project was conducted at the Teagasc Crop Research Centre, Oak Park, Carlow, Ireland. Potato breeding stocks produced at the Centre, were planted in the field and used for the experiment. The methodology used to test these potato plants for recombinant PVY genotypes was through reverse transcription polymerase chain reaction (RT-PCR). Initially all the samples were subjected to ELISA test and then to RT-PCR. The results from RT-PCR were compared with the symptoms observed in the field. In this experiment, the laboratory works followed the published procedures and protocols by Du *et al.*, (2006), Chick Ali., (2010), Rodriguez-Rodriguez *et al.*, (2020) and inhouse modified protocols.

2.1. Plant material

Potato plants were sampled at their two months of age (66 days from planting). One hundred and fifty-five potato leaf samples were collected from symptomatic and asymptomatic potato crops planted in Coolkenno and the Teagasc Crop Research Centre, Oak Park, Carlow. The potato plants at Coolkenno were breeding lines for variety development whereas the plants at Oak Park was part of an ongoing virus trial. List of all the sampled breeding lines and their result is presented in Appendix 1. In each sample, 5-8 leaves were collected and stored in a freezer at -4°C prior to processing.

2.2. Crude sample extraction

Crude samples were extracted using a leaf juice press (MEKU, Wennigsen, Germany). About 600 microliter (µl) was extracted in a 1.5 millilitre Eppendorf tube. These crude

samples were divided into two, one for ELISA and one for nucleic acid. These samples were kept at -20°C until used for ELISA and RNA extraction.

2.3. DAS-ELISA

The double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) test was conducted following the manufacturers protocol supplied with the kit (ADGEN Phytodiagnostics) for the most common potato viruses in Ireland, PVY, PVS and PVA. Polyclonal antibody for PVY and monoclonal antibody was used for PVS and PVA. The positive and negative results were then obtained from a spectrophotometer plate reader (Biotek, Winooski, VT, USA) at 405nm. The results were considered positive when the mean optical density (OD) values were greater than twice the value of the negative controls, as described by Della Bartola *et al.* (2020).

2.4. Nucleic acid extraction

Nucleic acid was extracted using a KingFisher robot with the MagMAX™ Plant RNA Isolation Kit- ©2016 (Thermo Fisher Scientific Inc) according to the manual. The extraction step of the protocol was modified and an inhouse protocol was used as follows: First, 100µl of leaf juice from a crude sample was added into 900µl of a preprepared buffer (described below). Then, 500µl of the mix was transferred into a well of a new 96 well plate. Water (double distilled) was used as a negative control. Thereafter, 100µl of Sarcosyl (10%) (to lyse the cells) was added to each well. The plate was then incubated at 56°C for 10 min, before centrifugation at 3500 rpm for 10 min. About 400µl of supernatant was then carefully removed without disrupting the pellet and transferred into a new deep well plate. Thereafter, the process followed the manufacturers' protocol as mentioned earlier and the kit MagMAX™ Plant RNA Isolation Kit supplied from applied biosystems by Thermo Fisher Scientific Inc. The

resulting RNA was eluted with 50 to 100µl RNA of double distilled water supplied in the kit.

RNA Extraction buffer: An RNA extraction buffer was prepared according to the protocol of Rott and Jelkmann (2001) by mixing 4.0 M guanidine thiocyanate, 0.2 M NaOAc pH 5.2, 25 mM EDTA, 1.0 M KOAc and 2.5% w/v PVP-40. Then the mix was topped up with water for the required final volume and kept on a shaker until properly mixed.

2.5. RNA Quantification

The extracted RNA samples were quantified using a Nanodrop 2000 spectrophotometer (ThermoFisher Scientific) to determine the concentration and the quality of the extracted RNA samples. The concentration was then standardised for the cDNA synthesis.

2.6. Reverse transcription (RNA to cDNA)

Complementary DNA (cDNA) was synthesized using the “MMLV reverse transcriptase-Sigma kit” following the manufacturer’s technical guidelines modified with inhouse protocol. Mixtures with enough material for two samples were prepared. The first mix was prepared using 7µl H₂O (nuclease free sigma water), 1µl random hexamer (250 ng/µl) and 2µl of extracted RNA (final quantity between 100 and 500 ng) for each sample. The plate was then incubated in a PCR machine ((Applied Biosystem by Life Technologies) at 90°C for 3 min to relax the strand, and then directly kept on ice to block the process. The second mix, with 6µl H₂O (nuclease free sigma water), 2µl 10x RT buffer, 1µl dNTPs (10mM) and 1µl R-transcriptase was prepared and 10µl of this new mix was added to the plate containing the 10µl of the first mix. The combined mix was then incubated in a PCR machine with program of 23°C for 10

min, 37°C for 50 min, 80°C for 10 min (to stop reaction) for 1 cycle and 10°C until removed from the machine.

2.7. Polymerase chain reaction (PCR)

Polymerase chain reaction was conducted following the methods of Du *et al.* (2006). The cDNA was amplified in two different multiplex PCR. First for the five potato viruses (PVY, PVA, PVS, PVX, PLRV) and 18S rRNA as an internal plant control. And secondly for the PVY recombinant strains. The primers used for the targeted potato viruses was based on Du *et al.* (2006), and the primers for PVY recombinant genotypes were prepared based on the protocol published by Chikh-Ali *et al.* (2010) for multiplex RT-PCR for simultaneous detection PVY isolates and modified by Rodriguez-Rodriguez *et al.*, (2020) as described in the Table 2.1 below. A master mix of a final volume of 25µl for each sample for the 155 samples was prepared by adding 18.8µl double distilled water, 2.5 10X Taq standard buffer, 0.5 dNTPs 10mM, 2µl primer mix (see table 2-1), 0.2µl Taq DNA Polymerase (ThermoPol®). Then 1µl cDNA from each sample was added to the wells of a PCR 96 well plate. One negative control (distilled water) was also added. After spinning down the plate for 30s, the PCR reaction was conducted on a thermocycler (Applied Biosystem by Life Technologies) and the program used was 95°C for 3 min for one cycle, 35 cycles at 94°C for 0.30 min, 58°C for 0.30 min and 72°C for 0.25 min, a final one cycle at 72°C for 5 min and left at 10°C forever.

Table 2. 1. List of reverse transcription–polymerase chain reaction (RT-PCR) primers used for five potato viruses and 18S rRNA (Internal control), and primers for PVY strains prepared from a volume of 100uM stock solution.

A. Primers used for five potato viruses and 18S rRNA			
Virus	Primer	Primer sequence (5' - 3')	Concentration in the PCR
PVA	PVY fw	TTTCTATGAGATCACTGCAACCACT	0.5µM
	PVY rev	TGACATTTCCGTCCAGTCCAA	0.5µM
PVX	PVX fw	AACTGGCAAGCACAAGGTTTCA	0.5µM
	PVX rev	CAGTTTGGGCAGCATTTCATTTC	0.5µM
PVY	PVA fw	ATACTCGRGCAACTCAATCACA	0.5µM
	PVA rev	CCATCCATCATAACCCAAACTC	0.5µM
PLRV	PVS fw	CCACTCCAACCTCCCCAGAAG	0.5µM
	PVS rev	TACATAGGGACGGCTTGCAT	0.5µM
PVS	PLRV fw	ACCRGATCCGACAAGCTCAGG	0.5µM
	PLRV rev	GCCATTTGCTCRGTGTTTCG	0.5µM
18S rRNA	18s fw	GAGAAACGGCTACCACATCCA	0.1µM
	18s rev	CGTGCCATCCCAAAGTCCAAC	0.1µM
B. Primers for PVY strains			
Primer		Primer sequence (5' - 3')	Concentration in the PCR
n156		GGGCAAACCTCTCGTAAATTGCAG'	0.2µM
o514		GATCCTCCATCAAAGTCTGAGC	0.2µM
n787		GTCCACTCTCTTTCGTAAACCTC	0.2µM
n2258		GTCGATCACGAAACGCAGACAT	0.2µM
o2172		CAACTATGATGGATTTGGCGACC	0.2µM
n2650c		TGATCCACAACCTTCACCGCTAACT	0.2µM
o2700		CGTAGGGCTAAAGCTGATAGTAG	0.2µM
S5585		GGATCTCAAGTTGAAGGGGAC	0.4µM
o6400		GTAACCTCCTAAACAAATGGTGGTTCG	0.4µM
SeroN-F		CTGGAACTCATACTGTGCCAC	0.2µM
SeroN-R		GTTTCTCCTATGTTCGTATGCAAGTT	0.2µM
SeroO-F		ATGCTGGTACATCTGGGACA	0.2µM
SeroO-R		TTGTTGTGGAGCATACTCAAGC	0.2µM

2.8. Gel electrophoresis

The PCR products were mixed with 5µl of loading dye (Gel Loading Dye, BioLabs, New England) and vortexed. Then, 3µl of the mix (PCR product and loading dye) was loaded to the wells of a 3% agarose gel in TBE buffer stained with GelRed (Biotium, Fremont, CA, USA). A 100pb ladder (NEB) was used. The bands were separated by applying 90 volts for 30 minute, 100 volts for an hour and 30 minutes and then 110 volts for an hour and visualized in a UV transilluminator (ENDURE™ DGS, Labnet International, Inc.). Based on the primers used, according to Du *et al.* (2006) and Chikh Ali *et al.* (2010) modified by Rodriguez-Rodriguez *et al.* (2020), the multiplex PCR assays for five potato viruses with the 18Sr (internal control) and the PVY strains were expected to produce bands as described in the following Table 2.2.

Table 2.2. Expected band sizes for PVY, PVX, PVA, PVS, PLRV viruses and PVY strains

Five viruses + internal control	Expected bands	References
PVY	166	Du <i>et al.</i> (2006)
PVX	145	Du <i>et al.</i> (2006)
PVA	116	Du <i>et al.</i> (2006)
PVS	342	Du <i>et al.</i> (2006)
PLRV	208	Du <i>et al.</i> (2006)
18S rRNA	255	Du <i>et al.</i> (2006)
PVY Strain	Expected bands	References
PVY ^{NTNa}	663, 441, 202	Rodriguez-Rodriguez <i>et al.</i> (2020)
PVY ^{NTNb}	441, 202	Rodriguez-Rodriguez <i>et al.</i> (2020)
PVY ^O	127 bp (1076bp)	Rodriguez-Rodriguez <i>et al.</i> (2020)
PVY ^N	202 bp (1307bp)	Rodriguez-Rodriguez <i>et al.</i> (2020)
NA-PVYN/NTN	202 bp	Chikh-Ali <i>et al.</i> (2010)
PVY ^{NTNa}	202 bp, 633, 441	Chikh-Ali <i>et al.</i> (2010)
PVY ^{NTNb}	202 bp, 441	Chikh-Ali <i>et al.</i> (2010)
PVY ^{NTN-NW} (SYR I)	127, 633, 441	Chikh-Ali <i>et al.</i> (2010)
PVY ^{NTN-NW} (SYR II)	127, 441	Chikh-Ali <i>et al.</i> (2010)
SYR III	127, 441, 278	Chikh-Ali <i>et al.</i> (2010)
PVY ^{N-W} (B)	853, 441	Chikh-Ali <i>et al.</i> (2010)

2.9. Assessment of foliar symptoms

Leaf samples were taken from symptomatic and randomly from healthy-looking leaves. During the sampling time, a picture of the sampled plant was taken for symptom description and comparison with the RT PCR results. The pictures were categorized according to the symptom observed and scored blindly to avoid any bias

from the genotype results. The results of the RT PCR were then compared to the described symptoms of the samples and the varieties.

Description of the symptom categories

Mosaic: Plant samples showing patchy or yellow spots and irregular greening of the leaf were categorized as mosaic symptoms.

Leaf crinkle: Plant samples showing a characteristic symptom of mottling, curling and distortion of the leaves were categorized as leaf crinkle.

Necrosis: Plant samples showing brown-black dotted spots and patches in the leaves were categorized as necrotic symptoms.

Table 2.3. Ratings given for severity of observed symptoms

Symptoms observed	Rating of severity		
Mosaic	1 = Mild	2 = Moderate	3 = Severe
Leaf crinkle	1 = Mild	2 = Moderate	3 = Severe
Necrosis	1 = Mild	2 = Moderate	3 = Severe

2.10. Comparison of crude sample extraction methods

A test on two different methods to extract the crude samples for the purpose of RT-PCR was conducted. This technique is, at the moment in use in Teagasc laboratory for samples destined for ELISA purpose only (personal communication with Jeanne Moore, Teagasc pathology laboratory technician). The objective of this comparison study was to determine the risk of contamination when using the MEKU Juicer so that using the same technique for RT_PCR would shorten the time of the diagnostic method. For this purpose, 34 samples were selected. Crude samples from these samples were further extracted using a single leaf of about 0.3g, ground with the aid of a tissue homogenizer (Bioreba extraction bags, Reinach, Switzerland) using 3ml of

RNA extraction buffer. The extraction buffer (as described in section 2.4) was prepared ahead on the extraction day.

MEKU leaf juice press



Bioreba Extraction bags



Figure. 2.1. Pictures of leaf juice extraction methods

2.11. Statistical analysis

The significance of the association between the virus strain groups of NTN and SYR and the observed symptom was conducted using a Fisher's exact test of independence on 2x2 contingency table. The statistical analysis computed using SPSS version 26.0 (IBM Corp, 2019). The level of significance was at $P \leq 0.05$.

3. RESULTS

3.1. Expression of PVY foliar symptoms

The most observed foliar symptom was mosaic with 57% of the leaf samples affected. Crinkling of the leaves was observed in 37% of the sampled plants. Whereas necrosis was observed in 8 plants (7%) showing brown to black dotted spots (Figure 3.1.A). Based on the rating given from one to three, most of the mosaic and necrosis symptoms were mild, whereas leaf crinkle showed almost equal distribution between mild and severe in all the infected plants (Figure 3.1.B). The captured observed symptoms are displayed in Figure 3.2.

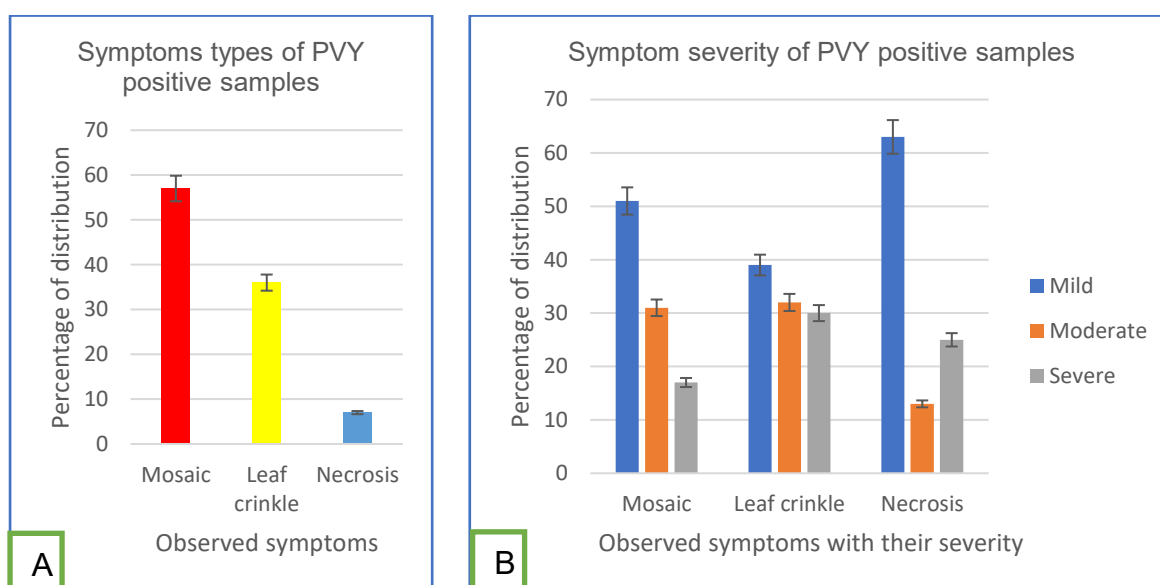


Figure 3.1. A. The symptoms observed in the field, B. Degree of symptom severity.

Table 3.1. The data in this table gives the number of plants displaying different symptom levels together with the values expressed on a percentage.

Symptoms	Mild		Moderate		Severe		Total	
	Number	%	Number	%	Number	%	Number	%
Mosaic	36	51	22	31	12	17	70	57
Leaf crinkle	17	39	14	32	13	30	44	36
Necrosis	5	63	1	13	2	25	8	7

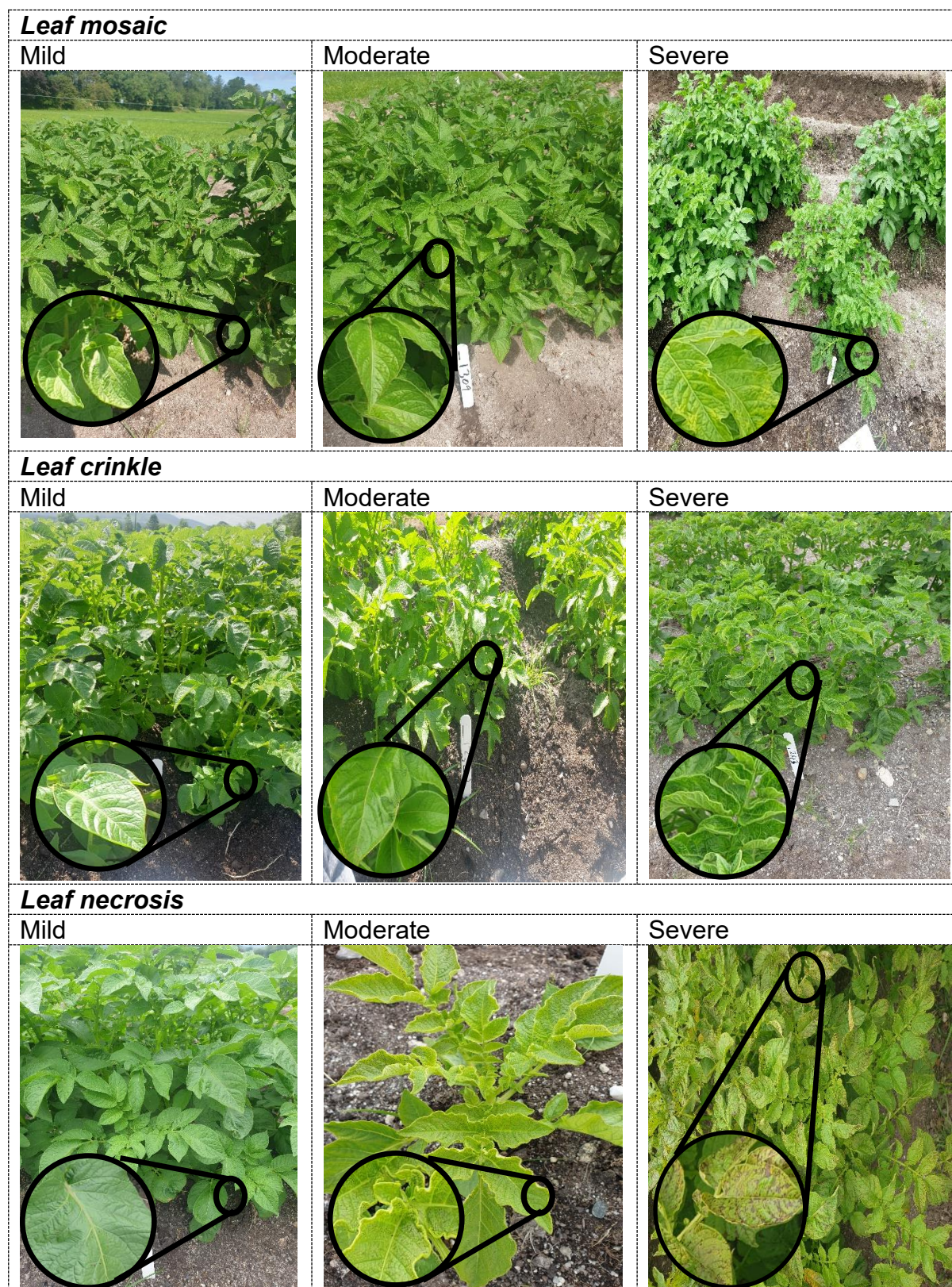


Figure 3.2. Representative images of symptoms recorded in the field and symptom severity (mild, moderate, and severe) was assigned to each the three groups, mosaic, crinkle and necrosis.

3.2. Serological (DAS-ELISA) of potato virus infections

The result of DAS-ELISA shows that PVY was the most predominant in the plants at 62%. A further 22% of the samples were positive for PVA and 10% were infected with PVS. Moreover, 58% of the plants were coinfecting with PVY, PVS and PVA (Table 3.2).

Table 3.2. DAS ELISA result of PVY, PVS and PVA viruses

Virus	PVY		PVS		PVA	
	No. of Samples	%	No. of Samples	%	No. of Samples	%
Positive	96	62	15	10	34	22
Negative	59	38	140	90	121	78

3.3. RT PCR

A. Multiplex RT PCR for PVY, PVS, PVA, PLRV and PVX

The multiplex PCR showed that 33 plants (21%) were positive for PVY, and 10 plants (6%) were infected with PVS. Even though not a single plant was found positive for PVA alone, 40% of the samples were found to be coinfecting with PVY and PVA, while the coinfection with PVY and PVS viruses accounted for 8% of the population. Overall, only 38 plants (25%) were found to be negative and the viruses PLRV and PVX was not found. The observed bands are described on Figure. 3.4.

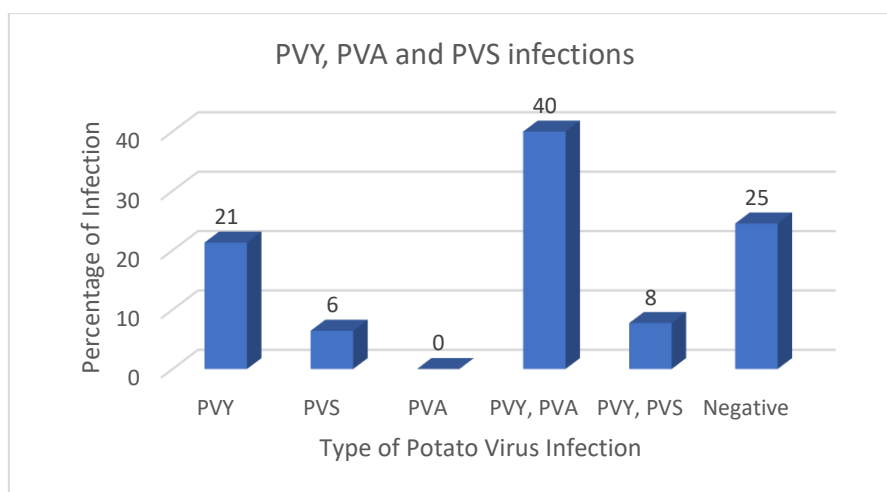


Figure 3.3. Identified potato viruses (PVA, PVS and PVY) through multiplex RT PCR expressed in percentage

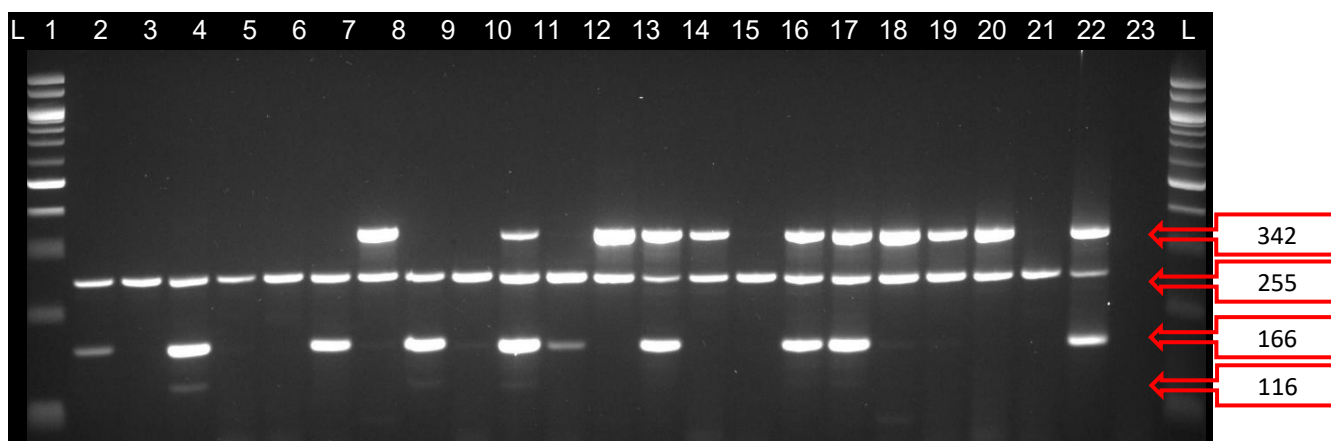


Figure 3.4. Representative PCR Products of potato viruses and internal control 18S_r (at 255bp) visualised in UV illuminator. Lane L; Ladder, Lane 1: PVY, Lane 2; Negative, Lane 3; PVY & PVA, Lane 4; Negative, Lane 5; Negative, Lane 6; PVY, Lane 7; PVS, Lane 8; PVY, Lane 9; Negative, Lane 10; PVY & PVS, Lane 11; PVY, Lane 12; PVS, Lane 13; PVY & PVS, Lane 14; PVS, Lane 15; Negative, Lane 16; PVY & PVS, Lane 17; PVY & PVS, Lane 18; PVY, Lane 19; PVY, Lane 20; PVY, Lane 21; Negative, Lane 22; PVY & PVS, Lane 23; Negative control

B. Multiplex RT PCR for the strains of PVY

The multiplex RT PCR for the strains of PVY showed that 44% of the samples were found to be infected with PVY^{NTNa} having three bands of 202, 441, 663bp. Some 10%

of the samples were PVY^{NTNb} and SYR III, each with a band size of 202, 441bp, and 127, 278, 441bp respectively. Then, 3% of the sample were PVY^{NTN-NW} (SYR II) and PVY^O each with bands size of 127, 441 and 127, 278 or 127, 532, 853bp respectively (Figure. 3.6). The remaining 30% of the samples was found to have a mixture of recombinant strains as described in Table 3.3. Of this mixed infection, 56% (18 samples) were found in 21% (34 samples) of the samples which are from the virus trial field (Figure. 3.5). Moreover, within the 40% of samples that were found coinfecting by PVY and PVA, 70% was with PVY^{NTNa}, 8% with PVY^{NTNb}, and the remaining 10% with the mixed infections. The recombinant strains of PVY identified in the experiment and the possible combinations of strains identified in the mixed infection are presented in table 3.3 and Appendix 2, respectively with their respective band sizes.

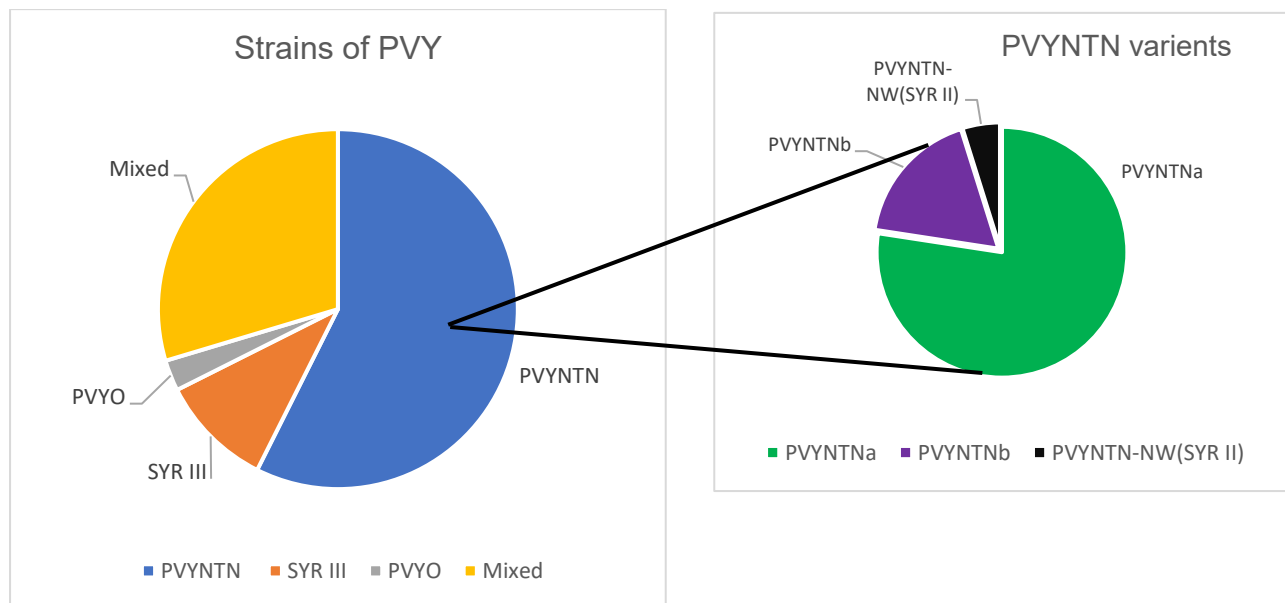


Figure 3.5. Frequency of dominant recombinant strains

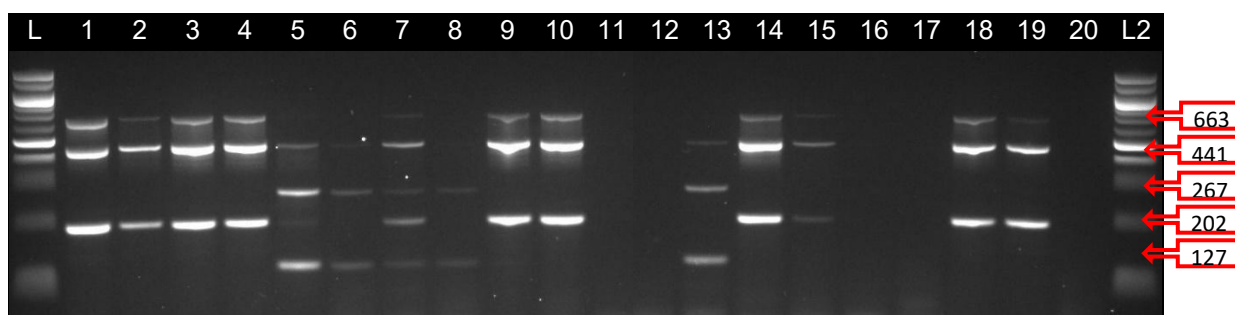


Figure 3.6. Representative PCR Products of PVY strains separated at 3% agarose gel visualised in UV illuminator. Lane L; Ladder, Lane 1: PVY^{NTNa}, Lane 2; PVY^{NTNa}, Lane 3; PVY^{NTNa}, Lane 4; PVY^{NTNa}, Lane 5; PVY^{SYR III}, Lane 6; PVY^{SYR III}, Lane 7; Mixed, Lane 8; PVY^O, Lane 9; PVY^{NTNa}, Lane 10; PVY^{NTNa}, Lane 11; Negative, Lane 12; Negative, Lane 13; PVY^{SYR III}, Lane 14; PVY^{NTNa}, Lane 15; PVY^{NTNa}, Lane 16; Negative, Lane 17; Negative, Lane 18; PVY^{NTNa}, Lane 19; PVY^{NTNa}, Lane 20; Negative control

Table 3.3. Strains of PVY identified in the study with their PCR product values.

Recombinant strains	Percentage (%)	Products in the multiplex PCR (bp)
PVY ^{NTNa}	44	202, 441, 663
PVY ^{NTNb}	10	202, 441
PVY ^{NTN-NW} (SYR II)	3	127, 441
SYR III	10	127, 278, 441
PVY ^O	3	127, 278/127, 532, 853
Mixed infection	30	Different band sizes*

* The different band sizes of the mixed infections are presented in Appendix 2.

3.4. Association between observed foliar symptoms and the strains

When comparing the symptoms with the virus and strains, the samples from the same variety which was observed to have symptoms were omitted from the association study because they were found to react negative in some samples and positive in the other samples. The mixed infections were not used in comparison of the symptoms with the virus strain. Out of the 53 recorded symptoms linked to the virus and/or strain

positive, only 37 were compared with the different recorded symptoms, after removing 16 recorded symptoms which were linked to mixed infections. PVY^{NTNa} were found to show mild to moderate mosaic in 10 samples, both mosaic and crinkle in 4 samples and a mixture of three symptoms (mosaic, crinkle, and necrosis) in one sample. PVY^{NTNb} was found to be associated with mild to moderate crinkle (3 samples) and moderate mosaic (1 sample). PVY^{NTN-NW}(SYR II) on the other hand was linked with one sample showing moderate mosaic. Samples positive for PVY^O were observed to show no symptom (1 sample), mild mosaic (1 sample) and a mix of severe mosaic and crinkle in one sample. The samples presenting as SYR III strain positive were found to show symptoms of moderate mosaic (2 samples), severe crinkle (2 samples), and 8 samples having a mix of moderate to severe mosaic and crinkle. Finally, 10 samples were observed to show a mix with PVA and one with PVS. The average rating on the severity of the symptoms in relation with the strains is presented on Figure 3.7 and a picture of a sample with a mixed infection and a healthy plant is presented on Figure 3.8. The statistical analysis shows that there is a significant association between the recombinant strains of NTN and SYR group and the observed symptoms on the recombinant strains with a P value of 0.016.

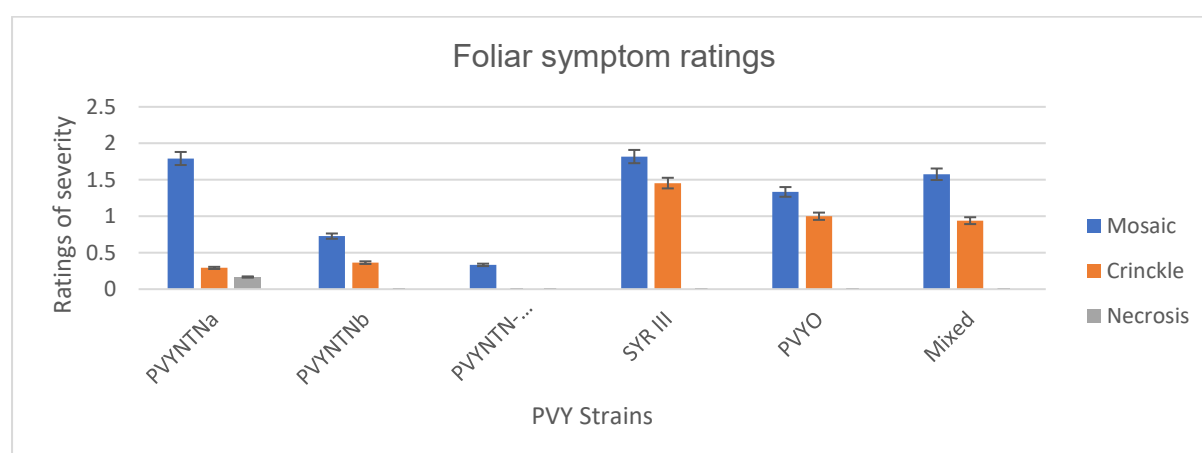


Figure 3.7. Average of the foliar symptom's severity ratings on relation to observed symptoms and the PVY strains

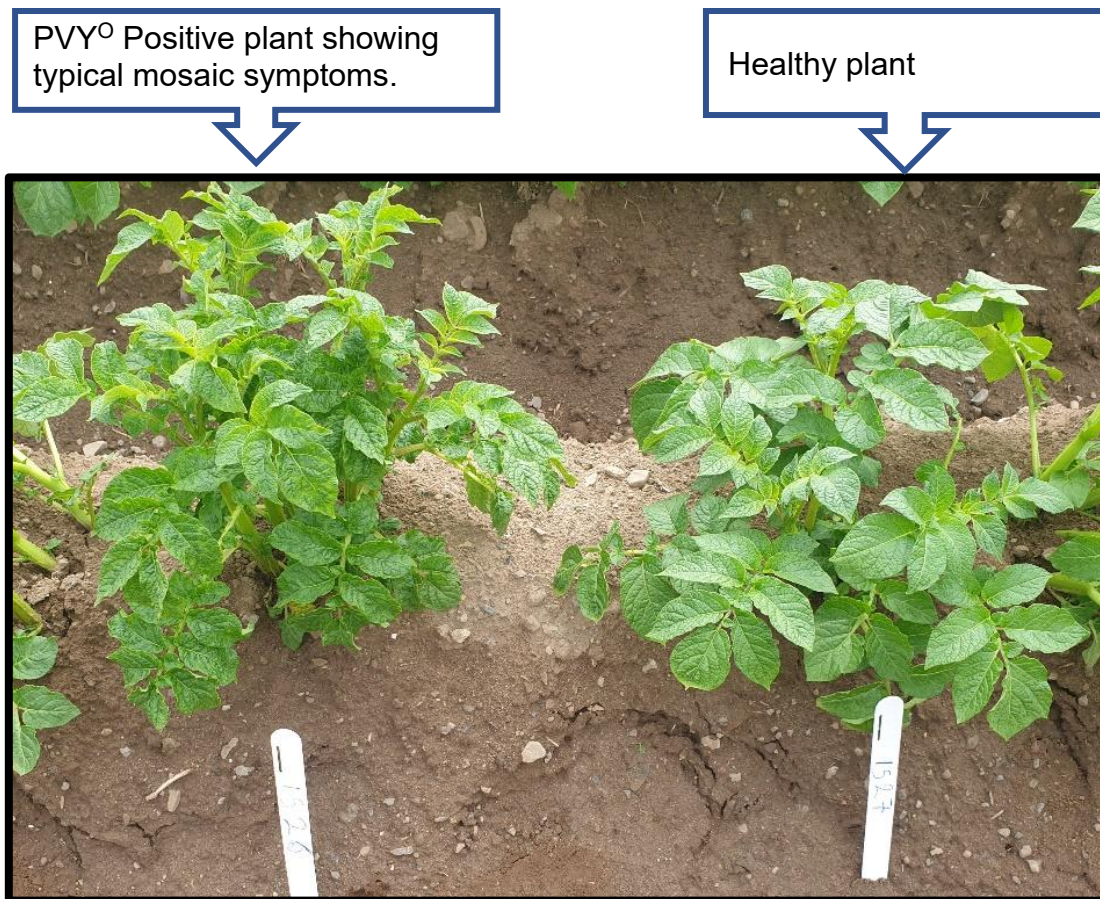


Figure 3.8. Picture of PVY positive and negative plants

3.5. Comparison of leaf juice extraction methods

The test conducted on the two different methods to extract the crude samples for the purpose of RT-PCR purpose shows that five samples (14%) had faint bands of contamination in the PCR product (Figure 3.9 A & B) from the leaf press extraction method. Two samples had different results in both extraction methods, as described in table below (Table 3.5).

Table 3.5. PCR result of the two extraction methods. The different possible combination of the mixed infections are presented in Appendix 2.

No.*	Leaf Press		No.*	Bioreba Extraction bags	
	Virus Type	PVY Strain		Virus Type	PVY Strain
28	PVY, PVA	Mixed 3	62	PVY, PVA	Mixed 3
29	PVS	PVY ^{NTN-NW} (SYR II)	63	PVS	PVY ^{NTN-NW} (SYR II)
30	PVY, PVA	Mixed 3 ^b	64	PVY	Mixed 6 ^b
31	Negative		65	Negative	
32	PVY, PVS	Mixed 2	66	PVY, PVS	Mixed 2
33	PVY	Mixed 2 ^a	67	PVS ²	
34	PVS		68	PVS	
35	PVY, PVS	Mixed 3	69	PVY, PVS	Mixed 3
36	PVS		70	PVS	
37	Negative		71	Negative	
38	PVY, PVS	Mixed 2	72	PVY, PVS	Mixed 2
39	PVY, PVS,	Mixed 3	73	PVY, PVS	Mixed 3
40	PVY, PVS	Mixed 2 ^a	74	PVS	
41	PVS		75	PVS	
42	PVS		76	PVS	
43	Negative		77	Negative	
44	PVY, PVS	Mixed 2	78	PVY, PVS	Mixed 2
45	PVY, PVS	Mixed 2	79	PVY, PVS	Mixed 2
46	PVS		80	PVS	
47	PVY, PVS	Mixed 6	81	PVY, PVS	Mixed 6
48	PVY	Mixed 5	82	PVY	Mixed 5
49	PVY, PVS	Mixed 5 ^a	83	PVS	
50	PVY, PVS	Mixed 5	84	PVY, PVS	Mixed 5
51	PVY	Mixed 6	85	PVY	Mixed 6
52	PVY	Mixed 5	86	PVY	Mixed 5
53	PVY	PVYO	87	PVY	PVYO
54	PVY, PVS	Mixed 5 ^b	88	PVY	Mixed 5 ^b
55	PVY, PVS	Mixed 2 ^a	89	PVY, PVS	PVY ^{NTNb}

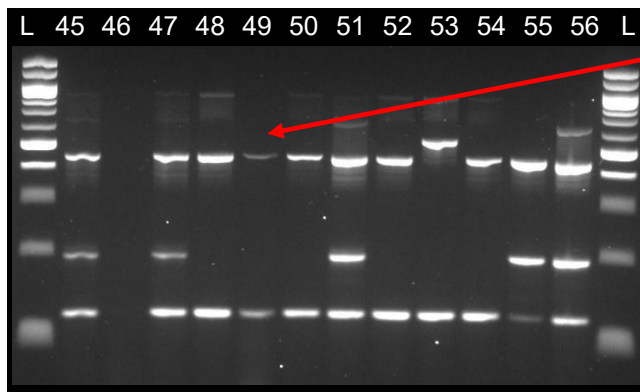
56	PVY, PVS	Mixed 3		90	PVY, PVS	Mixed 3
57	PVY	Mixed 2		91	PVY	Mixed 2
58	PVY, PVS	Mixed 6		92	PVY, PVS	Mixed 6
59	PVY, PVS	Mixed 2 ^a		93		
60	PVY, PVS	Mixed 5		94	PVY, PVS	Mixed 5
61	PVS			95	PVS	

*Sample ID in the PCR plate.

^a Contamination in leaf press

^b Different result observed in both methods

A. PCR product from leaf press juice extraction



Contaminated from the adjacent sample

Not contaminated from the adjacent sample

B. PCR product from Bioreba extraction bags

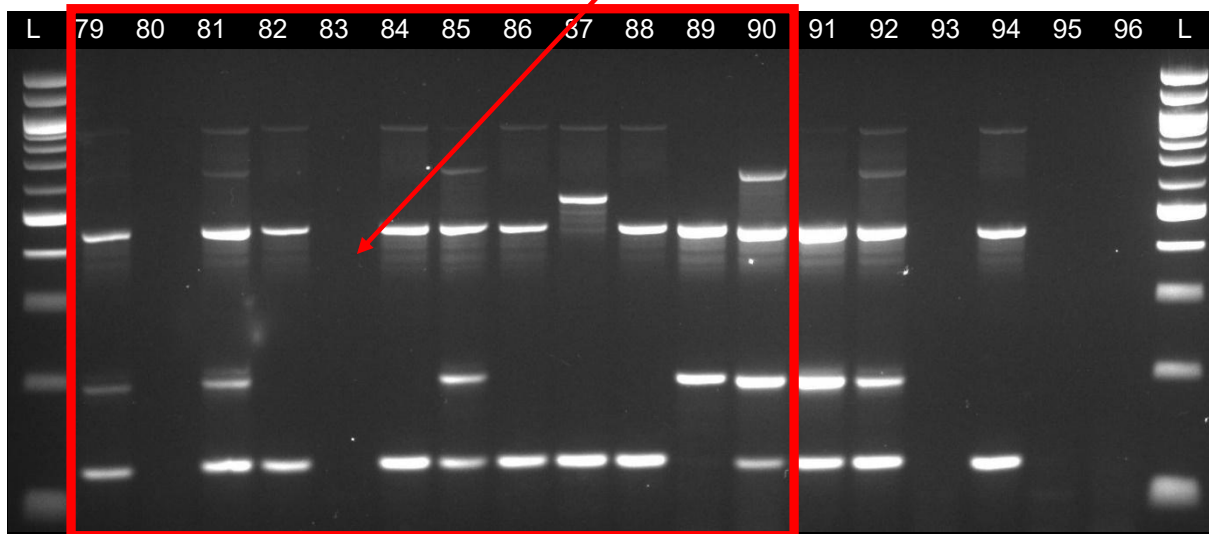


Figure. 3.9. PCR product of samples from leaf press juice and tissue homogenizer (Bioreba extraction bags) extraction methods

3.6. Comparison of Elisa and RT PCR

When comparing the results of the ELISA and PCR for the three viruses of potato, the positive samples for PVY was higher in PCR by 11%, PVS by 40% and PVA by 82% compared with ELISA (Figure. 3.10).

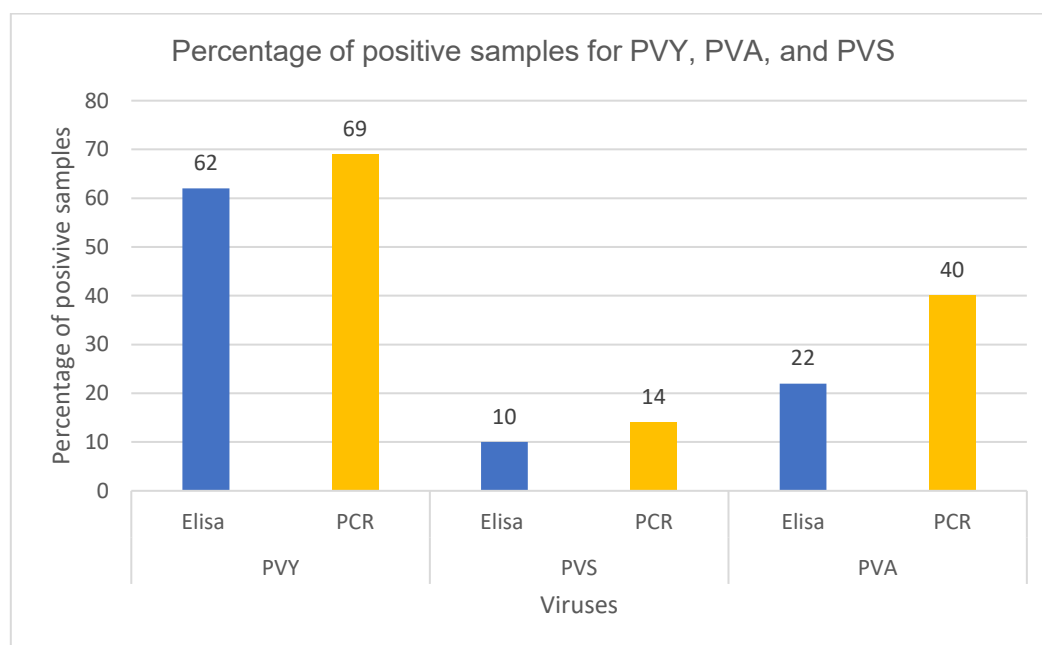


Figure 3.10. Percentage of samples found positive for PVY, PVS and PVA in Elisa and RT PCR test

4. DISCUSSION

The recombinant strain group of PVY^{NTN} is one of the most widespread viruses in the world (Onditi *et al.*, 2021). In Ireland, the most common recombinant strains of the PVY virus, as previous studies reported, were PVY^{NTN} and PVY^{N:O} (Hutton *et al.*, 2013; Hutton *et al.*, 2015; Della Bartola *et al.*, 2020). This study in the Teagasc potato crops has further confirmed the presence of the NTN recombinant variants circulating in the Irish potato industry. The Syrian variants of PVY, SYR II, and SYR III were also found to be prevalent in the tested potato samples.

The new recombinant strains of PVY are described as a virus strain that does not express unique symptoms in potato varieties (MacKenzie *et al.*, 2019). Symptoms are a manifestation of altered physiology and development, which potyviruses cause by interfering with host gene expression (Valkonen, 2007). The potato varieties planted in Teagasc Crop Research sites were observed to show different levels of disease symptom expression. The severity of the combined symptoms was observed to be linked with the recombinant strain SYR III showing more crinkling, mottling, or curling of potato leaves than the other identified recombinant strains. However, no veinal necrosis was observed, in contrast to a previous study by Chikh-Ali *et al.* (2010a) on which SYR-III was observed to show veinal necrosis in most of their samples. However, the mild mosaic observed linked with the PVY^{NTN-NW} (SYR II) is the same as that observed in their study.

The expression of mild to moderate foliar mosaic symptoms were mainly observed in the recombinant strain PVY^{NTNa} which supports the recent study by Whitworth *et al.* (2021) that the recombinant PVY strains (NTN, N:O, Nwi, and NE-11) induced milder foliar symptoms. On the other hand, PVY^O was observed to show mild mosaic to

moderate crinkling in the two positive PVY^O samples and no symptom in one sample. PVY^O is mainly characterized by severe symptoms of necrosis, mottling, stunting, mosaic, yellowing of leaflets and eventually leaf drops, and premature death of plants (Funke *et al.*, 2017; Della Bartola *et al.*, 2020). The different symptoms of PVY^O identified in this study may be due to a different expression of PVY^O subgroups, as identified by Nie *et al.* (2011), who recorded those two subgroups, designated as PVY^{O-RB} and PVY^{O-FL}, were found to be linked to mild and severe symptoms respectively. In addition, visual observation of one PVY^O positive plant showed dwarfing, coupled with the severe mosaic and with the mild crinkling of the leaf. This response is consistent with previous studies that found secondary symptoms result in a dwarf plant, with mottled or crinkled foliage (Wale *et al.*, 2008). The severe symptoms of PVY^O were observed on the samples taken from the field of potato planted using previously harvested crop from virus-positive fields. Therefore, the severity of the symptom could be due to secondary infection of the crop.

In several studies, it was found that the coinfection of PVY and PVX is a serious problem (Valkonen, 2007, Hameed *et al.*, 2014). In this experiment, PVA was found coinfecting only in the presence of PVY; mainly with PVY^{NTNa} (70%) and PVY^{NTNb} (8%). But there was no difference in the symptoms observed with the single virus-infected plants, in agreement with the finding of Hameed *et al.* (2014). The coinfection may indicate that the PVY can enhance the infection of the PVA, which supports the statement by Valkonen (2007) (and reference therein), that “PVY and other potyviruses may synergize unrelated viruses in co-infected plants and consequently symptoms and yield losses caused by the other viruses may be enhanced”. This is due to the overcoming of the RNA silencing during coinfection, a defense mechanism

by the host plant against viruses (Anandalakshmi *et al.*, 1998; Valkonen, 2007), thereby facilitating a better route for the proliferation of the virus in the host plant.

Moreover, in many parts of the world, multiple strains of PVY are found to simultaneously affect the same field and even individual plants (Blanchard *et al.*, 2008; Onditi *et al.*, 2021). Mixed infections are found to be high (30%), second to the PVY^{NTNa} in this study. These mixtures were mainly on the samples (21%) collected from the seeds planted from a tuber which reacted both positive and negative for viruses. As the previous study reports, the biggest losses and symptom severity are experienced from crops grown from PVY-infected seed (Valkonen, 2007; Wale *et al.*, 2008) which is termed as secondary infection. The severity of the symptoms was observed to be high in the thirty-four samples collected from the virus trial field where the crop was grown from seeds collected from a field which had PVY positive plants. In a study conducted by Nie *et al.* (2011) the mixed infection observed was counted individually for the possible strains within the mixed infection. But in this experiment, this was avoided because the possible coinfection could give a wrong count, if separated individually for the suspected possibilities, because most of the strains in this study do not have only one unique fragment size in the PCR products. Therefore, the observed variation in the symptoms in most of the samples being infected with the same strain could be due to the recombinant nature of the strain, as suggested by Chikh-Ali *et al.* (2010a) and the varietal differences in the Center, which confers different resistant abilities.

Furthermore, the symptom expression was studied at one stage, when the plant was aged at two months. A symptom expression of one pathogen is affected by different factors, where age and tolerance of the variety are important aspects. As the samples

were randomly taken from the different symptomatic and asymptomatic plants, this could show different results due to the various unique characteristics as observed in the potato varieties, Kerr's Pink and Sarpo Mira. Here, a curly leaf characteristic was observed in both negative and positive results in samples at the same time for the virus strains. This makes it difficult to distinguish the curly symptom, which is caused by a virus in these varieties, unlike the report by Hutton *et al.*, (2015) which describes this variety as having a mild mottling symptom.

The results from ELISA and PCR have shown differences. Using PCR, more samples were detected positive for PVY, PVS, and PVA. This result was also observed by Fageria *et al.* (2013), who compared both methods on PVY detection. It can be said therefore that PCR has a higher sensitivity than ELISA. This is because the detection of a virus through ELISA is dependent on the interaction between an antibody and the viral coat protein molecules and as it has been stated by Roos (2013) the viral coat protein amino acid composition can be altered through genetic mutation and adaptation. Such alterations mean that ELISA may be unable to successfully detect these viruses. Moreover, it was also observed that in one sample, coinfection with PVS and PVY was masked in the RT-PCR test for the five viruses. However, when the same sample was subjected to PVY strain multiplex RT PCR, a recombinant strain of PVY, SYR II was found. Even though the observed bands of this sample were very weak, which could have been due to the low titre of the virus, but it clearly shows that in such cases it can be masked by the other viruses or other strains in PCR products that have high titre.

The different results obtained in the extraction methods clearly indicate that the tissue homogenizers (Bioreba extraction bags) method gives clear PCR products by avoiding

cross-contamination, without the need of washing a machine between samples. However, the detection was better on the samples extracted using the leaf press machine. More samples were positive for different strains where there was no adjacent positive sample. The different result could have been due to less sensitivity of the method by Bioreba extraction bags because of the amount of homogenised tissue in the Bioreba bags is less (0.3g) compared to the leaf press. Moreover, because the samples (leaflets) used are not the same but from the same plant and the same leaf, this further shows that a different virus and/or strain can be expressed not only in the same plant but also in different parts of the leaf or leaflet.

In conclusion, it was observed that in the Teagasc potato variety development program, the recombinant strain PVY^{NTNa}, PVY^{NTNb}, and SYR III are more prevalent. This study is the first to report the prevalence of SYR III recombinant strain in Ireland. The potato plants have shown different characteristic foliar symptoms to the NTN and SYR strain groups. The foliar symptoms commonly observed in the plants, having identified positive for the same recombinant strain, gives a glimpse for further research on the symptom expression study. Moreover, as PVA was only found in connection with the recombinant strain PVY^{NTNa} only in this study, an in depth study on the coinfection of PVA with PVY and their importance is suggested. It is important to note that the samples in this study were collected from a small area, not enough to give a concluding result. This preliminary result of the association between the virus and symptom illustrates that mainly the PVY^{NTNa} and SYR III show a different common expression in most of the varieties. PVY^{NTNa} infection producing a mild mosaic symptom in most samples and SYR III showing a mix of moderate mosaic, with moderate to severe crinkling of leaves. The preliminary result of SYR III symptoms can be of help to Teagasc in field management of the potato viruses, through roguing,

before further spread to healthy plants. As this research was focused on the identification of the prevailing recombinant strains of the PVY virus and comparing them with observed symptoms, the strategy was blindly categorizing the symptoms without knowing the PCR results and later relating them with identified strains. So, a further in-depth research is recommended on the relationship of symptom expression, the recombinant strains, and the variety under development. This might be achieved by isolating and inoculating the virus strains in a selected healthy plant variety which may include the study of the tuber symptoms, because the NTN strain group was originally named after its necrotic symptoms in the tuber. Finally, a genotyping of the SYR III recombinant strain of PVY reported in this study and wider study on its distribution in Ireland is recommended.

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REFERENCES

- Anandalakshmi R, Pruss GJ, Ge X, Marathe R, Mallory AC, Smith TH, Vance VB** 1998. A viral suppressor of gene silencing in plants. *Proceedings of the National Academy of Sciences* **95**: 13079-13084.
- Anfoka G, Ahmad FH, Altaleb M, Al-Shhab M, Abubaker S, Levy D, Rosner A, Czosnek H.** 2014. First report of recombinant Potato virus Y strains infecting potato in Jordan. *Plant disease* **98**: 1017-1017.
- Avrahami-Moyal L, Tam Y, Sela N, Prakash S, Harel YM, Bornstein M, Shulchani R, Dar Z, Gaba V.** 2019. Characterization of potato virus Y populations in potato in Israel. *Archives of virology* **164**: 1691-1695.
- Basu A, Srikanta D.** 2003. Integrated management of potato (*Solanum tuberosum*) diseases in Hooghly area of West Bengal. *Indian Journal of Agricultural Sciences* **73**: 649-651.
- Blanchard A, Rolland M, Lacroix C, Kerlan C, Jacquot E.** 2008. Potato virus Y: a century of evolution. *Virology* **7**: 21-32.
- Boonham N, Walsh K, Hims M, Preston S, North J, Barker I.** 2002. Biological and sequence comparisons of Potato virus Y isolates associated with potato tuber necrotic ringspot disease. *Plant Pathology* **51**: 117-126.
- Burke, JJ.,** 2017. *Growing the potato crop*. Vita, equity house, upper Ormond quay, Dublin 7, Ireland.
- Chikh-Ali M, Alruwaili H, Vander Pol D, Karasev AV.** 2016. Molecular characterization of recombinant strains of Potato virus Y from Saudi Arabia. *Plant disease* **100**: 292-297.

- Chikh-Ali M, Maoka T, Natsuaki KT, Natsuaki T, 2010a.** PVYNTN-NW, a novel recombinant strain of Potato virus Y predominating in potato fields in Syria. *Plant Pathology* **59**: 31-41.
- Chikh-Ali M, Maoka T, Natsuaki KT, Natsuaki T. 2010.** The simultaneous differentiation of Potato virus Y strains including the newly described strain PVYNTN-NW by multiplex PCR assay. *Journal of Virological Methods* **165**: 15-20.
- Chikh-Ali M, Maoka T, Natsuaki KT. 2007.** The occurrence and characterization of new recombinant isolates of PVY displaying shared properties of PVYNW and PVYNTN. *Journal of Phytopathology* **155**: 409-415.
- Choi KH. 2012.** Viral Polymerases. In: Rossmann M, Rao V. eds. *Viral Molecular Machines*. Advances in Experimental Medicine and Biology, vol 726. Springer, Boston, MA. https://doi.org/10.1007/978-1-4614-0980-9_12
- Davie K, Holmes R, Pickup J, Lacomme C. 2017.** Dynamics of PVY strains in field grown potato: Impact of strain competition and ability to overcome host resistance mechanisms. *Virus research* **241**: 95-104.
- Delaney B. 2020.** Potatoes, Plant Health and Beyond. *Conference Presentations*, Department of Agriculture, Food & Marine, National Potato Conference, 18th February 2020 (<https://www.teagasc.ie/media/website/publications/2020/>) [accessed 18 June 2020]
- Della-Bartola M, Byrne S, Mullins E. 2020.** Characterization of potato virus Y isolates and assessment of nanopore sequencing to detect and genotype potato viruses. *Viruses* **12**: 478.
- Du Z, Chen J, Hiruki C. 2006.** Optimization and application of a multiplex RT-PCR system for simultaneous detection of five potato viruses using 18S rRNA as an internal control. *Plant Disease* **90**: 185-189.

- Dupuis B, Bragard C, Schumpp O. 2019.** Resistance of potato cultivars as a determinant factor of Potato virus Y (PVY) epidemiology. *Potato research* **62**: 123-138.
- Elwan EA, Abdel EE, Fattouh FA, Green KJ, Tran LT, Karasev AV. 2017.** Occurrence of diverse recombinant strains of Potato virus Y circulating in potato fields in Egypt. *Plant disease* **101**: 1463-1469.
- Fageria MS, Singh M, Nanayakkara U, Pelletier Y, Nie X, Wattie D. 2013.** Monitoring current-season spread of Potato virus Y in potato fields using ELISA and real-time RT-PCR. *Plant Disease* **97**: 641-644.
- Food and Agriculture Organization of the United Nations, 2020.** FAOSTAT statistical database. Rome: FAO.
- Funke CN, Nikolaeva OV, Green KJ, Tran LT, Chikh-Ali M, Quintero-Ferrer A, Cating RA, Frost KE, Hamm PB, Olsen N, Pavek MJ. 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.
- Gadhav KR, Gautam S, Rasmussen DA, Srinivasan R. 2020.** Aphid transmission of potyvirus: The largest plant-infecting RNA virus genus. *Viruses* **12**: 773.
- Galvino-Costa SBF, dos Reis Figureueira A, Camargos VV, Geraldino PS, Hu XJ, Nikolaeva OV, Kerlan C, Karasev AV. 2012.** A novel type of Potato virus Y recombinant genome, determined for the genetic strain PVYE. *Plant Pathology* **61**: 388-398.
- Glais L, Tribodet M, Kerlan C. 2002.** Genomic variability in Potato potyvirus Y (PVY): evidence that PVY N W and PVY NTN variants are single to multiple recombinants between PVY O and PVY N isolates. *Archives of virology* **147**: 363-378.

- Gray S, De Boer S, Lorenzen J, Karasev A, Whitworth J, Nolte P, Singh R, Boucher A, Xu H. 2010.** Potato virus Y: an evolving concern for potato crops in the United States and Canada. *Plant Disease* **94**: 1384-1397.
- Green KJ, Brown CJ, Karasev AV. 2018.** Genetic diversity of potato virus Y (PVY): sequence analyses reveal ten novel PVY recombinant structures. *Archives of virology* **163**: 23-32.
- Hameed A, Iqbal Z, Shaheen Asad SM. 2014.** Detection of multiple potato viruses in the field suggests synergistic interactions among potato viruses in Pakistan. *The plant pathology journal* **30**: 407.
- Hutton F, Kildea S, Griffin D, Spink J, Doherty G, Hunter A. 2013.** First report of potato tuber necrotic ringspot disease associated with PVY recombinant strains in Ireland. *New Disease Reports* **28**: 2044-0588.
- Hutton F, Spink JH, Griffin D, Kildea S, Bonner D, Doherty G, Hunter A. 2015.** Distribution and incidence of viruses in Irish seed potato crops. *Irish Journal of Agricultural and Food Research* **54**: 98-106.
- IBM Corp. 2019.** *IBM SPSS Statistics for Windows*, Version 26.0. Armonk, NY: IBM Corp.
- Jones RAC. 2014.** Virus disease problems facing potato industries worldwide: viruses found, climate change implications, rationalizing virus strain nomenclature, and addressing the Potato virus Y issue. In: Navarre R, Pavek MJ, eds. 2014. *The potato: botany, production and uses*. CABI, USA: 202-224.
- Kamangar SB, Smagghe G, Maes M, De Jonghe K. 2014.** Potato virus Y (PVY) strains in Belgian seed potatoes and first molecular detection of the N-Wi strain. *Journal of Plant Diseases and Protection* **121**: 10-19.

- Karasev AV, Gray SM. 2013.** Continuous and emerging challenges of Potato virus Y in potato. *Annual review of phytopathology* **51**: 571-586.
- Kassim NA, Nerway ZAA, Yousif KH. 2014.** Identification of Potato Virus Y (Pvy) and Its Economic Importance on Potato Crop. *Science Journal of University of Zakho* **2**: 304-309.
- Kehoe MA, Jones RAC. 2016.** Improving Potato virus Y strain nomenclature: lessons from comparing isolates obtained over a 73-year period. *Plant Pathology* **65**: 322-333.
- Lindner K, Trautwein F, Kellermann A, Bauch G. 2015.** Potato virus Y (PVY) in seed potato certification. *Journal of Plant Diseases and Protection* **122**: 109-119.
- MacKenzie TD, Lavoie J, Nie X, Singh M. 2018.** Differential spread of Potato virus Y (PVY) strains O, N: O and NTN in the field: Implications for the rise of recombinant PVY strains in New Brunswick, Canada. *American Journal of Potato Research* **95**: 301-310.
- MacKenzie TD, Nie X, Bisht V, Singh M. 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.
- Nie B, Singh M, Sullivan A, Singh RP, Xie C, Nie X. 2011.** Recognition and molecular discrimination of severe and mild PVYO variants of Potato virus Y in potato in New Brunswick, Canada. *Plant Disease* **95**: 113-119.
- Onditi J, Nyongesa M, van der Vlugt R. 2021.** Screening for PVY N-Wi Resistance in Kenyan Potato Cultivars. *Potato Research* **64**: 469–488.

- Quenouille J, Vassilakos N, Moury B. 2013.** Potato virus Y: a major crop pathogen that has provided major insights into the evolution of viral pathogenicity. *Molecular plant pathology* **14**: 439-452.
- Rodriguez-Rodriguez M, Chikh-Ali M, Johnson SB, Gray SM, Malseed N, Crump N, Karasev AV. 2020.** The Recombinant Potato virus Y (PVY) Strain, PVYNTN, Identified in Potato Fields in Victoria, Southeastern Australia. *Plant Disease* **104**: 3110-3114.
- Roos WG. 2013.** *An investigation of prevalence and the detection and race identification of South African potato viruses*. PhD Thesis, Stellenbosch University, Stellenbosch, South Africa.
- Rott ME, Jelkmann W. 2001.** Characterization and detection of several filamentous viruses of cherry: adaptation of an alternative cloning method (DOP-PCR), and modification of an RNA extraction protocol. *European Journal of Plant Pathology* **107**: 411-420.
- Scholthof KBG, Adkins S, Czosnek H, Palukaitis P, Jacquot E, Hohn T, Hohn B, Saunders K, Candresse T, Ahlquist P, Hemenway C. 2011.** Top 10 plant viruses in molecular plant pathology. *Molecular plant pathology* **12**: 938-954.
- Singh RP, McLaren DL, Nie X, Singh M. 2003.** Possible escape of a recombinant isolate of Potato virus Y by serological indexing and methods of its detection. *Plant Disease* **87**: 679-685.
- Singh RP, Valkonen JP, Gray SM, Boonham N, Jones RAC, Kerlan C, Schubert J. 2008.** Discussion paper: The naming of Potato virus Y strains infecting potato. *Archives of virology* **153**: 1-13
- Singh RP. 1999.** Development of the molecular methods for potato virus and viroid detection and prevention. *Genome* **42**: 592-604.

- Valkonen JP. 2007.** Viruses: economical losses and biotechnological potential. In: Vreugdenhil D, Bradshaw J, Gebhardt C, Govers F, Taylor M, MacKerron D, Ross H, eds. *Potato biology and biotechnology*. Elsevier Science BV, 619-641.
- Varma A. 1993.** Integrated management of plant viral diseases. In: Chadwick DJ, Marsh J, eds. *Crop protection and sustainable agriculture*. John Wiley & Sons, 140-157.
- Visser JC, Bellstedt DU, Pirie MD. 2012.** The recent recombinant evolution of a major crop pathogen, Potato virus Y. *PLoS One* **7**: 50631.
- Visser JC. 2012.** *A study of the strain evolution and recombination of South African isolates of Potato virus Y*. PhD Thesis, Stellenbosch University, Stellenbosch, South Africa.
- Wale SJ, Platt HW, Cattlin ND. 2008.** *Diseases, pests and disorders of potatoes: a color handbook*. London, UK: Manson Publishing.
- Whitworth JL, Gray SM, Ingram JT, Hall DG. 2021.** Foliar and Tuber Symptoms of US Potato Varieties to Multiple Strains and Isolates of Potato virus Y. *American Journal of Potato Research* **98**: 93-103.

APPENDICES

Appendix 1. The table show the list of varieties used in the experiment with their identified virus and/strain and the observed symptom ratings given.

Sample ID	Variety/ breeding line	Virus	PVY strain	Mosaic	Crinkle	Necrosis	No Symptom
1201	17011/01	PVY	PVY ^{NTNa}	1	1		
1202	17056/03	PVY, PVA	PVY ^{NTNa}	2	1	2	
1206	17160/01	PVY, PVA	PVY ^{NTNa}	2	2		
1214	17/42/02	PVY, PVA	PVY ^{NTNa}	2			
1230	17107/04	PVY	Mixed 1*	3	3		
1231	17107/04	PVY	PVY ^{SYR III}	1	1		
1234	17107/04	PVY	Mixed 4*	3	3		
1246	17103/05	PVY	PVY ^O	1			
1251	8296/05	PVY, PVA	PVY ^{NTNa}	2			
1259	9156A/02	PVY, PVA	PVY ^{NTNa}	1	1		
1276	T18007/04	PVY	PVY ^{SYR III}		3		
1279	18377/09	PVY, PVA	PVY ^{NTNa}	1			
1280	18377/09	PVY	PVY ^{NTNa}				0
1285	18292/01	PVY, PVA	PVY ^{NTNa}	2	2	3	
1286	18292/01	PVY, PVA	PVY ^{NTNa}	1			
1287	18292/01	PVY, PVA	PVY ^{NTNa}	2	2	3	
1288	18292/01	PVY	Mixed 1*	1			
1292	18366/17	PVY, PVA	PVY ^{NTNa}	2			
1302	Kerr's Pink	PVY	PVY ^O				0
1318	Sarpo Mira	PVY, PVA	PVY ^{NTNb}		1		
1319	T7080/01	PVY	PVY ^{SYR III}	2			
1320	T7080/01	PVY	Mixed 1*		2		
1321	T7080/01	PVY	PVY ^{SYR III}	3	2		
1322	T7080/01	PVY	PVY ^{SYR III}	3	2		
1323	T7080/01	PVY, PVA	Mixed 1*	2	2		
1324	T7080/01	PVY, PVA	Mixed 1*	3	3		
1325	T7080/01	PVY	PVY ^{SYR III}	2	1		
1326	T7080/01	PVY	PVY ^{SYR III}	2	1		
1327	T7080/01	PVY, PVA	Mixed 2*	1	2		
1328	T7080/01	PVY, PVA	PVY ^{NTNb}		1		
1329	T7080/01	PVY, PVA	Mixed 1*		2		
1330	T7080/01	PVY	PVY ^{SYR III}	2	2		

1331	T7080/01	PVY, PVA	PVY ^{NTNb}	1			
1332	T7080/01	PVY, PVA	Mixed 1*	1			
1333	T7080/01	PVY, PVA	Mixed 1*	1			
1334	T7080/01	PVY, PVA	Mixed 1*	1			
1335	T7080/01	PVY, PVA	PVY ^{SYR III}	3	2		
1336	17075/05	PVY, PVA	PVY ^{NTNb}	1			
1337	17075/05	PVY, PVA	PVY ^{NTNb}	1			
1339	17075/05	PVY, PVA	PVY ^{NTNb}	1			
1340	17075/05	PVY, PVA	PVY ^{NTNb}	1	1		
1341	17075/05	PVY, PVA	PVY ^{NTNb}	1			
1342	17075/05	PVY	PVY ^{NTNb}	1			
1344	17075/05	PVY	PVY ^{NTNb}		1		
1351	17103/05	PVY, PVA	PVY ^{NTNa}	2	1		
1352	17103/05	PVY, PVA	PVY ^{NTNa}	2			
1353	17103/05	PVY	PVY ^{NTN-NW(SYR II)}				0
1354	17103/05	PVY, PVA	PVY ^{NTNa}	1	1		
1355	17110/12	PVY	PVY ^{SYR III}		2		
1356	17110/12	PVY, PVA	PVY ^{NTNa}	1			
1357	17110/12	PVY	PVY ^{NTNa}	2			
1358	17110/12	PVY, PVA	PVY ^{NTNa}	2			
1359	17110/12	PVY, PVA	PVY ^{NTNa}	1			
1360	17110/12	PVY	PVY ^{NTN-NW(SYR II)}	1			
1361	17110/12	PVY, PVA	Mixed 3*	2	1		
1362	17110/12	PVY, PVA	Mixed 3*	1	1		
1363	17110/12	PVY	Mixed 3*	2			
1364	17110/12	PVY	Mixed 3*	1			
1365	17108/15	PVY, PVA	PVY ^{NTNa}	2	2		
1366	17108/15	PVY, PVA	PVY ^{NTNa}	2			
1367	17108/15	PVY, PVA	PVY ^{NTNa}	2			
1368	17108/15	PVY, PVA	PVY ^{NTNa}	2			
1369	17108/15	PVY, PVA	PVY ^{NTNa}	2			
1370	17108/15	PVY, PVA	PVY ^{NTNa}	2			
1371	17108/15	PVY, PVA	PVY ^{NTNa}	2			
1372	17108/15	PVY, PVA	PVY ^{NTNa}	2			
1373	17108/15	PVY, PVA	PVY ^{NTNa}	2			
1374	17108/15	PVY, PVA	PVY ^{NTNa}	2			
1375	17108/15	PVY, PVA	PVY ^{NTNa}	2			
1376	17108/01	PVY, PVA	PVY ^{NTNa}	2			
1377	17108/01	PVY, PVA	PVY ^{NTNa}	2			
1378	17108/01	PVY, PVA	PVY ^{NTNa}	2			
1379	17108/01	PVY, PVA	PVY ^{NTNa}	2			
1380	17108/01	PVY, PVA	PVY ^{NTNa}	2			

1381	17108/01	PVY, PVA	PVY ^{NTNa}	2			
1382	17108/01	PVY, PVA	PVY ^{NTNa}	2			
1383	17108/01	PVY, PVA	PVY ^{NTNa}	2			
1384	17108/01	PVY, PVA	PVY ^{NTNa}	2			
1385	17108/01	PVY, PVA	PVY ^{NTNa}	2			
1386	17108/01	PVY, PVA	PVY ^{NTNa}	2			
1387	17108/01	PVY, PVA	PVY ^{NTNa}	2			
1388	17108/01	PVY, PVA	PVY ^{NTNa}	2			
1389	17108/01	PVY, PVA	PVY ^{NTNa}	2			
1390	17108/01	PVY	PVY ^{NTNa}	2			
1391	17108/01	PVY	PVY ^{SYR III}	2			
1392	17108/01	PVY	PVY ^{NTNa}	2			
1393	17108/01	PVY, PVA	PVY ^{NTNa}	2			
1394	17108/01	PVY, PVA	PVY ^{NTNa}	1	1		
1501	T8304/08	PVY, PVA	Mixed 3*	1			
1502	T8304/08	PVS	PVY ^{NTN-NW} (SYR II)				0
1503	T6952/18	PVY	Mixed 6*	3			
1505	T6952/18	PVY, PVS	Mixed 2*		2		
1508	Gravity	PVY, PVS	Mixed 3*	2	1		
1511	Imagine	PVY, PVS	Mixed 2*	2	1		
1512	Spunta	PVY, PVS	Mixed 3*	2	2		
1517	T8310/03	PVY, PVS	Mixed 2*	3	3		
1518	T7326/24	PVY, PVS	Mixed 2*	1			
1520	Eersteling	PVY, PVS	Mixed 6*	3	3		
1521	T5653/08	PVY	Mixed 5*	1			
1523	T8247/04	PVY, PVS	Mixed 5*	3			
1524	T8560/07	PVY	Mixed 6*	1			
1525	T8032/04	PVY	Mixed 5*				0
1526	T8486/02	PVY	PVY ^O	3	3		
1527	T8486/02	PVY	Mixed 5*				0
1528	Commando	PVY, PVS	PVY ^{NTNb}	1			
1529	British Queen	PVY, PVS	Mixed 3*	2			
1530	British Queen	PVY	Mixed 2*	1			
1531	T8561/01	PVY, PVS	Mixed 6*	2			
143A	Eersteling	PVY, PVS	Mixed 5*	3			

* The different possibilities of combined infection in the mixed infection and their band sizes are presented in Appendix 2.

Appendix 2. The possibilities of combined infection in the mixed infection found in the same sample

Mixture	PVY strains	Products in the multiplex PCR(bp)	Percentage
Mixed 1	PVY ^O , PVY ^{SYR III} , PVY ^{NTNb} , or it can be a Natural mixed infection with PVY ^{NTN-NW} (SYR II) and PVY ^{NTNb} (or/and NA-PVYN)	127, 202, 267, 441	8
Mixed 2	Natural mixed infection with PVY ^{NTN-NW} (SYR II) and PVY ^{NTNb} (or/and NA-PVYN)	127, 202, 441	6
Mixed 3	Infection which can be between PVY ^{NTNa} , PVY ^{NTN-NW} (SYR I), PVY ^{NTN-NW} (SYR II) and/or PVY ^O	127, 202, 441, 663	6
Mixed 4	Mixed of PVY ^{NTNa} , PVY ^{NTNb} and SYR III, PVY ^{NTN-NW} (SYR I), PVY ^{NTN-NW} (SYR II), PVY ^N , PVY ^O	127, 202, 267, 441, 663	1
Mixed 5	Mixed of PVY ^{NTN-NW} (SYR I) and PVY ^{NW} (B)	127, 441, 853	5
Mixed 6	PVY ^{NTNa} , PVY ^{NTN-NW} (SYR I), PVY ^{NTN-NW} (SYR II) and/or PVY ^O , and PVY ^{NW} (B)	127, 202, 441, 663, 853	4