

**DOCTORAL THESIS**

# Occurrence and Characterization of Potato- Infecting Viruses and Their Strains in Estonia

Piret van der Sman

TALLINN UNIVERSITY OF TECHNOLOGY  
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**Declaration:**

Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree.

Piret van der Sman

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# **Kartulit nakatavate viiruste ja nende erinevate tüvede esinemine ning iseloostamine Eestis**

PIRET VAN DER SMAN







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## List of Publications

The list of the author's Publications, on which this thesis is based:

- I van der Sman, P., Pata, P., Järvekülg, L., & Sõmera, M. (2025). Long-term monitoring of six potato viruses in Estonian seed potatoes and an assessment of susceptibility of potato varieties to PVY. *Potato Research*, 68(3), 3123–3145. <https://doi.org/10.1007/s11540-025-09862-0>
- II van der Sman, P., Järvekülg, L., Stražev, A., Puurand, T., Pata, P., Sarmiento, C., Adams, I., & Sõmera, M. (2026). A survey of Potato virus Y strains in Estonian seed potatoes. *Plant Health Progress*, 27(1), 93–100. <https://doi.org/10.1094/PHP-06-25-0175-S>
- III van der Sman, P., Stražev, A. & Sõmera, M. (2026). First Report of Potato Virus S Andean Isolates Infecting Potato in Estonia. *Plant Disease, First look* <https://doi.org/10.1094/PDIS-05-26-0980-PDN>
- IV Just, K., Allika, R., van der Sman, P., Arif, U., Ilau, B., Koidumaa, R., et al. (2022). First report of Tomato leaf curl New Delhi virus infecting tomato and cucumber in Estonia. *New Disease Reports*, 45, e12083. <https://doi.org/10.1002/ndr2.12083>

## **Author's Contribution to the Publications**

Contribution to the papers in this thesis are:

- I The author conducted most of the virus monitoring experiments and supervised the rest, prepared the data sets, participated in statistical analysis, generated the illustrative graphics (if not generated by the used programs), drafted the manuscript, and participated on writing, reviewing and editing of the manuscript.
- II The author conducted part of the experiments (virus testing and strain assignment) and supervised the rest, prepared the data sets, analysed most part of the data, co-supervised part of the bioinformatic analysis, generated graphics (if not generated by the used programs), drafted the manuscript, and participated in writing, reviewing and editing of the manuscript.
- III The author participated in conceptualization of the study, supervised the experiments (virus testing, sequencing and strain assignment), drafted the manuscript, and participated in writing, reviewing and editing of the manuscript.
- IV The author conducted part of the experiments (virus testing and sequencing), analysed most part of the data, and participated in reviewing and editing of the manuscript.

## Introduction

The health status of potato seed tubers is a key factor in determining the productivity, quality and sustainability of potato production. Potato is one of the most important food crops worldwide. It is widely known and cultivated across a broad range of geographic regions and environmental conditions, from Andean highlands to the temperate regions of Northern Europe, as well as in diverse agroecological zones of Africa and Asia. Although the cultivated area of potato has diminished over recent decades in Europe, potato production continues to be a significant agricultural activity. Ensuring seed tuber health remains crucial for domestic food security and international trade. Among the numerous pathogens affecting potato, viruses represent a major concern because of their efficient transmission through tubers and vectors, and due to their genetic variability, what complicates the detection. In vegetatively propagated crops such as potato (*Solanum tuberosum* L.), viruses are major concern due to their ability to accumulate from one generation to the next that causes progressive yield decline, reduction of tuber quality, and downgrading seed lots, resulting in significant economic losses. Therefore, detection, identification and monitoring of potato viruses are central components of seed potato certification system, and in plant health management programs. Therefore, understanding the prevalence of viruses, viral diversity, host susceptibility, and the environmental factors influencing disease epidemiology are essential for maintaining high phytosanitary standards and supporting potato production.

Crop production systems are becoming increasingly vulnerable to emerging viruses that may spread through trade and vectors or become problematic due to changing environmental conditions. Globally, there are more than 60 viruses that may infect potatoes and nine viruses that are considered economically important. Those potato viruses are: A (PVA), Y (PVY), X (PVX), S (PVS), M (PVM), and V (PVV), potato leaf roll virus (PLRV), potato mop top virus (PMTV), and tobacco rattle virus (TRV). PVA, PVY, PVX, PVS, PVM, and PLRV are monitored in most European Union (EU) countries within the framework of the seed certification system.

Development of serological and molecular diagnostic methods has considerably improved the detection and characterization of potato viruses and their strains over the last decades. While incidence data reveal how frequently viruses occur, strain identification provides deeper insights into virus population structure, spread, and potential risks. These advances have enabled the large-scale characterization of viruses, being an important extension of routine virus monitoring in certification systems. Moreover, in addition to viruses already implemented in the seed certification systems, the broader phytosanitary context must also be considered. This is particularly relevant for viruses emerging from related hosts in agricultural production or from wild weeds that may act as reservoirs for viruses with expanding host range.

This study builds on more than 20 years of professional experience in the Plant Health Laboratory at the Centre of Estonian Rural Research and Knowledge (METK), where I have been involved in the routine testing, identification, and monitoring of plant viruses, including potato viruses, in Estonia. This extensive practical work has provided both the diagnostic foundation and scientific motivation for the present research, enabling the integration of routine laboratory testing with long term data analysis and the molecular characterization of virus strains. The study has also benefited from my active participation in international expert networks, including European and Mediterranean

Plant Protection Organization (EPPO) Panel on Diagnostics in Virology and Phytoplasmaology and the European Union Reference Laboratories (EURL) in Plant Health. Through this involvement, I have contributed and benefited from the exchange of knowledge, the harmonization of diagnostic methods, and increased awareness of emerging plant health risks.

This thesis is structured around four main parts. First, examination of the long-term monitoring data of six potato viruses in Estonian seed potatoes and evaluation of the susceptibility of cultivated potato varieties to PVY, including the influence of climate factors on PVY spread under local conditions. The second part focuses on the identification and distribution of PVY strain groups in Estonian seed potatoes using the multiplex reverse transcription polymerase chain reaction (RT-PCR) as the main tool. The third part introduces high-throughput sequencing (HTS), which complements conventional virus testing by enabling the detection of all viruses present in the sample and allowing a more detailed analysis of viral genomic diversity and strain variation in seed potato. The fourth part broadens the phytosanitary perspective by including viruses on other hosts, that may become relevant to potato production in the future. Together these parts combine analysis of long-term monitoring data, molecular characterization of detected potato viruses by traditional and advanced sequencing approaches, and epidemiological interpretations to improve the understanding of virus occurrence, diversity, host resistance, and potential phytosanitary risks to Estonian potato production. The overall aim of this work is to provide new knowledge based on data analysis and modernize the methodological approaches to support producers, breeders, policymakers, and the wider community in securing sustainable potato production.

The results of this study have been published in scientific phytopathology journals indexed in Scopus and Web of Science and have been presented orally at the 18th Triennial Meeting of the Virology Section of the European Association for Potato Research in 2025.

## Abbreviations

|                    |                                                                                                                         |
|--------------------|-------------------------------------------------------------------------------------------------------------------------|
| +ssRNA             | Positive-sense single-stranded RNA                                                                                      |
| Cap                | 7-methylguanosine (m7G) cap structure at the 5` viral mRNA                                                              |
| CP                 | Coat protein                                                                                                            |
| CRISPR-Cas systems | RNA-guided CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated) nucleases systems |
| DNA                | Deoxyribonucleic acid                                                                                                   |
| EFSA               | European Food Safety Authority                                                                                          |
| ELISA              | Enzyme-Linked Immunosorbent Assay                                                                                       |
| EPPO               | European and Mediterranean Plant Protection Organization                                                                |
| ER                 | Extreme resistance                                                                                                      |
| EU                 | European Union                                                                                                          |
| EUR                | Euro (currency code)                                                                                                    |
| EURL               | European Union Reference Laboratories                                                                                   |
| extra-A            | Extra adenosine nucleotide                                                                                              |
| gRNA               | Genomic ribonucleic acid                                                                                                |
| HC-Pro             | Helper component-proteinase                                                                                             |
| HR                 | Hypersensitive response                                                                                                 |
| HTS                | High-throughput sequencing                                                                                              |
| ICTV               | International Committee on Taxonomy of Viruses                                                                          |
| kb                 | Kilobase                                                                                                                |
| m7G                | 7-methylguanosine cap structure                                                                                         |
| METK               | Centre of Estonian Rural Research and Knowledge                                                                         |
| ML                 | Machine Learning                                                                                                        |
| MP                 | Movement protein                                                                                                        |
| mRNA               | Messenger ribonucleic acid                                                                                              |
| nm                 | Nanometre                                                                                                               |
| nt                 | Nucleotide                                                                                                              |
| ORF                | Open reading frame                                                                                                      |
| P3-PIPO            | A transframe movement protein (MP) for potyviruses                                                                      |
| PB                 | Pre-basic                                                                                                               |
| PBTC               | Pre-basic tissue culture plants                                                                                         |
| PCR                | Polymerase chain reaction                                                                                               |
| PLRV               | Potato leafroll virus                                                                                                   |
| PMTV               | Potato mop top virus                                                                                                    |
| poly-A tail        | Polyadenylated tail                                                                                                     |
| Pro                | Viral protease                                                                                                          |
| PSTVd              | Potato spindle tuber viroid                                                                                             |



|         |                                                 |
|---------|-------------------------------------------------|
| PTNRD   | Potato tuber necrotic ringspot disease          |
| PVA     | Potato virus A                                  |
| PVM     | Potato virus M                                  |
| PVS     | Potato virus S                                  |
| PVV     | Potato virus V                                  |
| PVX     | Potato virus X                                  |
| PVY     | Potato virus Y                                  |
| QP      | Quarantine pests                                |
| qPCR    | Quantitative polymerase chain reaction          |
| Rap     | Receptor-associated protein                     |
| RBP     | RNA-binding protein                             |
| RdRp    | RNA-dependent RNA polymerase                    |
| RNA     | Ribonucleic acid                                |
| RNQP    | Regulated non-quarantine pests                  |
| RT-PCR  | Reverse transcription polymerase chain reaction |
| sgRNA   | Subgenomic mRNA                                 |
| TGB     | Triple gene block                               |
| ToLCNDV | Tomato leaf curl New Delhi virus                |
| TRV     | Tobacco rattle virus                            |
| VPg     | Viral genome-linked protein                     |

# 1. Literature review

## 1.1 Potato production

Potato is considered one of the five most important food crops worldwide. The principal food crops like rice, wheat, and maize serve as the primary source of energy for billions of people, followed by root and tuber crops such as potatoes and cassava, which are especially significant in Europe, South America, and sub-Saharan Africa. Based on the information from Food and Agriculture Organization of the United Nations, the potato production area in the world has diminished in the last decade from 18 million hectares in 2014 to 16.8 million hectares in 2023 (the statistics collected on 07.07.2025). At the same time the yield of potatoes has increased from 20.5 t/ha to 22.8 t/ha. In Europe, the change has been even more drastic; the production area has dropped from 5.6 million hectares in 2014 to 4.1 million hectares in 2023, with over 25% decrease in production areas. Meanwhile, the yield has increased from 22.2 t/ha to 24.6 t/ha (FAOSTAT, 2025). In 2023, the biggest potato producers in Europe were Germany (24%), France (17.9%), the Netherlands (13.4%), and Poland (11.6%), forming almost 70% of total EU potato production (Eurostat, 2024).

Estonian potato production forms only 0.2% of European production but is an important economic sector locally. Historically, potato was introduced to Estonia in middle of eighteen century and was grown in gardens. Since 1860s, it became widely cultivated and important agronomic crop (Kahk, 1994). The peak of Estonian potato cultivation was in 1952, when the potato growing area was over 100 000 hectares (Kotkas, 2006). Since then, the industrial potato growing area has decreased drastically. Similarly to the world and Europe, the production areas have also shrunk remarkably in the last ten years. In Estonia, it has diminished approximately 45% (from 6350 ha in 2014 to 3457 ha in 2023), whereas the average yield has increased from 18.5 t/ha to 24.5 t/ha. Highest recorded yield in Estonia was in year 2020 with yield of 25.9 t/ha (Statistics Estonia, 2025). Compared to Europe and the world, the average yield has increased and reached the same level. Therefore, the improvement of Estonian potato production has been significant.

Potato yield is dependent on soil quality and fertility, weather conditions (temperature and rainfall), as well as pests and viral, bacterial, and fungal diseases. Resistance to diseases, adaption to local growing conditions, and a high seed tuber quality are crucial for good yields. Other key factors that improve potato production are irrigation and water availability, fertilizer use and nutrient management, crop rotation, general field management practices, and the timing of planting and harvesting.

## 1.2 Seed potato certification system in the EU and Estonia

The potato certification system plays a crucial role in the production and marketing of high-quality, healthy seed potatoes, thereby ensuring high yields and cost-effective potato production. There are standards that regulate seed potato production and phytosanitary procedures to ensure the quality of seed potatoes: United Nations Economic Commission for Europe (UNECE) *STANDARD S-1 concerning the marketing and commercial quality control of seed potatoes*, and European and Mediterranean Plant Protection Organization (EPPO) *PM 4/28 (2) Certification scheme for seed potatoes*. Those standards set the unified terminology and quality requirements of the high-quality seeds for planting and marketing all over the world. In the EU, seed potato production

and phytosanitary status are governed by *Regulation (EU) 2016/2031 on protective measures against pests of plants* and *Council Directive 2002/56/EC of 13 June 2002 on the marketing of seed potatoes*. Regulation establishes the general framework for the regulation of quarantine pests (QP) and union regulated non-quarantine pests (RNQP). QPs are not allowed in the EU plant trade or production system and the mother plants for pre-basic seed potatoes have to be free of following RNQPs: potato leaf roll virus (PLRV), potato virus Y (PVY), potato virus A (PVA), potato virus M (PVM), potato virus S (PVS), and potato virus X (PVX). Whereas council directive lays down specific certification and marketing requirements for seed potatoes including standards for varietal identity, varietal purity and phytosanitary quality. Its Annex I defines infection thresholds for blackleg disease (caused by *Dickeya* or *Pectobacterium*), *Candidatus Liberibacter solanacearum*, *Candidatus Phytoplasma solani*, potato spindle tuber viroid (PSTVd) and some viruses in seed potatoes. In addition, national authorities are responsible for the enforcement of the directive and may implement legislative standards exceeding EU basic requirements, reflecting local agronomic conditions and export objectives.

The economic losses due to those diseases are considered significant. For example, economic impact of potato blackleg and soft rot can be about 46 M EUR yearly in the EU (Dupuis et al., 2021). The annual economic losses caused by potato virus Y (PVY) are evaluated to be around 187 M EUR (Dupuis et al., 2024).

EU directives set the baseline tolerance for virus presence (mainly based on viral symptoms) in seed potatoes, but national authorities may use stricter standards. Virus tolerance limits are set based on the seed tuber categories. Seed potato classes are divided into three major categories: pre-basic, basic and certified. In Estonian official regulations, pre-basic is called Super Elite, and basic is called Elite. Finer classification distinguishes Super Elite seed potatoes (pre-basic tissue culture plants, marked as PBTC, and pre-basic potatoes grown in fields for the subsequent 3 years—marked as PB2, PB3, PB4, accordingly), Elite seed potatoes (marked as S, SE, and E) originating from Super Elite seed potatoes or higher-class Elite seed potatoes, and Certified seed potatoes (marked as A and B). There is a zero tolerance for viruses in the pre-basic tissue-culture (PBTC) potatoes and a 0.5% allowance in pre-basic (PB) seed potatoes (Table 1). Six potato viruses (PVA, PVY, PVM, PVS, PVX and PLRV) are monitored in Super Elite categories (PBTC and PB). Exceptionally, in the Netherlands, the PBTC is tested for additional viruses such as potato virus V (PVV), potato mop top virus (PMTV) and tobacco rattle virus (TRV), while the PB is only tested for PLRV and PVY. In lower class seeds (i.e. Elite and certified), the tested viruses and their limits are more variable in different member states depending on the national rules. For example, in France and Germany, all seed classes are tested for six viruses with some exceptions for some federal states in Germany where PVA and PVX are not tested. In Poland, Elite seed classes (S, SE and E) are tested for four viruses (PVM, PLRV, PVS and PVY) while certified seed classes (A and B) are tested only for PVM and PVY. Other countries, like the Netherlands, Latvia and Lithuania monitor the Elite and certified seed classes for PVY and PLRV, while in Estonia, those lower classes are tested only for PVY. In most EU countries, PVY is monitored and regulated in all seed potato categories. PVY levels are still the main reason why seed lots are often rejected during certification. PVY can be found in fields in various strains and variants, which lead to symptoms ranging from mild to severe. Most damaging strains are considered those that cause tuber necrotic ringspot disease (PTNRD) (Chikh-Ali et al., 2020). Currently, discrimination between the PVY strains is not mandatory in the certification system.

*Table 1. The seed potato classes, regulated viruses, and tolerated virus levels in the EU, and national requirements established in the biggest potato production countries (Germany, France, the Netherlands, and Poland), and in the Baltic countries. Personal communication with: Claudia Pöhler (Landwirtschaftskammer Niedersachsen, Germany); Sylvain Halftermeyer (French Interprofessional Organisation for Seeds and Plants, France); Inge van Duivenbode (Nederlandse Algemene Keuringsdienst, Netherlands); Justyna Pięcińska (Referencyjne Laboratorium Fitosanitarne, Poland); Jana Mihailova (State Plant Protection Service, Latvia) and Simona Žižienė (State Plant Service under the Ministry of Agriculture, Lithuania).*

| County          | Tolerated virus levels in seed classes <sup>1</sup> (%) |     |                     |      |     |                         |    | Tested viruses in Super Elite category (PBTC and PB)                                                                                           | Tested viruses in Elite and certified categories (S, SE, E, A, B) |
|-----------------|---------------------------------------------------------|-----|---------------------|------|-----|-------------------------|----|------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------|
|                 | Super Elite seed category                               |     | Elite seed category |      |     | Certified seed category |    |                                                                                                                                                |                                                                   |
|                 | PBTC                                                    | PB  | S                   | SE   | E   | A                       | B  |                                                                                                                                                |                                                                   |
| EU              | 0                                                       | 0.5 | 1                   | 2    | 4   | 8                       | 10 | PVA, PVX, PVM, PVS, PVY, PLRV                                                                                                                  |                                                                   |
| Germany         | 0                                                       | 0.5 | 1                   | 2    | 2   | 8                       | 10 | PVA <sup>1</sup> , PVX <sup>1</sup> , PVM, PVS, PVY, PLRV                                                                                      | PVA <sup>2</sup> , PVM, PVS, PVX <sup>2</sup> , PVY, PLRV         |
| France          | 0                                                       | 0.5 | 0.5                 | 1    | 2   | 5                       | 10 | PVA, PVX, PVM, PVS, PVY, PLRV                                                                                                                  | PVA, PVX, PVM, PVS, PVY, PLRV                                     |
| The Netherlands | 0                                                       | 0.5 | 0.5                 | 0.75 | 1.5 | 6                       | 10 | PVA <sup>3</sup> , PVX <sup>3</sup> , PVM <sup>3</sup> , PVS <sup>3</sup> , PVY, PLRV, PVV <sup>3</sup> , PMTV <sup>3</sup> , TRV <sup>3</sup> | PVY, PLRV                                                         |
| Poland          | 0                                                       | 0.5 | 1                   | 2    | 4   | 8                       | 10 | PVA, PVX, PVM, PVS, PVY, PLRV                                                                                                                  | PVM, PVS <sup>4</sup> , PVY, PLRV <sup>4</sup>                    |
| Latvia          | 0                                                       | 0.5 | 1                   | 2    | 4   | 8                       | 10 | PVA, PVX, PVM, PVS, PVY, PLRV                                                                                                                  | PVY, PLRV                                                         |
| Lithuania       | 0                                                       | 0.5 | 1                   | 2    | 4   | 8                       | 10 | PVA, PVX, PVM, PVS, PVY, PLRV                                                                                                                  | PVY, PLRV                                                         |
| Estonia         | 0                                                       | 0.5 | 1                   | 2    | 4   | 8                       | 10 | PVA, PVX, PVM, PVS, PVY, PLRV                                                                                                                  | PVY                                                               |

1 Seed potato classes: PBTC and PB in Super Elite category; S, SE, E in Elite category and certified A and B

2 not tested in some federal states

3 not tested in PB

4 not tested in the A and B classes

### 1.3 Potato viruses

Plant viruses are diverse groups of non-cellular obligate pathogens that infect wide spectrum of plant species, including potatoes. Plant viruses are composed of genetic material, either RNA or DNA, and surrounded by a protein coat. Plant viruses depend on their host cellular machinery for protein translation and for suitable conditions of their genome replication. Different viruses can cause diseases with mild or severe symptoms, depending on plant species and variety, and might lead to significant crop losses. Virus-infected wild plants stay often symptomless (Hasiów-Jaroszewska et al., 2021).

Potato is highly susceptible to viral diseases, and more than 60 viruses and one viroid have been reported worldwide to infect potato (Table 2). Most potato-infecting viruses are with positive-sense single-stranded RNA (+ssRNA) genomes. Out of all viruses infecting potatoes, only nine viruses and one viroid are considered to have global economic importance (Palukaitis, 2012). This list includes the viruses that are regulated in different EU countries by the potato certification system, i.e. PVA, PVY, PVM, PVS, PVX and PLRV but also three other viruses, i.e. PVV, PMTV, and TRV, and potato spindle tuber viroid (PSTVd) which are commonly not part of the certification system and, thus, are not widely monitored.

The nine mentioned viruses belong to following families: *Potyviridae* (PVY, PVA, PVV), *Alphaflexiviridae* (PVX), *Betaflexiviridae* (PVM and PVS), *Solemoviridae* (PLRV) and *Virgaviridae* (PMTV and TRV). Of those viruses, most damaging and the biggest threat to

potato industry are PVY and PLRV, with PVY overtaking the PLRV importance lately. Those two viruses can cause yield losses up to 80% either in single infection or if combined with other viruses (Kreuze et al., 2020a; Palukaitis, 2012). PVX in single infections can cause yield losses up to 40%, but in combinations with potyviruses (especially PVA and PVY) can cause yield losses up to 80%. Also, PVA can cause yield loss up to 40% but is considered less damaging than PVY, PVX or PLRV. PVS and PVM are usually responsible for only minor tuber yield losses but can be more severe in combination with other viruses (Kreuze et al., 2020a). Notably, PVY and PVX have been listed as Top 10 plant viruses in molecular plant pathology, while also PLRV has been mentioned among other important viruses (Scholthof et al., 2011). However, PVY was included in the Top 10 due to its significant economic impact and extensive genome variability, while PVX was involved because of being an important research model for virus-host interactions despite its diminished agricultural relevance due to effectiveness of seed certification systems (Scholthof et al., 2011; Úbeda et al., 2025).

Table 2. Potato-infecting viruses belonging to the species recognized by the International Committee on Taxonomy of Viruses (ICTV). Compiled from Kreuze et al. (2020a), Petrova et al. (2023), EFSA (Bragard et al., 2020d, van Gemert et al., 2024). Grey background indicates the viruses with worldwide importance, and bold font indicates viruses widely included to testing within the frame of seed certification system.

| Family            | Genus        | Binomial name                         | Virus                        | Acronym    | Transmission                                   | Distribution                            | Occurrence in potato                             |
|-------------------|--------------|---------------------------------------|------------------------------|------------|------------------------------------------------|-----------------------------------------|--------------------------------------------------|
| Alphaflexiviridae | Potexvirus   | <i>Potexvirus pepini</i>              | Pepino mosaic virus          | PepMV      | Mechanical, seeds                              | worldwide                               | Infective in experimental studies                |
|                   |              | <i>Potexvirus marmoracuba</i>         | Potato aucuba mosaic virus   | PAMV       | Mechanical, virus dependent aphid transmission | Worldwide                               | Sporadic or rare in potatoes                     |
|                   |              | <b><i>Potexvirus ecspotati</i></b>    | <b>Potato virus X</b>        | <b>PVX</b> | <b>Mechanical, tuber</b>                       | <b>Worldwide</b>                        | <b>Worldwide distributed viruses in potatoes</b> |
| Betaflexiviridae  | Carlavirus   | <i>Carlavirus chisolani</i>           | Potato virus H               | PVH        | Mechanical, tuber, aphids?                     | Asia                                    | Common or occasional viruses in potatoes         |
|                   |              | <b><i>Carlavirus misolani</i></b>     | <b>Potato virus M</b>        | <b>PVM</b> | <b>Aphids, mechanical, tuber</b>               | <b>Worldwide</b>                        | <b>Worldwide distributed viruses in potatoes</b> |
|                   |              | <i>Carlavirus pisolani</i>            | Potato virus P               | PVP        | Mechanical, tuber, aphids?                     | Brazil & Argentina                      | Common or occasional viruses in potatoes         |
|                   |              | <b><i>Carlavirus sigmasolani</i></b>  | <b>Potato virus S</b>        | <b>PVS</b> | <b>Mechanical, aphids, tuber</b>               | <b>Worldwide</b>                        | <b>Worldwide distributed viruses in potatoes</b> |
|                   |              | <i>Carlavirus trifolii</i>            | Red clover vein mosaic virus | RCVMV      | Aphids, mechanical                             | Worldwide                               | Single reports                                   |
|                   |              | <i>Carlavirus latensolani</i>         | Potato latent virus          | PotLV      | Mechanical, tuber, aphids                      | North America, rare                     | Sporadic or rare in potatoes                     |
|                   | Tepovirus    | <i>Tepovirus tafsolani</i>            | Potato virus T               | PVT        | Tuber, TPS, pollen and mechanical              | Southern Andean region                  | Common or occasional viruses in potatoes         |
| Bromoviridae      | Alfavirus    | <i>Alfavirus AMV</i>                  | Alfalfa mosaic virus         | AMV        | Aphids, mechanical, tuber                      | Worldwide                               | Sporadic or rare in potato                       |
|                   | Ilarvirus    | <i>Ilarvirus TSV</i>                  | Tobacco streak virus         | TSV        | Thrips assisted pollen, mechanical, tuber      | Worldwide, reported in potato in Brazil | Common or occasional viruses in potatoes         |
|                   | Cucumovirus  | <i>Cucumovirus CMV</i>                | Cucumber mosaic virus        | CMV        | Aphids, mechanically, tuber                    | Worldwide                               | Sporadic or rare in potatoes                     |
| Caulimoviridae    | Caulimovirus | <i>Caulimovirus tessellobrassicae</i> | Cauliflower mosaic virus     | CaMV       | Aphids, mechanical (experimental)              | Worldwide                               | Single reports                                   |

To be continued...

|                        | Genus              | Binomial name                           | Virus                                   | Acronym    | Transmission                                                       | Distribution                               | Occurrence in potato                                 |
|------------------------|--------------------|-----------------------------------------|-----------------------------------------|------------|--------------------------------------------------------------------|--------------------------------------------|------------------------------------------------------|
| <i>Closteroviridae</i> | <i>Crinivirus</i>  | <i>Crinivirus cucurbitae</i>            | Cucurbit yellow stunting disorder virus | CYSDV      | whitefly                                                           | Worldwide (warm and arid regions)          | Single reports                                       |
|                        |                    | <i>Crinivirus flaviorolani</i>          | Potato yellow vein virus                | PVVV       | Whiteflies, tuber                                                  | Northern Andean region, Panama             | Common or occasional viruses in potatoes             |
|                        |                    | <i>Crinivirus tomatichlorosis</i>       | Tomato chlorosis virus                  | ToCV       | Whiteflies                                                         | Worldwide                                  | Common or occasional viruses in potatoes             |
| <i>Geminiviridae</i>   | <i>Begomovirus</i> | <i>Begomovirus chilliapsici</i>         | Chilli leaf curl virus                  | ChiLCV     | whitefly, tuber                                                    | Asia                                       | Common or occasional viruses in potatoes             |
|                        |                    | <i>Begomovirus tuberosi</i>             | Potato yellow mosaic virus              | PYMV       | Whiteflies, tuber                                                  | Caribbean and South America                | Sporadic or rare in potatoes                         |
|                        |                    | <i>Begomovirus solanumdelhiense</i>     | Tomato leaf curl New Delhi virus        | ToLCNDV    | Whiteflies, tuber, low frequency transmission via seeds or pollen  | Asia, Europe and North Africa              | Common or occasional viruses in potatoes             |
|                        |                    | <i>Begomovirus solanumhavanaense</i>    | Tomato mosaic Havana virus              | ToMHaV     | whitefly, tuber                                                    | Central America                            | Sporadic or rare in potatoes                         |
|                        |                    | <i>Begomovirus solanumtainoense</i>     | Tomato mottle Taino virus               | ToMoTV     | Whiteflies, tuber                                                  | Cuba                                       | Common or occasional viruses in potatoes             |
|                        |                    | <i>Begomovirus solanumseverugosi</i>    | Tomato severe rugose virus              | ToSRV      | Whiteflies, tuber                                                  | Brazil                                     | Common or occasional viruses in potatoes             |
|                        |                    | <i>Begomovirus coheni</i>               | Tomato yellow leaf curl virus           | TYLCV      | Whiteflies, seeds (in tomatoes)                                    | Worldwide                                  | Single reports                                       |
|                        |                    | <i>Begomovirus solanumflavusvenae</i>   | Tomato yellow vein streak virus         | ToYVSV     | Whiteflies, tuber                                                  | South America                              | Common or occasional viruses in potatoes             |
|                        | <i>Curtovirus</i>  | <i>Curtovirus betae</i>                 | Beet curly top virus                    | BCTV       | Leaf hopper                                                        | America, Europe, Asia under dry conditions | Common or occasional viruses in potatoes             |
| <i>Pospiviroidae</i>   | <i>Pospiviroid</i> | <i>Pospiviroid fusituberis</i>          | Potato spindle tuber viroid             | PSTVd      | Mechanical, tuber, TPS, pollen, virus dependent aphid transmission | Worldwide                                  | Worldwide occurrences, mostly eradicated in potatoes |
| <i>Potyviridae</i>     | <i>Potyvirus</i>   | <i>Potyvirus phaseovulgaris</i>         | Bean common mosaic virus                | BCMV       | Aphids, mechanical, seeds                                          | Worldwide                                  | Single reports                                       |
|                        |                    | <i>Potyvirus papayanuli</i>             | Papaya ringspot virus                   | PRSV       | Aphids, mechanical                                                 | Worldwide                                  | Single reports                                       |
|                        |                    | <b><i>Potyvirus atuberosi</i></b>       | <b>Potato virus A</b>                   | <b>PVA</b> | <b>Aphids, mechanical, tuber</b>                                   | <b>Worldwide</b>                           | <b>Worldwide distributed viruses in potatoes</b>     |
|                        |                    | <i>Potyvirus vetuberosi</i>             | Potato virus V                          | PVV        | Aphids, mechanical, tuber                                          | Europe, South America                      | Common or occasional viruses in potatoes             |
|                        |                    | <b><i>Potyvirus yituberosi</i></b>      | <b>Potato virus Y</b>                   | <b>PVY</b> | <b>Aphids, mechanical, tuber</b>                                   | <b>Worldwide</b>                           | <b>Worldwide distributed viruses in potatoes</b>     |
|                        |                    | <i>Potyvirus tuberosiflavi</i>          | Potato yellow blotch virus              | PYBV       | Mechanical, aphid?, tuber (experimentally transmittable)           | United Kingdom                             | Single reports                                       |
|                        |                    | <i>Potyvirus cucurbitaflaviteselati</i> | Zucchini yellow mosaic virus            | ZYMV       | Aphids, mechanical, seeds                                          | Worldwide                                  | Single reports                                       |
|                        |                    | <i>Potyvirus citrulli</i>               | Watermelon mosaic virus                 | WMV        | Aphids, mechanical                                                 | Worldwide                                  | Single reports                                       |
|                        |                    | <i>Potyvirus muricati</i>               | Wild potato mosaic virus                | WPMV       | Aphids, mechanical                                                 | Andes                                      | Only reported in wild potatoes                       |

To be continued...

|                      |                               |                                              |                                              |             |                                                         |                                                |                                                  |
|----------------------|-------------------------------|----------------------------------------------|----------------------------------------------|-------------|---------------------------------------------------------|------------------------------------------------|--------------------------------------------------|
| <i>Rhabdoviridae</i> | <i>Alphanucleorhabdovirus</i> | <i>Alphanucleorhabdovirus tuberosum</i>      | Potato yellow dwarf virus                    | PYDV        | Leafhoppers (natural), mechanical, tuber (experimental) | North America                                  | Sporadic or rare in potatoes                     |
|                      | <i>Nucleorhabdovirus</i>      | <i>Alphanucleorhabdovirus melongenae</i>     | Eggplant mottle dwarf alphanucleorhabdovirus | EMDV        | Leafhoppers, mechanical (experimental)                  | Europe, Africa, Asia                           | Sporadic or rare in potatoes                     |
| <i>Secoviridae</i>   | <i>Cheravirus</i>             | <i>Cheravirus arracaciae</i>                 | Arracacha virus B                            | AVB         | TPS, pollen, tuber, mechanical                          | Andes                                          | Common or occasional viruses in potatoes         |
|                      |                               | <i>Cheravirus avii</i>                       | Cherry rasp leaf virus                       | CRLV        | Nematode, mechanical, seed                              | North America, China                           | Single reports                                   |
|                      | <i>Comovirus</i>              | <i>Comovirus andesense</i>                   | Andean potato mottle virus                   | APMoV       | Beetles, mechanically, tuber                            | Andean region, Brazil                          | Common or occasional viruses in potatoes         |
|                      | <i>Nepovirus</i>              | <i>Nepovirus betae</i>                       | Beet ringspot virus                          | BRSV        | Nematod, pollen, seeds                                  | Primarily Europe                               | Single reports                                   |
|                      |                               | <i>Nepovirus avii</i>                        | Cherry leaf roll virus                       | CLRV        | Seeds, pollen, mechanical (experimental)                | Worldwide                                      | Common or occasional viruses in potatoes         |
|                      |                               | <i>Nepovirus solani</i>                      | Potato black ringspot virus                  | PBRV        | Mechanical, tuber, TPS, pollen                          | Peru                                           | Common or occasional viruses in potatoes         |
|                      |                               | <i>Nepovirus betasolani</i>                  | Potato virus B                               | PVB         | Mechanical, tuber, nematodes?                           | Peru, recently reported, relatively common     | Common or occasional viruses in potatoes         |
|                      |                               | <i>Nepovirus nigranuli</i>                   | Tomato black ring virus                      | TBRV        | Nematodes, seeds, pollen, mechanical, tuber             | Europe, Asia, Africa                           | Sporadic or rare in potatoes                     |
|                      |                               | <i>Nepovirus usolani</i>                     | Potato virus U                               | PVU         | Nematodes, mechanical, tubers                           | Peru                                           | Single reports                                   |
| <i>Solemoviridae</i> | <i>Polerovirus</i>            | <b><i>Polerovirus PLRV</i></b>               | <b>Potato leaf roll virus</b>                | <b>PLRV</b> | <b>Aphids, tuber</b>                                    | <b>Worldwide</b>                               | <b>Worldwide distributed viruses in potatoes</b> |
| <i>Tombusviridae</i> | <i>Alphanecrovirus</i>        | <i>Alphanecrovirus solani</i>                | Potato necrosis virus                        | PoNV        | Mechanical? (experimentally)                            | United Kingdom                                 | Single reports                                   |
|                      | <i>Betanecrovirus</i>         | <i>Betanecrovirus nicotianae</i>             | Tobacco necrosis virus D                     | TNV D       | Olpidium brassicae                                      | Worldwide (prevalent in moist soils)           | Sporadic or rare in potatoes                     |
|                      | <i>Luteovirus</i>             | <i>Luteovirus phaseoli</i>                   | Bean leafroll virus                          | BLRV        | Aphids                                                  | Worldwide                                      | Single reports                                   |
| <i>Tospoviridae</i>  | <i>Orthotospovirus</i>        | <i>Orthotospovirus arachinecrosis</i>        | Groundnut bud necrosis orthotospovirus       | GBNV        | Thrips, mechanical (experimental)                       | Asia                                           | Common or occasional viruses in potatoes         |
|                      |                               | <i>Orthotospovirus arachianuli</i>           | Groundnut ringspot orthotospovirus           | GRSV        | Thrips, mechanical (experimental)                       | Americas                                       | Sporadic or rare in potatoes                     |
|                      |                               | <i>Orthotospovirus impatiensnecromaculae</i> | Impatiens necrotic spot virus                | INSV        | Thrips, mechanical                                      | Worldwide                                      | Sporadic or rare in potatoes                     |
|                      |                               | <i>Orthotospovirus tomatoflavi</i>           | Tomato chlorotic spot orthotospovirus        | TCSV        | Thrips, mechanical (experimental)                       | South America, Caribbean, southern part of USA | Common or occasional viruses in potatoes         |
|                      |                               | <i>Orthotospovirus tomatomaculae</i>         | Tomato spotted wilt orthotospovirus          | TSWV        | Thrips, tuber                                           | Worldwide                                      | Common or occasional viruses in potatoes         |
|                      |                               | <i>Orthotospovirus tomatanuli</i>            | Tomato yellow ring orthotospovirus           | TYRV        | thrips, tuber, mechanical                               | Iran, Kenya, Poland                            | Single reports                                   |

To be continued...



|                     |                    |                                     |                                   |       |                                         |                                                       |                                           |
|---------------------|--------------------|-------------------------------------|-----------------------------------|-------|-----------------------------------------|-------------------------------------------------------|-------------------------------------------|
| <i>Tymoviridae</i>  | <i>Tymovirus</i>   | <i>Tymovirus latandigenum</i>       | Andean potato latent virus        | APLV  | Beetles, mechanically, TPS, tuber       | Andean region                                         | Common or occasional viruses in potatoes  |
|                     |                    | <i>Tymovirus mosandigenum</i>       | Andean potato mild mosaic virus   | APMMV | Beetles, mechanically, TPS, tuber       | Andean region                                         | Common or occasional viruses in potatoes  |
|                     |                    | <i>Tymovirus belladonnae</i>        | Belladonna mottle virus           | BeMV  | Beetles, mechanically, tuber            | Europe                                                | Single reports                            |
| <i>Virgaviridae</i> | <i>Pomovirus</i>   | <i>Pomovirus colombiense</i>        | Colombian potato soil-borne virus | CPSbV | Spongospora subterranea                 | Colombia                                              | Common or occasional viruses in potatoes  |
|                     |                    | <i>Pomovirus solani</i>             | Potato mop-top virus              | PMTV  | Spongospora subterranea, tuber          | Americas, Europe, Asia in cool and humid environments | Common or occasional viruses in potatoes  |
|                     | <i>Tobamovirus</i> | <i>Tobamovirus tabaci</i>           | Tobacco mosaic virus              | TMV   | Mechanical, tubers                      | Worldwide, rare on potato                             | Sporadic or rare in potatoes              |
|                     |                    | <i>Tobamovirus tomatotessellati</i> | Tomato mosaic virus               | ToMV  | Mechanical, tubers, seeds (in tomatoes) | Worldwide                                             | Sporadic or rare in potatoes              |
|                     | <i>Tobravirus</i>  | <i>Tobravirus tabaci</i>            | Tobacco rattle virus              | TRV   | Nematodes, mechanical                   | Worldwide                                             | Worldwide distributed viruses in potatoes |

### 1.3.1 Family *Potyviridae*

Realm: *Riboviria*, Kingdom: *Orthornavirae*, Phylum: *Pisuviricota*, Class: *Stelpaviricetes*, Order: *Patatavirales*.

*Potyviridae* is the largest family of known plant RNA viruses. There are 13 genera with more than 259 virus species, including economically important potato viruses like PVY, PVA and PVV (all three belong to the biggest genus *Potyvirus*). Members of this family have mostly monopartite filamented flexuous particles with 11 to 20 nanometre (nm) in diameter and with various lengths between 650 to 950 nm, depending on the genera. Virions are made of one type of coat protein (CP), and these contain usually single molecules of +ssRNA genome with the length between 8.2 kb to 11.5 kb. Exceptionally, viruses belonging to the genus *Bymovirus* are bipartite, having two particles in different length: 250 to 300 nm, and 500 to 600 nm. *Bymoviruses* have two genomic RNA molecules (RNA1: 7.3 to 7.6 kb and RNA2: 3.5 to 3.7 kb). *Potyvirid* genome has a VPg (viral genome-linked protein) attached in the 5' end and a poly-A tail in 3' end (Figure 1). The +ssRNA genome usually encodes one big polyprotein and smaller polyprotein that are identical in their 5' end and are processed to mature, functional proteins. The gene order is conserved in the family. Transmission of viruses belonging in *Potyviridae* family occurs through various vectors; viruses belonging to *Potyvirus* genus are transmitted by aphid vectors in non-persistent manner. Other potyvirid transmission vectors are reported to be mites, whiteflies and plasmodiophorids (Inoue-Nagata et al., 2022).

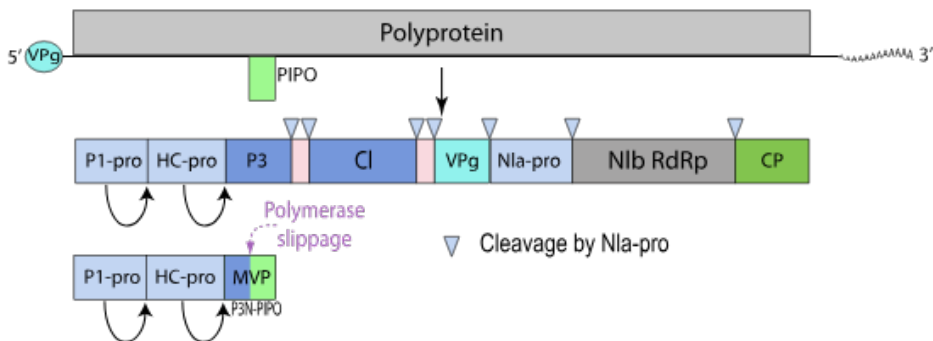


Figure 1. *Potyvirus* genome organization. Genome is monopartite linear +ssRNA of 10 kb in size. The 3' terminus has a poly-A tail. The 5' terminus has a genome-linked protein (VPg). Virion RNA is infectious and serves as both the genome and viral messenger RNA. The genomic RNA is translated into polyproteins, and the polyproteins are then processed into functional products by the action of three virus-encoded proteinases. P3-PIPO is expressed from genomic RNA that includes an extra A-nucleotide generated by polymerase slippage. The figure taken from the ViralZone web page.

#### 1.3.1.1 Potato virus Y

Family: *Potyviridae*, Genus: *Potyvirus*, Species: ***Potyvirus yituberosi***, known as potato virus Y (PVY).

Potato virus Y (PVY) is a well-known plant virus considered as one of the most damaging viruses in the potato industry (Kreuze et al., 2020a). PVY genome exhibits high rates of mutation and recombination events, which has led to numerous strain lineages and variants (Karasev and Gray, 2013; Lacomme et al., 2017; Green et al., 2017; 2018; Glasa et al., 2021, Samarskaya et al., 2025). PVY particles are flexuous, filamentous rod-shaped virions typical to genus *Potyvirus*. The particle measures approximately 730 nm

in length and 12 to 13 nm in diameter and is coated by approximately 2000 copies of viral CP. PVY has a +ssRNA genome with approximate length of 9704 nt covalently attached to VPg at the 5' end, and it has a poly-A tail at the 3' end (Quenouille et al., 2013). The genome encodes one large and one small polyprotein. The large polyprotein is subsequently processed into 10 functional proteins, including the viral CP and VPg. Smaller polyprotein is cleaved to three functional proteins: P1, HCPro and P3-PIPO. RNA transcript for smaller polyprotein expression is generated by addition of extra-A nucleotide during transcription due to a polymerase slippage (Olspert et al., 2015).

PVY has a wide host range, it has been reported to infect almost 500 plant species (Kerlan and Moury, 2008). The cultivated species which can be infected mostly belong to Solanaceae family, while weeds that could be infected can be both solanaceous and non-solanaceous species. PVY is causing severe diseases in cultivated hosts such as potato, tobacco, tomato, and pepper (Quenouille et al., 2013, Lacomme et al., 2017). PVY is transmitted in non-persistent manner by over 40 different aphid species and vegetatively through tubers. Symptoms, both foliar and tuber, caused by PVY vary greatly and depend on the host (species and variety), virus strain, environmental conditions, and infection origin (tuber-borne or vector-borne). Typical foliar symptoms include mosaic patterns, veinal necrosis, leaf drop, chlorotic spots, and leaf roughness. Some PVY strains induce PTNRD in susceptible varieties. Yield losses due to PVY are up to 80% or more, highly depending on virus strain and potato variety (Kreuze et al., 2020a).

PVY has strains and genetic variants which are identified and classified mostly based on their genomes and recombination patterns (Glais et al., 2017; Green et al., 2018). The strains of PVY are variable on biological, serological and molecular levels (Lacomme et al., 2017). Overall, there are more than 40 PVY strains reported, with five non-recombinant variants such as PVY<sup>O</sup>, PVY<sup>N</sup>, PVY<sup>Na-N</sup>, PVY<sup>O-05</sup> and PVY<sup>C</sup>. Out of those variants, PVY<sup>O</sup>, PVY<sup>N</sup> and PVY<sup>C</sup> are considered as parental strains for recombinants with PVY<sup>O</sup> and PVY<sup>N</sup> being the most common parental strains (Glais et al., 2002; Lorenzen et al., 2006; Green et al., 2018; Lacomme et al., 2017). More common and widespread recombinants are considered to belong to following strain groups: N:O, N-Wi, NTNa, NTNb, NE11, E, SYR-I, -II, and -III (Karasev and Gray, 2013; Green et al., 2017). Not so common strains are, for example: PVYN-Wi-156var, PVYN-Wi-261-4, PVY-SCRI-N, PVY-FrN, PVY-Nicola, PVY-T13, and PVY-nnp (Green et al., 2017). The most common recombination junctions (RJ) shown in Figure 2, are remarkably conserved in their positions, despite the variety of the recombinant strains that have been described (Lorenzen et al., 2006; Green et al., 2017). To date, the recombinant strains have mostly replaced the parental strains in potatoes, probably due to their less apparent foliar symptoms lowering the effectiveness of rogueing during phytosanitary inspections (Funke et al. 2017; Gray and Power, 2018, MacKenzie et al., 2018; Rushton et al., 2024).

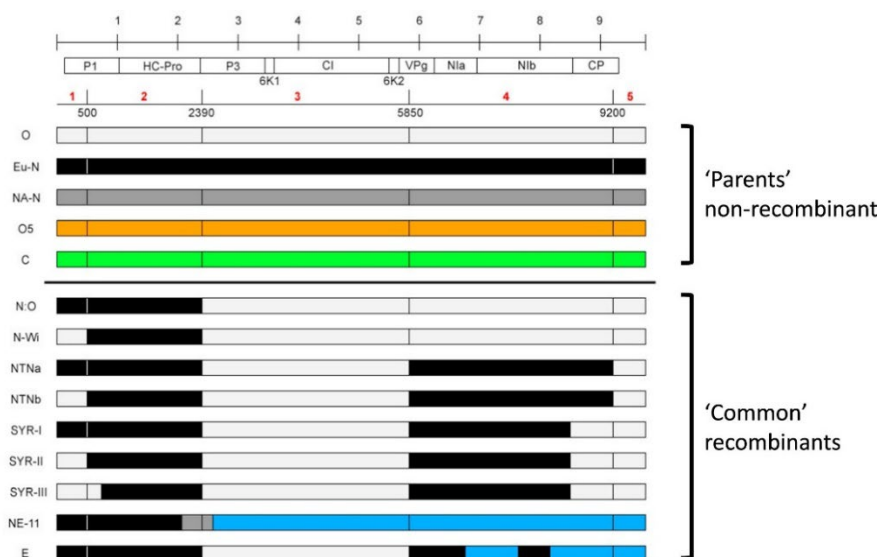


Figure 2. A schematic diagram of most common potato virus Y (PVY) recombinant genomes. The ruler at the top represents the PVY genome (ca. 9.7-kb); individual cistrons are presented as rectangles below that with corresponding protein names. Parental sequences are grouped above the black horizontal dividing bar, with different parents coloured differently. Recombinants are below the black bar, and fragments originating from different parents are coloured accordingly. The blue parent is a yet unknown strain which has never been found as a non-recombinant. The five major recombinant sections, based on common breakpoints, are designated in red numbers and the locations of breakpoints are given below that. Figure has been modified from Green et al. (2017).

Resistance to PVY in potato is primarily categorized as a hypersensitive response (HR) or an extreme resistance (ER). HR, mediated by strain specific genes, such as *Ny*, *Nc* and *Nz*, involves localized cell death (necrotic lesions) at the infection site to restrict viral spread. In contrast, ER provides broad-spectrum, durable protection by suppressing viral replication in the initially infected cells. Key ER genes incorporated from wild relatives of potato include *Ry<sub>adg</sub>*, *Ry<sub>sto</sub>* and *Ry<sub>chc</sub>* (Chebte et al., 2025; Valkonen, 2015; Kneib et al., 2017). While key loci for resistance to PVY have been molecularly characterized in some potato varieties, the presence and allelic status of these genes remain unidentified across the vast majority of the thousands of potato cultivars existing globally (Chebte et al., 2025).

### 1.3.1.2 Potato virus A

Family: *Potyviridae*, Genus: *Potyvirus*, Species: ***Potyvirus atuberosi***, known as potato virus A (PVA).

Potato virus A is an important plant pathogen that belongs to genus *Potyvirus* and has a close relationship to potato virus Y. The virus has flexuous filamentous helically constructed virions which are 730 nm in length and 15 nm in diameter, composed of virus encoded CP. Genome is +ssRNA that is 9565 nt long and with genome organization typical to genus *Potyvirus* (Fuentes et al., 2021a). PVA host range is limited to the family Solanaceae, and it has been reported to infect potato crops worldwide. Transmission of the virus occurs by aphids in non-persistent matter, although it can be transmitted also mechanically. The virus usually causes mild foliage symptoms or symptomless infection

but only on the cultivars lacking PVA hypersensitivity genes *Na* and *Na<sub>KE</sub>* (Valkonen et al., 1995). Yield losses caused by PVA might be up to 40% or even greater if combined with other potato viruses like PVY, PVS or PLRV (Kreuze et al., 2020a).

Like PVY, PVA has several strain groups, initially identified based on their reaction on potato cultivars (Valkonen et al., 1995; Rajamäki et al., 1998) but re-grouped later according to phylogenetic relationships. Three main recognized phylogroups are: A, including isolates found only in the Andes; W, including isolates distributed worldwide, but also in the Andes; and T, including isolates from tamarillo (*Solanum betaceum*) (Fuentes et al., 2021a).

### 1.3.1.3 Potato virus V

Family: *Potyviridae*, Genus: *Potyvirus*, Species: ***Potyvirus vetuberosi***, known as potato virus V (PVV).

Potato virus V is a plant virus belonging to the genus *Potyvirus*. PVV originates from Andean region but is now spread in South and Central America, Europe, and also in Middle East. In Europe, it has sporadically been reported from Ireland, Finland, France, Germany, Norway, Sweden, the Netherlands, and the United Kingdom (Fuentes et al., 2022). The characteristics of the PVV virions are typical to potyviruses, being flexuous filaments with 760 nm in length. The genome is +ssRNA, like all other potyviruses, and with a length of 9851 nt (Oruetebarria et al., 2000). Natural infection of PVV has only been reported on cultivated and wild potatoes, meaning that the host range of PVV is limited (Jones and Fribourg, 1986; Shiel et al., 2004; Gutiérrez et al., 2016). The virus is reported to be transmitted by aphid vectors in non-persistent manner (Fuentes et al., 2022). The virus causes mild or no foliar symptoms but can cause tuber symptoms when coinfecting with PVA (Carnegie and McCreath, 2010). Current sources do not offer reliable information on yield losses.

There exist several reports about different strains and variances of PVV with suggestions that strain diversity is geographically structured, showing relatively little variation within the regions but pronounced divergence between the continents. Phylogenetic studies show that there are two major phylogroups further divided into five minor phylogroups: the Andean specific A1 to A4, and the World (W) group (Fuentes et al., 2022).

### 1.3.2 Family *Alphaflexiviridae*

Realm: *Riboviria*, Kingdom: *Orthornavirae*, Phylum: *Kitrinoviricota*, Class: *Alsuviricetes*, Order: *Tymovirales*.

*Alphaflexiviridae* is a family comprising plant and fungus-infecting viruses. The family includes 72 species across six genera: *Allexivirus*, *Lolavirus*, *Platypuvirus*, *Potexvirus* (plant-infecting species only), and *Botrexvirus* and *Sclerodarnavirus* (fungus-infecting species only) (Úbeda et al. 2025). PVX (*Potexvirus ecspotati*) is considered the most damaging potato-infecting virus within the members of *Alphaflexiviridae* and it belongs to genus *Potexvirus* that is the biggest in the family with more than 50 species. Viruses belonging in this family have flexuous filaments with variable length between 470 and 800 nm having a diameter around 12 to 13 nm. In most members of the family, the viral capsid is composed of a single polypeptide, and it contains one linear molecule of +ssRNA. Genome size is variable and it ranges from 5.4 to 9 kb; the genome possesses 7-methylguanosine cap structure (m7G) at the 5' and poly(A) tail at 3' end. Most members of the family have five to seven open reading frames (ORFs), and they share unique

lineage of alphavirus-like replication proteins (Figure 3). In plant-infecting members of the family (except *Platypuvirus*), a conserved set of genes called triple gene block (TGB) is present. All plant-infecting viruses in the family are easily transmitted by mechanical inoculation and many of them have no known invertebrate or fungal vectors (Kreuze et al., 2020b; Úbeda et al., 2025).

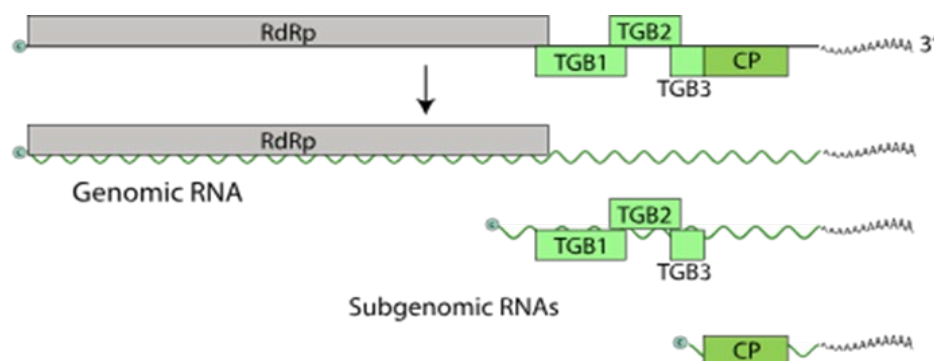


Figure 3. Potexvirus genome organization. Linear +ssRNA genome is 5.9-7 kb in size. The 5' end is capped, and the 3' terminus is polyadenylated. Encodes for 5 proteins. The virion-packed RNA is infectious and serves as both the genome and a viral messenger RNA. RNA-dependent RNA polymerase (RdRp) is translated directly from the genomic RNA. The other ORFs are expressed from subgenomic mRNAs (sgRNAs). SgRNA1 serves as a polycistronic template that expresses all TGBs via a leaky scanning mechanism of ribosomes. In addition, sgRNA2 (not shown) expresses TGB2 and TGB3 in lower abundances. CP is expressed from its own subgenomic RNA. The figure taken from the ViralZone web page.

### 1.3.2.1 Potato virus X

Family: *Alphaflexiviridae*, Genus: *Potexvirus*, Species: ***Potexvirus ecspotati***, known as potato virus X (PVX).

Potato virus X is globally distributed plant virus primarily infecting potatoes, and it is a nominal species in the genus *Potexvirus*. PVX was previously considered a major concern in seed potato production, but due to the certification system effectiveness the impact of PVX damage has diminished (Bragard et al., 2020c). Virions are relatively short flexuous filaments, with 460 to 480 nm in length and 13 nm diameter, consisting approximately 1300 copies of CP. Genome of the virus is +ssRNA with the length of 6400 nt having a cap structure at the 5' end and a poly-A tail at the 3' end (Verchot, 2022; Kreuze et al., 2020b). The genome contains five ORFs that encode five proteins. While potato is the primary host, PVX also infects several other solanaceous plants such as tomato and tobacco. PVX is commonly spread through pollen, true seeds or via mechanical transmission and it does not have known invertebrate vector (Verchot, 2022). Usually, PVX induces mild mosaic in a foliage of solanaceous plants, but it can cause tuber yield loss up to 40% in single infection and even more in combination with PVY or PVA (Kreuze et al., 2020a).

Potato virus X strains were initially classified into four distinct groups based on their ability to overcome host resistance genes (*Nx*, *Nb* and *Rx*). Similar classification was obtained by serology based on recognition of epitopes of the coat protein (Cockerham, 1970). At genomic scale, the phylogenetic study revealed divergence into two main clades: Phylogroup I and II, which further resolve into five smaller subgroups. In case of

PVX strains, biological and phylogenetical differentiation is not always in concordance (Fuentes et al., 2021b).

### 1.3.3 Family *Betaflexiviridae*

Realm: *Riboviria*, Kingdom: *Orthornavirae*, Phylum: *Kitrinoviricota*, Class: *Alsuviricetes*, Order: *Tymovirales*.

*Betaflexiviridae* is a family that includes plant-infecting viruses. This family includes two subfamilies, fifteen genera and 152 species. Out of known potato infecting viruses, five belong to *Carlavirus* and one to *Tepovirus* genera. The important potato infecting viruses PVM and PVS both belong to *Carlavirus* genus. Viruses in the family have filamentous virions with a diameter of 12 nm and within a length between 600 to 1000 nm. Genomes of these viruses are single linear molecules of +ssRNA with the length from 6.4 to 9.5 kb and with poly-A tail at the 3' end and likely a cap in the 5' end (Figure 4). ORFs number vary from two to six (Rubino, 2024). High genetic variability has been observed in populations of several *Carlavirus* species, including PVS and PVM (Cox and Jones, 2010; Tabasinejad et al., 2014; Plchová et al., 2014).

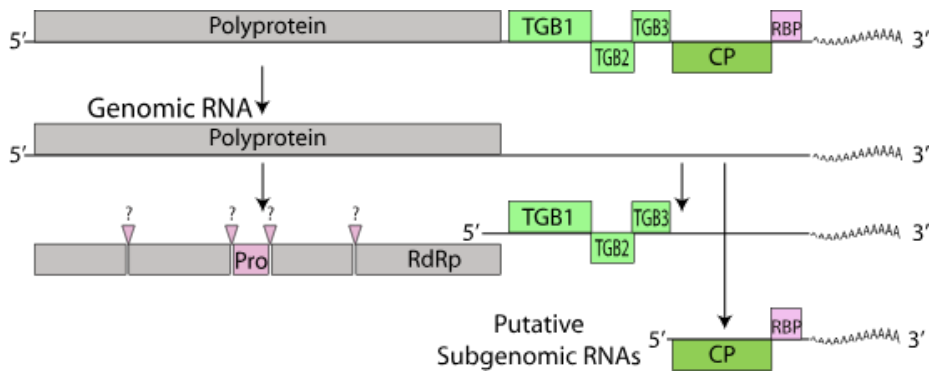


Figure 4. *Carlavirus* genome organization. Linear +ssRNA genome is of 5.8-9 kb in size. The 3' terminus is polyadenylated and, in some genera, the 5' end is capped. *Carlaviruses* encode 3 to 6 proteins. The virion RNA is infectious and serves as both the genome and viral messenger RNA. RNA-dependent RNA polymerase (RdRp) is translated directly from the genomic RNA as a part of polyprotein processed to several proteins with different functions (here: Pro marks a viral protease). The other ORFs are expressed from subgenomic mRNAs (sgRNAs), this includes TGBs (triple gene block proteins), CP (coat protein), and RBP (an RNA-binding protein). The figure taken from the ViralZone web page.

#### 1.3.3.1 Potato virus M

Family: *Betaflexiviridae*, Genus: *Carlavirus*, Species: ***Carlavirus misolani***, known as potato virus M (PVM).

Potato virus M has a global distribution (Bragard et al., 2020a). It has filamentous particles with length of 650 nm and diameter of 12 nm. Viral genome +ssRNA with the length of 8530 nt, having six individual ORFs. The genome has a cap structure in the 5' end, and with the poly-A tail in the 3' end (Tabasinejad et al., 2014). PVM host range is limited to solanaceous species where it causes mottling, mosaic, crinkling, leaflet deformation, and stunting of shoots (Xu et al., 2010). PVM causes mild to severe symptoms in potatoes, depending on viral strain, and potato variety. The virus is naturally transmitted in non-persistent manner by aphids (Robert and Bourdin, 2001). In general,

PVM causes minor tuber yield losses, except when co-infected with other more severe viruses such as PVX or PVY (Kreuze et al., 2020a).

PVM isolates groups into two phylogenetically divergent strain groups: PMV-O, the ordinary, and PVM-D, the divergent (Tabasinejad et al., 2014).

### 1.3.3.2 Potato virus S

Family: *Betaflexiviridae*, Genus: *Carlavirus*, Species: ***Carlavirus sigmasolani***, known as potato virus S (PVS).

Potato virus S is among the most widespread viruses affecting potato worldwide, and it is considered as the fourth most important potato virus after PVY, PLRV and PVX (Kreuze et al., 2020a). PVS virions are flexuous filaments in length of 610 to 710 nm and in diameter of 12 to 15 nm and the genome is a +ssRNA with the approximate length up to 8500 nt, having the cap structure in 5' end and poly-A tail in 3' end with six ORFs in-between (Gutiérrez 2013; Matousek et al., 2005). Aphids transmit PVS in non-persistent manner, while mechanical means and vegetative propagation are also highly effective (Robert and Bourdin, 2001). Host range is limited with susceptible species mostly belonging in the Solanaceae or Chenopodiaceae families. Infection with PVS usually results in either mild symptoms (leaf mottling or rugosity) or symptomless disease in potatoes, and yield losses are minor. In case of severe PVS strain, the yield losses can reach up to 20% and co-infection with other viruses can result in even higher losses (Kreuze et al., 2020a).

Similarly to PVM, PVS also has two strain classifications well established over several studies. Historically, the differentiation was done based on the observation that ordinary PVS isolates (PVS-O) caused only local lesions in *Chenopodium quinoa*, while PVS isolates from the Andean region (PVS-A) caused systemic infection (Matousek et al 2005). More detailed studies indicate that there are three phylogenetic lineages (PVS-O, PVS-A, and PVS-RVC; the latter represents the distinct lineage of the Andean isolates infecting *Solanum phureja* and *S. tuberosum* ssp. *andigena*), while also several recombinant isolates exist (Santillan et al. 2018; Topkaya et al. 2023).

### 1.3.4 Family *Solemoviridae*

Realm: *Riboviria*, Kingdom: *Orthornavirae*, Phylum: *Pisuviricota*, Class: *Pisoniviricetes*, Order: *Sobelivirales*

There are five genera, with 120 species of plant viruses in the *Solemoviridae* family: *Enamovirus*, *Polemovirus*, *Polerovirus*, *Sobemovirus* and *Hubsclerovirus* (Sömera et al., 2021; Simmonds et al., 2024). The first four genera include plant viruses, while the fifth accommodates fungal viruses. Viruses in the family *Solemoviridae* have icosahedral particles with 26 to 34 nm in diameter. The genomes are relatively small, usually varying from four to six kb in length, and are +ssRNA genomes with a VPg at the 5' end and without a poly-A tail in the 3' end. Number of ORFs vary between genera and is from four in genus *Enamovirus* to ten in genus *Polerovirus* (Figure 5). Transmission of the viruses can be mechanical, by vegetative propagation, through soil or by insects. *Polerovirus* transmission occurs almost always by aphids. Potato leafroll virus (PLRV), a nominal species belonging to genus *Polerovirus* is a serious threat to the potato industry.



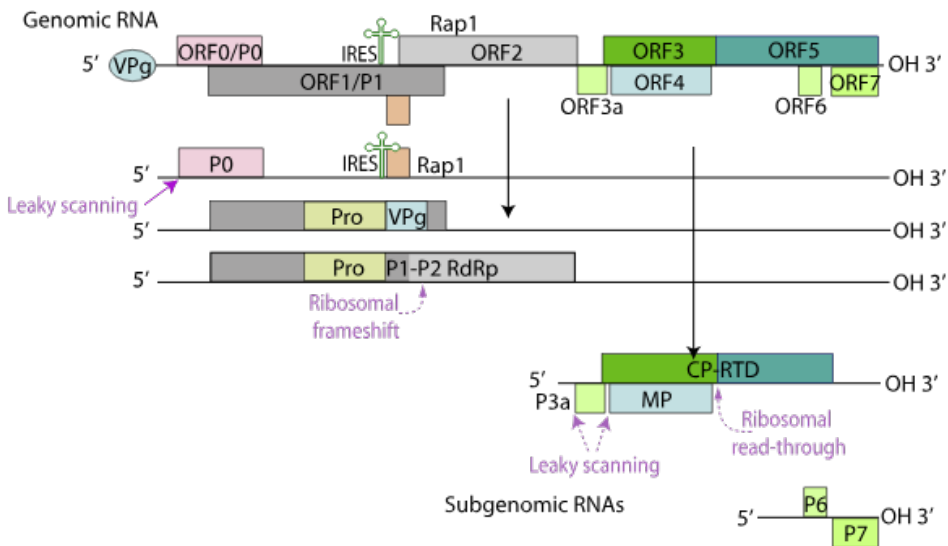


Figure 5. Polerovirus genome organization. Monopartite linear +ssRNA genome is of 5.3-5.7 kb in size with a viral genome-linked protein (VPg) bound at the 5' end. There is no poly-A tail or tRNA-like structure at the 3' end. The virion RNA is infectious and serves as both the genome and viral messenger RNA. ORF0 encodes P0, a viral suppressor of RNA silencing. ORF1 encodes a viral polyprotein processed to smaller functional proteins, including protease (Pro) and VPg. The polymerase is expressed by a ribosomal frameshifting near the end of ORF1 into ORF2 frame. Rap1 translation initiates about 1500 nt downstream of the 5' end of the genomic RNA (gRNA) by internal ribosome entry site (IRES). The P3a protein, movement protein (MP) and coat protein (CP) are expressed from the subgenomic mRNA (sgRNA) by a leaky scanning of ribosomes. Suppression of termination of the CP stop codon produces CP-RTD (CP extended), the capsid subunit with a read-through domain essential for aphid transmission. P6 and P7 proteins are expressed from separate sgRNA. The figure taken from the ViralZone web page.

#### 1.3.4.1 Potato leaf roll Virus (PLRV)

Family: *Solemoviridae*, Genus: *Polerovirus*, Species: ***Polerovirus PLRV***, known as potato leafroll virus (PLRV).

Potato leaf roll virus (PLRV) together with PVY are considered the most dangerous plant viruses threatening potato crops worldwide with PLRV importance as number one overtaken by PVY lately while PLRV still being serious threat in many countries (Kreuze et al. 2020a; Farooq et al., 2022).

PLRV virions are icosahedral particles with approximate diameter of 25 nm and consisting of two variants of CPs: the regular and the C-terminally extended low-frequency variant expressed via a CP stop codon readthrough. The C-terminal readthrough protein is associated with aphid transmission (Taliensky et al. 2003). The genome of the virus is +ssRNA, with approximate length of 5.9 kb with a VPg protein covalently attached to the 5' terminus. There is no poly-A tail (LaTourrette et al., 2021). The PLRV genome encodes ten ORFs while majority of known polerovirus genomes encode seven ORFs – the ORF encoding the Rap protein has been identified only in PLRV genome, as well, only a few poleroviruses encode ORF6 and ORF7 (Sömera et al., 2021). Transmission between plants occurs in non-replicative persistent manner by aphids (in particular, by *Myzus persicae*) (Cowan et al., 2025). Virus has a narrow host range, infecting plant species mostly belonging to Solanaceae family (Thomas et al., 2002).

PLRV isolates exhibit biological variability in symptoms, ranging from mild to extremely severe (Kreuze et al., 2020a). Global PLRV populations have high genetic diversity caused by high mutation and recombination rates. PLRV isolates generally cluster into two major phylogroups (O and N), with specific regional relationships (Cowan et al., 2025; Farooq et al., 2022).

### 1.3.5 Family *Virgaviridae*

Realm: *Riboviria*, Kingdom: *Orthornavirae*, Phylum: *Kitrinoviricota*, Class: *Alsuviricetes*, Order: *Martellivirales*.

Family *Virgaviridae* includes seven genera and 59 species including four species reported to infect potatoes, one (TRV) in genus *Tobravirus*, two (tomato mosaic virus and tobacco mosaic virus, ToMV and TMV, respectively) in genus *Tobamovirus*, and one (PMTV) in *Pomovirus* genus (Table 2). ToMV and TMV are reported rarely from potato while TRV and PMTV are considered having economic importance in certain regions of the world. Virions of the *Virgaviridae* family are non-enveloped, rod-shaped particles with 20 nm in diameter and in varying length from 46 nm to 310 nm, depending on the genus. Genomes vary between 6.3 to 13 kb and are +ssRNA, not segmented in *Tobamovirus* genus but segmented in other genera (Adams et al., 2017) (Figures 6-8).

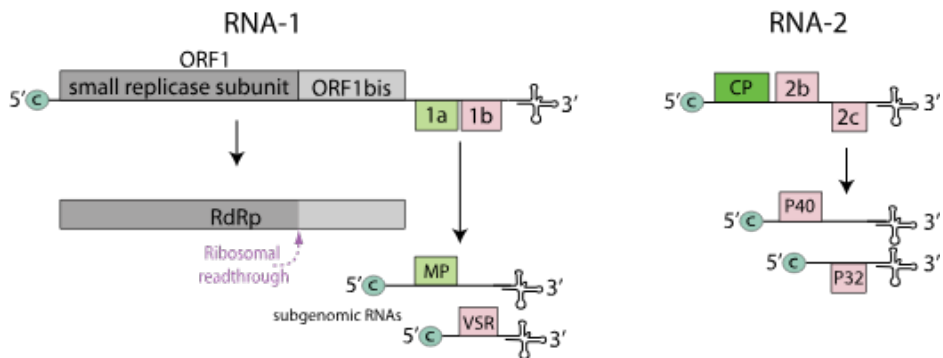


Figure 6. *Tobravirus* genome organization. Segmented, linear, +ssRNA genome. The two segments are of 6.8 and 4.5 kb in size, respectively. The genomic RNAs are capped, not polyadenylated in the 3' end. The virion RNA is infectious and serves as both the genome and viral messenger RNA. The RNA-1 5'proximal ORFs are directly translated to produce the viral constituents of the replicase complex. RNA-dependent RNA polymerase (RdRp) is translated through suppression of termination codon at the end of ORF1. Subgenomic RNAs are synthesized during replication of RNA-1 and RNA-2 and give rise to the movement protein (MP) and viral suppressor of RNA silencing (VSR). The figure taken from the ViralZone web page.

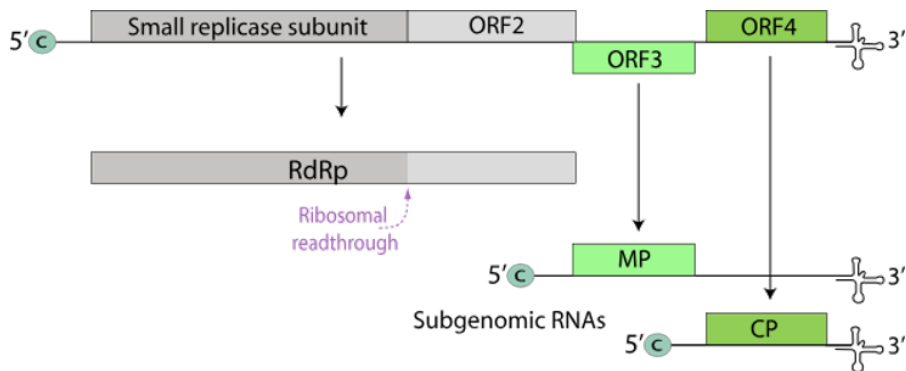


Figure 7. Tobamovirus genome organization. Monopartite linear +ssRNA genome is 6.3-6.5 kb. The 3'-terminus has a tRNA-like structure. The 5' terminus has a methylated nucleotide cap. The virion RNA is infectious and serves as both the genome and viral messenger RNA. The 5'-proximal ORFs are directly translated to produce the viral constituents of the replicase complex. RNA-dependent RNA polymerase (RdRp) is translated through suppression of termination at the end of ORF1. The small replicase is involved in replication and acts as a suppressor of RNA silencing. The movement proteins and the capsid protein are expressed from separate subgenomic mRNAs. The figure taken from the ViralZone web page.

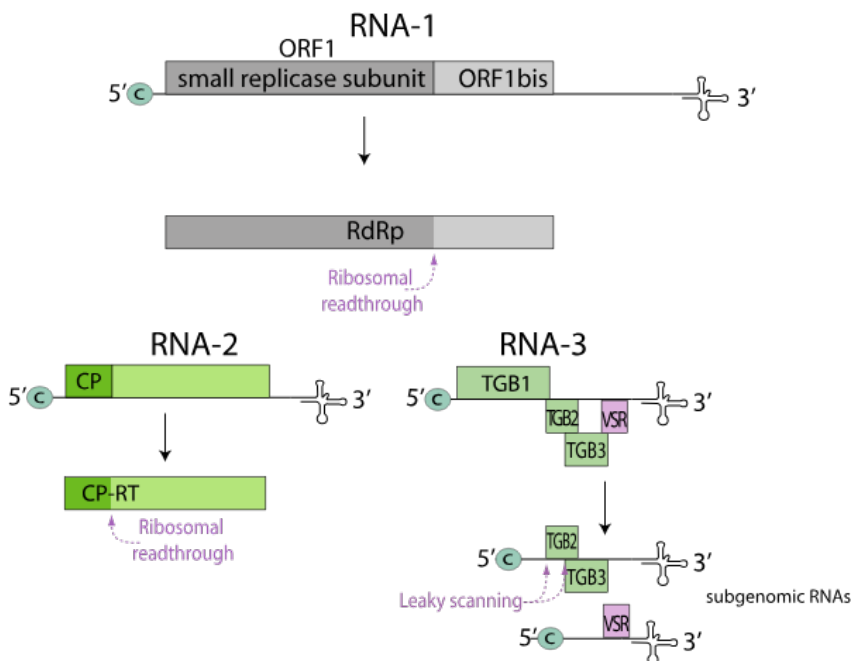


Figure 8. Pomovirus genome organization. Segmented linear +ssRNA genome. The three segments are of 6, 3.5 and 3 kb in size, respectively. The genomic RNAs are capped and display a tRNA-like structure in the 3' terminus. The virion RNA is infectious and serves as both the genome and viral messenger RNA. The RNA-1 5'-proximal ORFs are directly translated to produce the viral constituents of the replicase complex. RNA-dependent RNA polymerase (RdRp) is translated through suppression of termination at the end of ORF1. Suppression of translation termination in RNA-2 produces an extended capsid protein. TGB1, TGB2 and TGB3 are expressed from RNA-3, and function as movement proteins. TGB2 and TGB3 proteins are produced by leaky scanning. The figure taken from the ViralZone web page.

#### 1.3.5.1 Tobacco rattle virus (TRV)

Family: *Virgaviridae*, Genus: *Tobravirus*, Species: ***Tobravirus tabaci***, known as tobacco rattle virus (TRV).

Tobacco rattle virus is a plant virus capable of infecting a wide range of economically important crops. This virus is widely spread. The particles are rod-shaped with a diameter of 23 nm and mostly in two different lengths, longer one (L) is 185 to 196 nm, and shorter one (S) is between 50 to 115 nm (Robinson and Harrison, 1985). Particles L and S contain two +ssRNAs: RNA1 is approximately 6.79 and RNA2 1.8 to 4.5 kb, accordingly. Particle L is infectious alone while particle S is not. Host range of TRV is wide – it can infect more than 400 species of plants (Moyo et al., 2022). Symptoms depend on the plant host and the viral strain. On potato tubers, viruses are causing so called “spraing” symptoms that include necrotic arcing and corky ringspots inside the tuber’s flesh. If the infected tubers are planted, new shoots will be stunted and distorted, leaves having mottle or mosaic symptoms (Dale et al., 2004).

TRV exists as a complex of genetically and phenotypically distinct strains, that differ in serotypes, genomic sequences and in symptomatology. Based on serology, TRV was divided into three serotype groups (Robinson and Harrison, 1985). Phylogenetical studies showed that in the bipartite genome, RNA 1 remains relatively stable, while RNA 2 exhibits more variability (Mahillon et al., 2025; Moyo et al., 2022). CP gene analysis divided isolates into four lineages (alpha, beta, gamma and delta) (Mahillon et al., 2025; Yin et al., 2014).

#### 1.3.5.2 Potato mop-top virus (PMTV)

Family: *Virgaviridae*, Genus: *Pomovirus*, Species: ***Pomovirus solani***, known as potato mop-top virus (PMTV).

Potato mop-top virus is a destructive plant pathogen that causes significant economic losses in potato production worldwide due to distinctive symptoms on potato tubers (Savenkov et al., 2021). PMTV has been reported to be present in Europe, Asia, South and North America (Santala et al., 2010). The virus particles are straight and tubular, measuring 100 to 300 nm in length and 18 to 20 nm in diameter. It has a tripartite +ssRNA genome divided into three molecules with approximate sizes of 6 kb, 3.2 kb and 2.5 kb (Savenkov et al., 2021). PMTV symptoms vary greatly depending on potato variety and environmental conditions. In foliage, leaves have yellow blotches, rings or chevrons. In severe cases, shoots have shortened internodes and dwarfed “mop-top” growth is reported. In tubers, PMTV primary infection is causing raised brown lines or arcs (so called “spraing”), secondary infection can lead to deep cracks and reticulation or freckled/blotchy surface (Harrison and Jones, 1971). Symptoms are favored by cool, humid conditions and often lessen in warm, dry weather (Carnegie et al., 2010). Vector for PMTV is *Spongospora subterranea* f. *sp. subterranea* (Sss), that causes powdery scab in potatoes (Arif et al., 1995). PMTV can persist in the resting spores and remain infective up to 18 years (Calvert, 1968). PMTV has relatively narrow host range, and it is mainly infecting species in *Solanaceae*, *Tetragoniaceae* and *Chenopodiaceae* families (Andersen et al., 2002).

PMTV phylogenetic studies indicate that it originates from Andean region of South America. Two phylogroups can be distinguished: group 1 includes divergent Peruvian variants, and group 2 is further divided in three subclades, of which, clade I bears exclusively South American isolates, and clades II and III contain other global isolates with Europe as a primary hub for spread to North America and Asia (Fang et al., 2026). Isolates

are classified by symptoms they cause in the severe (S) and mild (M) strains and this differentiation is related to signature motif in CP-RT protein and conserved residues in TGB1 (Kalyandurg et al., 2017).

## **1.4 Plant virus detection methods in diagnostics**

Rapid and reliable diagnosis (i.e. detection and identification) of plant viruses is crucial to ensure good yield, avoid economic losses and guarantee effective disease management in agriculture. Plant virus diagnostics have evolved from visual inspection and inoculation of indicator plants to highly sensitive and accurate molecular methods and real-time digital sensing. Traditional laboratory methods like enzyme-linked immunosorbent assay (ELISA) or polymerase chain reaction (PCR) (with different configurations) continue to be “the golden standard” because of their accuracy and scalability. With the integration of high-throughput sequencing (HTS), CRISPR-Cas systems and Machine Learning (ML), the diagnostics of plant viruses is in change by shifting focus from slow, laboratory-dependent assays to fast on-site detections methods (Wang et al., 2022; Sun et al., 2022; Kanapiya et al., 2024; Jones et al., 2017).

### **1.4.1 Conventional diagnostic methods**

Traditional methods are well-established and widely used in laboratories for the detection of plant viruses as a part of seed potato certification systems. The most widely used serological method in plant virus diagnostic is ELISA. The method allows to detect viruses by using antibodies that specifically bind viral coat proteins (antigens). The presence of a virus is measured by colour change caused by an enzymatic reaction between the antibody-attached enzyme and a chromogenic substrate added to the plate (Regassa, 2022). Even though ELISA has already been replaced by quantitative PCR (qPCR) in many laboratories, it remains as favoured method in many others because of its simplicity, adaptability and cost effectiveness for screening a huge number of samples for seed certification systems.

In molecular detection, PCR and RT-PCR are the primary tools for detecting plant viruses, depending on the type of their genetic material (DNA or RNA, respectively) (Wang et al., 2022; Kanapiya et al., 2024). RT-PCR and its different variants are especially important in plant virus diagnostics because most of plant viruses are RNA viruses. Since RT-PCR can detect viral RNA at very low levels, it is generally much more sensitive than ELISA. The sensitivity of quantitative (RT)-PCR is even 10 to 100 times higher than that of regular (RT-)PCR. Both qualitative and quantitative (RT-)PCRs can also be used as the multiplex allowing simultaneous detection of different viruses or different variants (strains) of same virus. For example, several multiplex RT-PCR and RT-qPCR have been developed for PVY strain identifications (Rigotti et al., 2007; Nie et al., 2002; Lorenzen et al., 2006; Chikh-Ali et al., 2013). In addition, there are isothermal amplification methods such as loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) that operate on constant temperature and that makes it easier to use those at on-site testing (Kanapiya et al., 2024).

### **1.4.2 Advanced and emerging methods**

Newer technologies are mostly focused on increasing the speed of the analyses, making it portable for on-site testing, or on the ability to detect unknown pathogens. Particularly, the CRISPR-Cas systems are under fast development for virus detection to provide more

accurate and fast on-site testing methods (Mushtaq et al., 2021). Discovery of effector proteins Cas12a and Cas13a that exhibit collateral (trans) cleavage activity following target recognition has enabled a development of highly specific and sensitive nucleic acid-based diagnostic platforms that do not need expensive equipment or controlled laboratory environments. Cas12a-based platforms target single-stranded DNA, while Cas13a-based platforms have a potential to offer direct single-stranded RNA targeting (Kumar et al., 2026).

Nowadays, the most widely used advanced method in plant virus detection is HTS and its variations (Mushtaq et al., 2021; Sun et al., 2022; Wang et al., 2022; Kanapiya et al., 2024). Most common and widely used is Illumina sequencing, that uses “sequencing by synthesis” to generate short reads with very high accuracy. Nanopore sequencing, also called as the third-generation sequencing method, has made HTS portable and possible for on-site analyses (Sun et al., 2022; Kanapiya et al., 2024). Other platforms such as Ion Torrent or PacBio are available but not so commonly used due to different reasons (Sun et al., 2022; Kanapiya et al., 2024). Compared to conventional diagnostic tools such as ELISA or PCR, or novel CRISPR-Cas based systems, those methods are so called “hypothesis-free” because of allowing to detect all the viruses that could exist in the sample, i.e. known and unknown without predicting which primers or antibodies to use.

Recent advances in proximal and remote optical sensing, coupled with ML are revolutionizing non-destructive disease detection by monitoring physiological changes in plants before the symptoms are visible (Wang et al., 2022; Ghosh et al., 2022). Hyperspectral imaging and remote sensing by drones or satellites together with ML and deep learning are opening totally new ways for early-stage diagnostics in automated way achieving accuracy metrics comparable with human expertise providing critical data for precision farming and large-scale biosecurity surveillance (Ghosh et al., 2022; Wang et al., 2022; Kanapiya et al., 2024). Optical sensing methods are promising but in need of huge dataset for training the models to achieve accuracy.

## 2. Aims and significance of the study

Reliable detection and identification of potato viruses, together with an improved understanding of their epidemiology, remain key challenges in potato virology and crop protection. Effective virus management requires not only routine detection of known pathogens, but also knowledge of the strains present, the suitability of diagnostic methods, and awareness of emerging threats relevant to the potato production.

In Estonia, a regular annual monitoring within the frame of the seed potato certification system has generated a lot of valuable information on virus incidences. However, the scientific use of this data, particularly in combination with molecular characterization of viruses and the long-term epidemiological analysis, has remained limited. In addition, information on the strain composition of potato viruses in Estonia has been lacking, despite its importance for understanding virus epidemiology, varietal performance and disease management.

The aim of this thesis was to investigate the occurrence of potato viruses in Estonia, with particular emphasis on viral strain composition, assessment of varietal susceptibility, improvement of diagnostics, and identification of phytosanitary risks relevant to potato industry.

More specifically, the objectives were:

1. To determine the occurrence of potato viruses in Estonian seed potato production
2. To assess the factors influencing PVY occurrence and spread in Estonia
3. To evaluate the susceptibility of potato varieties to PVY under Estonian field conditions
4. To characterize the strain composition of potato viruses, present in Estonia, with particular emphasis on potato virus Y strains
5. To identify broader phytosanitary risks relevant to potato sector, including emerging viruses detected in non-potato hosts that may pose future threat to potato production

The significance of this thesis lies in combining the long-term virological monitoring data collected through the seed potato certification with weather and varietal information, providing a more comprehensive understanding of potato virus epidemiology in Estonia. The work presents the first characterization of PVY strains in Estonia and the Baltic region, provides a new information on other potato virus strains in the region, and demonstrates the value of certification data for improved diagnostics, update of seed potato certification system, varietal evaluation, breeding, and future surveillance, thereby strengthening the scientific basis for potato virus management and phytosanitary preparedness in Estonia.

## **3. Materials and methods**

### **3.1 Materials**

#### **3.1.1 Plant material**

The material used in this study originated from official plant health monitoring for potato seed tuber certification surveys organized by the Estonian Agriculture and Food Board. The testing was conducted at the METK laboratory mainly by the author of this thesis and other members of the laboratory. The study included potato tubers (or plants grown from the tubers) and leaf material from various plants. The exact material and the number of samples used for long-term virus monitoring of six viruses and PVY strain assignment were described in Publications I and II. Material used for HTS survey in 2025 were described in Publication III. In the case of other hosts, standard procedure for testing leaf material, was similar as described in Publication IV.

#### **3.1.2 Climatic, geographic, and varietal metadata**

To assess the influence of environmental and agronomic factors on PVY incidences, virus test results were linked with metadata on field location, county, seed class, and cultivated variety obtained from the Estonian Agricultural Board. Climatic data, focusing on temperature and precipitation were obtained from the Estonian Environmental Agency (Publications I and II).

### **3.2 Methods**

#### **3.2.1 Regular virus monitoring**

During the early period of the seed potato certification yearly surveys (until 2014), virus testing was based on serological detection. ELISA was used as described in Publication I. In 2015, the real-time TaqMan RT-PCR was implemented for PVY detection and from 2021 all routine testing for six viruses was done using this method (Publications I and II).

#### **3.2.2 Statistics**

Briefly, associations between PVY (and its strains) incidences and explanatory variables, including year, temperature, precipitation, field location and potato variety, were assessed. Statistical significance thresholds and detailed parameters are provided in original articles (Publications I and II).

#### **3.2.3 Assessment of varietal susceptibility to PVY**

A systematic assessment of potato varieties susceptibility to PVY under Estonian field conditions was not carried out previously. We designed a framework on how to perform the statistical evaluation based on the extensive dataset collected during the regular seed potato monitoring (Publications I and II).

#### **3.2.4 Strain identification methods for PVY**

For PVY strain identification survey, a multiplex RT-PCR developed by Lorenzen et al. (2006) and modified by us was used as described in Publication II. Additionally, HTS was applied for a low number of samples to validate the results.



### **3.2.5 HTS sequencing and bioinformatics**

In Publication II, the sequencing procedure was performed in collaboration with the FERA (Food and Environment Research Agency, UK), followed by a bioinformatic analysis pipeline developed in collaboration with the University of Tartu for the targeted PVY strain survey. The potato virus survey based on HTS in 2025 was done in the METK Laboratory and analysed as described in Publication III.

### **3.2.6 RT-PCR for amplifying coat protein region**

To confirm the viral finding from HTS, total RNA was extracted from potato plants as described in Publication III, samples were forwarded for additional RT-PCR using primers published by Rashid (2021). RT-PCR was carried out following the QIAGEN OneStep RT-PCR Kit protocol.

## 4. Results and discussion

The results presented in this chapter describe and discuss analysis of the data collected during virological surveys of Estonian seed potatoes held in 2007 to 2022 (Publications I and II), and the additional survey held in 2025 (Publication III and some yet unpublished data). As well, the data collected on other potential pathogenic viruses able to infect potato have been discussed to integrate it into a broader epidemiological and plant health framework relevant to potato virus research in Estonia (Publication IV and van der Sman et al., unpublished data).

The virological surveys have been analysed with particular emphasis on the occurrence of potato viruses in Estonia, the factors influencing PVY spread, the strain composition of Estonian PVY population, and the phylogenetic strain grouping of other potato viruses identified during this work, also, the discussion involves phytosanitary risks by potato-infecting viruses not yet detected in Estonian seed potatoes but in other hosts. These findings are relevant not only for plant virology and disease epidemiology, but also for practical plant health management, seed certification, and potato breeding. The data gathered and analysed in this work provide detailed overview of viral composition in Estonian seed potato, while also demonstrating the value of national monitoring systems for plant virus epidemiology research.

### 4.1 Occurrence of potato viruses in Estonian seed potato (Publications I)

A regular virus testing within the frame of seed certification systems has provided a valuable opportunity to assess virus incidences over long period and monitor the changes in the viral dynamics under regular production conditions in Estonia.

The long-term survey of seed potatoes in Estonia from 2007 to 2022 showed that PVY was the most prevalent virus threatening the seed production (Publication I). In Super Elite seed lots, the average PVY incidence over studied period was 2.43%. The highest annual level for PVY was as high as 6.56% on a year 2011, whereas in 2020 no PVY was detected (Figure 9a). In lower class seed lots, classified as Elite (S, SE, and E) and Certified (A and B), the average incidence of PVY was 3.92%, with highest incidence level on 2021 with 8.29%, while relatively low occurrence (under 1%) was recorded in 2013, 2014, 2017, and 2022 (Figure 9f). Among the other viruses tested in Super Elite seed lots, PVM and PVS were present at much lower levels, with average incidences of 0.48% and 0.11%, respectively. PVM was found to be the second most prevalent virus in Estonian seed potato with high incidence rates on 2008, 2016, and 2019. In other years, level of PVM stayed relatively low, and no PVM was found on the years 2017 and 2020 (Figure 9b). PVS overall averages stayed below 0.5% during the studied period (Figure 9c). PLRV and PVX were detected only on the early years of the study, with PLRV being present on 2007 and 2008, and PVX only in 2007 (Figure 9d and 9e, respectively). Notably, PVA was not detected during the monitoring period.

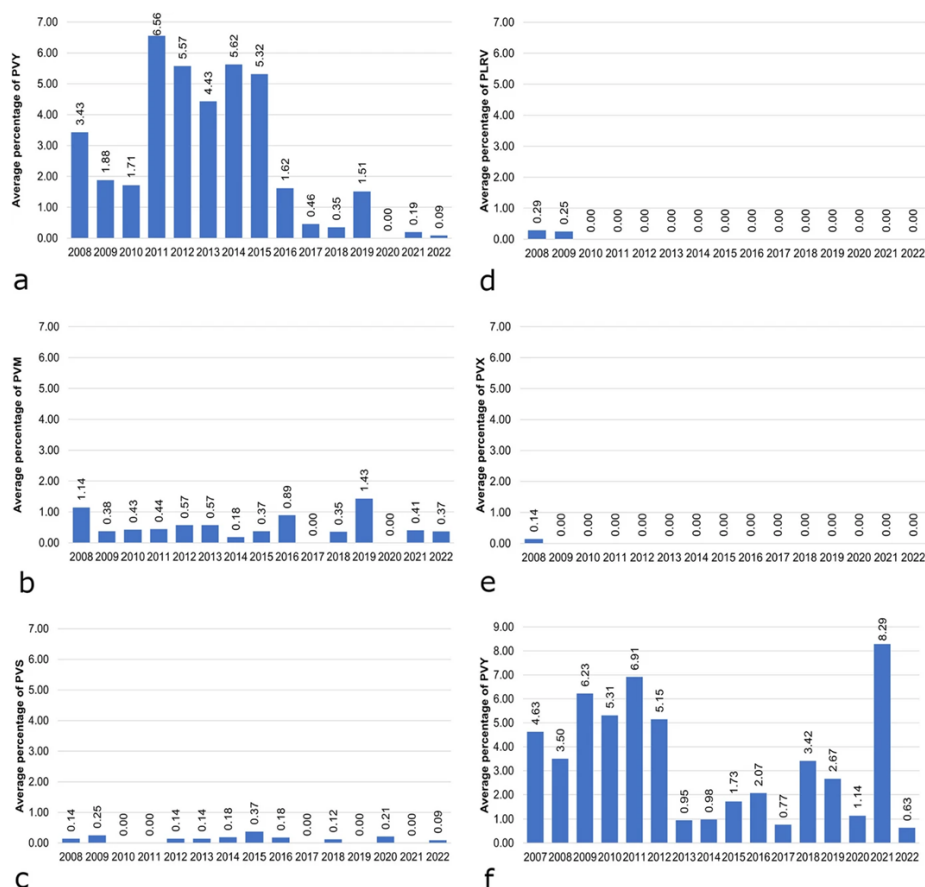


Figure 9 a–e. Yearly incidence (%) of PVY, PVM, PVS, PLRV, and PVX in super elite (SE until 2014; PBTC and PB from 2015) seed potato (2008–2022). f. Yearly incidence of PVY in lower categories (classes E and A until 2014; S, SE, E, A, and B from 2015) seed potatoes (2007–2022) (Publication I).

In Estonia, the routine monitoring of six potato viruses (PVY, PVA, PVX, PVM, PVS and PLRV) has been required only for Super Elite seed lots, and the number of these seed lots has remained relatively low compared with lower seed classes. In lower class, certification testing has been focused primarily on PVY and, until 2015, also on PVA; consequently, the incidences of PVM, PVX, PVS and PLRV in these seed classes have not been reported.

To address this gap, all seed lots submitted for certification in 2025 were also analysed by HTS. The results showed that, in analysed 13 lower class seed lots, PVY, PVM and PVS were present in considerable number of samples, and PLRV was also detected (Table 3). Single infections were identified in eight seed lots: PVS was detected in five lots, PVY in two and PLRV in one. Complex infection patterns were also observed, with PVY co-occurring with PVM in two lots and with PVS in one. Furthermore, one lot exhibited a triple infection involving PVY, PVS and PLRV, while another lot showed co-infection of PLRV and PVS. In the Super Elite seed lots, PVY was identified once and PVS twice as single infections, alongside with one case of PVM and PVS co-infection. All results obtained from HTS were subsequently validated through the amplification of the viral CP regions. These findings indicate that PVY, PVM and PVS are prevalent within Estonian

seed potato production. Notably, the detection of PLRV marks a significant discovery, representing the first confirmed presence of the virus in Estonia after 16 years.

*Table 3. Virus distribution in 2025 seed lots by HTS. Among 34 tested seed lots, 17 contained viruses (van der Sman et al., unpublished). Blue indicates lower seed categories (Elite and Certified) and green represent the Super Elite category seed lots.*

| METK sample number | Seed category | Variety  | Origin of the seed lot | County     | PVY | PVM | PVS | PLRV |
|--------------------|---------------|----------|------------------------|------------|-----|-----|-----|------|
| 4557               | lower class   | Julinka  | Germany                | Viljandi   |     |     | X   |      |
| 4559               | lower class   | Cordula  | Germany                | Viljandi   |     |     |     | X    |
| 4686               | lower class   | Red Lady | Germany                | Lääne-Viru | X   |     |     |      |
| 4729               | lower class   | Paroli   | Germany                | Viljandi   | X   |     | X   | X    |
| 4730               | lower class   | Jola     | Germany                | Viljandi   |     |     | X   |      |
| 4797               | lower class   | Paroli   | Germany                | Võru       |     |     | X   | X    |
| 4928               | lower class   | Maret    | Estonia                | Võru       | X   | X   |     |      |
| 5045               | lower class   | Soraya   | Germany                | Tartu      |     |     | X   |      |
| 5122               | lower class   | Islara   | Germany                | Järva      |     |     | X   |      |
| 5429               | lower class   | Madeira  | Germany                | Järva      | X   |     |     |      |
| 5598               | SE            | Varje    | Estonia                | Jõgeva     |     |     | X   |      |
| 5604               | SE            | Varje    | Estonia                | Jõgeva     |     |     | X   |      |
| 5614               | SE            | Maret    | Estonia                | Jõgeva     |     | X   | X   |      |
| 5615               | lower class   | Piret    | Estonia                | Jõgeva     | X   |     | X   |      |
| 5718               | lower class   | Maret    | Estonia                | Jõgeva     | X   | X   |     |      |
| 5720               | lower class   | Teele    | Estonia                | Jõgeva     |     |     | X   |      |
| 5863               | SE            | Maret    | Estonia                | Jõgeva     | X   |     |     |      |

During the long-term survey, the incidences of PLRV and PVX were low and observed only in the early years of the study while PVA was missing completely, indicating that these viruses are of limited importance in Super Elite seed category. The reduced importance of PVX has also been reported in other countries in association with effectiveness of certification systems (Bragard et al., 2020c). However, because routine monitoring of six viruses has historically been restricted to Super Elite seed lots, this picture has remained incomplete for lower seed classes. The additional testing carried out in 2025 helped address the virus incidences both in Super Elite and in lower-class seed potatoes. It was found that besides PVY, PVS was quite common, and PVM and PLRV were also detected. Compared with the historical report about PVX being the most prevalent potato virus in Estonia (Järvekülg et al. 1995), the data analysed in this study point to the shift in the dominant virus spectrum, with PVY being dominant together with the raising importance of PVS.

Routine seed certification system data suggested that PVY was the most prevalent virus, which is consistent with its global status as the most economically important potato virus with considerable economic losses (Palukaitis, 2012; Kreuze et al., 2020a; Dupuis et al 2024). However, the 2025 HTS survey, which provided more comprehensive overview of viral presence in lower seed classes, indicated that PVS may be even more

problematic within Estonian context. These findings suggest that the current seed potato certification system should be re-evaluated; specifically, testing protocol for lower seed classes should be expanded to include other viruses such as PVS, PVM, and PLRV as Poland, Germany and France are doing. Furthermore, incorporating HTS into formal certification process represents a valuable and effective option enhancing the accuracy of viral detection.

Importantly, it was observed that the quality of Estonian seed potatoes improved over the time. In both, Super Elite and lower-class seed potatoes, PVY incidence showed a decreasing trend, and this decline was statistically significant. Similar downward tendencies were also observed for PVM and PVS in Super Elite seed lots, although their trends were not statistically significant, likely, because of their already low incidence and year-to-year fluctuations (Figure 10). The absence of PVX in later years in Estonian Super Elite seeds further supports the conclusion that the general phytosanitary quality of certified seed potatoes has improved.

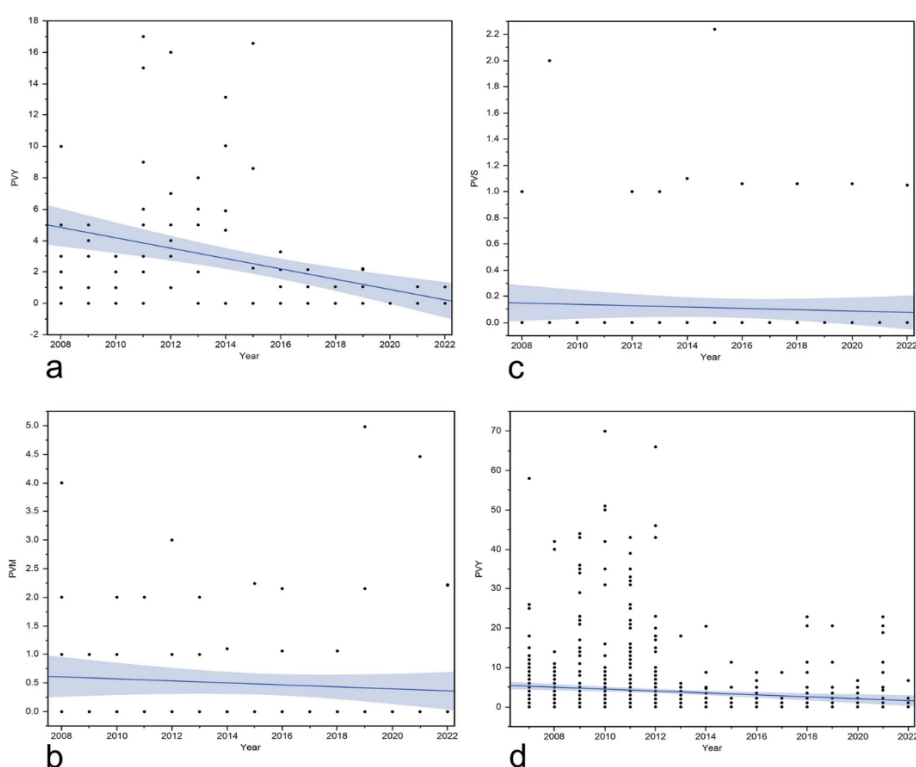


Figure 10 a–c. PVY, PVM, and PVS trends in Estonian Super Elite (SE until 2014; PBTC and PB from 2015) seed potatoes in 2008–2022. d PVY trends in Estonian seed potato lower categories (classes E and A until 2014; S, SE, E, A, and B from 2015) in 2007–2022. Virus incidence rates in each seed lot are shown as dots (the lots with same values are shown as one dot), whereas the linear regression trendline is shown with a 95% confidence interval (blue) (Publication I).

The continued presence of PVY and PVM exceeding the thresholds applied to seed potato certification demonstrates that those viruses, especially PVY, remain major challenges for the potato production sector in Estonia. This is particularly significant because certification thresholds for early-generation seed are strict, and even relatively

low infection rates may result in downgrading or rejection of seed lots, thus causing economic losses. Despite the overall progress in virus control, PVY remains the key target of potato virus surveillance and management in Estonia.

## 4.2 Factors affecting the spread of PVY in Estonia (Publication I)

As PVY was identified as the predominant potato virus in Estonia (based on analysis of more than thousand seed lots, including both Super Elite and lower-class seed tubers), the next objective of the study was to evaluate the factors influencing its spread under local production conditions by combining the virus monitoring data with information on cultivated potato varieties and regional monthly environmental conditions over vegetation period (May to August), namely temperature and precipitation.

Climatic variables were found to influence PVY incidence, although their overall effect was weaker than that of potato variety. In the analysis, temperature and precipitation data from each month of the potato growing seasons, from May to August, were evaluated in relations to PVY incidences (Figure 11). Multiple linear regression analysis showed that weather conditions are statistically significant ( $p = 0.0005$ ;  $r = 0.1675$ ). The strongest relationships were observed for July and August climate variables: higher temperatures in July ( $p < 0.0001$ ) and increased precipitation in July ( $p = 0.0266$ ) corresponded to higher PVY incidences, whereas higher temperatures in August ( $p = 0.0299$ ) were linked to lower PVY incidences. Among these factors, July temperature showed strongest effect, while the others were comparatively weak. The same variables were not significant for other months of the growing season (Table 4), suggesting that weather conditions influenced PVY spread but were probably not the main explanatory factor.

*Table 4. Relationship between PVY infection in Estonian seed potatoes and environmental variables (temperature and precipitation) during 2007-2022 (Publication I).*

| Month  | Temperature |          | Precipitation |         |
|--------|-------------|----------|---------------|---------|
|        | t Ratio     | p value  | t Ratio       | p-value |
| May    | -0.77       | 0.4393   | -1.18         | 0.2389  |
| June   | -0.26       | 0.7957   | -1.16         | 0.2464  |
| July   | 4.73        | <0.0001* | 2.22          | 0.0266* |
| August | -2.18       | 0.0299*  | -0.58         | 0.5588  |

\* = Statistically significant differences, significance level  $\alpha \leq 0.05$ ; positive t-Ratio values indicate higher PVY incidence than grand mean, and negative values indicate lower PVY incidence than grand mean

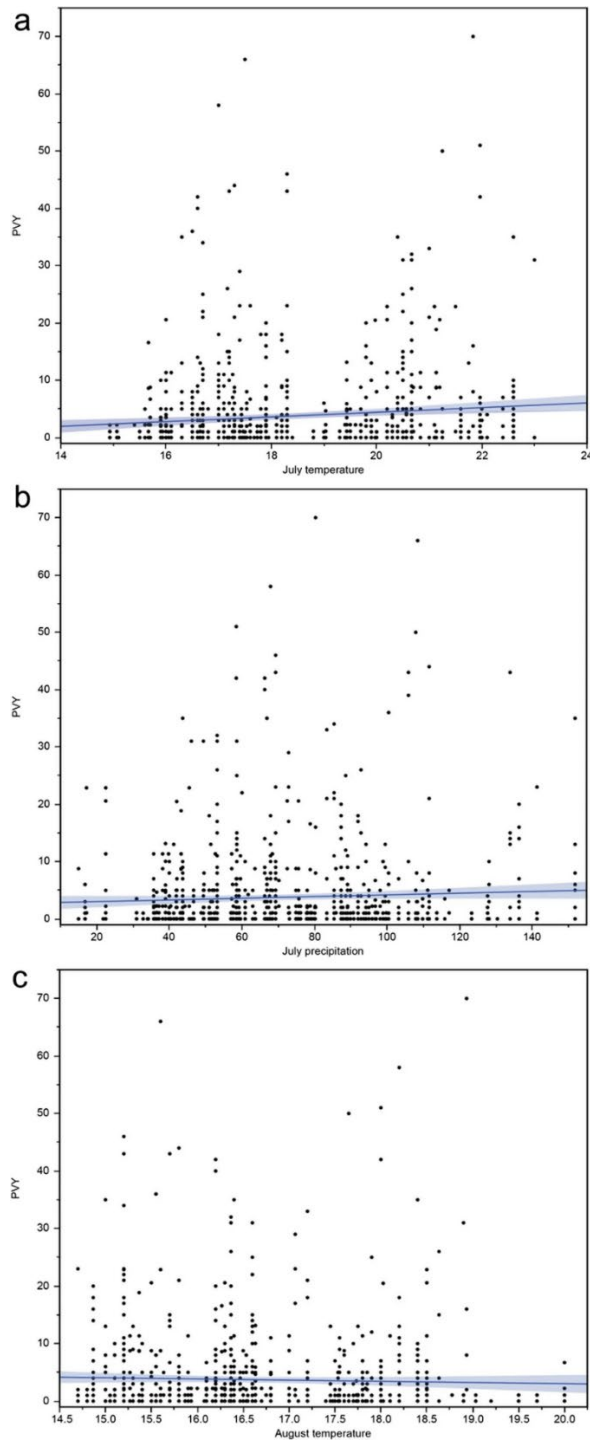


Figure 11. Relationships between PVY incidences and statistically important environmental variables during 2007–2022: a. temperature in July, b. precipitation in July, c. temperature in August. PVY incidence rates in each seed lot are shown as dots, whereas the linear regression trendline is shown with a 95% confidence interval (blue) (Publication I).

The observed relationships can be explained by the epidemiology of PVY. Previous studies have shown that cooler conditions increase the effectiveness of plant hypersensitivity-induced resistance to PVY while warmer temperatures promote the susceptibility and facilitate the systemic spread of the virus (Valkonen, 1997; Choi et al., 2017). In addition, PVY is transmitted in non-persistent manner by aphids, and vector abundance and flight activity are influenced by temperature and moisture conditions. In Estonia, aphid populations in potato fields have been reported to peak in mid-July (Muljar, 2009), which correlates with the period when temperature and precipitation showed the strongest relationship with PVY incidences. Warmer weather conditions in July may therefore enhance the aphid activity and transmission opportunities, leading the higher infection levels.

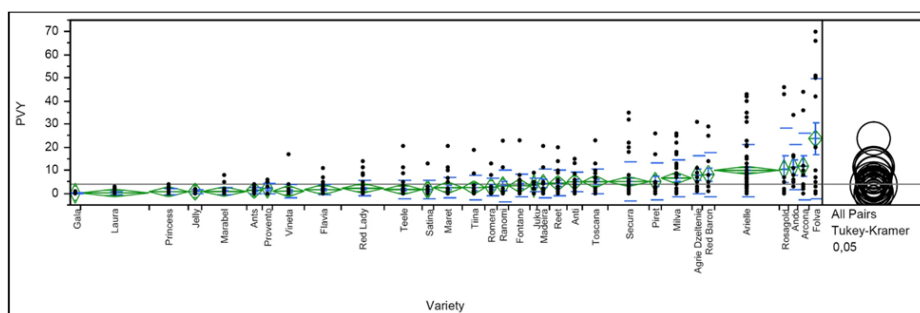


Figure 12. One-way ANOVA analysis of PVY incidences (% shown in y-axis) in tested individual seed lots by potato variety (shown in x-axis). The width of green diamonds represents the group size of the variety. The grey line shows a grand mean. Black dots indicate individual seed lots. Blue lines are at  $\pm$  one or two STDEV; dots exceeding the blue lines show the outliers (Publication I).

Analysis of the 32 most frequently cultivated potato varieties revealed highly significant differences in PVY infection rates ( $p < 0.0001$ ;  $r = 0.5193$ ) in One-way ANOVA analysis (Figure 12), allowing the classification of varieties, grown in local conditions, into resistance categories ranging from very resistant to very susceptible (Table 5). Varieties such as Laura, Jelly, Gala and Princess were categorised as with a very high resistance, based on both the low average PVY infection levels and low maximum infection rates recorded during the studied period. In contrast, varieties like Ando, Arielle, Arcona, Rosagold, and Folva were classified as very susceptible due to their high average infection levels and, in several cases, extremely high peak infection rates. Notably, in those categories, all varieties showed statistically significant support, with p-value under significance level  $\alpha \leq 0.05$ , and negative t-ratios for very high resistance varieties and positive t-ratios for very susceptible varieties, indicating constantly low and high PVY levels, respectively. By contrast, most of the varieties in between those groups, showed less clear statistical support, likely because greater variations in infection rates between the seed lots, and in some cases due to smaller number of samples (Table 6). These results confirm that potato variety is a major determinant of PVY epidemiology under field conditions.



Table 5. Suggested classification of potato varieties into PVY resistance groups according to our study (Publication I).

| Resistance group     | Average PVY percentage in field conditions (%) | Highest PVY infection rate in field conditions (%) | Variety                                                                                               |
|----------------------|------------------------------------------------|----------------------------------------------------|-------------------------------------------------------------------------------------------------------|
| Very high resistance | <1                                             | <5                                                 | Gala, Laura, Princess, Jelly                                                                          |
| High resistance      | 1 to 2.99                                      | 5 to 9.9                                           | Marabel, Ants, Provento                                                                               |
| Medium resistance    |                                                | 10 to 19.9                                         | Vineta, Flavia, Teele, Satina, Red Lady, Maret, Tiina, Romera                                         |
| Susceptible          | >3                                             | 20 to 34.9                                         | Ranomi, Fontane, Juku, Madeira, Reet, Anti, Toscana, Secura, Piret, Milva, Agrie Dzeltenie, Red Baron |
| Very susceptible     |                                                | >35                                                | Arielle, Rosagold, Ando, Arcona, Folva                                                                |

Table 6. Statistical support for variety susceptibility evaluation. \* Indicates  $p$ -value < 0.05 (Publication I).

| Variety         | Average percentage of PVY | Maximum percentage of PVY | t Ratio | p value  | Resistance group     |
|-----------------|---------------------------|---------------------------|---------|----------|----------------------|
| Gala            | 0,1                       | 1,0                       | -2,1    | 0,0358*  | very high resistance |
| Laura           | 0,2                       | 3,0                       | -5,02   | <0,0001* | very high resistance |
| Princess        | 0,8                       | 4,0                       | -3,45   | 0,0006*  | very high resistance |
| Jelly           | 0,8                       | 2,0                       | -2,11   | 0,0354*  | very high resistance |
| Marabel         | 0,8                       | 8,0                       | -3,57   | 0,0004*  | high resistance      |
| Ants            | 1,3                       | 4,0                       | -1,83   | 0,0671   | high resistance      |
| Provento        | 2,1                       | 6,0                       | -1,25   | 0,2101   | high resistance      |
| Vineta          | 1,0                       | 17,0                      | -2,94   | 0,0034*  | medium resistance    |
| Flavia          | 1,7                       | 11,0                      | -2,61   | 0,0094*  | medium resistance    |
| Teele           | 1,7                       | 20,6                      | -2,61   | 0,0093   | medium resistance    |
| Satina          | 1,7                       | 13,0                      | -1,43   | 0,1538   | medium resistance    |
| Red Lady        | 2,2                       | 14,0                      | -2,24   | 0,0257*  | medium resistance    |
| Maret           | 2,5                       | 20,6                      | -1,69   | 0,0908   | medium resistance    |
| Tiina           | 2,5                       | 18,9                      | -1,47   | 0,1411   | medium resistance    |
| Romera          | 2,8                       | 13,0                      | -0,98   | 0,3269   | medium resistance    |
| Ranomi          | 3,3                       | 22,9                      | -0,71   | 0,4784   | susceptible          |
| Fontane         | 3,3                       | 23,0                      | -0,92   | 0,358    | susceptible          |
| Juku            | 3,5                       | 9,0                       | -0,47   | 0,6381   | susceptible          |
| Madeira         | 4,3                       | 20,6                      | -0,22   | 0,829    | susceptible          |
| Reet            | 4,5                       | 20,0                      | -0,16   | 0,8739   | susceptible          |
| Anti            | 5,0                       | 15,0                      | 0,16    | 0,8724   | susceptible          |
| Toscana         | 5,1                       | 23,0                      | 0,25    | 0,8053   | susceptible          |
| Secura          | 5,1                       | 35,0                      | 0,36    | 0,7212   | susceptible          |
| Piret           | 5,2                       | 26,0                      | 0,22    | 0,8286   | susceptible          |
| Milva           | 6,7                       | 26,0                      | 1,51    | 0,1322   | susceptible          |
| Agrie Dzeltenie | 8,0                       | 31,0                      | 1,56    | 0,119    | susceptible          |
| Red Baron       | 8,2                       | 29,0                      | 1,71    | 0,0876   | susceptible          |
| Arielle         | 10,0                      | 43,0                      | 5,76    | <0,0001* | very susceptible     |

| Variety  | Average percentage of PVY | Maximum percentage of PVY | t Ratio | p value  | Resistance group |
|----------|---------------------------|---------------------------|---------|----------|------------------|
| Rosagold | 10,4                      | 46,0                      | 2,58    | 0,0100*  | very susceptible |
| Ando     | 11,4                      | 34,0                      | 2,88    | 0,0040*  | very susceptible |
| Arcona   | 11,8                      | 44,0                      | 3,38    | 0,0008*  | very susceptible |
| Folva    | 23,8                      | 70,0                      | 10,2    | <0,0001* | very susceptible |

Although susceptibility ratings given by the breeders are intended to provide an objective measure of varietal response, the resulting score depends to some extent on the used standards (Świeżyński et al., 2001) and may not fully reflect on the performance under local conditions. Moreover, varietal susceptibility under local growing conditions may differ from such evaluations, as the regional climate conditions are not identical to those at the original trial sites, and the varieties are exposed to the population of locally circulating viruses and their strains. In present study, the variety classification was done based on a long-term monitoring on a real field condition, which increases the relevance of the results for agricultural decision making and for breeders helping to select parental lines for new varieties. Unlike controlled resistance trials, our data integrate the combined effect of virus pressure, vector activity, weather conditions, and local agronomic practices. Consistent with this, several varieties in this study performed differently under Estonian conditions than suggested by earlier descriptions. Varieties such as Secura, Romera, Madeira, Toscana, and Milva appeared more susceptible than expected, whereas Laura and Princess showed better resistance than previously indicated.

The extensive long-term dataset, which combined information on PVY incidence, varietal susceptibility, and weather variables, made it possible to examine how the effect of temperature and precipitation varied among potato varieties. The results showed that the influence of climatic variables was not uniform across all varieties. Very resistant and resistant varieties did not show significant response to July temperature or precipitation and to August temperature, which were identified as influencing factors for overall PVY spread, whereas some susceptible and very susceptible varieties did exhibit significant association with these variables (Figure 13). In addition, the weather effect appeared to be more pronounced in varieties of non-local origin. This suggests that host resistance, and possibly also local adaption, can buffer environmental effects on virus spread, and has practical value, as it implicates that resistant varieties are not only less infected but may also provide more stable performance across years under different weather conditions.

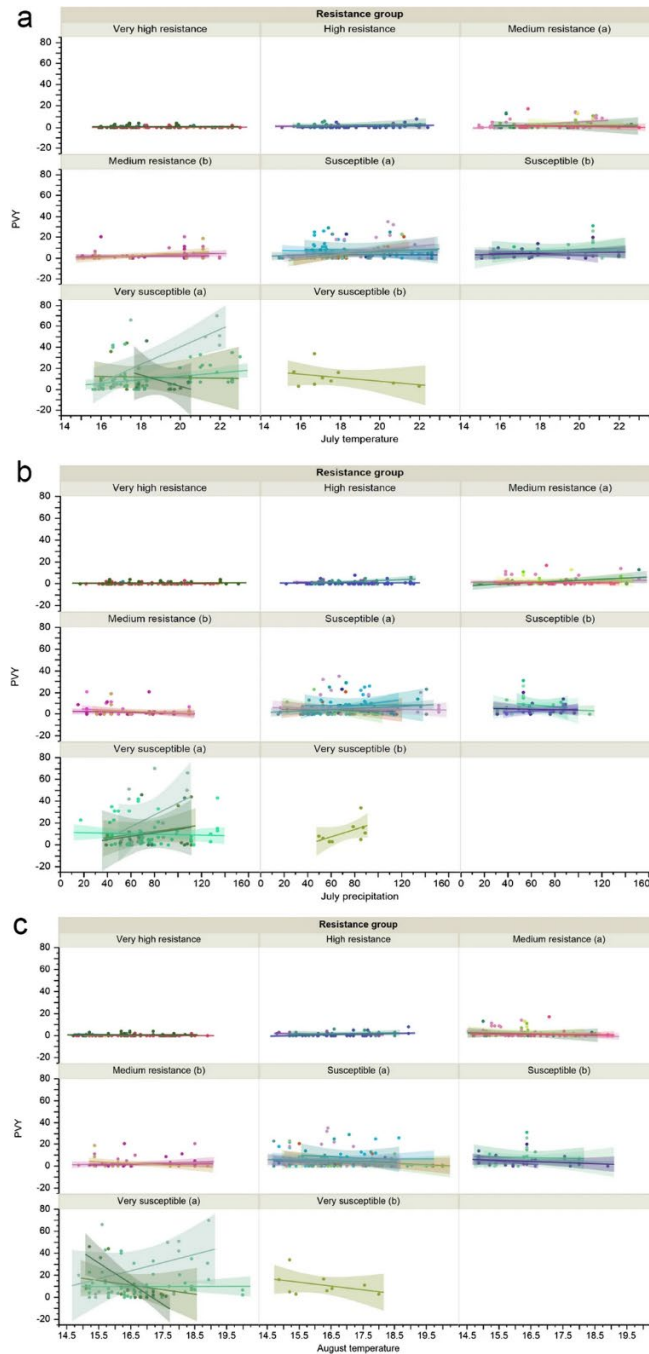


Figure 13. Effect of environmental variables on PVY incidence in potato varieties of different susceptibility groups: a. PVY vs. July temperature, b. PVY vs. July precipitation, c. PVY vs. August temperature. Varieties in medium resistance, susceptible, and very susceptible groups are presented in two subgroups, indicating their origin: (a) Central European, (b) Estonian or Latvian. Colours depict different varieties: their PVY incidence rates in each seed lot are shown as dots, whereas the linear regression trendline for each variety is shown with a 95% confidence interval (shaded area) (Publication I).

Regional differences in PVY incidences were also observed across Estonian counties. Tartu and Pärnu counties showed significantly higher PVY incidence rates than the national average, whereas some other counties exhibited comparatively low levels (Table 7). However, these geographical patterns were closely related to varietal composition. Counties with a higher proportion of susceptible and very susceptible varieties had higher PVY incidence rate, and counties where a high-resistant varieties were more common had lower PVY levels (Figure 14). Thus, the regional differences in PVY spread appeared to be influenced by the composition of cultivated varieties than by location itself.

*Table 7. Statistical analysis of PVY incidences in Estonian counties (Publication I).*

| <b>County</b> | <b>Average percentage of PVY</b> | <b>Maximum percentage of PVY</b> | <b>t Ratio</b> | <b>p value</b> |
|---------------|----------------------------------|----------------------------------|----------------|----------------|
| Harju         | 3,6                              | 66,0                             | -0,41          | 0,6833         |
| Jõgeva        | 3,6                              | 51,0                             | -0,67          | 0,5022         |
| Järva         | 2,2                              | 20,6                             | -1,78          | 0,0748         |
| Lääne-Viru    | 2,8                              | 36,0                             | -1,49          | 0,1356         |
| Põlva         | 3,3                              | 43,0                             | -0,69          | 0,4887         |
| Pärnu         | 9,8                              | 70,0                             | 4,13           | <0,0001*       |
| Rapla         | 2,6                              | 35,0                             | -1,74          | 0,0818         |
| Tartu         | 5,9                              | 58,0                             | 2,9            | 0,0038*        |
| Valga         | 4,5                              | 22,0                             | 0,19           | 0,8463         |
| Viljandi      | 2,8                              | 43,0                             | -1,88          | 0,0602         |
| Võru          | 3,1                              | 33,0                             | -0.86          | 0.3911         |



Figure 14. a. Proportions of 32 evaluated varieties of very susceptible (blue), susceptible to resistant (orange), and very resistant (grey) groups grown in different counties. b. Proportions of seed grades (SE, Super Elite (yellow); E, Elite (grey); A, class A (orange); NC, not certified (blue)) across the counties for the 32 evaluated varieties. Super Elite (SE) seed was cultivated only in Jõgeva county. Numbers of analysed seed lots are shown for each subset (Publication I).

Taken together, the results indicate that PVY spread in Estonia is influenced by climatic conditions, but primarily it is determined by cultivated potato varieties. Although environmental conditions and aphid populations cannot be controlled directly, the choices of the cultivars represent a practical and effective means of reducing PVY spread and maintaining seed potato quality. The findings therefore support the use of long-term, region-specific variety performance data in knowledge-based variety selection for seed potato production and breeding decisions.

### 4.3 PVY Strain Composition in Estonian seed potato (Publication II)

Characterisation of PVY strain composition in Estonia was essential for understanding its epidemiology and impact on seed potato production. PVY is one of the most genetically variable potato viruses due to its high recombination readiness. Nowadays, the recombinant strains have become dominant over the original parental strains in many potato-growing regions of the world. The properties of PVY strains differ in pathogenicity, symptom expression, transmission efficiency, and interaction with host resistance (Karasev and Gray, 2013; Green et al., 2017, 2018; Lacomme et al., 2017). Therefore, the spread of PVY in Estonia cannot be interpreted adequately based on virus incidences alone; it must be considered in relation to the structure of the circulating PVY populations together with an assessment of the susceptibility of the grown potato varieties.

The first survey of Estonian PVY strain composition in seed potatoes was conducted using material tested as virus-positive during seed potato certification testing from three

different periods: 2011 to 2013; 2016 to 2018; and 2020 to 2022. In total, 1041 PVY-positive samples were analysed by multiplex RT-PCR targeting the main well known recombination junctions of the PVY genome (Green et al., 2017; Lorenzen et al., 2006). The results showed that the Estonian PVY population was dominated by recombinant strain groups. The two main strain groups detected were PVY<sup>N-Wi</sup> and PVY<sup>NTN</sup>. Of the analysed samples, 466 contained PVY<sup>N-Wi</sup> and 395 contained PVY<sup>NTN</sup>, while in 63 samples contained both strains. By contrast, parental non-recombinant strains were detected rarely and mainly within mixed infections (Figure 15).

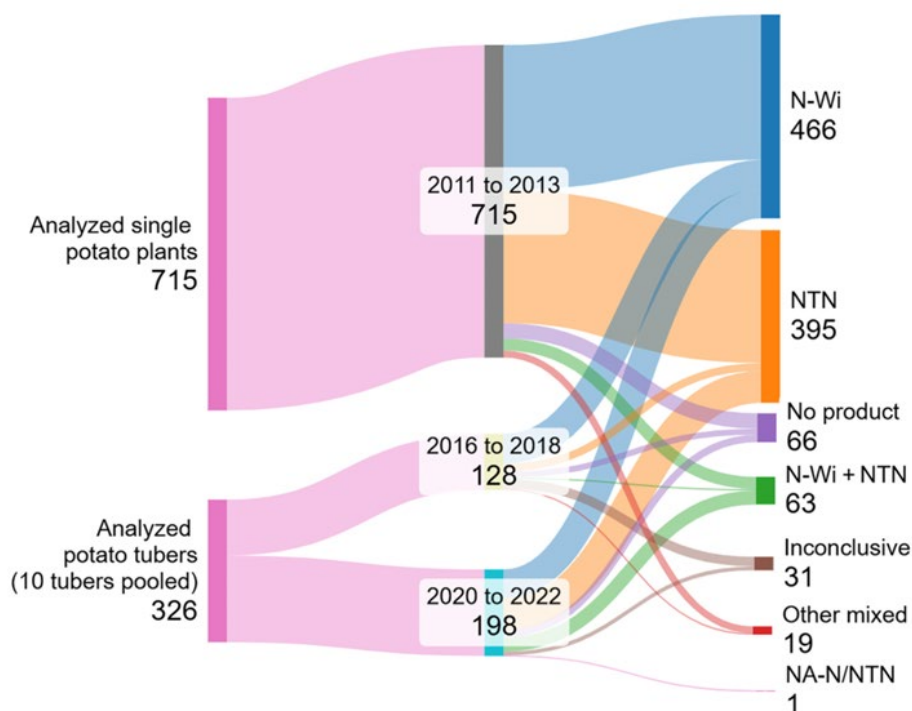


Figure 15. Sankey diagram visualizing the strain identity of different Estonian isolates according to multiplex RT-PCR results. The bars on the left represent the total number of potato virus Y (PVY)-positive samples identified in pre-screening, originating either from grown-out plants (identified by ELISA) or from tubers (identified by real-time RT-PCR). The bars in the middle represent the number of positive samples in each survey period; the bars on the right represent the RT-PCR results. Numbers under the text represent the count of samples (Publication II).

These findings indicate that the PVY population circulating in Estonian seed potatoes is dominated by recombinant strain lineages. This is consistent with international observations showing that recombinant PVY strains have largely replaced parental strains in potato production systems (Karasev and Grey, 2013; Green et al., 2018; Tran et al., 2022; Rushton et al., 2024; Ding et al., 2022). The predominance of recombinant strains is epidemiologically important because such strains may vary in symptom expression, aggressiveness, transmission, and ability to overcome resistance of the host (Funke et al., 2016; Gray and Power, 2018). Therefore, the characterization of PVY strains is not only important for understanding the diversity of the virus population, but also for informed selection of diagnostic approaches, supporting seed certification, improving

the evaluation of varietal performance under field conditions, and providing valuable information for potato breeders.

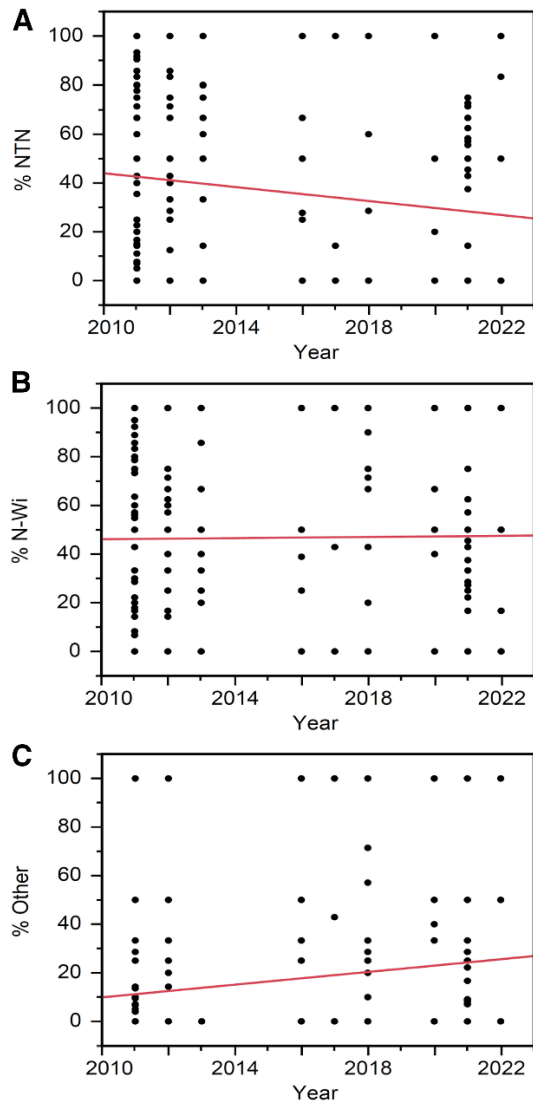


Figure 16. Potato virus Y (PVY) strain trends in Estonian seed potatoes in three survey periods (2011 to 2013, 2016 to 2018, and 2020 to 2022): A.  $PVY^{NTN}$ ; B.  $PVY^{N-Wi}$ ; C. other strains. PVY incidence rates in each seed lot are shown as dots, and the linear regression trendline is shown in red (Publication II).

A statistically significant decline was observed for  $PVY^{NTN}$ , whereas no significant trend was found for  $PVY^{N-Wi}$ . In addition, the proportion of samples assigned to category “other” increased over time (Figure 16). This category includes other strains than N-Wi or NTN, but also the inconclusive RT-PCR profiles, probably representing the isolates that could not be fully resolved with selected multiplex primer set. Although,  $PVY^{N-Wi}$  and  $PVY^{NTN}$  were the dominant strain groups throughout the study period, their relative

proportions varied between years (Figure 17). These results suggest that the PVY population in Estonia is dynamic, and subject to temporal change. Such shifts may arise from several interacting factors, including changes in cultivated varieties, differences in vector-mediated transmission, and selective process acting on the virus populations in the field.

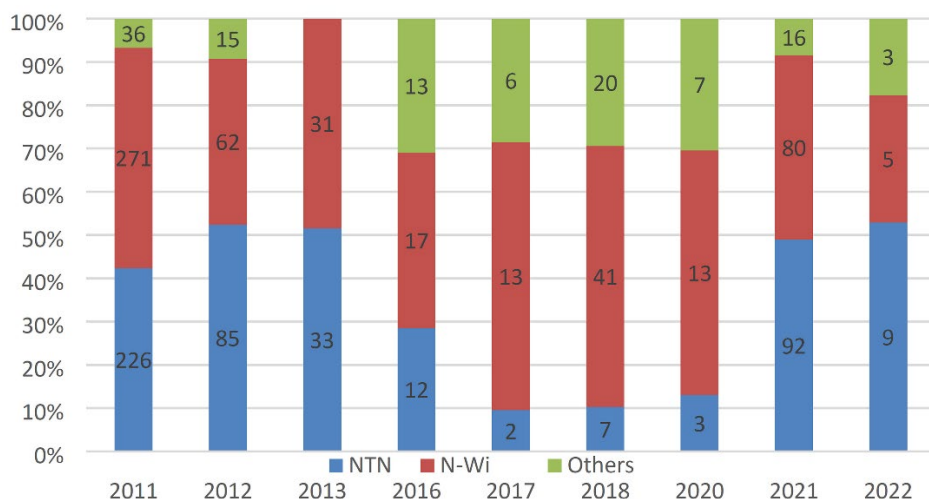


Figure 17. Proportion of potato virus Y (PVY) strains in analysed seed potato samples by year. Blue, NTN; red, N-Wi; green, other strains. The number of positive samples is indicated for each subset. NTN and N-Wi found in mixed infection were counted for the appropriate strain group. For the first period (2011 to 2013), the number represents individual samples; from 2016 onward, the number is the bulked samples (Publication II).

To complement the RT-PCR-based strain typing, selected samples from the survey were further analysed by HTS, which enabled the assembly of six full-length or nearly full-length PVY genomes and several shorter contigs. Those nearly complete and complete genomes were further analysed to identify recombination patterns using an in-house developed PVY Strainer program. Analyses revealed that the sequenced Estonian isolates belonged to the recombinant subgroups PVY<sup>N-Wi</sup> or PVY<sup>NTNa</sup> (Figure 18). This was particularly important because the multiplex RT-PCR assay used in this study enabled to identify the major recombinant strain groups, but it did not discriminate between the subgroups like NTNa and NTNb, or N-Wi and N:O. Sequencing filled this gap by providing a higher resolution characterization of the studied Estonian isolates. Importantly, while an RT-PCR-based strain typing suited well for large-scale monitoring of PVY strain groups providing relatively cheap approach, the sequencing provided more detailed information for a precise characterization of the circulating isolates and enabled also phylogenetic analyses.



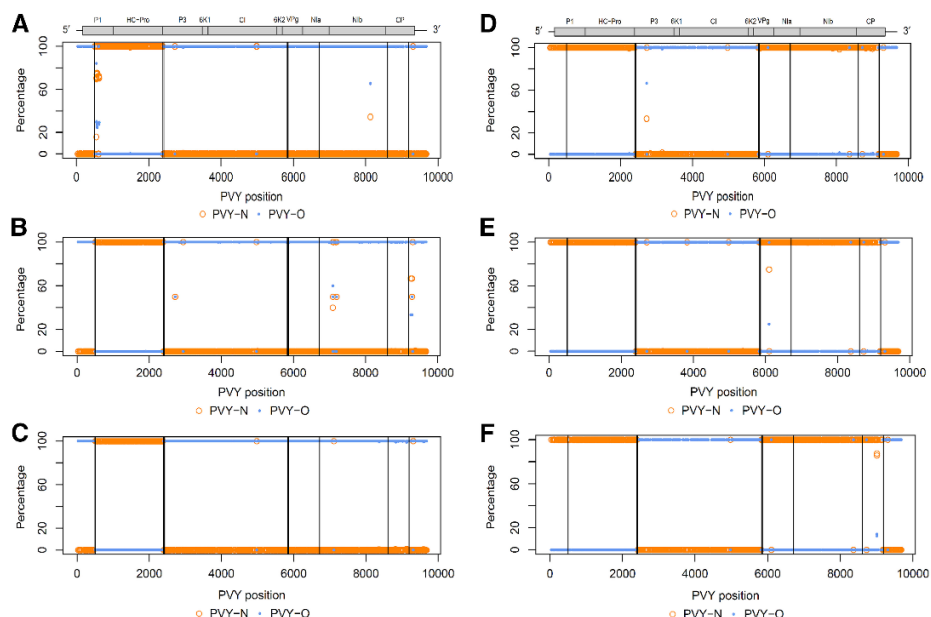


Figure 18. Recombination detection analysis by the GenomeTester package with the *gmer\_counter* function for assembled Estonian potato virus Y (PVY) isolates. HTS samples 9 (A) 10 (B); and 12 (C) showed the genome organization of PVY<sup>N-Wi</sup>, and samples 11 (D); 13 (E); and 14 (F) showed the typical genome organization of PVY<sup>NTNa</sup> (Publication II).

Additional information on Estonian PVY strain composition was further obtained from the 2025 seed potato survey involving 34 fields, from which all received seed potato samples were subjected to HTS as described in Publication III. The data on PVY findings are presented here, although not yet published. In sum, 7 of the analysed seed lots contained PVY (Table 3). Four full-length or nearly full-length genomes and several shorter contigs of PVY were assembled. Recombination analysis of the full-length genomes was performed using the same analytical tool as in the published PVY strain study (Publication II). Two of the genomes were identified as PVY<sup>N-Wi</sup> (Figure 19 B and C) with closest matches to Russian (OR480046) and German (ON604839) isolates in BLASTN analysis. In one library two different genomes were detected, one as PVY<sup>NTNa</sup> and one as PVY<sup>N:O</sup> (Figure 19 A) with closest matches to Russian isolates OR479975 and OR479975 in BLASTN analysis, respectively. The N:O strain isolate represents the first Estonian sequenced PVY<sup>N:O</sup> genome. Phylogenetic analysis of these four genome sequences from 2025 survey and the six PVY isolate genome sequences obtained from our first PVY strain study showed that the Estonian PVY sequences were most closely related to the isolates originating mostly from Russia and Slovenia, but also Germany, Slovakia, and Kazakhstan (Figure 20). The results of the GenomeTester analysis were in good agreement with the phylogenetic analysis, as the sequenced isolates grouped into the expected branches of the phylogenetic tree respective to their recombination patterns. This indicates that the PVY population present in Estonia forms a part of Eastern and Central European PVY population rather than representing unique local lineages. The similarity to German isolates is consistent with the movement of seed potatoes and other planting material in Europe. According to the World Integrated Trade Solution (WITS), Germany and the Netherlands have been the major import sources of seed potatoes in Estonia throughout

the last years (WITS, 2024). Similarly, the relationship with Russian, Slovenian, Slovak and Kazakh isolates can be explained by import-export activities. For example, Slovenia and Slovakia both import a large portion of their seed potatoes from Germany and the Netherlands. Kazakhstan imports seed potatoes mostly from Russia, Belarus, Germany, and the Netherlands (WITS, 2024). The Russian export and import rates are not known during the last four years, and are possibly reduced due to the trade restrictions, but in 2019, main origins of seed potatoes in Russia were the Netherlands, Germany, Finland, Belarus, Kazakhstan and India (WITS, 2019). In addition to the trade-related spread, the regional spread of virus populations could be connected to the mass-migration flights of aphid vectors (Nieminen et al 2000).

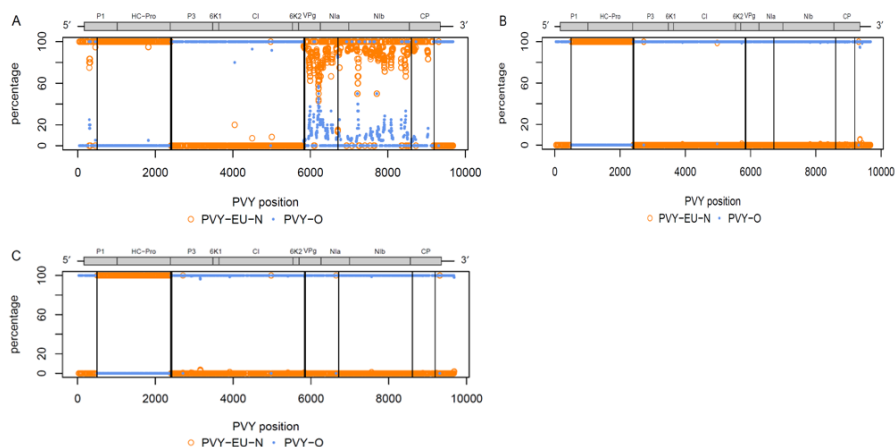


Figure 19. Recombination detection analysis by the GenomeTester package with the *gmer\_counter* function for assembled Estonian potato virus Y (PVY) isolates. Sample A represents a mixed infection of PVY<sup>NTNa</sup> and PVY<sup>N:O</sup>; samples B and C, indicate the genome organization characteristic of PVY<sup>N:O</sup>.

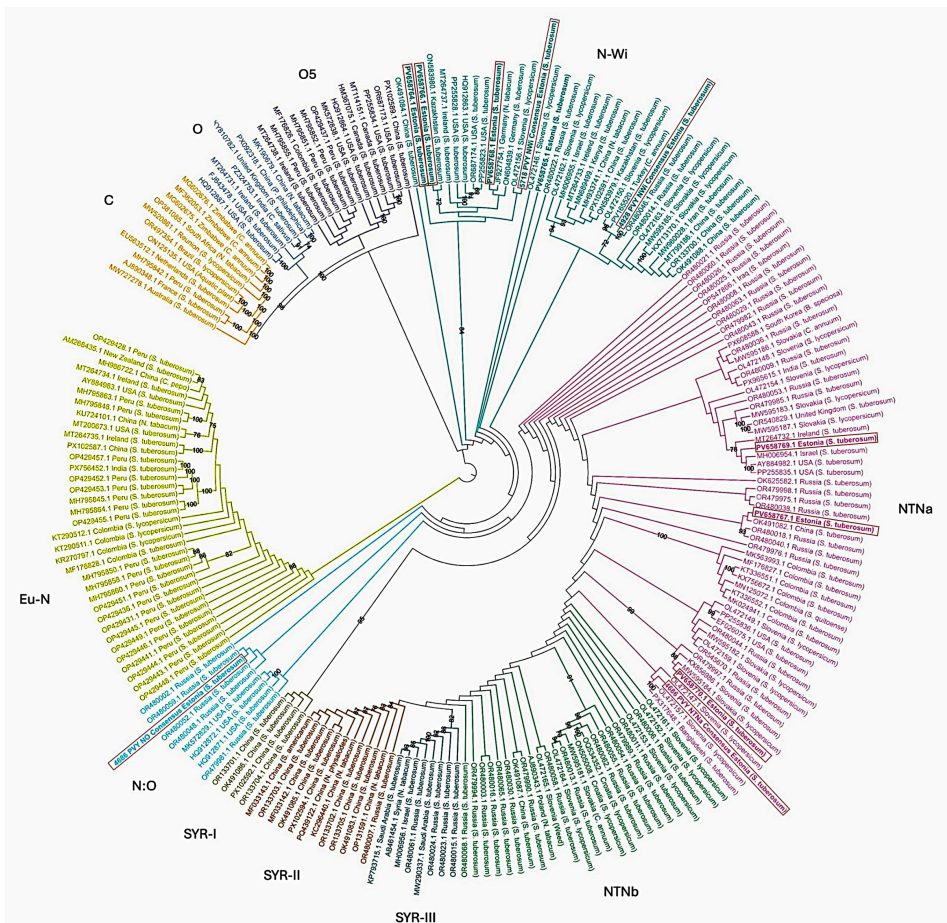


Figure 20. Phylogenetic tree of potato virus Y (PVY) isolates. Genome sequences were aligned in MEGA 12 using MUSCLE (1000 iterations), and maximum-likelihood phylogenetic trees were reconstructed using RAXML 2.0.1 with 1000 bootstrap replicates under the GTR+G+I model. Trees were visualized in iTOL. Estonian isolates are shown in bold, additionally surrounded by red squares.

Although limited in number, these additional sequences support the conclusion, that recombinant strains dominate in Estonia. However, it might be possible that the diversity of recombinant strains in the country may be broader than indicated by these first HTS-surveys. This underlines the importance of continued strain monitoring for detecting changes in the PVY populations and for improving understanding of its epidemiology. In this context, HTS is likely to become particularly suitable tool for the future strain surveillance, because it allows more detailed characterization of circulating isolates than the conventional strain-typing assays.

An important aspect of the performed PVY strain survey was the opportunity to examine the relationship between regional PVY strain composition and grown potato varieties. We demonstrated that prevalence of PVY<sup>N-Wi</sup> and PVY<sup>NTN</sup> differed remarkably among varieties. Some varieties, such as Laura, Madeira, and Jelly were not found to be infected with PVY<sup>NTN</sup> but rather with PVY<sup>N-Wi</sup>, likewise Romera, Maret, Teele, Secura and Anti that were predominantly infected with N-Wi but seldom with NTN. In contrast, Red Lady, Princess, Arielle, Marabel, Reet and Fontane showed higher proportion of NTN

infection and were only marginally targeted by N-Wi (Figure 21). The results indicate that potato variety's susceptibility or resistance may influence relative success of individual PVY strain groups. Consequently, the selection of cultivated varieties may contribute to shifts in the PVY strain composition over the time.

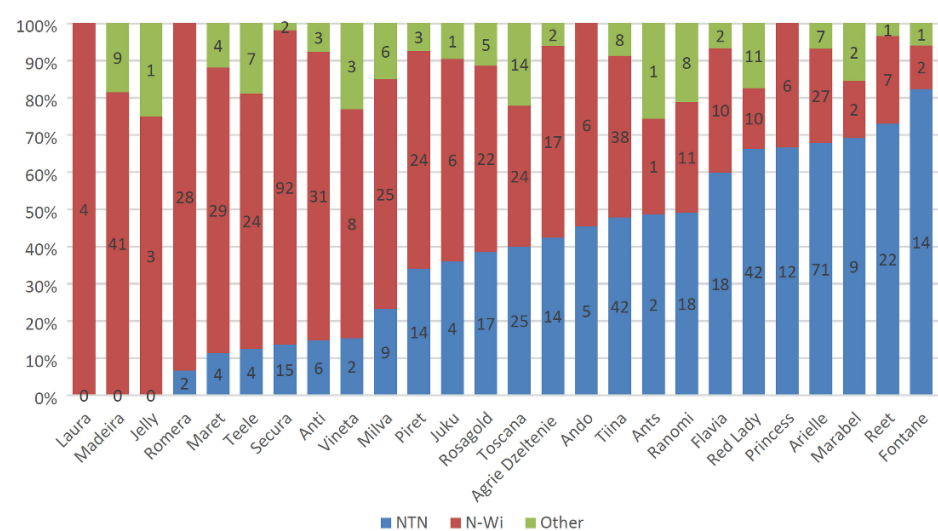


Figure 21. Proportion of potato virus Y (PVY) strain isolates in analysed PVY-infected seed potato samples by variety. Blue, NTN; red, N-Wi; green, other strains. Numbers indicate the number of positive samples (Publication II).

When interpreted together with the long-term monitoring data on varietal susceptibility presented in the previous chapter, the PVY strain composition results become particularly informative. The long-term dataset demonstrated that potato variety was the strongest determinant of PVY incidence in Estonia and the field resistance differed substantially among cultivars. The strain composition data add an important further dimension to this interpretation by indicating that varietal performance depends not only on general resistance to PVY, but also on the strain composition of the local virus population. This provides a plausible explanation for why some cultivars behaved differently in Estonian conditions than it would have been expected based on breeders' information. If resistance is more effective against one strain group than the other, then the apparent field performance of the variety will depend partly on the local prevalence of those strain groups. Conversely, the selection of cultivated varieties may also influence the dynamics of the PVY population itself by favouring the spread of certain strain groups over the time.

The interpretation is consistent with previous studies demonstrating that varietal resistance to PVY may be strain specific and the host resistance gap shape the relative abundance of PVY strains on commercial potato fields (Funke et al., 2016; Dupuis et al., 2019). In Estonia, the combination of long-term field monitoring and molecular strain characterization provides a particular informative framework for understanding PVY epidemiology. Together, these approaches, the rate of infection and the composition of the virus population must be considered when evaluating the susceptibility of potato varieties for seed production under local conditions.

The methodological aspects of the strain survey also merit consideration. Multiple RT-PCR proved initially as a suitable approach for large-scale surveillance of PVY strain groups, but a proportion of samples yielded with no amplification or inconclusive banding patterns, particularly in bulked tuber samples. Also, in some cases, unexpected PCR products appeared, most possibly as the result of non-specific primer binding to host genetic material. These observations underlie the value of combining targeted diagnostic assays with sequence-based approaches, particularly when detailed characterization of virus populations is required.

The current study represents the first systematic characterization of PVY strain composition not only in Estonia but also in the Baltic region. This gives the findings a significance beyond national level. PVY strain populations are known to vary geographically and temporally, and such differences may have important consequences for resistance durability, seed quality, and disease management (Green et al., 2017; Tran et al., 2022). The present study therefore provides a baseline for further studies of PVY population dynamics in the neighbouring countries.

Taken together, the results show that PVY population in Estonian seed potatoes is dominated by recombinant strain groups, principally PVY<sup>N-Wi/N:O</sup> and PVY<sup>NTNa</sup>. When interpreted together with long-term field data on varietal susceptibility, these findings demonstrate that PVY epidemiology in Estonia is shaped not only by overall infection pressure but also by interactions between specific virus strain groups and cultivated potato varieties. This has direct implications for diagnostics, varietal evaluations, seed potato production, and breeding. This emphasises the need to consider PVY as a genetically diverse and epidemiologically complex pathogen.

#### **4.4 Other potato virus strains identified in Estonian seed potato (Publication III)**

In addition to the long-term survey, the further information on potato virus diversity was obtained from the 2025 seed potato survey. In 2025, all seed lots submitted for certification, including all seed categories, were analysed by the HTS. This provided the opportunity to detect and characterize viruses beyond the scope of routine targeted testing and yielded in additional complete or near-complete genome sequences of PVY, PVS, PVM and PLRV present in Estonian seed potato material. Of those, new PVY isolates were described in previous chapter.

For PVS, one full genome sequence was known from the long-term study (Publication II); this belonged to the PVS-O (ordinary) strain group and showed the closest match to German isolate OM471986 with the nucleotide identity almost 99% in BLASTN analysis (Publication II). The 2025 survey yielded in five additional full-length or nearly full-length sequences of PVS genomes, of which two were identified as PVS-A, the Andean strain isolates (Figure 22; Publication III). The genetic analysis of Estonian PVS-A isolates (PZ397343-44) showed closest matches to the isolates reported from Kazakhstan (MN095414.1) with nucleotide identity near 99% in BLASTN analysis. As mentioned earlier, Kazakhstan is also an importer of German seed potatoes according to the WITS data. The phylogenetic analysis confirmed their grouping within PVS-A strain group (Figure 22). The PVS-A strain isolates are generally considered more severe than PVS-O strain isolates. The isolates representing PVS-A strain have been rarely found in the EU, except these that have occasionally reported in material originating from Germany (Bragard et al. 2020b). The non-European PVS-A has a quarantine status in the EU, while

the quarantine status of PVS-A with European origin still needs to be assessed by epidemiological research (Bragard et al. 2020b). The PVS-A positive seed lots discovered in the 2025 survey originated from the neighbouring fields in Viljandi county, but the seed potato itself was imported from Germany, therefore, indicating a putative introduction.

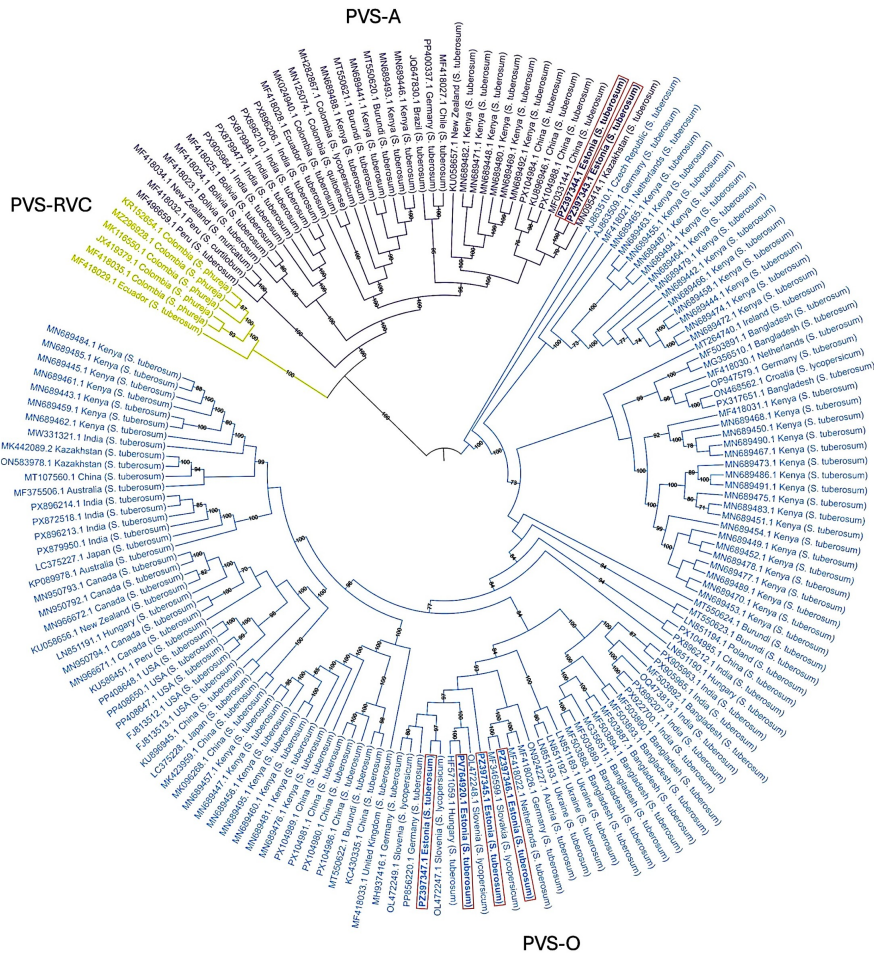


Figure 22. Phylogenetic tree of potato virus S (PVS) isolates. Genome sequences were aligned in MEGA 12 using MUSCLE (1000 iterations), and maximum-likelihood phylogenetic trees were reconstructed using RAXML 2.0.1 with 1000 bootstrap replicates under the GTR+G+I model. Trees were visualized in iTOL (modified from Publication III). Estonian isolates are shown in bold, surrounded by red squares.

The three other PVS isolates, the one from potato grown from German seed potato and the two grown from Estonian seeds, belonged to the PVS-O strain group, and those were most similar to German, Slovakian and Slovenian isolates (PP856220, MF346599 and OL472247, respectively) with nucleotide identity over 98% in BLASTN analysis (Publication III). The phylogenetic analysis confirmed their grouping within PVS-O strain group and with European isolates (Figure 22). Two samples from which the near-complete or complete PVS genomes (including PVS-A genomes) were assembled originated from lower class seed potatoes; therefore, under routine monitoring, the



presence of the virus would likely have remained undetected, and the occurrence of two distinct PVS strains in Estonia would not have been discovered.

In the case of PVM, three complete or near-complete genome sequences were assembled from the samples of 2025 survey. All these seed lots, one from Super Elite category while other two from lower categories, represented seed potatoes with Estonian origin (Table 3), and all isolates belonged to PVM-O (ordinary) strain group with highest similarity to Slovenian isolates (OL472243 and OL472245) with identity over 97% in BLASTN analysis. The phylogenetic analysis confirmed their grouping within PVM-O strain group and the Estonian isolates indicated close phylogenetic relationships (Figure 23).

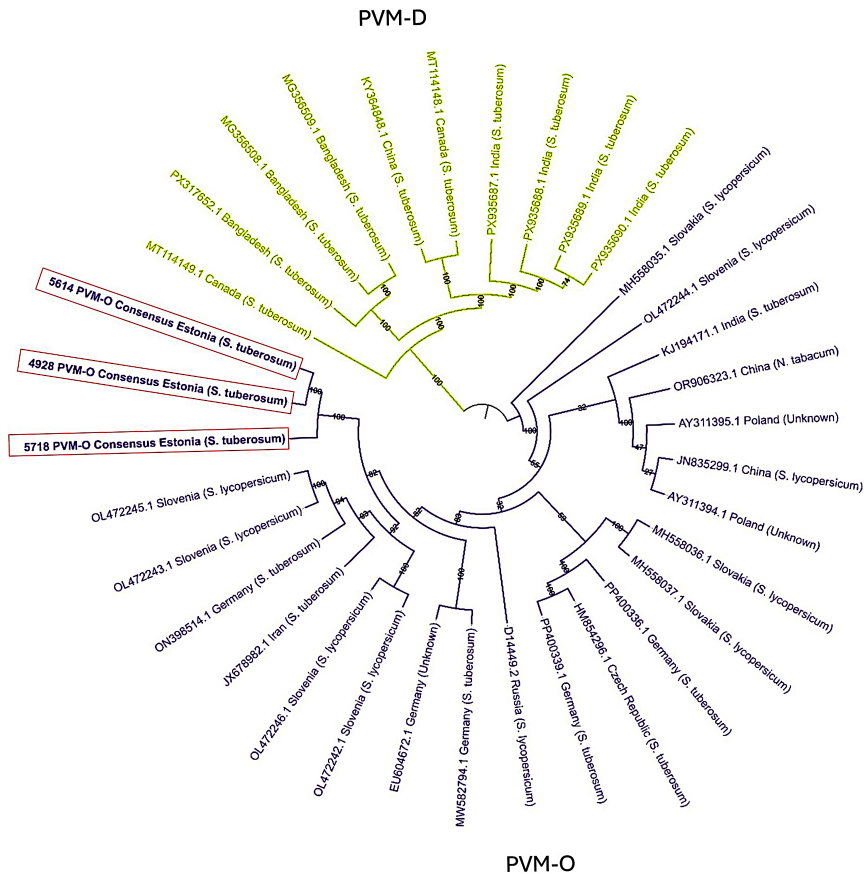


Figure 23. Phylogenetic tree of potato virus M (PVM) isolates. Genome sequences were aligned in MEGA 12 using MUSCLE (1000 iterations), and maximum-likelihood phylogenetic trees were reconstructed using RAXML 2.0.1 with 1000 bootstrap replicates under the GTR+G+I model. Trees were visualized in iTOL. Estonian isolates are shown in bold, surrounded by red squares.

For PLRV, two full-length or nearly full-length genomes were obtained from seed samples originally coming from Germany, and both represented PLRV-O (ordinary) strain with the closest matches to German isolate (MH937415) showing almost 99% of identity in BLASTN. The phylogenetic analysis confirmed their belonging in the PLRV-O group

(Figure 24). As with PVS, the seed lots with PLRV infection, were from lower category seeds and without HTS survey the occurrence of PLRV would have not been detected.

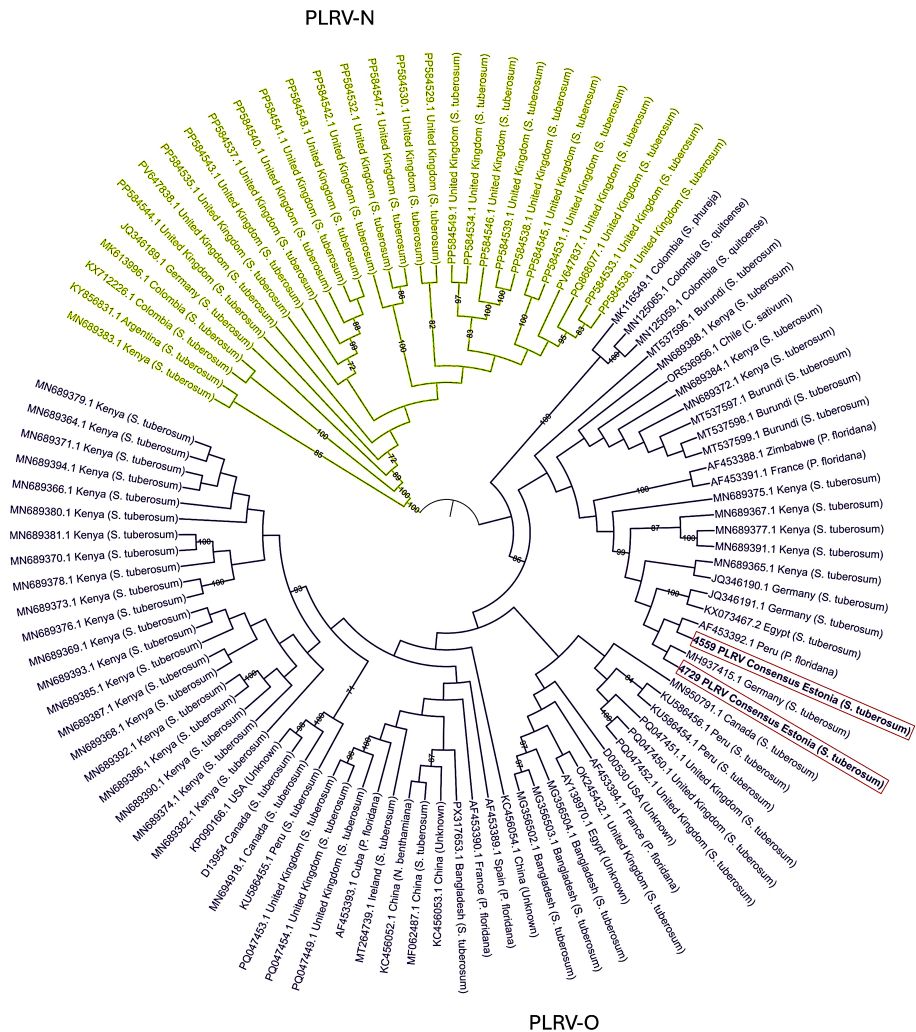


Figure 24. Phylogenetic tree of potato leafroll virus (PLRV) isolates. Genome sequences were aligned in MEGA 12 using MUSCLE (1000 iterations), and maximum-likelihood phylogenetic trees were reconstructed using RAXML 2.0.1 with 1000 bootstrap replicates under the GTR+G+I model. Trees were visualized in iTOL. Estonian isolates are shown in bold, surrounded by red squares.

In conclusion, the 2025 HTS-based survey revealed a broader diversity of potato viruses in Estonian seed potatoes than could be detected by routine certification testing. By analysing all seed categories, the survey enabled the detection and identification of viral strains of PVS, PVM and PLRV that would have likely remained undetected under standard monitoring. Notably, the detection of both PVS-A and PVS-O provided evidence for the presence of two distinct PVS strain groups in Estonia. Phylogenetic analyses further suggested that part of the detected viral diversity is linked to the international movement of seed potatoes, particularly trade with Germany. Overall, these results



demonstrate the value of HTS for improving virus surveillance in seed certification systems.

#### **4.5 Phytosanitary risks due to emerging virus threats (Publication IV)**

In addition to the potato viruses established in Estonian seed potato production, potential phytosanitary risks associated with emerging viruses must also be considered. Viruses first detected in non-potato hosts may nevertheless be relevant to potato production if they possess a broad host range or are capable persisting in the agricultural environment through alternative reservoir plants. The findings published in article IV and obtained during the routine testing of different host plants (van der Sman et al., unpublished data), therefore, also contribute to a broader assessment of virus-related phytosanitary risks in Estonia.

In 2019, tomato leaf curl New Delhi virus (ToLCNDV) that has a status of quarantine pathogen was detected for the first time in Estonia from cucumber and tomato samples originating from Halinga, Pärnu county (Publication IV). Molecular analysis of amplified genome region showed that the Estonian isolates were identical to each other and closely related to the ToLCNDV-ES strain previously reported from Spain. The first detection of ToLCNDV in Estonia was important because the virus is known to have wide host range and considerable epidemic potential (Moriones et al., 2017). Following confirmation of the detection, the plants were destroyed and the greenhouse was disinfected, and no further infection was detected in the subsequent year, indicating successful eradication. Nevertheless, this incidence demonstrated that imported seeds may pose a potential risk to Estonian agriculture, highlighting the importance of strict phytosanitary controls and continued monitoring of seed imports. ToLCNDV is a virus that can infect potato causing potato apical leaf curl disease (Kreuze et al., 2020a; Zaidi et al., 2017).

Among the viruses with the potential to infect potato, tobacco rattle virus (TRV) was detected in peony and astilbe in Estonia (van der Sman et al., unpublished data). TRV is a threat to potato production because it can cause corky ringspot disease in tubers (Moyo et al., 2022; Robinson and Harrison, 2003). Its detection in ornamental hosts indicates that the virus is present in Estonia although yet outside potato production systems. Because TRV is transmitted by nematodes (soil-borne infection), its epidemiology differs substantially from that of aphid-transmitted potato viruses and may be more difficult to manage through certification measures alone once the problems occur.

Taken together, the findings on ToLCNDV and TRV demonstrate that phytosanitary risks to the Estonian potato sector are not limited to the viruses currently monitored in the frame of seed certification. Although both viruses were detected in non-potato hosts and not detected in 2025 HTS survey from seed potatoes, both viruses can infect potato and may therefore pose a future threat. These observations underscore the importance of broad surveillance, early detection, and consideration of alternative hosts in national plant health preparedness.

## 5. Conclusions and future perspectives

This thesis provides an integrated overview of the occurrence, and strain composition of potato viruses in Estonia, with particular emphasis on PVY and its epidemiological significance in seed potato production.

The long-term monitoring data showed that among the six viruses included in routine certification testing of Super Elite seed potatoes, PVY was consistently the most prevalent, whereas PVM and PVS occurred at much lower levels, PLRV and PVX were rare, and PVA was not detected. Additional analyses conducted in 2025 further showed that in addition to PVY, also PVM, PVS, and PLRV are present in lower seed classes (Elite and Certified), and it can happen in higher incidences than expected. Thus, while the potato virus epidemiology in Estonia is based on the knowledge obtained from routine monitoring, the presence of other viruses than PVY can be underestimated by the routine seed potato production system monitoring.

The usage of HTS in the 2025 survey enabled the determination of the strain composition of identified viruses. All sequenced isolates of PVM, PLRV and PVS, except the two isolates of PVS, belonged to the ordinary strains and had the closest phylogenetic relationships with the isolates mostly found in Europe. Notably, two PVS isolates belonged to the strain group PVS-A (the Andean phylogroup) that is rarely found in Europe. More detailed analysis showed that the introduction of these isolates is most possibly caused by import of German seed potatoes. Indeed, there exist earlier reports indicating that Germany acts as a source of PVS-A, which is considered as a severe risk to potato production. Introduction of lower-class seed potato from the regions with the presence of severe virus strains but not tested for any other viruses than PVY and PVA creates an epidemiological risk.

Moreover, the work showed that phytosanitary risks relevant to potato production are not limited to viruses currently included in routine certification testing. The detection of other viruses (ToLCNDV, TRV) capable of infecting potato in Estonia, although from non-potato hosts, further illustrates the potential risks for the sector.

A central contribution of this thesis is the first detailed characterization of PVY strain composition in Estonia and the Baltic region. The research indicated that PVY population in Estonian seed potatoes is dominated by recombinant strain groups, primarily PVY<sup>NTNa</sup> and PVY<sup>N-Wi</sup>. Identification of these strains by the widely used multiplex RT-PCR assay was generally working but the used assay also produced some inconclusive data. Further HTS analysis confirmed that the detected variability in multiplex RT-PCR patterns was most probably created by unspecific annealing of viral primers to the host genomes with different genetic backgrounds. The next survey, based on HTS, confirmed the continuing presence of PVY<sup>NTNa</sup> and PVY<sup>N-Wi</sup>, although one isolate of PVY<sup>N:O</sup> was found too. Together, these findings demonstrated that the real strain composition of PVY (or any other virus) population could not be resolved with RT-PCR based survey only. Indeed, the thesis demonstrated the value of combining targeted molecular diagnostic methods with HTS. Whereas RT-qPCR and RT-PCR are suitable for large-scale monitoring, HTS enables more precise characterization of strains, also providing the basis for phylogenetic analyses. HTS is likely to become an important tool in surveillance, as it enables a detection and identification of known and unknown viruses, helping to identify the appropriate diagnostic targets timely.

The thesis also demonstrated that long-term certification data have a value beyond their regulatory purpose. When combined with information on cultivated varieties and

regional weather conditions, these data made it possible to examine the epidemiological drivers of PVY incidences under real-life production conditions. The results showed that potato variety is the most important factor in PVY spread. Climatic variables also influenced PVY incidences, but their effect was weaker than that of variety. It was seen that resistant varieties were less affected by weather factors than susceptible ones, highlighting the importance of cultivar selection. The combination of PVY strain data together with varietal information provided further epidemiological insights. Difference in the susceptibility of potato varieties to different PVY strains, together with selection of cultivated varieties, plays a role in shaping PVY strain composition.

Overall, this thesis demonstrated that potato virus epidemiology in Estonia is shaped by the interactions between virus occurrence, strain composition, varietal susceptibility, and environmental conditions. The results are directly relevant to diagnostics, seed potato certification, varietal evaluation, breeding, and future phytosanitary surveillance.

Future work should continue to make use of monitoring data for comprehensive scientific analyses in combination with varietal information and weather-related factors. If possible, the aim should be involvement of a broader range of potato viruses in routine monitoring, also in lower seed classes. Continued strain-level surveillance is needed periodically, because as the present study demonstrated that strain populations are dynamic and may change over time. In addition, future monitoring should address alternative hosts, such as other crops, ornamentals, and weeds, as potential reservoirs for potato-infecting viruses, as well as the insect vectors to obtain a complete epidemiological overview.

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## **Abstract**

### **Occurrence and Characterization of Potato-Infecting Viruses and Their Strains in Estonia**

Potato is an important agricultural crop in Estonia, and the production of high-quality seed potatoes is essential for maintaining yield, and competitiveness of the sector. Potato viruses represent a persistent challenge for the production because they reduce tuber quality, lower yield, and compromise the phytosanitary status. In vegetatively propagated material, such as potato, reliable detection and identification of the viruses, together with the knowledge of their strain composition and epidemiology, are especially essential for effective disease management. However, although annual virus monitoring is routinely conducted within the Estonian seed potato certification system, its broader scientific use has remained limited, and information on the strain composition of potato viruses in Estonia has been lacking. In addition, the susceptibility of potato varieties and other factors influencing virus spread under Estonian conditions has not been evaluated systematically.

The aims of this thesis were to investigate the occurrence and identity of potato viruses in Estonia, with emphasis on varietal susceptibility, improvement of diagnostic approaches, identification of strain compositions, and phytosanitary risks relevant to potato industry. More specifically, the work aimed to determine which potato viruses are present in Estonian seed potatoes, to improve the accuracy of detection and virus characterization, to characterize the strains of the viruses, especially PVY, to evaluate the susceptibility of potato varieties to PVY, to assess the factors influencing PVY spread, and to address wider risks posed by emerging viruses.

To achieve those aims, the thesis combined several complementary approaches. Long-term monitoring data collected through the Estonian seed potato certification system from 2007 to 2022 was used to assess the incidences of six potato viruses routinely monitored in certification: PVY, PVA, PVX, PVM, PVS, and PLRV. These data were further linked with information on cultivated potato varieties, and regional weather variables, namely temperature and precipitation during the potato growing season. Targeted diagnostic methods, including ELISA, (RT)-PCR, and real-time RT-PCR methods were used for virus detection and strain typing. In addition, high throughput sequencing was applied to selected virus-positive material and later to all seed potato lots included in the 2025 survey, enabling deeper characterization of virus strains and phylogenetic relationships.

The long-term monitoring data showed that PVY is the predominant virus in Estonia. Among the six viruses routinely monitored in Super Elite seed potatoes, PVY was consistently present, whereas PVM and PVS were detected at considerably lower levels, PVX and PLRV were detected only in the early years of the study, and PVA was not found. Additional survey from 2025 demonstrated that PVS, PVM and PLRV are also present in lower seed classes, for which corresponding information had previously been unavailable.

The long-term dataset provided an opportunity to analyse the factors affecting PVY spread under Estonian conditions. Potato variety was identified as most important determinant of PVY incidences, and differences in infection levels allowed the classification of varieties into resistance groups ranging from very high resistance to very susceptible. Several cultivars performed differently in Estonian conditions than expected based on breeders' information, indicating that varietal susceptibility was context dependent. Climatic variables, particularly in July, also influenced the spread of PVY,

although their effect was weaker than that of potato variety. The further data analysis showed that resistant varieties were less affected by weather-related variation.

A substantial part of the thesis focused on the strain composition of PVY in Estonia. Multiplex-RT-PCR analysis of 1041 PVY positive samples collected during 2011 to 2013, 2016 to 2018, and 2020 to 2022 showed that the Estonian PVY population was dominated by recombinant strain groups, primarily PVY<sup>N-Wi</sup> and PVY<sup>NTN</sup>. Sequencing of selected isolates confirmed that analysed Estonian isolates belonged to PVY<sup>N-Wi</sup> and PVY<sup>NTNa</sup> subgroups. Additional sequencing on 2025 also revealed one isolate with PVY<sup>N:O</sup> genome, representing the first Estonian isolate with that genomic structure. Phylogenetic analysis demonstrated that Estonian PVY isolates were closely related to isolates from Russia, Slovenia, Germany, Slovakia, and Kazakhstan, placing them within a broader geographic perspective.

The PVY strain composition data together with varietal information demonstrated differences in the susceptibility of potato varieties to individual PVY strain groups. Therefore, selection of potato varieties might play a role in shaping PVY strain dynamics under field conditions. Moreover, it was shown that local PVY composition can affect varietal performance in local conditions. This helps to explain why some varieties performed differently in Estonia than expected based on breeders' description. Several varieties performed here as very susceptible and were, hence, not suitable for cultivation under Estonian conditions.

High-throughput sequencing provided additional strain-level information also on other potato viruses detected in Estonian seed potatoes. Full-length or nearly full-length genomes of PVS, PVM and PLRV were assembled and characterized. The PVS isolates belonging to two different strain groups (A, the Andean, and O, the ordinary), flagged important information, that PVS-A, considered rare in Europe, is possibly freshly introduced from Germany due to limited virus control in lower class seed potato. PVM and PLRV isolates both belonged to the O group of respective viruses. Phylogenetic analyses showed that these isolates grouped mainly with European isolates.

Finally, this thesis addressed broader phytosanitary risks relevant to potato production. The first detection of ToLCNDV virus in tomato and cucumber, together with yet unpublished findings of TRV in ornamental hosts, demonstrates that viruses capable of infecting potato occur outside potato production systems. These findings underlie the importance of monitoring alternative hosts and broad-scope consideration of natural and trade-related transmission pathways in strengthening national capacity to respond to the plant health threats.

In conclusion, this thesis provides the first comprehensive overview of potato viruses occurrence and PVY strain composition in Estonia and the Baltic region. It demonstrates the value of combining long-term certification data with varietal and weather information, and of applying molecular methods, including HTS, for deeper virus characterization. The results are relevant to diagnostics, seed potato certification, varietal evaluation, breeding, and future surveillance. Importantly, it also contributes to strengthening the scientific basis for potato virus management and phytosanitary preparedness in Estonia.



## Lühikokkuvõte

### Kartulit nakatavate viiruste ja nende erinevate tüvede esinemine ning iseloomustamine Eestis

Kartul on Eestis oluline põllukultuur ning kvaliteetse seemnekartuli tootmine on vajalik saagikuse ja sektori konkurentsivõime säilitamiseks. Kartuliviirused on kartulitootmisele suureks väljakutseks, kuna need vähendavad mugulate kvaliteeti ning saagikust ja mõjutavad fütosanitaarset staatust. Vegetatiivselt paljundatava materjali, nagu kartul, puhul on haigustõrje jaoks oluline viiruste usaldusväärne tuvastamine ja identifitseerimine. Koos teadmistega ringlevate viirustüvede ning nende epidemioloogia kohta võimaldab see tõhusamat tõrjet. Eesti seemnekartuli sertifitseerimissüsteemi osana teostatakse iga-aastaselt viiruste monitooringut, kuid saadud informatsiooni laiem teaduslik kasutamine on jäänud piiratuks ning teave ringlevate kartuliviiruste tüvede kohta Eestis on puudulik. Lisaks sellele ei ole varem süstemaatiliselt hinnatud Eesti kartulisortide vastuvõtlikkust ega muid viiruse levikut mõjutavaid tegureid.

Selle töö laiemaks eesmärgiks oli uurida kartuliviiruste esinemist Eestis, pöörates erilist tähelepanu diagnostiliste meetodite täiustamisele, viirustüvede koosluste määramisele, kartulisortide vastuvõtlikkuse hindamisele ning sektorile oluliste fütosanitaarsete riskide tuvastamisele. Täpsemalt oli töö eesmärk välja selgitada, millised kartuliviirused esinevad Eesti seemnekartulis, parendada viiruste tuvastamise ja identifitseerimise meetodeid, iseloomustada viiruste, eriti kartuliviirus Y (PVY), tüvesid, hinnata kartulisortide vastuvõtlikkust mainitud viirusele ning tuvastada teisi PVY levikut mõjutavaid tegureid ja käsitleda laiemaid taimeviirustega seotud riske sektorile.

Nende eesmärkide saavutamiseks ühendati selles töös mitu teineteist täiendavat lähenemisviisi. Kartuliviiruste esinemissageduse hindamiseks kasutati Eesti seemnekartuli sertifitseerimissüsteemi raames aastatel 2007 kuni 2022 kogutud seireandmeid kuue rutiinselt monitooritava kartuliviiruse kohta: PVA, PVY, PVX, PVM, PVS ja PLRV. Andmed seoti omakorda kasvatatud kartulisortide ja piirkondlike ilmastikuteguritega, eeskätt kartuli kasvuperioodi temperatuuri ja sademete hulga. Viiruste tuvastamiseks ja nende tüvede määramiseks kasutati viirusspetsiifilisi diagnostilisi meetodeid, sealhulgas ELISA-t, RT-PCR-i ja reaalaaja RT-PCR-i. Lisaks rakendati valitud hulga perioodi 2007 kuni 2022 viiruspositiivsete proovide analüüsimiseks ka kõrge läbilaskevõimega sekveneerimist ning 2025-ndal aastal tehti mass-sekveneerimine juba kõikidele seemnepartiidele. See võimaldas põhjalikumalt iseloomustada viirustüvesid ja nende fülogeneetilisi seoseid.

Pikaajalised seireandmed näitasid, et PVY on Eestis enamlevinud kartuliviirus. Kuue kartuliviiruse, mida määratakse rutiinselt „supereliit“ seemnekartuli kategooriast, analüüsitulemused näitasid järjepidevalt PVY olemasolu. Lisaks tuvastati ka PVM ja PVS viiruseid, kuid neid esines märksa madalamal tasemel. PVX ja PLRV-d tuvastati ainult uurimisperioodi alguses ning PVA viirust ei leitudki. 2025-ndal aastal läbiviidud täiendav analüüs näitas, et PVS, PVM ja PLRV esinevad ka madalamates seemnekategooriates, mille kohta varasem sellekohane teave puudus.

Pikaajaline andmestik võimaldas analüüsida PVY levikut mõjutavaid tegureid Eesti tingimustes. Kõige olulisemaks PVY esinemissagedust määravaks teguriks osutus kartulisort ning nakkustasemete erinevuse põhjal oli võimalik sordid liigitada resistentsusklassidesse alates väga kõrge resistentsusega sortide klassist kuni väga vastuvõtlikeni. Mitme sordi käitumine Eesti tingimustes erines aretajate esitatud andmetest, mis viitab sellele, et sortide vastuvõtlikkus sõltub konkreetsest

kasvukeskkonnast. Ka kliimategurid, eeskätt juulikuu ilmastikutingimused, mõjutasid PVY levikut, kuigi nende mõju oli väiksem kui kartulisordil. Edasine andmeanalüüs näitas, et resistentsed sordid olid ilmastikutingimuste muutlikkuse suhtes vähem tundlikud.

Oluline osa tööst keskendus PVY tüvede koosseisu uurimisele Eestis. Aastatel 2011-2013, 2016-2018 ja 2020-2022 kogutud 1041 PVY positiivse proovi multipleks-RT-PCR tulemused näitasid, et Eestis on PVY populatsioonis enamlevinud rekombinantset tüverühmad, peamiselt PVY<sup>N-Wi</sup> ja PVY<sup>NTN</sup>. Valitud isolaatide sekveneerimine kinnitas, et analüüsitud Eesti isolaadid kuulusid alamrühmadesse PVY<sup>N-Wi</sup> ja PVY<sup>NTNa</sup>. 2025-ndal aastal tehtud täiendav sekveneerimine tuvastas ka ühe PVY<sup>N-O</sup> genoomiga isolaadi, mis oli esimene sellise genoomistruktuuriga isolaat Eestis. Fülogeneetiline analüüs näitas, et Eesti PVY isolaadid olid enamasti lähedaselt suguluses Venemaalt, Sloveeniast, Saksamaalt, Slovakiast ja Kasahstanist pärit isolaatidega, mis paigutas need leiud laiemasse geograafilisse konteksti.

PVY tüvede koosluste andmed koos sorditeabega näitasid, et kartulisortide vastuvõtlikus erinevate PVY tüvegruppide suhtes on erinev. Seetõttu võib kartulisortide valik mõjutada PVY tüvede levimise dünaamika kujunemist põllutingimustes. Ühtlasi ilmnes, et kohaliku PVY populatsiooni koosseis võib mõjutada ka sortide toimivust kohalikes tingimustes. See aitab selgitada, miks mõned sordid Eesti põldudel ei vastanud aretaja kirjeldustele vastuvõtlikkuse osas. Mõned sordid käitusid Eesti tingimustes väga vastuvõtlikena ning osutusid seetõttu siin kasvatamiseks sobimatuteks.

Kõrge läbilaskevõimega sekveneerimine võimaldas saada täiendavat tüve tasandil informatsiooni ka teiste Eesti seemnekartuli proovidest tuvastatud viiruste kohta. PVS-i, PVM-i ja PLRV täispidad või peaaegu täispidad genoomid assambleeriti ja iseloomustati. Kahte erinevasse tüverühma kuuluvate PVS isolaatide (A ehk Andide ja O ehk tavalise) tuvastamine osutas olulisele aspektile, et Euroopas haruldaseks peetav PVS-A on tõenäoliselt äsja Saksamaalt sisse toodud. Nii PVM kui PLRV isolaadid kuulusid vastavate viiruste O-rühma. Fülogeneetiline analüüs näitas, et need isolaadid rühmitusid peamiselt Euroopa isolaatidega.

Lõpuks käsitleti töös ka kartulikasvatuse seisukohalt laiemaid fütosanitaarseid riske. ToLCNDV esmaleid Eestis tomatist ja kurgist koos seni avaldamata tulemustega TRV leidude kohta ilutaimedest näitab, et potentsiaalseid kartulit nakatavaid viiruseid esineb ka väljaspool kartulikasvatust. Need leiud rõhutavad alternatiivsete peremeestaimede seire ning nii loodusliku kui kaubandusega seotud levikuteede laiapõhjalise käsitlemise tähtsust, et tugevdada riigi suutlikkust reageerida taimetervise ohtudele.

Kokkuvõttes annab käesolev töö esimese tervikliku ülevaate kartuliviiruste esinemisest ning PVY tüvede koostisest Eestis ja laiemalt Baltimaade piirkonnas. Töö näitab pikaajalise sertifitseerimisandmete, sorditeabe ja ilmastikuandmete ühendamise väärtust ning molekulaarsete meetodite, sealhulgas kõrge läbilaskevõimega sekveneerimise, olulisust viiruste põhjalikumal iseloomustamisel. Saadud tulemused on olulised diagnostika, seemnekartuli sertifitseerimise, sortide hindamise, aretuse ja tulevaste seirete seisukohalt. Ühtlasi annab töö olulise panuse kartuliviiruste leviliku kontrollimise ja fütosanitaarse valmisoleku teadusliku aluse tugevdamisse Eestis.



## Appendix

### Publication I

van der Sman, P., Pata, P., Järvekülg, L. et al. Long-Term Monitoring of Six Potato Viruses in Estonian Seed Potatoes and an Assessment of Susceptibility of Potato Varieties to PVY. Potato Res. 68, 3123–3145 (2025). <https://doi.org/10.1007/s11540-025-09862-0>





# Long-Term Monitoring of Six Potato Viruses in Estonian Seed Potatoes and an Assessment of Susceptibility of Potato Varieties to PVY

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## Abstract

Determination of virus incidence rates of PVY, PVA, PVX, PVM, PVS, and PLRV is crucial for certifying the EU's Super Elite seed potatoes. Typically, lower-class seed potatoes are categorized by PVY and PVA rates. Our analysis covered 1023 seed lots tested for certification in Estonia from 2007 to 2022. We found that in 112 Super Elite potato seed lots, PVY incidence was 2.43%, PVM 0.48%, and PVS 0.11% over 16 years, with PLRV and PVX being rare. No PVA was detected. In 911 lower-class seed lots, PVY incidence was 3.92%, and PVA was not detected. PVY incidence from 2007 to 2022 showed a weak positive correlation with July temperatures and precipitation, and a weak negative correlation with August temperatures. One-way ANOVA analyses of the 32 most frequently cultivated varieties revealed significant infection rate differences ( $p < 0.0001$ ), allowing for the categorization of potato varieties into susceptibility/resistance groups. Laura, Jelly, Gala, and Princess were placed among very highly resistant varieties, whereas Ando, Arielle, Arcona, Rosagold, and Folva were placed among very susceptible varieties. Varieties like Secura, Romera, Folva, and Rosagold were more susceptible than reported earlier. Climatic variables did not affect very resistant or resistant varieties, while PVY incidences were significantly influenced in some of the susceptible or very susceptible varieties. This impact was more pronounced on varieties of non-local origin. The study suggests that long-term monitoring data collected via the national seed potatoes certification system can provide valuable and independent insights into varieties' susceptibility/resistance under regional conditions.

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**Keywords** Potato varieties · Potato viruses · PVY · Resistance · Susceptibility

## Introduction

The potato (*Solanum tuberosum*) is one of the most important agronomic crops worldwide and the leading vegetable crop in Estonia, introduced in the middle of the eighteenth century when it was initially grown in gardens. Gradually, it established itself as a crucial food crop, and by the 1860s, it became extensively cultivated (Kahk 1994). In 1926, regulations were implemented to control the transport of potatoes, aiming to prevent the spread of dangerous plant diseases and pests (“The Control of the Transport” 1929).

The Estonian Potato Growers Association played a key role in promoting potato cultivation and export. Starting in 1932, Estonian seed potatoes were exported to Italy, Spain, Switzerland, Great Britain, Norway, Portugal, Palestine, Argentina, Uruguay, and other destinations. Potato cultivation peaked in 1952 when the cultivation area expanded to 104,800 hectares (Kotkas 2006). However, since the 1980s, the industrial potato growing area has significantly decreased, shrinking from 72,300 to 3381 hectares by 2022. Simultaneously, there has been a shift in average yields from 15.9 t/ha in 1980 to 23.1 t/ha in 2022, with the highest recorded yield of 25.9 t/ha in 2020 (Statistics Estonia, <http://www.stat.ee>).

Over the last 16 years, the overall arable land used for potato cultivation has decreased to approximately one third of its original value. However, the portion dedicated to seed potatoes has remained relatively stable, averaging approximately 266 hectares. The quality of seed potatoes is consistently monitored to ensure the production of propagation material free or nearly free of infectious microorganisms and viruses that may cause plant diseases.

The most important globally spread potato viruses are the potyviruses potato virus Y (PVY), and potato virus A (PVA), the carlaviruses potato virus M (PVM), and potato virus S (PVS), the potexvirus potato virus X (PVX), and the polerovirus potato leafroll virus (PLRV). Most of these are primarily aphid-transmitted, whereas PVX is transmitted mechanically (Kreuze et al. 2020).

In Estonia, the observed symptoms of viral infections in potatoes most possibly corresponding to PVY and PVX were first described in the 1930s (Lepik 1939). At that time, test plants such as tobacco, bell pepper, and thorn apple were inoculated to characterize virus infection symptoms in combination with the determination of molecular masses of virus particles. PVX was considered the prevalent virus, whereas PVY was considered to have the greatest economic impact (Ümarik 1946). Soon after, a combined use of bioassays, electron microscopy, and polyclonal antisera enabled the starting of the regular monitoring and testing of the susceptibility/resistance of potato varieties grown in Estonia (Agur 1995). The first country-wide survey using monoclonal antibodies in enzyme-linked immunosorbent (ELISA) analysis to study the spread of potato viruses X, M, Y, and S, along with potato leafroll virus (PLRV), was carried out in 1986–1987 (Järvekülg et al. 1995). Among the 5987 potato tubers analyzed, 13.8% were infected by at least one virus. The incidence rates of the tested five viruses were the following: PVX 6.7%, PVS 4.7%,

PVY 4.0%, PVM 3.6%, and PLRV 3.5%. The annual seed potato virus monitoring based on serological detection was established in 2002. Unfortunately, the monitoring data collected between 2002 and 2006 is inaccessible for research purposes.

The current study describes the incidence of potato viruses Y, A, X, M, S, and PLRV revealed during the regular seed potato monitoring from 2007 to 2022 in Estonia. We employ serological detection combined with molecular methods. We focus on climatic factors and potato variety susceptibility influencing the spread of PVY, which remains the predominant potato virus in Estonia.

## Materials and Methods

### Weather Information

The weather information was received from the Estonian Environment Agency.<sup>1</sup> We collected temperature and precipitation data based on field locations, using the county average if multiple locations within a county were involved (Suppl. Table 1).

### Samples

The seed potato samples were collected for virus testing as part of the potato seed certification system by inspectors from the Estonian Agriculture and Food Board (EAFB). The seed potato certification system in Estonia adheres to domestic laws and regulations aligned with EU legislation. Before 2015, seed potato tubers were classified as follows: Super Elite (SE), Elite (E1–E3), and certified class A. Since 2015, seed potatoes have been categorized as follows: Super Elite seed potatoes (pre-basic tissue culture plants, marked as PBTC, and pre-basic potatoes grown in fields for the subsequent 3 years—marked as PB2, PB3, PB4, accordingly), Elite seed potatoes (marked as S, SE, and E) originating from Super Elite seed potatoes or higher-class Elite seed potatoes, and Certified seed potatoes (marked as A and B). The incidence of the six potato viruses is crucial in determining the category of Super Elite seed potato lots, and the incidence of PVY + PVA is the basis for the categorization of Elite and Certified classes.<sup>2</sup>

Identification of potato viruses was conducted at the Centre of Estonian Rural Research and Knowledge (METK) in the Plant Health and Microbiology Laboratory from 2007 to 2022. The analyses were performed without prior data on sampling sites (the cultivar and field location data were made available later by the EAFB

<sup>1</sup> The data are available in the Estonian Environment Agency's environmental monitoring database KESE as of 20.02.2024.

<sup>2</sup> Before 2015, the acceptable virus percentages were 0% for PLRV, PVM, PVS, and PVX, and 0.5% for PVY + PVA in SE seed potatoes; 2% for PVY + PVA in E1/E2 seed potatoes; 4% for PVY + PVA in E3; and 10% for PVY + PVA in A class seed potatoes. Starting from 2015, none of six viruses is allowed in PBTC, and a 0.5% virus presence is permissible in PB classes. The allowed PVY percentage in Elite seed potatoes is 1% for S class, 2% for SE class, and 4% for E classes. In the lower-class Certified seed potatoes, the PVY percentage may exceed up to 8% in A class and 10% in B class.



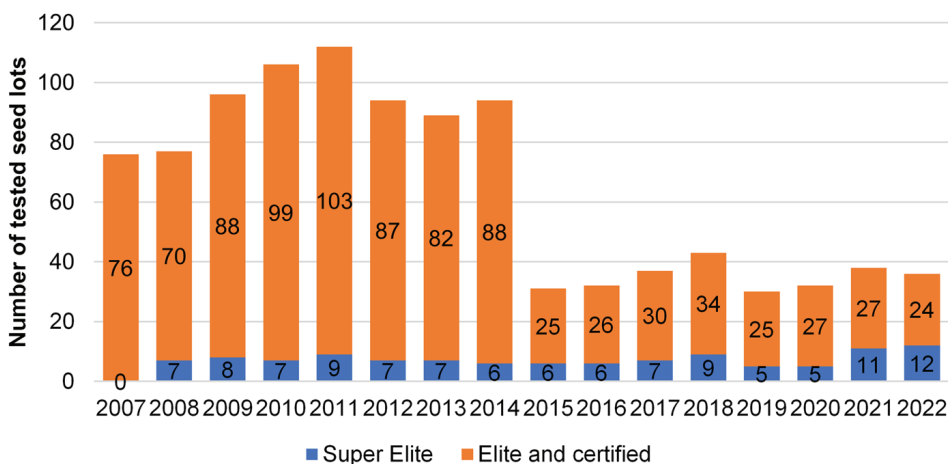
for preparing the current publication). Samples were collected from multiple locations across Estonia, covering 11 out of 15 counties. Each sampling lot consisted of 130 tubers, with 96 or 100 being involved in testing, depending on the method used for virus monitoring. From 2007 to 2014, the number of seed lots per year varied between 76 and 115. From 2015 onward, the number of seed lots decreased and ranged from 30 to 43 due to legislative change.

In total, 1023 seed lots were collected for further analysis over the period from 2007 to 2022. One hundred twelve Super Elite tuber seed lots were tested for the six most common potato viruses (PVA, PVY, PVX, PVM, PVS, PLRV). Six hundred ninety-three lower-class (Elite and Certified) tuber seed lots were tested for two viruses, PVA and PVY (until 2014), and 218 lower-class seed lots were tested for PVY only (since 2015). The numbers of seed potato lots tested in different years are shown in Fig. 1.

At the beginning of the surveys, ELISA-based virus detection analyses were used for both Super Elite and Elite/Certified class potatoes. From 2015 onward, Real-Time RT-PCR was implemented for PVY detection in Elite and Certified classes, whereas ELISA continued to be used for six virus detection in Super Elite potatoes. Since the 2021 season, only Real-Time RT-PCR has been used for all.

## ELISA

For ELISA analyses, plants were grown from tubers in quarantine greenhouse facilities at the METK. Leaves from newly grown plants were collected after 4 weeks and ground to obtain sap. This sap was used for detecting six viruses (PVY, PVA, PVM, PVS, PVX, and PLRV) using Bioreba ELISA antibodies or complete kits, following the manufacturer's instructions. Single plants were analyzed until 2014. Later, the bulking of four samples was employed. In the case of sample bulking, the



**Fig. 1** The number of tested super elite seed potato lots (SE until 2014; PBTC and PB from 2015; shown in blue) compared to lower categories (E and A until 2014; S, SE, E, A, and B from 2015; shown in orange)

UNECE Seed Potato Standard S-1 (<https://unece.org/trade/wp7/SeedPotatoes-Standards>) guidelines were followed to calculate the most probable value of the virus present in the sample.

### Real-Time TaqMan RT-PCR

The sampling process involved bulking 10 tubers together in a single extraction. From each tuber, pieces were collected from both ends. Subsequently, the total nucleic acid was extracted, and Real-Time RT-PCR runs were performed for the detection of PVY, PVX, and PLRV following the protocol described by Boonham et al. (2009) with slight modifications (the final concentration of reverse transcriptase was increased to 0.16 U/ $\mu$ l). The same protocol was applied for the detection of PVS, PVM, and PVA with primers specific to these viruses. Primer sequences and their origin are shown in Table 1. The UNECE Seed Potato Standard S-1 guidelines were used to calculate the most probable value of the virus present in the sample.

### Statistical Analyses

Statistical analyses were performed using JMP10.0 and Microsoft Excel software. The datasets were tested using the least squares multiple linear regression model to determine the statistical significance between PVY incidences and years, PVY incidences and monthly temperatures, PVY incidences and precipitation, PVY incidences and location of the fields, PVY incidences and grown varieties, and the resistance groups of cultivated varieties. To illustrate the differences between the PVY incidence % and varieties, one-way ANOVA and the Tukey–Kramer post hoc tests were used.

### Evaluation of Variety Resistance/Susceptibility to PVY

The virus monitoring involved the seed potato tubers of 132 varieties grown in Estonia during the studied period. Of those, the resistance/susceptibility to PVY was evaluated for the 32 most frequently grown foreign and Estonian varieties (Suppl. Table 2). Among popular foreign varieties, only those with 10 or more seed lots involved in monitoring were included in resistance/susceptibility evaluation. Since the susceptibility/resistance of Estonian varieties (Ando, Anti, Ants, Juku, Maret, Piret, Reet, Teele, and Tiina) has previously not been evaluated systematically, all local potato varieties were analyzed to get the laboratory analysis-based evidence about their status, even if the final count was less than ten seed lots per variety.

In assessing the variety's susceptibility/resistance in local growing conditions, we considered two key parameters: the average and the highest PVY percentages found over the studied period. The average PVY percentage provides a mean level of PVY incidence across the studied period in Estonia. The highest PVY rate reflects the peak susceptibility of a variety during periods of intense virus pressure, and it represents the highest recorded PVY incidence rate per seed lot over the studied period.

**Table 1** Sequences and origin of primers and probes

| Assay name | Primer/probe name   | Sequence                                            | Reference                    |
|------------|---------------------|-----------------------------------------------------|------------------------------|
| COX        | COX F               | CGT CGC ATT CCA GAT TAT CCA                         | Boonham et al. (2009)        |
|            | COX RW              | CAA CTA CGG ATA TAT AAG RR C C RR AAC TG            |                              |
|            | COXSOL 1511 T probe | JOE – AGG GCA TTC CAT CCA GCG TAA GCA – TAMRA       |                              |
| PLRV       | PLRV-F              | GGC AAT CGC CGC TCA A                               | Boonham et al. (2009)        |
|            | PLRV R              | TGT AAA CAC GAA TGT CTC GCT TG                      |                              |
|            | PLRV Probe          | FAM—CCT CGT CCT CGG GGA ACT CCA GTT – TAMRA         |                              |
| PVX        | PVX- 1F             | ACA CAG GCC ACA GGG TCA A                           | Boonham et al. (2009)        |
|            | PVX- 1R             | GGG ATG GTG AAC AGT CCT GAA G                       |                              |
|            | PVX- 2F             | GGA TCC ACC AAA TCA ACT ACC AC                      |                              |
|            | PVX 2R              | GGT ATG GTG AAT AGC CCT GAA TTG                     |                              |
|            | PVX Probe           | FAM—ACT GCA GGC GCA ACT CCT GC – TAMRA              |                              |
| PVY        | PVY411 F            | GGG CTT ATG GTT TGG TGC A                           | Boonham et al. (2009)        |
|            | PVY477R             | CCG TCA TAA CCC AAA CTC CG                          |                              |
|            | PVY probe           | FAM – TGA AAA TGG AAC CTC GCC AAA TGT CA – TAMRA    |                              |
| PVA        | PVA F               | TCT GAA GGT AGG AAG AAA GAA GGA GA                  | This study                   |
|            | PVA R               | GAG TCC CAG CAG TAC CTA AAT CG                      |                              |
|            | PVA probe           | FAM – TGT CCT TCA CGG CAA CAG CTT TGC TAC T – TAMRA |                              |
| PVM        | PVM F               | GGG ATT GAT CAG GCG ACC TA                          | This study                   |
|            | PVM R               | TGA ACA CCT GAT TGC GAT TTG                         |                              |
|            | PVM probe           | FAM –TAG CTC ACA ATA CGC ACA AAG ACA TCG CA–TAMRA   |                              |
| PVS        | PVS- 1 for          | AAG TGG TGA TCA TGT GTG CAA GCG                     | Mortimer-Jones et al. (2009) |
|            | PVS- 1 rev          | ATT GCA ATG ATC GAG TCC AAG GGC                     |                              |
|            | PVS- 1 probe        | Cy5-ACT GTG GAG TTC CCA ACA GGC GCA GT-BHQ- 2       |                              |

## Results and Discussion

### The Yearly Incidence of Six Potato Viruses in Super Elite Seed Potatoes

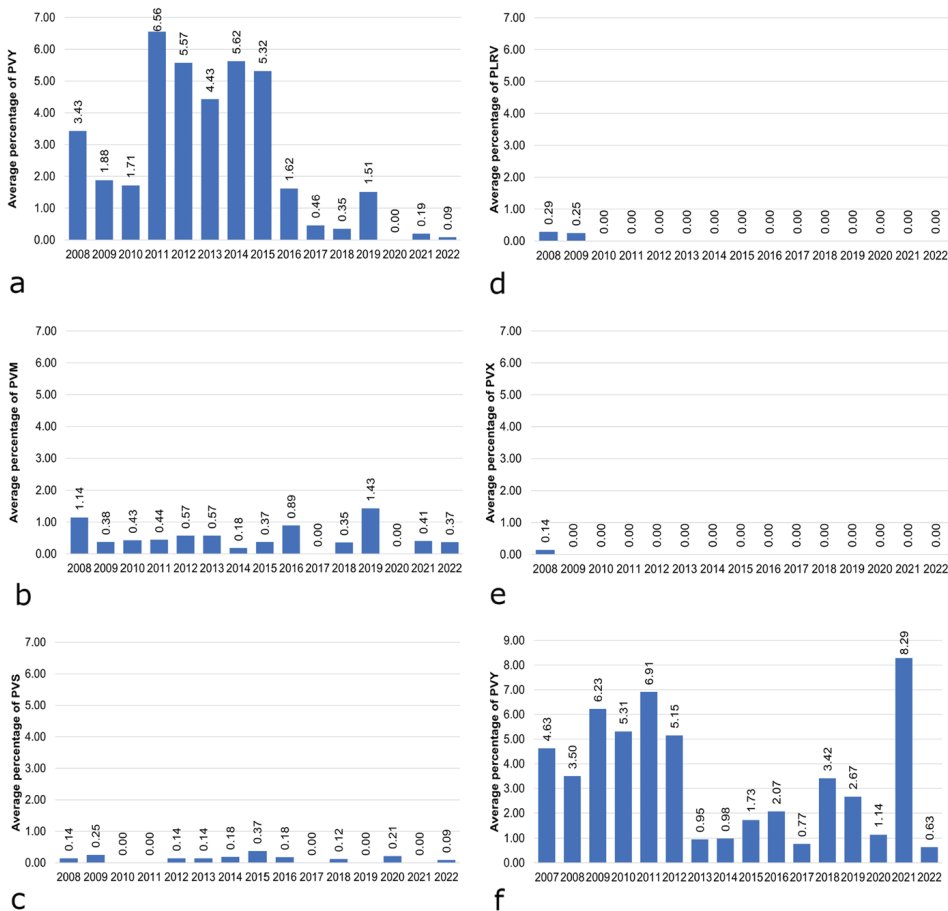
Super Elite seed potatoes are exclusively cultivated at the METK Jõgeva Station, primarily consisting of varieties of Estonian origin. In 2007, no Super Elite seed potatoes were produced as all seed lots from 2006 failed to meet the Super Elite standards, with virus levels exceeding the established limits (Fig. 1). Until 2014, the proportion of Super Elite PB seed potatoes among all tested tubers was under 10%. This proportion has been on the rise since 2015, reaching 33.3% in 2022.

The Super Elite seed tubers were tested for six viruses: PVA, PVY, PVX, PVM, PVS, and PLRV. PVY was the most prevalent virus through the years. The overall average level of PVY for all Super Elite seed lots from 2007 to 2022 was 2.43%, with the highest level recorded at 6.56% in 2011 while no PVY was detected in 2020 (Fig. 2a). PVM was the second most prevalent virus, with an overall average of 0.48% (Fig. 2b). The level of PVM was notably high in 2008, 2016, and 2019. In other years, PVM percentages were negligible, with no PVM detected in the tested samples in 2017 and 2020. The annual average incidence of PVS remained below 0.5% during the study period, with the overall average as low as 0.11% (Fig. 2c). PLRV and PVX were detected only at the beginning of the potato virus monitoring with low incidence rates (Fig. 2d and e). Our virus monitoring did not reveal any PVA infections. The proportion of detected five potato viruses has been drastically changed compared to the previous study at Jõgeva county, when the predominant potato virus was PVX (5.4%), followed by PVS (1.1%), and PVM (0.6%), with no incidences of PVY or PLRV (Järvekülg et al. 1995).

Throughout the observed period, the quality of Estonian seed potatoes has been on the rise, evidenced by diminishing virus incidences. The trends for PVY, PVM, and PVS are negative, as illustrated in Fig. 3a–c. However, only the trend for PVY is statistically significant ( $p = 0.0021$ ), suggesting a genuine decline. In contrast, the negative trends of PVM ( $p = 0.3933$ ) and PVS ( $p = 0.5229$ ) may be attributed to random variations due to fluctuating incidence rates across different years and generally low infection rates.

### The Yearly Incidence of PVA and PVY Virus Infection in Elite (S, SE, and E) and Certified (A and B) Seed Potato

Analyses of the yearly incidence of PVA and PVY were performed on seed potatoes sourced from 11 counties. Notably, no PVA incidence was detected (tests were conducted up to 2015). The average PVY incidence over the studied period was 3.92%, with the lowest percentages (under 1%) recorded in 2013, 2014, 2017, and 2022, as shown in Fig. 2f. Above-average PVY percentages ranging from 4.63 to 6.91% were observed in 2007, and from 2009 to 2012, whereas the year 2021 had the highest PVY prevalence (8.29%) of all (Fig. 2f). A statistical analysis of PVY incidences in Estonian seed potatoes over the studied period indicates that the health of

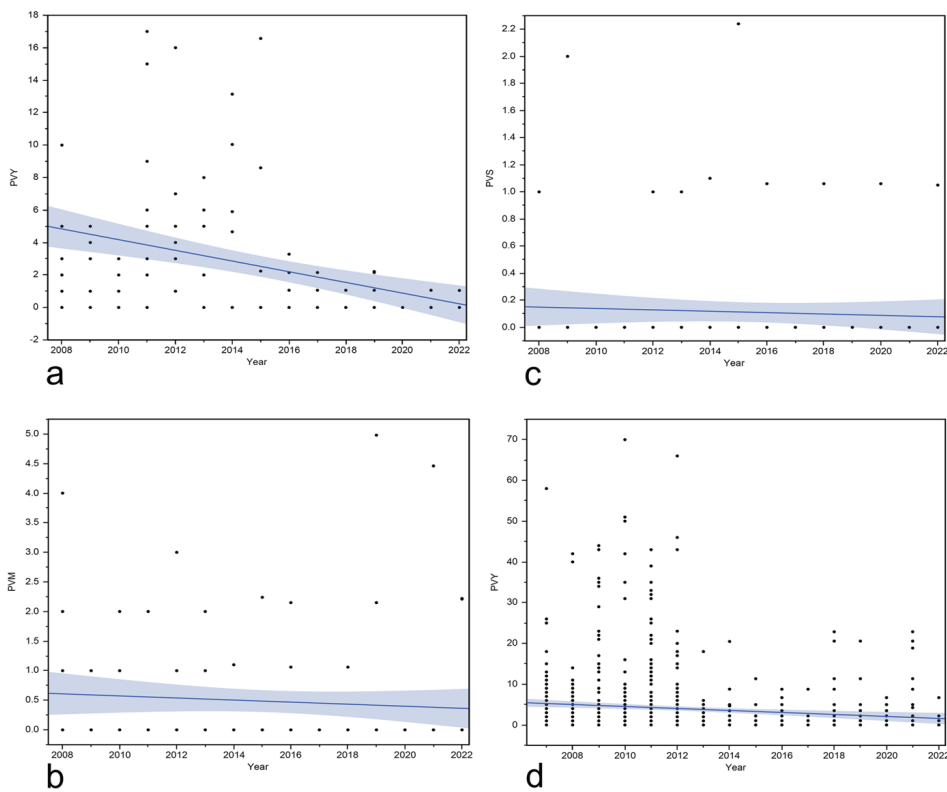


**Fig. 2** **a–e** The yearly incidence (%) of PVY, PVM, PVS, PLRV, and PVX in super elite (SE until 2014; PBTC and PB from 2015) seed potato (2008–2022). **f** The yearly incidence of PVY in lower categories (classes E and A until 2014; S, SE, E, A, and B from 2015) seed potatoes (2007–2022)

lower-class seed potatoes has been improving, similar to Super Elite seed potatoes, as evidenced by the decreasing PVY incidences ( $p = 0.0003$ ) (Fig. 3d).

### Impact of Climate Conditions on PVY Incidence

Estonia is situated in the northern segment of the temperate climate zone, being in the transitional area between maritime and continental climates. Influenced by the maritime air warmed by the northern Atlantic Ocean, Estonia has a comparatively mild climate for its northern latitude. The Baltic Sea contributes to the differences observed between the coastal regions and inland areas. The potato growing season is from May to August, with an average temperature of 15.4 °C and a variation of 3.3 °C (Suppl. Table 1). The average monthly temperatures of the growing season in 2007–2022 were 11.1 °C, 15.5 °C, 18.0 °C, and 16.9 °C for May through August, respectively, with variations between years of around four to five degrees per month



**Fig. 3** **a–c** PVY, PVM, and PVS trends in Estonian super elite (SE until 2014; PBTC and PB from 2015) seed potatoes in 2008–2022. **d** PVY trends in Estonian seed potato lower categories (classes E and A until 2014; S, SE, E, A, and B from 2015) in 2007–2022. Virus incidence rates in each seed lot are shown as dots (the lots with same values are shown as one dot), whereas the linear regression trendline is shown with a 95% confidence interval (blue)

(Suppl. Table 1). Estonia falls within a humid zone where precipitation exceeds total evaporation. Among the four vegetation months, the heaviest average precipitation (93 mm) occurred in August. The average precipitation during the whole growing season across the studied period was 69.7 mm, ranging from 44.3 to 87.9 mm.

Climate factors vary notably between the counties, considering the small size of Estonia. The greatest variation in temperature in the same year and same month was up to 4.8 °C in May 2013, when the average temperature in Võru county was 15.3 °C, whereas it was 10.5 °C in Harju county. The difference in precipitation in different locations in the same month was as big as 183.8 mm in August 2010, when the precipitation was 243.2 mm in Võru county and 59.5 mm in Ida-Viru county. Therefore, to study the climate impact on PVY spread, we analyzed the influence of temperatures and precipitation data individually for all months of the growing season in each county. Multiple linear regression showed that weather conditions are statistically significant ( $p = 0.0005$ ;  $r = 0.1675$ ). Weather in July and August had the biggest effect, more precisely temperatures in July ( $p < 0.0001$ ) and August ( $p = 0.0299$ ), and precipitation in July ( $p = 0.0266$ ) was significant for PVY incidence

**Table 2** Relationship between PVY infection in Estonian seed potatoes and environmental variables (temperature and precipitation) during 2007–2022

| Month  | Temperature     |                 | Precipitation   |                 |
|--------|-----------------|-----------------|-----------------|-----------------|
|        | <i>t</i> -ratio | <i>p</i> -value | <i>t</i> -ratio | <i>p</i> -value |
| May    | – 0.77          | 0.4393          | – 1.18          | 0.2389          |
| June   | – 0.26          | 0.7957          | – 1.16          | 0.2464          |
| July   | 4.73            | < 0.0001*       | 2.22            | 0.0266*         |
| August | – 2.18          | 0.0299*         | – 0.58          | 0.5588          |

\*Statistically significant differences, significance level  $\alpha \leq 0.05$ ; positive *t*-ratio values indicate higher PVY incidence than the grand mean, and negative values indicate lower PVY incidence than the grand mean

(Table 2). In July, higher temperatures and higher precipitation resulted in a higher PVY infection rate, indicating a weak positive correlation (Fig. 4a, b). Conversely, in August, we observed a reversed effect on temperatures: higher temperatures resulted in lower PVY incidences, demonstrating a weak negative correlation (Fig. 4c).

Earlier studies suggest that lower potato growth temperatures induce a greater efficiency of hypersensitive resistance to PVY, whereas higher growth temperatures enhance the susceptibility and systemic spread of PVY (Valkonen 1997; Choi et al. 2017). The spread of PVY is dependent on its aphid vectors. Studies on the seasonal dynamics of aphids in Estonian potato fields in 2008 indicated the arrival of aphids at the end of May, with their abundance peaking in mid-July and remaining relatively high until mid-August (Muljar 2009). *Aphis fabae*, the most abundant aphid species in Estonian potato fields, has been suggested to be the main contributor to the successful spread of PVY in the neighboring country, Finland, due to its flight activity at early potato growth stages (Muljar 2009; Kirchner et al. 2013).

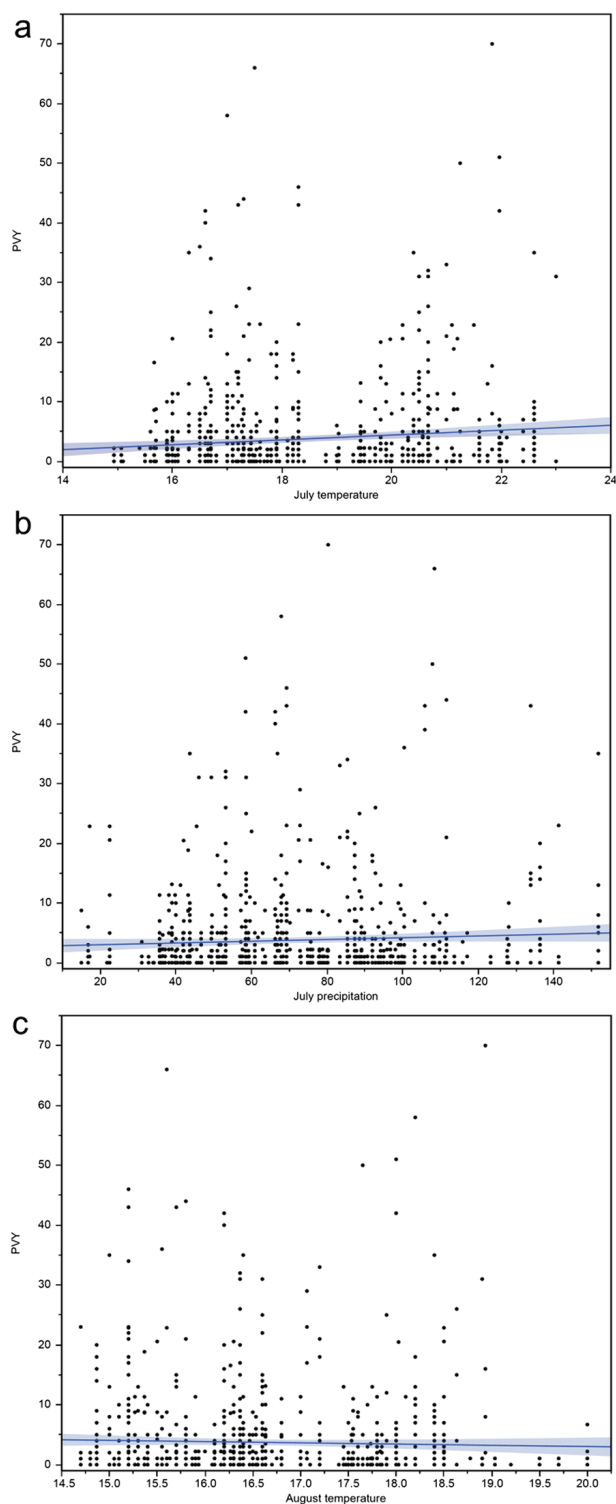
### Susceptibility of Different Potato Varieties to PVY Infection in Estonia

Susceptibility assessment is expected to provide an objective and reliable characteristic of the variety, but a scoring of susceptibility depends on the breeder standards (Świeżyński et al. 2001). On the other hand, in real life, susceptibility may vary because regional climatic and environmental conditions differ from the trial site but also because of exposure to variances in the spread of virus strains with different severity (Davie et al. 2017; Funke et al. 2017; Della Bartola et al. 2020; Onditi et al. 2022; Sutula et al. 2022; Samarskaya et al. 2023).

Long-term monitoring data collected via the seed potatoes certification system can provide independent insight into varieties' susceptibility/resistance. In the current study, 1023 seed potato lots were tested for PVY infection over the period from 2007 to 2022. The monitoring involved 132 potato varieties, of which 32 were assessed for PVY susceptibility.

One-way ANOVA analysis on the 32 varieties confirmed that the variety played a crucial role in PVY infection rates ( $p < 0.0001$ ;  $r = 0.5193$ ). There was a clear difference between resistant and susceptible varieties. For example, very susceptible

**Fig. 4** Relationships between PVY incidences and statistically important environmental variables during 2007–2022: **a** temperature in July, **b** precipitation in July, **c** temperature in August. PVY incidence rates in each seed lot are shown as dots, whereas the linear regression trendline is shown with a 95% confidence interval (blue)





varieties, such as Ando, Arcona, Arielle, Folva, and Rosagold mean diamonds, were above the grand mean, while very highly resistant varieties like Gala, Jelly, Laura, and Princess were below that level (Fig. 5).

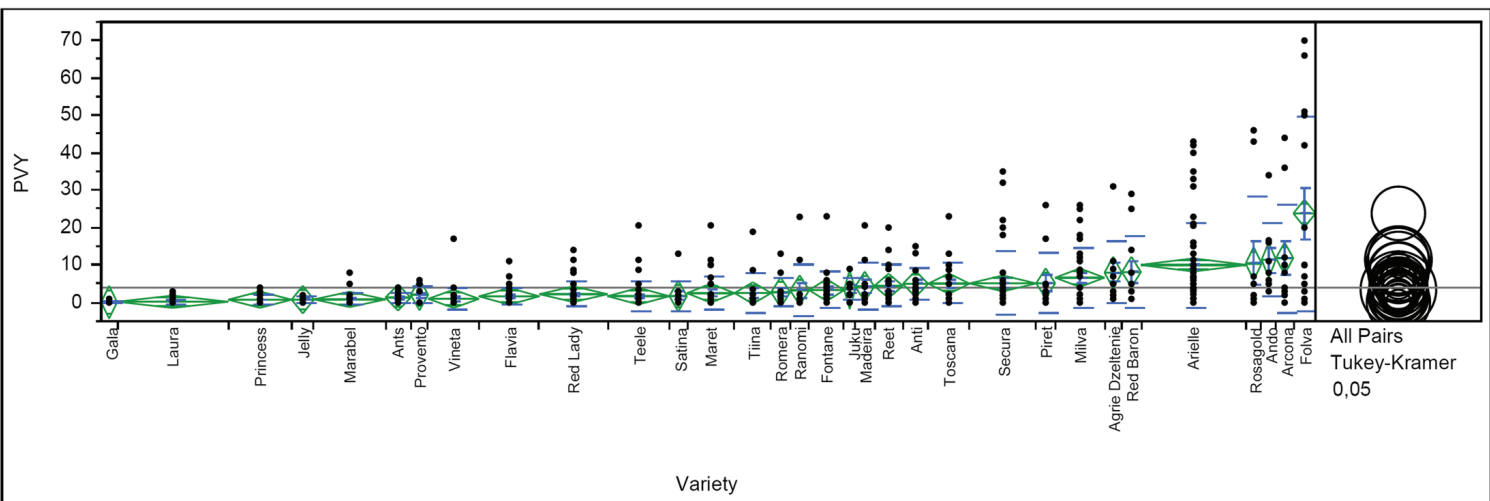
Our results on the average and highest PVY percentage demonstrated that among the 32 assessed varieties, there was a considerable difference in infection levels, allowing us to categorize potato varieties into resistance groups based on these two parameters (Table 3). Varieties with an overall virus presence of less than 1% and their highest PVY rate under 5% were considered very resistant. In the medium to high resistance groups, the average PVY percentage ranged from 1 to 2.99%, with high resistance varieties showing the highest PVY percentage between 5 and 9.99%, and varieties with medium resistance had the highest PVY percentage over 10%. Varieties with an average PVY rate over 3% and the highest PVY percentages ranged from 20 to 34.9% were considered susceptible, while those with the highest PVY percentage over 35% were deemed very susceptible.

Four varieties—Gala, Jelly, Laura, and Princess—indicated *very high resistance* to PVY. This resistance was also statistically significant, with  $p$ -values of  $p = 0.0358$  for Gala,  $p = 0.0354$  for Jelly,  $p < 0.0001$  for Laura, and  $p = 0.0006$  for Princess, respectively (Table 4). According to previous information (Suppl. Table 2), Gala and Jelly were known to have very high resistance to PVY, whereas Laura was known to have high resistance, and Princess had medium to high resistance. Notably, previously described as highly resistant, Laura demonstrated very high resistance in Estonian conditions, with an average infection level of only 0.2% over 16 years (Table 4). With 70 tested seed lots, Laura has been the most popular seed potato variety grown in Estonia. As well, Princess exhibited better resistance than expected in Estonian growing conditions.

Three potato varieties—Ants, Provento, and Marabel—were categorized by us as the group of *high resistance* to PVY because differently from the varieties being extremely resistant, these had slightly higher maximum infection rates sometimes (Table 4). The Estonian variety Ants positioned on the borderline between very high and high resistance groups. The highest PVY infection rate for Ants was 4%, which would typically indicate a very high resistance, but its average PVY was 1.3%, leading us to place it in the high resistance group. The  $p$ -value for Ants was 0.0671 which is close to the significance level  $\alpha \leq 0.05$ . Statistical analyses supported the classification of Marabel as a resistant variety ( $p = 0.0004$ ;  $n = 45$ ). In the case of Provento ( $p = 0.2101$ ), the difference is not statistically significant, presumably, due to the highly variable infection rate and relatively small group size ( $n = 11$ ) (Table 4). The variety representatives have classified Marabel into the *high resistance* group and Provento into the *medium to high resistance* group (Suppl. Table 2).

Eight varieties—Flavia ( $p = 0.0094$ ), Satina ( $p = 0.1538$ ), Romera ( $p = 0.3269$ ), Red Lady ( $p = 0.0257$ ), Vineta ( $p = 0.0034$ ), Tiina ( $p = 0.1411$ ), Teele ( $p = 0.0093$ ), and Maret ( $p = 0.0908$ )—were categorized into the *medium resistance* group by us (Table 3). Out of those eight varieties, the grouping of six varieties—Flavia, Maret, Red Lady, Satina, Teele, and Tiina—was in concordance with previously available data. Only Vineta and Romera, which were previously stated to have medium to high resistance or high resistance, respectively, showed lower-rank resistance than expected (Suppl. Table 2).

Out of 32 varieties tested, twelve—Juku ( $p = 0.6381$ ), Anti ( $p = 0.8724$ ), Reet ( $p = 0.8739$ ), Piret ( $p = 0.8286$ ), Madeira ( $p = 0.8290$ ), Ranomi ( $p = 0.4784$ ),



**Fig. 5** One-way ANOVA analysis of PVY incidences (% shown in y-axis) in tested individual seed lots by potato variety (shown in x-axis). The width of green diamonds represents the group size of the variety. The grey line shows a grand mean. Black dots indicate individual seed lots. Blue lines are at  $\pm$  one or two STDEV; dots exceeding the blue lines show the outliers

Fontane ( $p = 0.3580$ ), Toscana ( $p = 0.8053$ ), Milva ( $p = 0.1322$ ), Red Baron ( $p = 0.0876$ ), Agrie Dzeltnie ( $p = 0.1190$ ), and Secura ( $p = 0.7212$ )—were placed in the *susceptible* group according to our estimation (Table 4). All these varieties showed  $p$ -values higher than the significance level  $\alpha \leq 0.05$  because their PVY infection rates varied below and above the grand mean value.

Previous suggestions indicated that Anti, Juku, Madeira, and Toscana have medium resistance to PVY, and Milva has medium to high resistance to PVY. However, when grown in Estonia, all these varieties showed susceptibility to PVY. It should be noted that the number of tested tubers of Juku was less than for other varieties, being only 700; therefore, further investigations are needed for a final evaluation of that variety. Ranomi, Fontane, and Red Baron were reported to be susceptible to the PVY<sup>N</sup> strain but minimally susceptible or quite resistant to the PVY<sup>NTN</sup> strain (Suppl Table 2). Thus, their susceptibility in our survey may have been related to the circulating PVY strains (which were not determined here). In concordance with our study, Reet and Piret were previously assessed as susceptible (Suppl Table 2). Contrary to our data, Secura has been considered highly resistant to PVY according to the variety representative (Suppl. Table 2). Secura's average infection rate over the studied period was high, being 5.1% (Table 4) as it was influenced by the extreme examples of infection rates that were recorded over several years. For instance, all four tested seed lots were infected by over 20% in 2011, and in one lot, the infection rate was even 35%. Agrie Dzeltnie has been evaluated as very susceptible; our analysis indicated it is a susceptible variety.

Five varieties—Ando ( $p = 0.0040$ ), Arielle ( $p < 0.0001$ ), Arcona ( $p = 0.008$ ), Rosagold ( $p = 0.0100$ ), and Folva ( $p < 0.0001$ )—were identified as *very susceptible* in our study (Table 4). The variety Rosagold, previously described as quite resistant or resistant to PVY<sup>N</sup> and PVY<sup>NTN</sup> strains, was surprisingly very susceptible, with the highest PVY infection rate of 46% in 2012, and an average PVY rate of 10.4% over the years. Folva, which has been categorized as a medium resistance variety earlier, exhibited the highest PVY infection level recorded in Estonia over the studied period—its infection rate peaked at 70% in one of the seed lots in 2010. The previously stated susceptible varieties Arielle, Arcona, and Ando were also classified as very susceptible by us (Table 3). In our study, their PVY occurrence was relatively

**Table 3** Classification of potato varieties into PVY resistance groups according to our study

| Resistance group     | Average PVY percentage in field conditions (%) | Highest PVY infection rate in field conditions (%) | Variety                                                                                              |
|----------------------|------------------------------------------------|----------------------------------------------------|------------------------------------------------------------------------------------------------------|
| Very high resistance | < 1                                            | < 5                                                | Gala, Laura, Princess, Jelly                                                                         |
| High resistance      | 1 to 2.99                                      | 5 to 9.9                                           | Marabel, Ants, Provento                                                                              |
| Medium resistance    |                                                | 10 to 19.9                                         | Vineta, Flavia, Teele, Satina, Red Lady, Maret, Tiina, Romera                                        |
| Susceptible          | > 3                                            | 20 to 34.9                                         | Ranomi, Fontane, Juku, Madeira, Reet, Anti, Toscana, Secura, Piret, Milva, Agrie Dzeltnie, Red Baron |
| Very susceptible     |                                                | > 35                                               | Arielle, Rosagold, Ando, Arcona, Folva                                                               |

high in all years. The extreme susceptibility to PVY is likely the reason why the varieties Rosagold, Folva, and Arcona are no longer grown in Estonia as seed potatoes.

Notably, all varieties within the *very high resistance* group and the *very susceptible* group had statistically significant *p*-values (Table 4). Varieties with very high PVY resistance had negative *t*-ratios, and very susceptible varieties had positive *t*-ratios in the linear regression analysis. This indicates that highly resistant varieties usually remain uninfected, whereas very susceptible varieties consistently exhibit high PVY levels. Their group sizes (number of seed lots) did not influence their

**Table 4** Statistical support for variety susceptibility evaluation. \* indicates *p*-value < 0.05

| Variety         | Average percentage of PVY | Highest percentage of PVY | <i>t</i> -ratio | <i>p</i> -value | No. of tested seed lots | Resistance group     |
|-----------------|---------------------------|---------------------------|-----------------|-----------------|-------------------------|----------------------|
| Gala            | 0.1                       | 1.0                       | − 2.1           | 0.0358*         | 10                      | Very high resistance |
| Laura           | 0.2                       | 3.0                       | − 5.02          | < 0.0001*       | 70                      | Very high resistance |
| Princess        | 0.8                       | 4.0                       | − 3.45          | 0.0006*         | 40                      | Very high resistance |
| Jelly           | 0.8                       | 2.0                       | − 2.11          | 0.0354*         | 14                      | Very high resistance |
| Marabel         | 0.8                       | 8.0                       | − 3.57          | 0.0004*         | 45                      | High resistance      |
| Ants            | 1.3                       | 4.0                       | − 1.83          | 0.0671          | 16                      | High resistance      |
| Provento        | 2.1                       | 6.0                       | − 1.25          | 0.2101          | 11                      | High resistance      |
| Vineta          | 1.0                       | 17.0                      | − 2.94          | 0.0034*         | 32                      | Medium resistance    |
| Flavia          | 1.7                       | 11.0                      | − 2.61          | 0.0094*         | 38                      | Medium resistance    |
| Red Lady        | 2.2                       | 14.0                      | − 2.24          | 0.0257*         | 43                      | Medium resistance    |
| Teele           | 1.7                       | 20.6                      | − 2.61          | 0.0093*         | 39                      | Medium resistance    |
| Satina          | 1.7                       | 13.0                      | − 1.43          | 0.1538          | 11                      | Medium resistance    |
| Maret           | 2.5                       | 20.6                      | − 1.69          | 0.0908          | 30                      | Medium resistance    |
| Tiina           | 2.5                       | 18.9                      | − 1.47          | 0.1411          | 23                      | Medium resistance    |
| Romera          | 2.8                       | 13.0                      | − 0.98          | 0.3269          | 12                      | Medium resistance    |
| Ranomi          | 3.3                       | 22.9                      | − 0.71          | 0.4784          | 12                      | Susceptible          |
| Fontane         | 3.3                       | 23.0                      | − 0.92          | 0.358           | 22                      | Susceptible          |
| Juku            | 3.5                       | 9.0                       | − 0.47          | 0.6381          | 7                       | Susceptible          |
| Madeira         | 4.3                       | 20.6                      | − 0.22          | 0.829           | 12                      | Susceptible          |
| Reet            | 4.5                       | 20.0                      | − 0.16          | 0.8739          | 18                      | Susceptible          |
| Anti            | 5.0                       | 15.0                      | 0.16            | 0.8724          | 16                      | Susceptible          |
| Toscana         | 5.1                       | 23.0                      | 0.25            | 0.8053          | 26                      | Susceptible          |
| Secura          | 5.1                       | 35.0                      | 0.36            | 0.7212          | 42                      | Susceptible          |
| Piret           | 5.2                       | 26.0                      | 0.22            | 0.8286          | 12                      | Susceptible          |
| Milva           | 6.7                       | 26.0                      | 1.51            | 0.1322          | 31                      | Susceptible          |
| Agrie Dzeltenie | 8.0                       | 31.0                      | 1.56            | 0.119           | 11                      | Susceptible          |
| Red Baron       | 8.2                       | 29.0                      | 1.71            | 0.0876          | 12                      | Susceptible          |
| Arielle         | 10.0                      | 43.0                      | 5.76            | < 0.0001*       | 66                      | Very susceptible     |
| Rosagold        | 10.4                      | 46.0                      | 2.58            | 0.0100*         | 10                      | Very susceptible     |
| Ando            | 11.4                      | 34.0                      | 2.88            | 0.0040*         | 9                       | Very susceptible     |
| Arcona          | 11.8                      | 44.0                      | 3.38            | 0.0008*         | 11                      | Very susceptible     |
| Folva           | 23.8                      | 70.0                      | 10.2            | < 0.0001*       | 14                      | Very susceptible     |

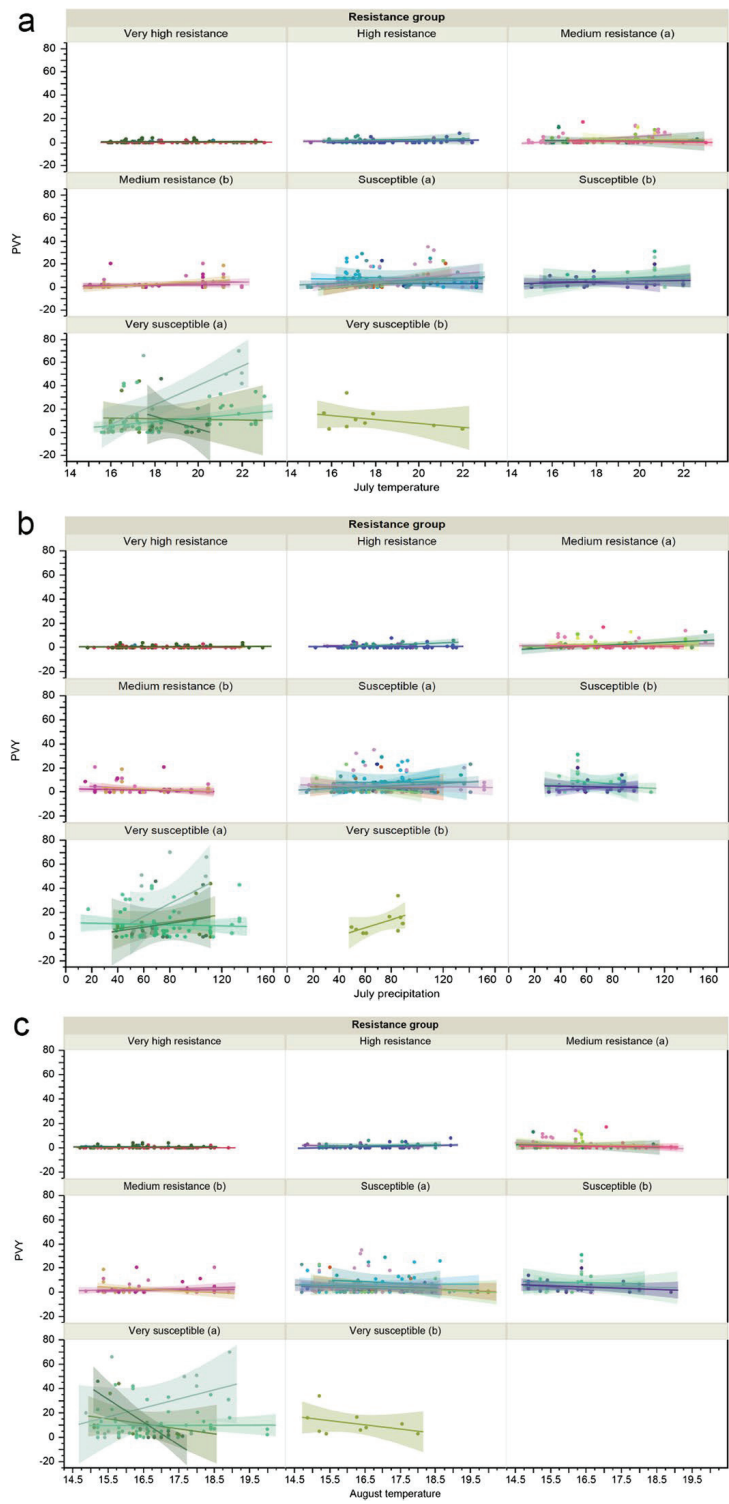
statistical support. The varieties in groups ranging from *high resistance* to *susceptible* did not show clear statistical support for smaller group sizes, probably because of the variable infection rates between the seed lots. The final infection rates may also be influenced by the proportion of tuber-born infection in the field and by the viral load in planted tubers, which have a significant impact on the local PVY spread (Rosenman et al. 2019). However, the initial PVY rates in the surveyed seed potatoes were presumably not high as all were grown from certified Super Elite or Elite seeds.

### The Relationships Between Variety's Susceptibility and Environmental Variables

Next, we sought to understand the relationship between variety's susceptibility and environmental variables influencing PVY spread. The analysis indicated that neither temperature in July and August nor precipitation in July had a statistically important effect on overall PVY rates in very resistant and resistant varieties. However, these environmental variables did affect some susceptible and very susceptible varieties on different levels and with different effects (Fig. 6a–c). Surprisingly, the weather effect was more pronounced on non-Estonian origin varieties than those of Estonian or Latvian origin. The biggest effect was observed in the case of Folva with a strong positive correlation between July temperature and PVY incidences ( $p = 0.0020$ ;  $r = 0.7950$ ), and in the case of Rosagold, despite of small group size  $n = 10$ , a strong negative correlation was observed between August temperature and PVY incidences ( $p = 0.0048$ ;  $r = 0.8714$ ). The maturity of these two varieties might be the reason why Folva is more affected by July and Rosagold by August temperature, as Folva is an early variety and Rosagold is a midseason variety. Interestingly, comparative molecular research on the PVY-susceptible variety Chicago and the PVY-resistant variety Gala suggested that responses to heat stress and PVY infection in potatoes have some common underlying mechanisms, controlling plant sensitivity to multiple stresses in a variety-specific manner (Makarova et al. 2018; Spechenkova et al. 2021).

The significant difference in PVY levels in medium-resistant and susceptible variety groups throughout the years suggests that infection rates can vary under different growing conditions. In some cases, the reason for deviations may lie in varying resistance to different PVY strains prevalent in different years (Funke et al. 2017). To date, differentiation of PVY strains has not been required as part of the Estonian seed potato certification system, although there is a clear need to provide growers with accurate, region-specific epidemiological information for the choice of suitable potato varieties. Also, such data would be useful for local breeders, helping them to select the best breeding material.

**Fig. 6** Effect of environmental variables on PVY incidence in potato varieties of different susceptibility groups: **a** PVY vs. July temperature, **b** PVY vs. July precipitation, **c** PVY vs. August temperature. Varieties in medium resistance, susceptible, and very susceptible groups are presented in two subgroups, indicating their origin: (a) Central European, (b) Estonian or Latvian. Colors depict different varieties; their PVY incidence rates in each seed lot are shown as dots, whereas the linear regression trendline for each variety is shown with a 95% confidence interval (shaded area)



## The Incidence of PVY in Seed Potatoes by County

Seed potato cultivation was practiced to varying degrees in 11 of 15 counties of Estonia during the period from 2007 to 2022. The highest number of samples originated from Jõgeva county (268), which is also the location of the METK potato breeding center, followed by Tartu (170) and Viljandi (157) counties. The smallest number of samples was received from Valga county (8) (Table 5). No samples were collected from four counties without seed potato production: Hiiu, Saare, Lääne, and Ida-Viru.

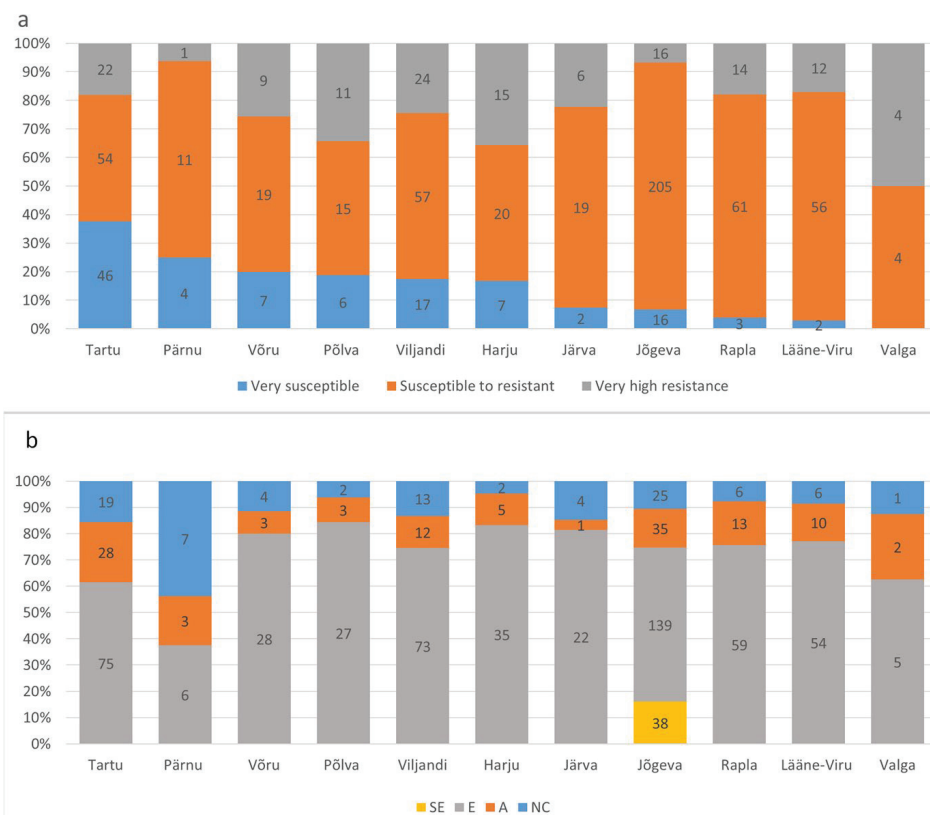
The analysis revealed that average PVY levels were relatively low in Järva, Lääne-Viru, Rapla, and Viljandi counties, all staying below 3%. Statistically, Järva ( $p = 0.0748$ ), Rapla ( $p = 0.0818$ ), and Viljandi ( $p = 0.0602$ ) counties exhibited results nearing the significance level of  $\alpha \leq 0.05$ , with negative  $t$ -ratio values indicating lower PVY levels in these regions. On the other hand, Tartu ( $p = 0.0038$ ) and Pärnu ( $p < 0.0001$ ) counties experienced higher PVY infection rates, with average PVY rates of 5.9% and 9.8%, respectively, and positive  $t$ -ratio values confirming the elevated PVY levels in these counties. PVY rates in the remaining counties did not show statistically significant deviations from the average (Table 5).

Since our previous data on variety susceptibility showed that varieties in very high resistance and very high susceptibility groups had statistically important  $p$ -values (Table 4), we further analyzed varieties grown in different counties by that information. Tartu and Pärnu counties had a higher proportion of very susceptible varieties than other counties and the proportion of very high resistant varieties was rather low (Fig. 7a). Accordingly, the proportion of seed lots graded as Elite class was lower in these counties (Fig. 7b). In Tartu county, the very susceptible variety Arielle was the most prevalent. In Pärnu county, Milva and Arielle, which belong to the susceptible and very susceptible groups, were the most favored varieties. It should be noted that the record high PVY percentage (around 70%) registered in 2010 was attributed to the variety Folva grown in Pärnu county (Table 5). The percentage of cultivated very high resistance varieties was highest in Põlva and Harju counties yielding the highest proportions of Elite seed (Fig. 7).

**Table 5** Statistical analysis of PVY incidences in Estonian counties. \* indicates  $p$ -value  $< 0.05$

| County     | Number of tested seed lots | Average percentage of PVY | Maximum percentage of PVY | $t$ -ratio | $p$ -value   |
|------------|----------------------------|---------------------------|---------------------------|------------|--------------|
| Harju      | 50                         | 3.6                       | 66.0                      | − 0.41     | 0.6833       |
| Jõgeva     | 268                        | 3.6                       | 51.0                      | − 0.67     | 0.5022       |
| Järva      | 57                         | 2.2                       | 20.6                      | − 1.78     | 0.0748       |
| Lääne-Viru | 92                         | 2.8                       | 36.0                      | − 1.49     | 0.1356       |
| Põlva      | 51                         | 3.3                       | 43.0                      | − 0.69     | 0.4887       |
| Pärnu      | 27                         | 9.8                       | 70.0                      | 4.13       | $< 0.0001^*$ |
| Rapla      | 100                        | 2.6                       | 35.0                      | − 1.74     | 0.0818       |
| Tartu      | 170                        | 5.9                       | 58.0                      | 2.9        | $0.0038^*$   |
| Valga      | 8                          | 4.5                       | 22.0                      | 0.19       | 0.8463       |
| Viljandi   | 157                        | 2.8                       | 43.0                      | − 1.88     | 0.0602       |
| Võru       | 43                         | 3.1                       | 33.0                      | − 0.86     | 0.3911       |





**Fig. 7** **a** Proportions of 32 evaluated varieties of very susceptible (blue), susceptible to resistant (orange), and very resistant (grey) groups grown in different counties. **b** Proportions of seed grades (SE, super elite (yellow); E, Elite (grey); A, class A (orange); NC, not certified (blue)) across the counties for the 32 evaluated varieties. super elite (SE) seed was cultivated only in Jõgeva county. Numbers of analyzed seed lots are shown for each subset

Based on this data, it can be suggested that differences in PVY rates between the counties are likely related to the proportion of cultivated PVY-susceptible or PVY-resistant varieties. However, it seems that the growth location also played a role in PVY incidence for a few specific varieties. For example, Vineta, which was mostly tested to be virus-free (22 out of 32 seed lots) or had a low (less than 2%) infection rate, experienced a 17% infection rate in Pärnu county in 2009 (hence, classified as medium resistant to PVY).

## Conclusions

According to our study, PVY was the most prevalent of six potato viruses involved in regular Super Elite seed potato monitoring conducted from 2007 to 2022 as part of the seed potato certification process. Additionally, PVM and PVS



were commonly detected at low levels. PLRV and PVX have not been detected since 2008–2009. Remarkably, PVA was not detected at all. The quality of Estonian seed potatoes has improved over the study period, as evidenced by decreasing infection rates. However, managing PVY and PVM infections continues to pose challenges for Super Elite seed potato growers. The acceptable virus percentage for the Super Elite category is less than 0.5%, and each year, several Super Elite seed lots are not certified as such due to these two viruses. In Elite and Certified seed potatoes, the incidence trend of PVY has been close to that of the Super Elite class, and PVY rates have decreased.

Statistical analyses revealed that the most important factor in PVY spread is the cultivated variety, while environmental variables such as temperature and precipitation are also significant. Results indicated that PVY incidence positively correlates with temperatures and precipitation rates in July, and negatively with temperatures in August. The analysis of the average and the highest PVY incidence rates of the 32 assessed varieties demonstrated that it is possible to categorize potato varieties into resistance groups based on these two parameters. The statistics reliably supported the categorization of highly resistant and very susceptible varieties. Further statistical analysis confirmed that resistance to PVY in very resistant or resistant varieties remained stable, unaffected by July and August temperatures or July precipitation. At the same time, these environmental variables substantially affected PVY incidence in some susceptible or very susceptible varieties. Interestingly, the weather effect was more pronounced on non-local origin varieties than on those of local origin.

Our study demonstrated that long-term seed potato monitoring data can be used for the evaluation of the variety's susceptibility/resistance. Comparisons of previous resistance evaluations of potato varieties to our long-term monitoring data revealed that several varieties were more susceptible to PVY than suggested by the variety representatives. For instance, Secura and Romera, previously considered highly resistant to PVY, were categorized as either susceptible or having only medium resistance in our study. Madeira, Toscana, and Milva appeared to be rather susceptible to PVY. Folva and Rosagold, once considered to have medium resistance, showed extreme susceptibility to PVY and are no longer grown in Estonia as seed potatoes. In this study, we revealed that two counties—Tartu and Pärnu—have higher PVY rates than others. Not surprisingly, it relates to the popularity of more susceptible varieties in these regions during the surveyed period.

The susceptibility/resistance evaluation of varieties serves as a valuable knowledge source for seed potato growers and breeders, helping to improve production efficiency by controlling virus spread. Among the Estonian varieties, Ants ranked the most resistant to PVY, being at the borderline of highly resistant and resistant groups, while Ando exhibited the highest susceptibility to PVY. Evaluation of Estonian varieties is valuable information for Estonian potato breeders. Among foreign varieties, Gala, Jelly, Laura, and Princess showed a very high resistance in our monitoring data analyses, meaning that their average PVY incidence rate was under 1%, and their highest infection always remained under 5%.

A conscious choice of very resistant and resistant varieties for cultivation would help to reduce PVY-related economic losses, which are estimated for the EU region

at 187 million EUR annually. Of that, 96 million counts for seed potatoes, consisting of chemical treatment against PVY aphid vectors, but also due to the downgrading of seed certificates (Dupuis et al. 2024). However, it is complicated by the very high number of varieties available without resistance evaluation or genetic profiling data helping to suggest their susceptibility. On the other hand, monitoring of seed potatoes for certification is required in the EU (and in many other countries) to keep the spread of diseases under control. Such an enormous amount of monitoring data is commonly used only for the certification process, but it could also be useful for evaluating additional varieties resistance under different regional climatic conditions and circulating virus strains.

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11540-025-09862-0>.

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**Data Availability** Data supporting the findings of this study are available upon request from PvdS.

## Declarations

**Conflict of Interest** The authors declare no competing interests.

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## Survey

## A Survey of Potato Virus Y Strains in Estonian Seed Potatoes

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## Abstract

Potato virus Y (PVY) is the most economically significant virus affecting potato crops worldwide. In the current study, we performed three surveys using multiplex reverse transcriptase PCR (RT-PCR) to analyze PVY strain compositions in Estonian seed potatoes collected for seed certification. Altogether, 1,041 samples of PVY were analyzed: 715 from the first survey (2011 to 2013), 128 from the second survey (2016 to 2018), and 198 from the third survey (2020 to 2022). The results indicated that recombinant strains PVY<sup>NTN</sup> and PVY<sup>N-Wi</sup> are dominant, whereas identification of several circulating strain variants was inconclusive with the chosen primers. Linear regression analysis

showed that there was a statistically significant ( $P = 0.00334^*$ ) negative trend for PVY<sup>NTN</sup>. High-throughput sequencing revealed that assembled genome sequences represented either PVY<sup>NTNa</sup> or PVY<sup>N-Wi</sup>. Notably, the data analysis suggested that the presence and spread of specific PVY strains varied depending on the potato variety. Knowledge of PVY epidemiology trends and variety responses to PVY strains is valuable for growers, inspectors, and future breeding programs. This was the first survey of PVY strains in Estonia and in the region of the Baltic states.

**Keywords:** NTN, N-Wi, potato, PVY, strain, susceptibility, variety

Potato (*Solanum tuberosum*) is one of the most important crops worldwide. More than 50 viruses and one viroid can infect it naturally. Potato virus Y (PVY) is the most prevalent potato virus globally, causing the highest yield losses (Kreuze et al. 2020). PVY is prone to high mutation and recombination rates; hence, many strain lineages and variants have been described (Glasa et al. 2021; Green et al. 2017, 2018; Karasev and Gray 2013; Lacomme et al. 2017; Samarskaya et al. 2025). Historically, PVY strain groups were sorted by host response and resistance gene interactions (Lacomme et al. 2017; Singh et al. 2008). Lately, though, molecular criteria are becoming more important. Phylogenetic analysis of complete PVY genomes has revealed that the strain lineage N (necrotic), the lineage O (ordinary, non-necrotic), and the lineage C (a branch of lineage O, more commonly infecting other *Solanaceae* than potato) are three lineages that are considered non-recombinant. In addition, a number of recombinants have been described formed between N and O (Gibbs et al. 2017). During the last 10 years, recombinant strains have largely replaced parental strains, capable of overcoming earlier host resistance. The most common recombinants belong to the strain groups of N:O, N-Wi, NTN, NTNb, NE11, E, SYR-I, -II, and -III (Green et al. 2017; Karasev and Gray 2013). Some

recombinants cause only mild foliar symptoms, reducing rogueing success during phytosanitary inspections (Ding et al. 2022; Funke et al. 2017; Gray and Power 2018; MacKenzie et al. 2018; Onditi et al. 2022; Rushton et al. 2024). The studies indicate that the success of recombinants may lie also in their preferential acquisition and inoculation by aphid vectors. For example, the better transmission efficiency of PVY<sup>NTN</sup> over PVY<sup>O</sup> in potato has been reported by the green peach aphid *Myzus persicae* (Carroll et al. 2016; Syller and Grupa 2014). Also, it has recently been shown that recombinant strains NTN and N-Wi outcompete the parental O strain in potato co-infections but tend to have synergistic interactions with each other (Niraula et al. 2024).

Historically, the most common and widely used method for the detection of PVY has been enzyme-linked immunosorbent assay (ELISA) due to its relatively low cost and accessibility. Although several companies provide antibodies for PVY detection and strain identification by serological methods such as ELISA, the limitations in identifying the recombinant strains hinder the proper classification of recombinant isolates. Therefore, a clear shift from serological to PCR-based molecular methods has happened during the last decade. Differentiation between the main strain groups has been made possible by multiplex RT-PCR tests targeting the known recombination junction sites in the genome (Chikh-Ali et al. 2013; Lorenzen et al. 2006b). Such strain profiling has enabled the characterization of PVY populations worldwide (Alves et al. 2024; Bai et al. 2019; Crosslin et al. 2006; Davie et al. 2017; Elwan et al. 2021; Funke et al. 2017; Green et al. 2017; Kamangar et al. 2014; Lorenzen et al. 2006a; MacKenzie et al. 2018; Onditi et al. 2022; Rodriguez-Rodriguez et al. 2020; Rushton et al. 2024; Samarskaya et al. 2025; Tran et al. 2022).

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PVY is the prevalent virus in Estonian seed potato. The long-term monitoring of PVY incidence rate indicated an average of 2.4% in potato lots tested for certification of the highest quality class seed potato and 3.9% in lower-class seed from 2007 to 2022. Detailed analysis showed significant infection rate differences between the cultivated varieties (van der Sman et al. 2025). However, this study did not determine the composition of circulating PVY strains. In the current study, we analyzed the PVY strain composition in Estonian seed potato over three periods: 2011 to 2013, 2016 to 2018, and 2020 to 2022. The study revealed that the recombinant strains PVY<sup>NTN</sup> and PVY<sup>N-Wi</sup> are prevalent. Notably, the PVY strain composition differed greatly among the analyzed potato varieties.

Materials and Methods

PVY prescreening

Five hundred and eighty-five seed lots sampled in the frame of the seed potato certification over three periods (2011 to 2013, 2016 to 2018, and 2020 to 2022) were chosen for PVY strain profiling. In the first period, the presence of PVY was pre-evaluated in all individual seed tubers using the grow-out method, followed by DAS-ELISA using commercial broad-spectrum PVY antibodies from Bioreba AG. No pre-selection based on symptoms was made. In the second and third survey periods, the initial detection of PVY was performed in the bulk of 10 tubers by a TaqMan real-time RT-PCR assay (van der Sman et al. 2025). Samples that tested positive for PVY from all three periods were further assessed by multiplex RT-PCR to identify recombination junction profiles and assign PVY isolates to strain groups.

Molecular typing of PVY strains by multiplex RT-PCR

Molecular typing was performed for samples previously identified as PVY positives. RNA extraction from plant sap obtained from 3- to 4-week-old aerial parts was performed using the CTAB protocol (EPPO 2004), and RNA extraction from tubers was done as described in the protocol adapted from Boonham et al. (2009). Strain genotyping was done using the primer set of multiplex RT-PCR targeting the most common recombination junctions in the PVY genome (Lorenzen et al. 2006b). The main recombination junctions are highly conserved, and, usually, differences between strain subgroups lie in additional recombination at the 5' or 3' termini (Green et al. 2017). The chosen multiplex RT-PCR

method allows for the identification of the parental strains PVY<sup>N</sup> (by amplification of 328- and 398-bp products) and PVY<sup>O</sup> (by amplification of 267- and 689-bp products), which lack the recombination junctions. The North American-type N produces only one band with a size of 328 bp. In addition, multiplex RT-PCR enables the identification of different recombinants between O and N based on primers annealing to the two most common recombination junctions. Good separation between recombinant strain groups can be seen between the PVY<sup>NTN</sup> strain group (by amplification of 181- and 542-bp products) and the PVY<sup>N-Wi/N:O</sup> strain group (by amplification of 181- and 689-bp products). However, the chosen multiplex RT-PCR does not discriminate the subgroups of PVY<sup>NTN</sup> (NTNa, NTNb, SYR-I, -II, and -III), nor the subgroups of PVY<sup>N-Wi</sup> and PVY<sup>N:O</sup> (of those two, the first is spread worldwide, whereas the latter is mainly spread in North America) (Lorenzen et al. 2006b). The chosen primers do not detect PVY<sup>C</sup> strain not common in potato due to a lack of primer binding sites.

The original protocol was modified as follows: oligo dT and primer concentrations were optimized (0.14 μM) to obtain the best results using the QIAGEN OneStep RT-PCR Kit (cat. no. 210212). One-step RT-PCR was performed in a 25-μl reaction volume that contained 1 μl of extracted RNA. First, reverse transcription was performed at 50°C for 30 min, deactivation of revertase was done at 95°C for 15 min, followed by 35 cycles of PCR: 94°C for 30 s, 50°C for 30 s, and 72°C for 90 s, ending with final extension for 7 min at 72°C. PCR products were separated on a 1% agarose gel in TBE buffer.

Statistical analyses

Statistical analyses were performed using JMP10.0 and Microsoft Excel software. The datasets were tested using the least squares multiple linear regression model to determine the statistical significance between PVY strain incidences and years.

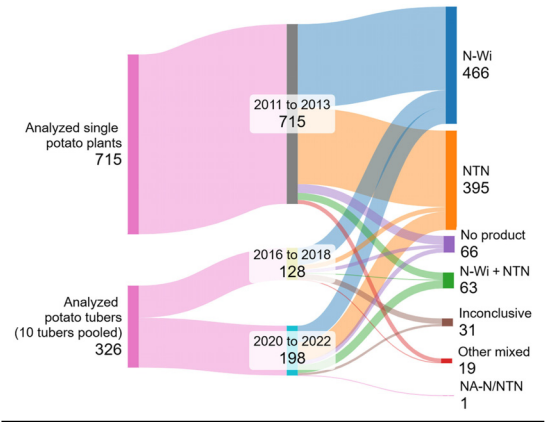
High-throughput sequencing

Total RNAs of six leaf samples originating from individual seed tubers grown out for further studies during the first survey period and nine total RNAs of tuber samples from later periods were sequenced at Fera Science Ltd, United Kingdom. Library preparation and sequencing followed the protocol described by Fowkes et al. (2021). Ribosomal RNA was selectively removed using the Illumina TruSeq Complete Plant Leaf kit to enrich for viral sequences. For

| TABLE 1<br>Identification of potato virus Y (PVY) strain groups based on the multiplex RT-PCR analysis of 715 individual PVY-positive potato plants grown out from seed potato tubers collected for the seed certification process in 2011 to 2013 and 128 and 198 bulked samples collected for the seed certification process in 2016 to 2018 and 2020 to 2022, respectively |      |     |                         |             |              |            |        |                                |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------|-----|-------------------------|-------------|--------------|------------|--------|--------------------------------|
| Year                                                                                                                                                                                                                                                                                                                                                                          | N-Wi | NTN | Mixture of NTN and N-Wi | Other mixed | Inconclusive | No product | NA-NTN | Total number of tested samples |
| 2011                                                                                                                                                                                                                                                                                                                                                                          | 254  | 201 | 15                      | 12          | 0            | 24         | 0      | 506                            |
| 2012                                                                                                                                                                                                                                                                                                                                                                          | 51   | 74  | 9                       | 4           | 0            | 11         | 0      | 149                            |
| 2013                                                                                                                                                                                                                                                                                                                                                                          | 27   | 29  | 4                       | 0           | 0            | 0          | 0      | 60                             |
| 2016                                                                                                                                                                                                                                                                                                                                                                          | 16   | 11  | 1                       | 1           | 7            | 5          | 0      | 41                             |
| 2017                                                                                                                                                                                                                                                                                                                                                                          | 12   | 1   | 1                       | 0           | 2            | 4          | 0      | 20                             |
| 2018                                                                                                                                                                                                                                                                                                                                                                          | 40   | 6   | 1                       | 2           | 13           | 5          | 0      | 67                             |
| 2020                                                                                                                                                                                                                                                                                                                                                                          | 13   | 3   | 0                       | 0           | 3            | 4          | 0      | 23                             |
| 2021                                                                                                                                                                                                                                                                                                                                                                          | 49   | 62  | 31                      | 0           | 3            | 13         | 1      | 159                            |
| 2022                                                                                                                                                                                                                                                                                                                                                                          | 4    | 8   | 1                       | 0           | 3            | 0          | 0      | 16                             |
| Number of samples                                                                                                                                                                                                                                                                                                                                                             | 466  | 395 | 63                      | 19          | 31           | 66         | 1      | 1,041                          |

each sample, an IDT unique dual index was incorporated during library construction to enable accurate demultiplexing and eliminate cross sample contamination issues. Following library preparation, the indexed samples were pooled and sequenced using the Illumina MiSeq platform with V3 chemistry (2 × 300-bp paired-end reads). Raw reads were separated according to their indices using MiSeq's onboard demultiplexing software. A negative control plant sample, no-template library, and index combination not used in run were included in the analysis. No PVY was detected in any sample other than those from this study. De novo assembly of contigs was performed with Velvet assembler, optimizing k-mer size to maximize assembly quality. Cleaned paired-end reads, previously trimmed

and filtered for quality, were used as input. The assembled contigs were screened using BLAST against a custom PVY database compiled from 705 full-length PVY genomes retrieved from NCBI GenBank. A local BLAST database was built using the *makeblastdb* tool. BLAST results enabled the selection of the most appropriate reference genomes (having the longest contig with the highest identity) for further read mapping based on sequence identity and coverage. Mapping of trimmed reads to selected reference genomes was conducted using BWA-MEM, followed by conversion, sorting, and indexing of alignment files with SAMtools. Consensus sequences were generated to facilitate downstream analyses, such as variant calling and coverage assessment. A local consensus genome was reconstructed using SAMtools' consensus function. For strain identification, we used the program PVY strainer developed by us, which is publicly available and archived on Zenodo (<http://doi.org/10.5281/zenodo.15656790>). This approach not only facilitated the localization of N- and O-type regions within the genome but also allowed for the identification of potential PVY strain co-infections. The specificity of primers used in multiplex RT-PCR were assessed by primer mapping to the assembled genomes using the Geneious Prime 2025.1.2 (<https://www.geneious.com>). In addition, the presence of other potato viruses was explored by mapping the high-throughput sequencing-assembled contigs to the downloaded NCBI virus database.



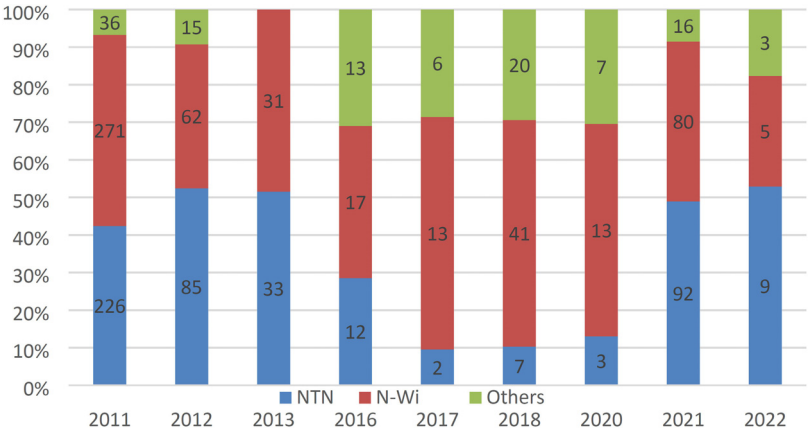
**FIGURE 1** Sankey diagram visualizing the strain identity of different Estonian isolates according to multiplex RT-PCR results. The bars on the left represent the total number of potato virus Y (PVY)-positive samples identified in prescreening, originating either from grown-out plants (identified by DAS-ELISA) or from tubers (identified by real-time RT-PCR). The bars in the middle represent the number of positive samples in each survey period; the bars on the right represent the RT-PCR results. Numbers under the text represent the count of samples.

Results and Discussion

Molecular typing by multiplex RT-PCR

Out of the 1,041 analyzed samples, 466 contained PVY<sup>N-Wi</sup> strain, 395 had PVY<sup>NTN</sup> strain, 63 had mixed infections of PVY<sup>NTN</sup> and PVY<sup>N-Wi</sup> strains, 19 consisted of a mixed infection of PVY<sup>NTN</sup> or PVY<sup>N-Wi</sup> and other PVY strains, 66 did not produce any PCR amplicon with the used multiplex primer set, and 31 produced only one out of two PCR amplicons or the amplicons with incorrect size, being inconclusive for PVY strain identification (Table 1). No non-recombinant O or N strains were detected in single infections. In one sample, PVY<sup>N</sup> was present in a mixture with PVY<sup>NTN</sup>. PVY<sup>O</sup> was found in combination with PVY<sup>NTN</sup>, PVY<sup>NA-N/NTN</sup>, or PVY<sup>N-Wi</sup> (Table 1). A remarkably high portion of samples identified as PVY-positive in real-time RT-PCR prescreening did not produce any RT-PCR products with the chosen primer set. The rates

**FIGURE 2** Proportion of potato virus Y (PVY) strains in analyzed seed potato samples by year. Blue, NTN; red, N-Wi; green, other strains. The number of positive samples is indicated for each subset. NTN and N-Wi found in mixed infection were counted for the appropriate strain group. For the first period (2011 to 2013), the number represents individual samples; from 2016 onward, the number is the bulked samples.



of such samples were the highest in 2016, 2017, and 2020 (12.2, 20.0, and 17.4%, respectively). Inconclusive results (when only one PCR product was amplified instead of the required two or a product with incorrect size) not enabling accurate strain identification were also higher for the second and third surveys, amounting to approximately 10 to 25% (Fig. 1). No product or inconclusive results might be due to bulking of the samples that diluted the virus amount in the final sample and because the real-time RT-PCR used in prescreening is more sensitive than RT-PCR, enabling the detection of PVY even if present in a very low concentration. This might be the reason RT-PCR analysis did not always produce the proper results.

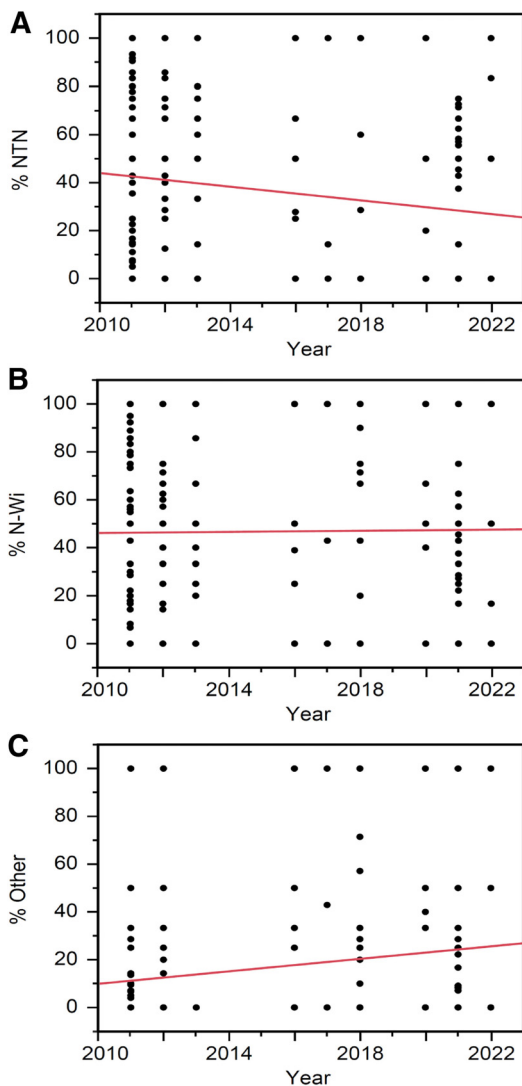
The multiplex RT-PCR results showed that around 90% of Estonian isolates are recombinant isolates from the PVY<sup>NTN</sup> or PVY<sup>N-Wi</sup> group. Non-recombinant strains of PVY were rarely found in the survey; parental strain type PVY<sup>O</sup> was only found in 13 mixed infections with PVY<sup>N-Wi</sup>, PVY<sup>NTN</sup>, or in the mix of PVY<sup>NA-N/NTN</sup>, PVY<sup>NTN</sup>, and possibly PVY<sup>N-Wi</sup>; three samples had mixed infections of PVY<sup>NTN</sup> and PVY<sup>NA-N/NTN</sup>; and only one sample had the parental PVY<sup>N</sup> together with PVY<sup>NTN</sup>. Once, PVY<sup>NA-N/NTN</sup> was present in a single infection (Fig. 1).

To understand the dynamics of the most common PVY strain groups in Estonia, we divided the tested isolates into three groups—PVY<sup>N-Wi</sup>, PVY<sup>NTN</sup>, and “Other”—based on RT-PCR results (Fig. 2). If N-Wi and/or NTN were identified from mixed infection, these were added to the respective group. If infection was caused by any other (identified or unidentified) strains, these were counted as “Other.” Further, their proportions and dynamics trends were analyzed.

PVY<sup>N-Wi</sup> and PVY<sup>NTN</sup> strains were prevalent during the first survey in 2011 to 2013, constituting 90 to 100% of all identified strains; levels were 70% during the second survey and 70 to 90% in the third survey (Fig. 2). N-Wi was the dominant strain in 2011, during the second survey in 2016 to 2018, and at the beginning of the third survey in 2020, whereas NTN was the most favorable PVY strain in 2012 and 2013 and in 2021 to 2022. Notably, the proportion of PVY<sup>NTN</sup> was considerably low in 2017, 2018, and 2020. The proportion of “Other” varied annually, being relatively low in the first part of the survey, higher in the middle part (2016 to 2018 and 2020), and lower again in the last 2 years (Fig. 2). The pattern of type change is probably real, as the pattern seemed to return to similar to the early samples (ELISA) in the later samples (qPCR) and, so, was unlikely to be bias-induced by the method change. Statistical analysis showed that there was a statistically significant ( $P < 0.05$ ) negative trend for PVY<sup>NTN</sup> ( $t$  ratio  $-2.14$ ;  $P = 0.0334^*$ ), no statistically significant trend for PVY<sup>N-Wi</sup> ( $t$  ratio  $0.17$ ;  $P = 0.8645$ ), and a statistically significant positive trend for “Other” ( $t$  ratio  $2.51$ ;  $P = 0.0127^*$ ) over the period from 2011 to 2022 (Fig. 3). The prevalence of PVY<sup>N-Wi</sup> over the PVY<sup>NTN</sup> strain has, for example, also lately been reported in Switzerland, where it was suggested to be related to the fitness and resistance profile of the grown potato varieties (Dupuis et al. 2019).

To understand the susceptibility of different potato varieties to the prevalent strains PVY<sup>N-Wi</sup> and PVY<sup>NTN</sup> in Estonia, we analyzed the relationships between these strains and 26 commonly grown potato varieties previously evaluated for PVY susceptibility in field conditions (van der Sman et al. 2025). The selection criterion was at least two infected seed lots, with more than 10 isolates collected. Exceptions were made for the samples from varieties Ants, Laura, and Jelly, with only four tested PVY-positive isolates. Those varieties showed a high or very high PVY resistance in our previous variety analysis, which is the reason for having a small number of infected seed lots (van der Sman et al. 2025).

The current study revealed that several cultivars were more often infected by PVY<sup>N-Wi</sup>, whereas others were more often infected by PVY<sup>NTN</sup> (Fig. 4). Notably, three varieties (Laura, Madeira, and Jelly) were not found to be infected by PVY<sup>NTN</sup>. The proportion of PVY<sup>N-Wi</sup> infected samples was also high in varieties Romera, Maret, Teele, Secura, and Anti, reaching from 69% (Teale) to 93% (Romera), showing that those varieties are prone to be infected with N-Wi and rarely infected with other strain variants. On the other end of the scale were the varieties such as Red Lady, Princess, Arielle,



**FIGURE 3**

Potato virus Y (PVY) strain trends in Estonian seed potatoes in three survey periods (2011 to 2013, 2016 to 2018, and 2020 to 2022): **A**, PVY<sup>NTN</sup>; **B**, PVY<sup>N-Wi</sup>; **C**, other strains. PVY incidence rates in each seed lot are shown as dots, and the linear regression trendline is shown in red.

Marabel, Reet, and Fontane, with a high PVY<sup>NTN</sup> proportion (from 66% for Red Lady to 82% for Fontane).

High-throughput sequencing

Next, six individual samples originating from the first survey (2011 to 2013) and nine bulked samples (each containing material of 10 tubers from the same seed lot) from later periods (2016 to 2022) were sequenced using high-throughput sequencing to characterize the examples of Estonian PVY isolates and to address the lack of accurate strain identification seen in the multiplex RT-PCR. Samples were chosen based on data received from RT-PCR to cover different PVY strain variants circulating in Estonia. Three chosen samples produced only one PCR fragment of 181 bp, representing the inconclusive results; five chosen samples produced 181- and 689-bp RT-PCR products, indicating the PVY<sup>N:O/N-Wi</sup> strain group; two chosen samples produced 181- and 452-bp RT-PCR products, indicating the PVY<sup>NTN</sup> strain group; two chosen samples did not produce any RT-PCR product; and three chosen samples produced combinations of RT-PCR products, indicating mixed infections (Table 2).

Sequencing of 6 out of 15 samples resulted in seven nearly full PVY genome sequences (samples 9 to 14) (GenBank accession numbers PV658764 to PV658770). In these samples, the average number of reads was around 1.4 million paired-end reads per sample (varying from 0.9 million to 2.5 million), and the average number of PVY-mapped reads was 33,800 reads per sample (varying from around 2,000 to 80,000). The other samples had a higher average number of paired-end reads (1.7 million) per sample, but only a few reads (3 to 388) mapped to PVY. Although the majority of successfully assembled genomes originated from leaf material, two PVY genomes were also assembled from sample 9, originating from seed tuber material. In the current study, the real-time RT-PCR Ct value lower than 20 was a good indicator of a PVY concentration suitable for further full genome assembly from high-throughput sequencing data (Table 2).

The PVY strains in samples 9, 10, and 12 were identified as PVY<sup>N-Wi</sup>, whereas samples 11, 13, and 14 had PVY<sup>NTN</sup>, according to the closest relative in BLASTN analysis (Table 2). In samples 9 and 10, two and one near-full genome of PVY<sup>N-Wi</sup> was assembled, respectively, agreeing with the RT-PCR results by producing the expected 181- and 689-bp amplicons. Sample 12 consisted of the PVY<sup>N-Wi</sup> genome, and it produced three different amplicons (181, 689, and the additional 267-bp product) in RT-PCR. Among samples 11, 13, and 14, which contained the NTN- strain, only sample 11 produced the expected amplicons (181 and 425 bp) in RT-PCR analysis; sample 13 had one additional RT-PCR product of 328 bp, and sample 14 had three extra RT-PCR products of 267, 328, and 689 bp. Mapping of the primer sequences to the assembled genomes using the Geneious Prime 2025.1.2 primer mapping function did not find any primer displacement, unexpected annealing to new regions, or mismatches in primer binding sites, even if three mismatches were allowed for primer binding. In addition, no evidence of mixed infections was observed in samples with inconclusive RT-PCR results. Although not confirmed by amplicon sequencing, we assume that the extra amplicons in RT-PCR were a result of unspecific binding of primers to the host genome.

Other samples (1 to 8 and 15) did not produce full-length consensus sequences to PVY, although samples 2 and 6 had a relatively high number of reads mapping to PVY (388 and 82, respectively), resulting in the longest contigs in length of 2,543 nt for sample 2 and 1,432 nt for sample 6. Samples 1, 3, 5, 7, 8, and 15 had low numbers of reads mapping to PVY (from 3 reads in the case of sample 1 to 15 reads in sample 5). BLASTN analysis of their longest PVY-hitting contigs (163 to 461 nt) resulted in a match to PVY with high identity (Table 2). The high-throughput sequencing results together with earlier real-time RT-PCR and conventional multiplex RT-PCR results led to the presumption that PVY was present in the samples in a low concentration, not enough to assemble full genomes. No reads mapped to PVY from sample 4 that did not produce any

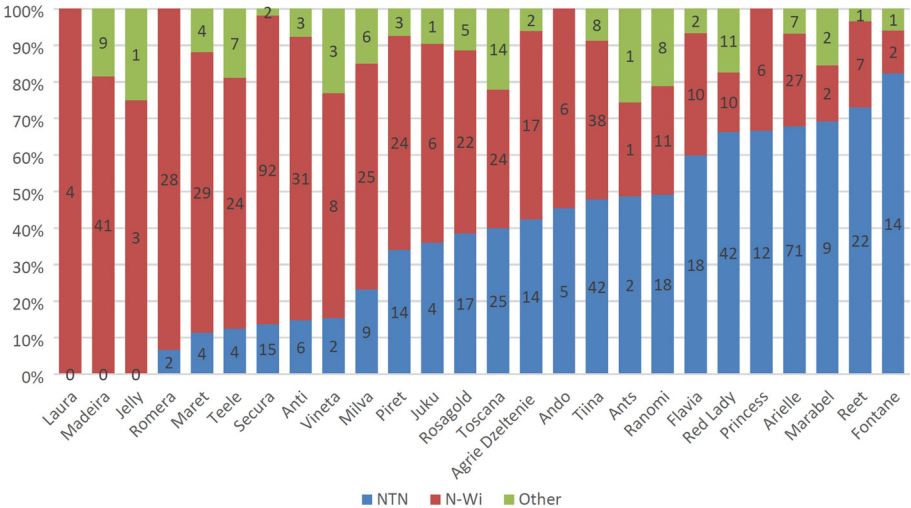


FIGURE 4 Proportion of potato virus Y (PVY) strain isolates in analyzed PVY-infected seed potato samples by variety. Blue, NTN; red, N-Wi; green, other strains. Numbers indicate the number of positive samples.

**TABLE 2**  
**Analysis of high-throughput sequencing (HTS) data from bulked potato tuber samples (samples 1 to 9) and from potato leaf samples (samples 10 to 15)**  
**previously analyzed in real-time and multiplex RT-PCR<sup>a</sup>**

| Sample number    | Variety   | Analyzed material | Multiplex RT-PCR fragment size (bp) | RNA concentration | Ct value in real-time RT-PCR | Total reads | Reads mapping to PVY | Number of viral contigs | Maximum contig length (nt) | Mean sequence depth | Genome coverage (%) | Best BLAST match to longest contig: (country) ID % | PVY strain |
|------------------|-----------|-------------------|-------------------------------------|-------------------|------------------------------|-------------|----------------------|-------------------------|----------------------------|---------------------|---------------------|----------------------------------------------------|------------|
| 1                | Lucilla   | Tubers            | 181, 689                            | 23.2              | 29.4                         | 1,623,000   | 3                    | 2                       | 197                        | 2                   | 2.4                 | MH006955.1 (Israel) 9.49%                          | ND         |
| 2                | Lucilla   | Tubers            | 181, 689                            | 42.8              | 24.6                         | 1,591,996   | 388                  | 7                       | 2,543                      | 7.5                 | 84.8                | JF927753.1 (Poland) 99.14%                         | ND         |
| 3                | Arielle   | Tubers            | 181                                 | 235.7             | 27                           | 4,222,956   | 13                   | 8                       | 306                        | 1.8                 | 13.5                | JN936427.1 (South Africa) 99.67%                   | ND         |
| 4 <sup>b</sup>   | Red Lady  | Tubers            | No product                          | 66.4              | 40                           | 2,037,595   | 0                    | 0                       | 0                          | 0.0                 | 0.0                 | No PVY                                             | None       |
| 5                | Tiina     | Tubers            | 181                                 | 8.7               | 32.3                         | 790,924     | 15                   | 8                       | 163                        | 1.4                 | 9.4                 | KY848000.1 (USA) 100%                              | ND         |
| 6                | Red Lady  | Tubers            | 181, 452                            | 335.7             | 40                           | 2,357,852   | 82                   | 8                       | 1,432                      | 4.8                 | 33.1                | OQ102071.1 (Germany) 99.79%                        | ND         |
| 7                | Lucilla   | Tubers            | 181                                 | 6.2               | 29.7                         | 1,130,988   | 13                   | 9                       | 234                        | 1.6                 | 7.1                 | HQ912872.1 (USA) 99.57%                            | ND         |
| 8                | Albertine | Tubers            | 181, 689                            | 1                 | 28.8                         | 927,234     | 13                   | 8                       | 461                        | 1.6                 | 14.6                | OR133702.1 (China: Inner Mongolia) 99.78%          | ND         |
| 9.1 <sup>c</sup> | Teele     | Tubers            | 181, 689                            | 25.4              | 19.1                         | 867,580.2   | 9,672                | 1                       | 9,688                      | 202.3               | 99.9                | ON604839.1 (Germany) 99.14%                        | N-Wi       |
| 9.2 <sup>c</sup> | Teele     | Tubers            | 181, 689                            | 25.4              | 19.1                         | 578,386.8   | 6,448                | 1                       | 9,688                      | 134.9               | 99.9                | AM113988.1 (Germany) 99.89%                        | N-Wi       |
| 10               | Arielle   | Leaves            | 181, 689                            | 17.1              | 12.1                         | 982,576     | 64,690               | 1                       | 9,694                      | 1416.9              | 99.9                | ON583980.1 (Kazakhstan) 99.78%                     | N-Wi       |
| 11               | Arielle   | Leaves            | 181, 452                            | 1.1               | 19.9                         | 2,504,611   | 16,388               | 1                       | 9,686                      | 273.6               | 99.9                | OR480036.1 (Russia) 99.85%                         | NTNa       |
| 12               | Milva     | Leaves            | 181, 267, 689                       | 708.1             | 11.7                         | 1,404,645   | 23,171               | 1                       | 9,687                      | 337.1               | 99.9                | ON604839.1 (Germany) 99.72%                        | N-Wi       |
| 13               | Manitou   | Leaves            | 181, 328, 425                       | 35                | 15.2                         | 1,200,310   | 80,140               | 1                       | 9,580                      | 1433.0              | 98.8                | JF927763.1 (Poland) 99.76%                         | NTNa       |
| 14               | Anuschka  | Leaves            | 181, 267, 328, 425, 689             | 38.9              | 16.5                         | 1,067,161   | 2,048                | 1                       | 9,675                      | 27.8                | 99.6                | JF927761.1 (Poland) 99.72%                         | NTNa       |
| 15               | Arielle   | Leaves            | No product                          | 0.6               | 27.6                         | 214,104     | 11                   | 10                      | 241                        | 1.1                 | 5.8                 | KY848016.1 (USA: Colorado) 89.52%                  | ND         |

<sup>a</sup> PVY, potato virus Y; ND, not determined, as there were not enough PVY sequences in the sample. Analyzed material is potato tubers and potato leaves.

<sup>b</sup> Sample 4 contained potato virus S, with 3,629 reads mapping to its full-length genome; the maximum contig length was 8,445 nt with 112.2-fold sequence depth; the best BLASTN match was to OM471986.1 (Germany) with 98.88%.

<sup>c</sup> Sample 9 contained two slightly different N-Wi isolates, shown here in two lines to indicate it.



amplicons in the multiplex RT-PCR and had an inconclusive Ct value result (40 cycles) in the initial PVY prescreening. However, sample 4 contained potato virus S, the genome of which was assembled in full length (GenBank accession number PV764920).

Further, an in-house-developed PVY strainer program, was used to perform recombination analyses of the near full-length PVY genomes. It allows for quantification of the read distribution among PVY parental strain types and enables identification of the genome organization of the isolates. Analysis confirmed that all Estonian PVY genomes were recombinants between PVY<sup>N</sup> and PVY<sup>O</sup>. Samples identified earlier as PVY<sup>N:O/N-Wi</sup> (samples 9, 10, and 12) had a recombination pattern of PVY<sup>N-Wi</sup>, and samples identified as PVY<sup>NTN</sup> (samples 11, 13, and 14) had a recombination pattern typical of PVY<sup>NTNa</sup> (Fig. 5).

Conclusions

The prevalent PVY strains in Estonia belong to the PVY<sup>NTN</sup> and PVY<sup>N-Wi</sup> groups based on RT-PCR results. The sequenced PVY isolates belonged to PVY<sup>NTNa</sup> and common PVY<sup>N-Wi</sup> subgroups, with typical recombination patterns. Statistical analysis of PVY strain incidences compared with years indicated that PVY<sup>NTN</sup> has negative trends. Analyses of potato varieties’ susceptibility to different strains showed that there were some varieties (Laura, Madeira, Jelly, Romera, Maret, Teele, Secura, and Anti) prone to be infected with the PVY<sup>N-Wi</sup> strain group, whereas others (such as Red Lady,

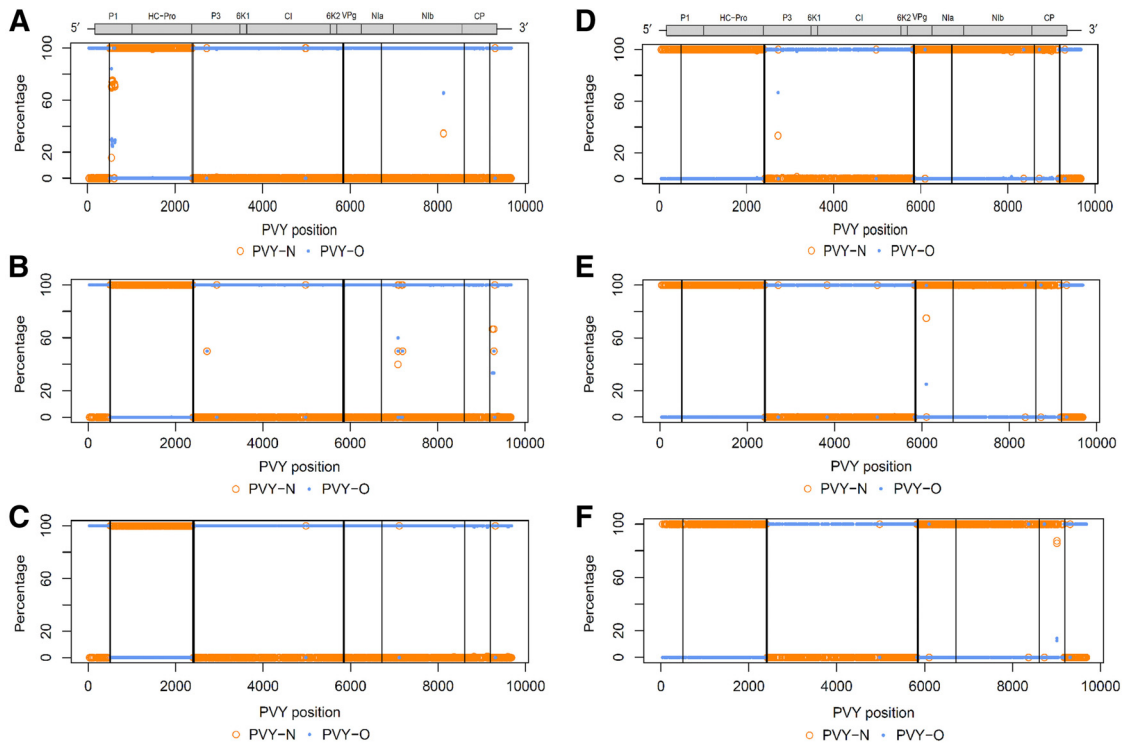
Princess, Arielle, Marabel, Reet, and Fontane) were more likely to be infected with PVY<sup>NTN</sup>. This was the first survey of PVY strains in the region of the Baltic states. The gathered data on local PVY strain prevalence is important for choosing efficient detection protocols and disease management. Furthermore, understanding varietal responses to PVY strains is valuable for growers, inspectors, and future breeding programs.

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**FIGURE 5**  
Recombination detection analysis by the GenomeTester package with the gmer\_counter function for assembled Estonian potato virus Y (PVY) isolates. Samples **A**, **9**; **B**, **10**; and **C**, **12** showed the genome organization of PVY<sup>N-Wi</sup>, and samples **D**, **11**; **E**, **13**; and **F**, **14** showed the typical genome organization of PVY<sup>NTNa</sup>.

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### **Publication III**

van der Sman, P., Stražev, A. & Sõmera, M. (2026). First Report of Potato Virus S Andean Isolates Infecting Potato in Estonia. Plant Disease, First look  
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## First Report of Potato Virus S Andean Isolates Infecting Potato in Estonia

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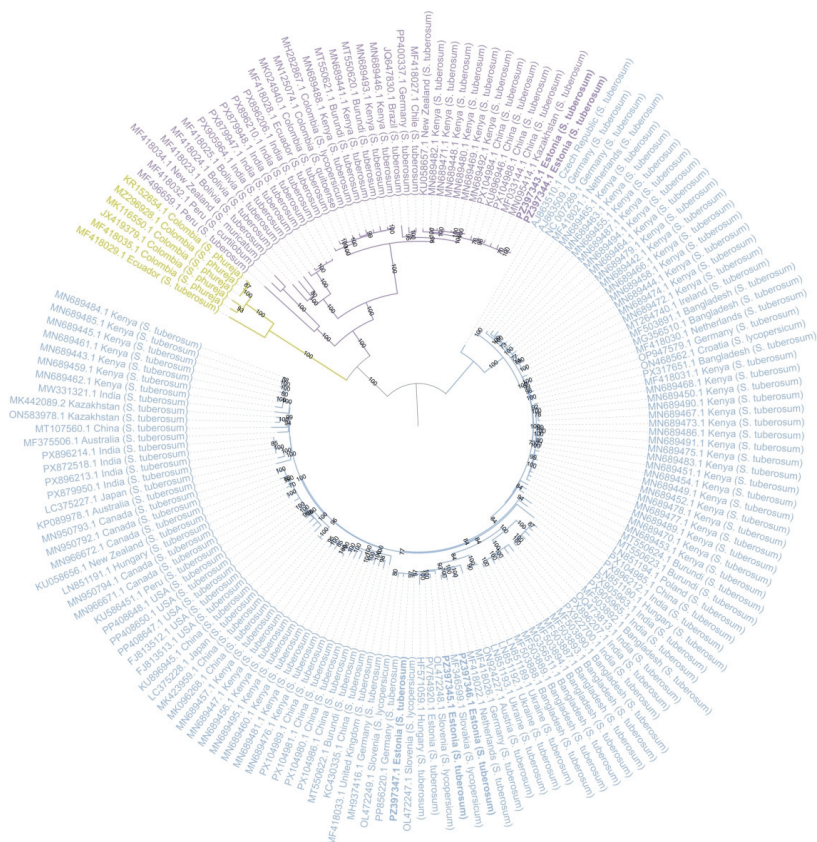
Potato virus S (PVS, *Carlavirus sigmasolani*) is a regulated pathogen in EU seed potato (*Solanum tuberosum* L.) production programs. Phylogenetic studies have identified three lineages: ordinary (–O; spread worldwide), and two Andean phylogroups (–A, mainly found in Latin America, Middle East, and East Africa; and –RVC with a limited spread in the Andes region) (Topakaya et al. 2023). Recently, we reported the first complete genome of a PVS-O isolate in Estonia (PV764920; van der Sman et al. 2026).

In September 2025, seed lots from 34 potato fields (100 tubers per lot) were analysed using high-throughput sequencing (HTS). Plants were grown out and pooled into ten subsamples for each lot. Total RNA was extracted using a Thermo Scientific KingFisher Flex system with the MagMAX™ Plant RNA Isolation Kit (Applied Biosystems). The subsamples from the same seed lot were subsequently pooled for library preparation using the TruSeq Stranded Total RNA with Ribo-Zero Plant kit (Illumina) and sequenced on an Illumina iSeq100 platform using an iSeq 100 i1 Reagent v2 (300-cycle) cartridge, producing 2 × 75 bp paired-end (PE) reads. Reads were demultiplexed according to the instrument workflow. All bioinformatic analyses were performed in Geneious Prime 2026.0.2. Raw reads were quality trimmed and filtered using BBDuk, then PE reads were merged and duplicate reads were removed using default settings. De novo assembly was carried out with SPAdes. Resulting contigs were screened by BLASTn against the NCBI virus database, revealing 71 contigs, ranging from 297 to 8547 nucleotides in length, with the highest nucleotide identity with PVS in 11 out of 34 libraries. Five complete or near-complete genomes were assembled. Two genomes (GenBank Acc. No. PZ397343-44) represented PVS-A isolates, showing the highest nucleotide identity with the Kazakhstan isolate MN095414 (99%), while three genomes (GenBank Acc. No. PZ397345-47) represented PVS-O isolates showing the highest nucleotide identity (98%) with European isolates PP856220, MF346599, and OL472247. PVS-A was once detected in the same library with PVS-O, and once with PVY and PLRV. In other libraries, PVS-O was detected alone (in seven cases), or together with PVY and PVM (once), or with PVY (once). No other viruses were found. Phylogenetic analysis of PVS genome sequences was performed in MEGA 12 using MUSCLE (1000 iterations), and maximum-likelihood phylogenetic tree reconstruction in RAXML 2.0.1 GTR+G+I model, 1000 bootstrap replicates tree visualized in iTOL, showing

clustering of Estonian PVS genomes consistent with BLASTn results (Suppl. Fig. S1). In parallel, seed samples were tested for potato virus A, Y, X, M, S and potato leafroll virus, using RT-qPCR as described in van der Sman et al. (2025). RT-qPCR results confirmed the findings of HTS. PVS incidence ranged from 1% to more than 20%. Our two PVS-A findings originated from the neighbouring fields located in Viljandi county where the seed potato was grown from the seed tubers originated from Germany. Given that Andean PVS-A isolates have been mainly reported in association with material originating from Germany (Bragard et al. 2020), this finding may suggest a possible introduction pathway linked to imported seed material.

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Maximum-likelihood phylogenetic analysis of potato virus S genome sequences. Sequences were aligned in MEGA 12 using MUSCLE (1000 iterations), and the phylogenetic trees were reconstructed using RAXML 2.0.1 with 1000 bootstrap replicates under the GTR+G+I model. Trees were visualized in iTOL. Blue: PVS-O isolates, purple: PVS-A isolates, green: PVS-RVC isolates. Bold: Estonian isolates identified in current study.

84x88mm (600 x 600 DPI)



#### **Publication IV**

Just, K., Allika, R., van der Sman, P., Arif, U., Ilau, B., Koidumaa, R. et al. (2022) First report of Tomato leaf curl New Delhi virus infecting tomato and cucumber in Estonia. *New Disease Reports*, 45, e12083. <https://doi.org/10.1002/ndr2.12083>



# First report of *Tomato leaf curl New Delhi virus* infecting tomato and cucumber in Estonia

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## KEYWORDS

*Begomovirus*, *Cucumis sativus*, *Solanum lycopersicum*, ToLCNDV

*Tomato leaf curl New Delhi virus* (ToLCNDV; genus *Begomovirus*; family *Geminiviridae*) is an emerging virus in the Mediterranean, where it is causing disease outbreaks in cucurbit crops (Moriones et al., 2017). It is transmitted by the whitefly *Bemisia tabaci* as well as by seed and mechanical inoculation (Kil et al., 2020). In 2019, symptoms resembling infection with ToLCNDV were observed in cucumber (*Cucumis sativus* cvs. 'Kostas' and 'Torreón') in a commercial greenhouse (0.1 ha) in Halinga, Pärnu county, Estonia. The diseased plants showed interveinal chlorosis and yellowing (Figure 1). No visible symptoms were observed on tomato (*Solanum lycopersicum*) in a neighbouring greenhouse (0.1 ha). Five samples were collected from different cultivars of tomato (two) and cucumber (three) plants. Each sample consisted of 20 leaves and included both asymptomatic and symptom-bearing leaves collected from 20 different plants. DNA was extracted using a CTAB extraction method and the presence of DNA-A of ToLCNDV was tested by PCR amplification of 741 bp of the coat protein (CP) gene using the ToLCNDV-specific primers AV30F and AV31R (Phaneendra et al., 2011). The PCR test revealed infection of ToLCNDV in the tomato cv. Sakura and cucumber cv. Kostas samples. However, ToLCNDV does not cause unique symptoms and the non-infected cucumber cultivar (Torreón) in the greenhouse had the same symptoms as the infected

cultivar (Kostas). Thus, there is no clear evidence that ToLCNDV solely caused the interveinal chlorosis and yellowing observed in cucumber.

Sequences of the amplified PCR fragments from cucumber and tomato were identical to each other and to a Spanish isolate of the ToLCNDV-ES strain from cucumber (Accession No. MW219696). In addition, they shared 99.9% identity with ToLCNDV-ES isolates from Spain, Tunisia and India. Although the identity with the ToLCNDV-ES isolates based on the CP gene fragment is unambiguous, a partial sequence may limit the conclusions from phylogenetic analysis. However, the phylogenetic analysis indicated the close relationship of the Estonian ToLCNDV isolates with those of the strain ToLCNDV-ES (Figure 2). The sequence data has been submitted to GenBank (OM031061-OM031062). The seeds were acquired from a retailer in Estonia. After confirmation of infection, phytosanitary measures were applied to eradicate the disease, i.e. the plants were burned, and the greenhouses disinfected. In 2020, all cultivars growing in the same greenhouses (six tomato and five cucumber cvs.) were tested and no infection was detected. Thus, the infection was considered to be eradicated. To limit the establishment of ToLCNDV into new areas, it is important to regularly screen imported plants and planting material, as well as to undertake surveillance for the presence of the virus.

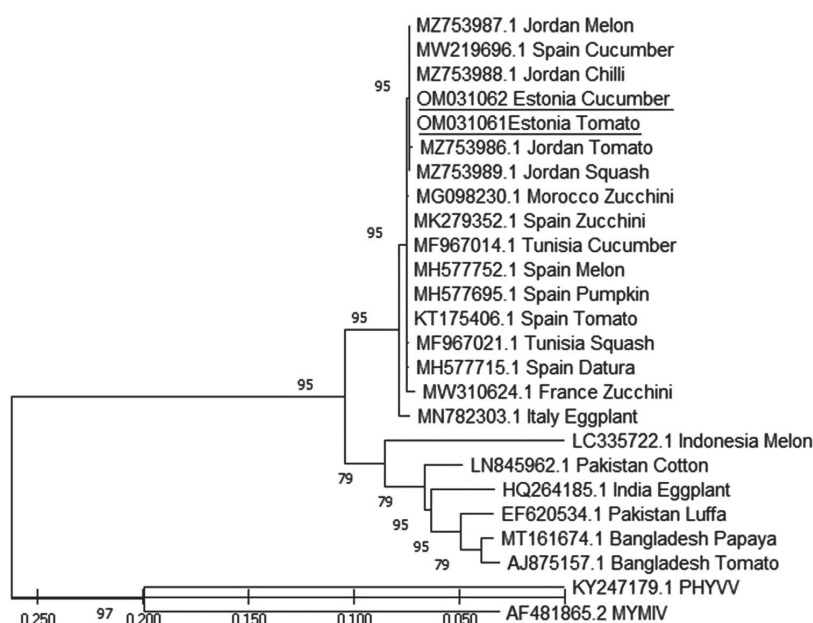
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**FIGURE 1** Cucumber cv. Kostas infected with ToLCNDV in a commercial greenhouse in Estonia showing interveinal chlorosis and yellowing symptoms



**FIGURE 2** Phylogenetic analysis of 741 nt CP gene sequences of ToLCNDV DNA-A. The evolutionary history was inferred using the Maximum Likelihood method and Jukes-Cantor model while evolutionary analyses were conducted in MEGA X

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| Russian  | Basic         |

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| 2022-2022    | Agricultural Research Centre, Deputy head of the Plant<br>Health and Microbiology Laboratory                           |
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## Research projects

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| 2024-2027 | Improving detection of quarantine rust fungi PA1-RUP-IDQRusts                                                                                            |
| 2016-2018 | Eupresco Network „Ringtest for improved Potato virus Y strain detection “                                                                                |
| 2015-2019 | COST Action FA1407, Food and Agriculture, "Application of next generation sequencing for the study and diagnosis of plant viral diseases in agriculture" |
| 2011-2015 | Breeding for disease resistance in plants. Estonian Biotechnology Programme AR11121                                                                      |

## Supervised Thesis

*Anette Stražev, MSc, 2025.* Application of High-Throughput Sequencing for Virus Detection in Estonian Seed Potato and Identification of Potato Virus Y Strains Using

Bioinformatic Methods. University of Tartu, Faculty of Science and Technology, Institute of Molecular and Cell Biology.

*Anette Stražev, BSc, 2023.* Comparison and evaluation of DNA/RNA extraction methods from potato. Estonian University of Life Sciences, Chair of Plant Health.

*Kristiina Ennemuist, BSc, 2015.* Potato virus Y strains identification and characterisation in Estonian seed potatoes in 2011-2013. Tallinn University of Technology, Faculty of Science, Department of Gene Technology.

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| Eesti keel    | Emakeel  |
| Hollandi keel | Algtase  |
| Vene keel     | Algtase  |

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| 2022–2022     | Põllumajandusuuringute Keskus, Taimetervise ja mikrobioloogia laborijuhataja asetäitja |
| 2005–2022     | Põllumajandusuuringute Keskus, Viroloog-peaspetsialist                                 |

### Teadusprojektid

|           |                                                                                                                                                           |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------|
| 2024–2027 | Uued meetodid karantiinsete roosteseente tuvastamiseks ja määramiseks PA1-RUP-IDQRusts                                                                    |
| 2016–2018 | Euphresco „Ringtestide läbiviimine kartuli viiruse Y tüvede määramise paremaks muutmiseks”                                                                |
| 2015–2019 | COST Action FA1407, Toit ja Põllumajandus, Järgmise põlvkonna sekveneerimise rakendamine taime viirushaiguste uurimisel ja diagnoosimisel põllumajanduses |
| 2011–2015 | Põllukultuuride resistentsusaretus. Eesti Biotehnoloogia programm AR11121                                                                                 |

### Juhendatud lõputööd

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