UNIVERSITY OF RWANDA

MONITORING OF MAJOR VIRUSES AFFECTING POTATO EARLY GENERATION SEEDS IN RWANDA. CASE STUDY: “FROM TISSUE CULTURE TO CERTIFIED SEEDS”.

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NIYONTEZE Ghislain

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By

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College of Science and Technology

at

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Supervisor: Dr. Anastase Nduwayezu

Co-Supervisor: Prof. Antoine Nsabimana

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Declaration of independent work

I, **NIYONTEZE Ghislain** hereby declare that this research project submitted to the University of Rwanda, Rwanda for the degree Master of Science in Biotechnology is my own original work and has not been submitted before to any Institution by myself or any other person in fulfilment of the requirements to the award of any degree or any other qualification.

NIYONTEZE Ghislain

DEDICATION

First, I give all glory to God almighty and Jesus Christ, savior of my soul whose guidance and grace carried me through this academic Journey.

To my family, relatives and friends; thank you for love, patience, sacrifice. Your endless support have been my anchor.

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LIST OF ABBREVIATIONS and ACRONYMS

PVX	Potato Virus X
PVY	Potato Virus Y
PVM	Potato Virus M
PVS	Potato virus S
PVA	Potato virus A
PLRV	potato leaf roll virus
EGS	Early generation seed
CIP	International center of Potato
ORF	open reading frame
CP	coat protein
Vg	viral genome
HC-Pro	helper component protein
Rs	Ralstonia Solanaceaum
DAS-ELISA	Double antibody sandwich -enzyme linked immunosorbent Assay
RT-LAMP	Reverse transcription Loop-mediated isothermal amplification
HTS	High throughput sequencing
ONT	Oxford Nanopore technology
CRISPR	Clustered regularly interspaced sequence short palindromic repeats

ABSTRACT

Potato is an important crop in rwanda for food security and income generation. However, its production remains low about 9t/ha compared to its potential around 50t/ha. This is due to virus infections which compromise seed quality across the early generation seeds (EGS) system. This study presents a comprehensive epidemiological assessment of six major potato viruses including *Potato virus X* (PVX), *Potato virus Y* (PVY), *Potato virus M* (PVM), *Potato virus S* (PVS), *Potato virus A* (PVA) and *Potato leafroll virus* (PLRV) throughout EGS production pathway from in *vitro* tissue culture to certified seeds. A total of 284 composite leaf samples were collected from in vitro tissue culture laboratories, screen houses and seed multiplication fields and were screened from viral diseases using the double antibody sandwich enzyme-linked immunosorbent assay (DAS ELISA). Results revealed that most of tissue culture derived plantlets were free from viruses, PVX (10%) and of PVY (1.67%) were detected, suggesting contamination during in vitro multiplication. In screen houses, PVY ($p < 0.0001$) and PVX (41.1%) were significantly prevalent, highlighting vector transmission and mechanical spread risks. Seed multiplication fields showed high incidences of PVY ($p=0.0002$) and PLRV ($p=0.042$) signaling virus accumulation and inadequate vector control. Varietal susceptibility was evident with Kinigi, Gikungu and Kirundo being particularly affected. The findings underscore systemic gaps in Rwanda's potato system where operational lapses, varietal susceptibility and region disparities amplify viral pressure. Based on these findings, this study recommends to enhance aseptic conditions in potato tissue culture facility and envisage to implement virus clean up protocols for high-value potato varieties like Kinigi and establishing mandatory virus testing at critical stages of EGS value chain to ensure phytosanitary integrity.

Keywords: *solanum tuberosum* , aphid, *Potato virus X*, *Potato virus Y*, *Potato leafroll virus*, early generation seed, seed degeneration, prevalence, DAS ELISA.

CONTENTS

1. INTRODUCTION

1.1. Introduction and Background

Globally, potato (*Solanum tuberosum L.*) is ranked at the third place due to its importance after wheat and rice (Okonya et al., 2021) and is the fourth crop in human consumption, the first in tuber and root crop production (Muhinyuza et al., 2022)**,** and the third crop after banana and cassava in Rwanda (MINAGRI, 2022). It is ranked among the top ten priority crops of food and income in the East African Community (EAC) while Rwanda is ranked the third largest potato producer in Sub-Sahara Africa and the second in East Africa after Kenya (Urinzwenimana Clement, 2024).

In Rwanda potato is cultivated in different parts mostly about 1800 m.a.s.l and household production are for food security and for income generation (Muhinyuza et al., 2022). As one of the top ten countries in Africa in Irish potato production, throughout years the production was highly increased around 908,700 metric tons and the production increment is estimated around 8,5% (FAO start 2024), this confers potato to be the second cash crop after cassava in the country's agriculture sector to secure income generation and food security stability (HAVUGIMANA et al., 2023a).

North and West provinces for their rich volcanic soil and cool weather favor potato production. Gicumbi, Burera, Musanze, and Nyabihu districts located in the Northern and Western parts of the country, accounts for more than 90% on potato production in the country (Vries & Limited, 2020). The average consumption rate is 80, 250, and 150 kg per year, respectively in urban areas, zones usually producing potatoes, and in other country areas (Issues, 2020).

According to CIP database, there are over 30 officially released potato varieties in Rwanda. Presently the four popular varieties (Kinigi, Gikungu, Kirundo and Cruza) were released between 1984 and 1992. Four varieties namely Kazeneza, Twihaze, Ndamira and Cyerekezo released between 2019 and 2020 are slowly gaining popularity among the farmers and buyers.

Despite the crop importance in Rwanda, its production remains low, estimated at 9 t/ha (K. Sharma et al., 2021) against the potential yield of the crop is about 50 t/ha (Shimira et al., 2020a). Quality seed potato is the most important agricultural input that determines the final tuber yield and drives the productivity of other agricultural inputs in potato production systems. Much of the yield gap currently constraining productivity in the country is attributed to poor seed quality.

The low yields have largely been attributed to high prevalence of seed-borne pests and diseases, notably late blight, bacterial wilt, nematodes, and viruses. Pests and diseases contribute to production loss of up to 33% (Okonya et al., 2019) and viruses are reported as important diseases affecting potato in Rwanda (Okonya et al., 2021).

1.2. Problem statement

Potato seed degeneration due to viruses can reduce production by 70% to 90% in Rwandan fields (Okonya et al.2019). Incidence and severity are not investigated regularly to evaluate viruses in early generation seeds and their associated vectors in Rwanda (Okonya et al., 2021). Therefore, being cognizant that any virus free planting material has been produced locally, prior to engaging in my research is very important. Rwandan potato seed multipliers propagate it both sexually by direct planting of potato seeds and vegetatively (Chuntale, 2018). Clonal propagation of potato is associated with viruses, bacterial and fungal diseases (Gong et al., 2019). Viral status helps to predict adaptation to dynamics of climate change and understanding of potato viral diseases prevalence helps to put appropriate mitigation strategies and to strengthen introduction of potato virus free planting materials within potato seed system.

1.3. Justification of the study

Potato plays an important role in food security and income generation worldwide, in Rwanda particularly. For few years ago, potato production continue to decrease because of low quality and health status of planting materials. The traditional farming is continue to spread and accumulate disease especially viruses which significantly cause yield losses and reduce tuber quality over successive planting cycles. As the solution from government, the production of early generation seeds under tissue culture offered more reliable solutions. This technique enabled the rapid virus free plantlets production under suitable and controlled climate conditions fostering production of pre-basic and basic seeds categories. Micropropagation under tissue culture enhanced to produce clean planting material and ending up the deterioration of potato seed that was propagated through informal seed systems.

Knowing the benefits and capacity of tissue culture and other pipeline of seed multiplication, limitations of key information on performance, efficiency and challenges encountering from tissue culture to certified seeds pathway including mostly viral infection status, quality management and

access to certified seed remain uncertain. It is in this regard, this study aims to analyze the prevalence and distribution of potato viral diseases across the early generation seed production and multiplication schemes in order to draw improvement strategies in facilitation of certification procedures for sustainable supply of high quality and virus-free planting material to farmers. Outcomes from this study will be used as to guide researchers, policy makers, extension services and seed inspectors together to improve accessibility to clean planting material which will contribute to a sustainable potato productivity and food security.

1.4. Objective

The overall objective is to assess the prevalence and distribution of potato viruses across the potato early generation seeds production chain from tissue culture derived seed stock to certified seed stage.

1.5. Specific objectives

a. Sampling in different agro-ecological zones where potato crop is grown;

b. To determine prevalence of potato viruses across early generation seeds from tissue culture (G0 stage) to certified seeds (G4 stage);

c. To measure virus infection rate within the potato early generation seed production stages.

1.6. Hypothesis

As a staple food and cash crop in Rwanda, potato plays a crucial role in food security and rural livelihood. It is grown mainly in northern and western provinces regions of the country. Its production remains low by estimation around 9 tons per hectare compared to their potential production estimated around 50 tons per hectare, partly due to virus infections coupled with limited access to quality seed and adoption of good agricultural practices. Among the main viruses, PVX, PVS, PVA, PVY and PLRV are prominent viruses in Rwanda and are spread via seed tubers and aphid vectors causing significant yield losses and seed degeneration. Virus infections tend to increase with seed generation stage, stressing the importance of producing virus-free EGS to reduce disease pressure in certified seed stocks. Recent farmer training and quality seed initiative have improved yields, but virus pressure remains a major constraint to sustainable potato production in Rwanda.

2. LITERAURE REVIEW

2.1. Origin and description of potato

Irish Potato belongs in the plantae kingdom, magnoliophyte division, Magnoliopsida class, Asteridae sub class, in the order of solanales, family of Solanaceae, genus solanum and S.tuberosum species (Reddy et al., 2018).

Cultivated Potato came from Peru (Andes) and Bolivia. Bolivians domesticated at the first round for more than 800 years ago. The Mexican adopted it where Spanish explorers exported it into Europe, north America, Asia and Africa. In Rwanda, it came with German missionaries in the beginning of 19^{th} century, where at the first sight was not envied, later appreciated because of the famine in 1940's (Muhinyuza et al., 2016).

The potato (Solanum tuberosum) is a member of Solanaceae family in tuberosum genus. This genus comprises more than 200 species grouped into Pachystemonum and Leptostemonum sub genuses. Pachystemonum is grouped into five divisions of which Pelota division where belong tuber bearing species. Pelota division contains Estolonifera and potatoe subdivisions. Potatoe subdivision, Tuberosa part is all cultivated species are classified. Around 72 % of the species are diploid (2n=24) and nearly 12% tetraploid (2n=48) and the remaining are triploid (2n=36), pentaploid (2n=60) and hexaploid (2n=72). A tetraploid species called Solanum tuberosum L. is the most widely cultivated potato (Reddy et al., 2018).

Tuberosum genus consists of seven domesticated species: *Solanum ajanhuiri* (2x), *Solanum chaucha* (2x), *Solanum curtilobum* (5x), *Solanum juzepczukii* (3x), *Solanum phureja* (2x), *Solanum stenotomum* (2x), and *Solanum tuberosum* (4x) with *andigenum* and *tuberosum* subspecies (Spooner, 2013). S. ajanhuiri (2n = 2x = 24) and s. juzepczukii (2n = 3x = 36) came from the unique ancestry. S. ajanhuiri only came from Bolivian- Peruvian origins of hybridized between Solanum stenotomum(diploid) and wild type Solanum megistacrolobum (De Haan & Rodriguez, 2016).

Globally, potatoes are disturbed and cultivated in more than 158 countries which confers its significance at fourth place with the 92 billion USD of income (Issues, 2020b).

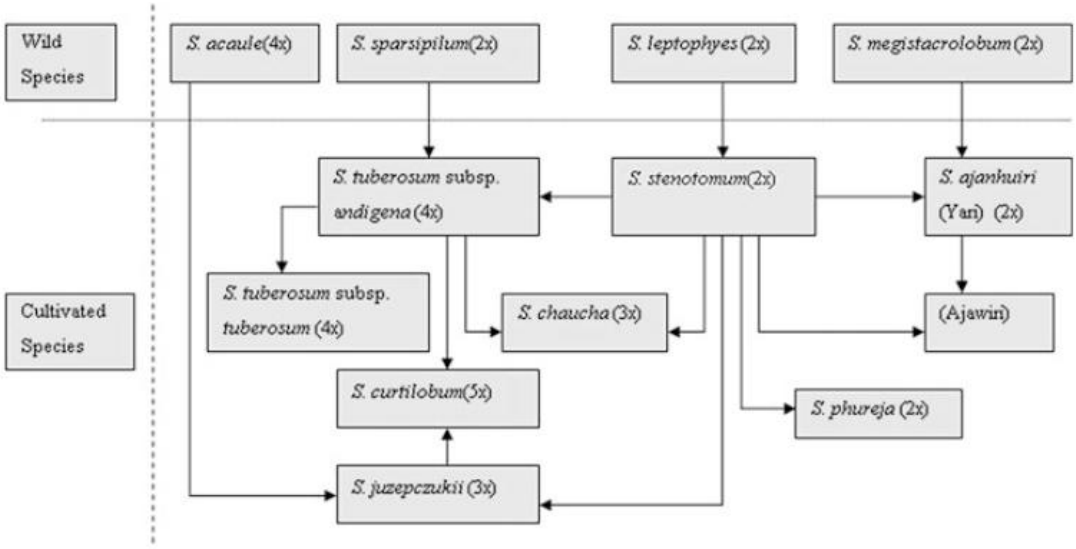

Figure 1: Potato diversity and its genetic enhancement. Srivastava et al., 2016

2.2. Nutritional attributes and medicinal properties of potato

Potato in many countries is essential because of its variety of low fatty contents, vitamins and minerals. It contains ascorbic acid, vitamin B6, vitamin B9, vitamin B1 and both microminerals and microminerals (including potassium, iron, copper, manganese, phosphorus). In 100g of potato, contains 90 kcal, energy component composed of water (75g), carbohydrates (19g), fat (0.1g), ascorbic acid (0.25g), CHO (19g), calcium (12mg), iron (1.8mg), phosphorus (57mg), magnesium (23mg), sodium (6mg), pyridoxine (0.25mg), vitamins B1(0.08mg) and B2 (0.03mg) (Waseem khalid1, 2020).

Potato contains carbohydrates especially starch and other sugars important source of energy for the brain and the whole body. Potato consists of fibers for cellulose, pentosane, pectin and other dietary fibers. Fibers are essential for human body in regulating blood glucose, blood lipid levels and accelerate weight loss. It has been reported that these fibers are important in fighting against colon cancer. Potato are cholesterol free and is made for macro (calcium, potassium, phosphorus, sodium, magnesium, sulfur and chloride) required by the body not more than 100mg per day and micronutrients (copper, iron, zinc, chromium, cobalt, iodine, molybdenum, and selenium) required by the body less than 100mg per day; all of them are vital for maintaining the organism in normal conditions and biochemical pathways (Ndungutse, 2019).

Beside its source of energy, potato promotes health and intervene in chronic diseases management and prevention (Zaheer & Akhtar, 2016). Potato proteins are coequal with animal proteins (whole eggs) and are of high quality better than of beans and peas proteins because of its high content of methionine substantially rich in lysine (BERINDEAN, 2022).

Potato is a source of secondary metabolites that are essential to human health. Phenolic compounds from potato are secondary metabolites responsible for red and purple colors in the potato, plays a main role in reducing cancer risks, heart and diabetes diseases. Carotenoids from potato are essential in defense against degenerative genetic disorders from oxidation source (Ngoda et al., 2019).

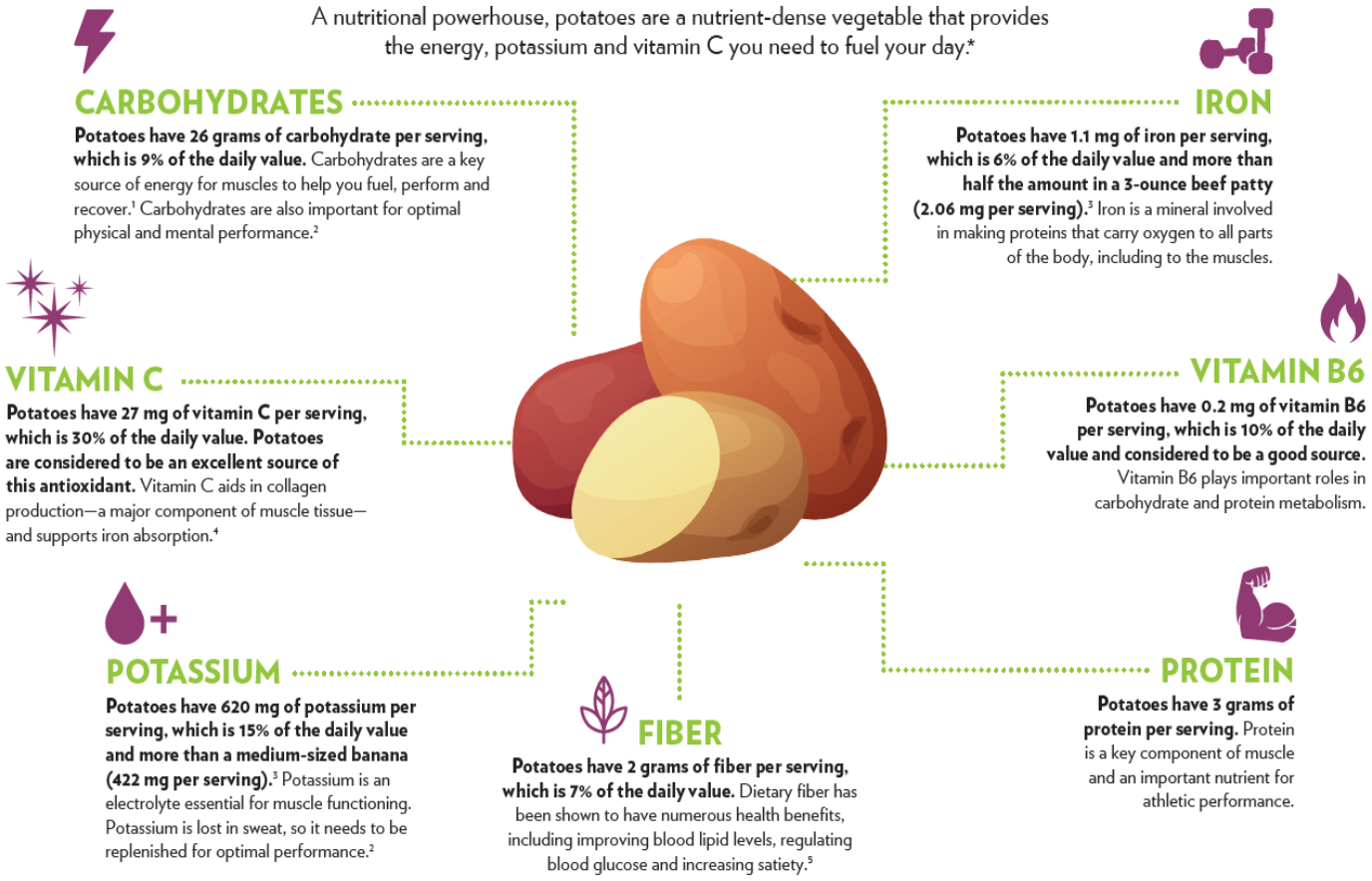

Figure 2: Potato USA (2021)

2.3. Potato production constraints

Potato is infected by several pest and diseases which cause significant economic losses. Pests which cause significant losses in potato including aphids species (*Myzus persicae, Macrosiphum euphorbiae, and Aulacorthum solani*), thrips (*Frankliniella schultzei*), nematodes (*Meloidogyne spp*), cutworm (*Agrotis spp*), potato tuber moth (*Phthorimaea operculella*). The yield losses caused by potato pests is up 100% depending upon several interacting factors (Otieno, 2019).

Diseases are also causing significant effect on both potato produce and quality (Tessema et al., 2024). Viral diseases (PVY, PLRV, etc.), bacterial wilt (*Ralstonia solanacearum*), late blight (*Phytophthora infestans*), Early Blight (*Alternaria solani*), Verticillium Wilt (*Verticillium albo-atrum/Verticillium dahlia*), Common Scab (*Streptomyces scabies*) are the most common diseases that affect potato production (Otieno, 2019). Potato diseases severity attributed to late blight in yield losses can reach 75%, for bacterial wilt, the losses is estimated between 75% to 100%, and losses attributed to viruses in production degeneration can reach 90% (Okonya et al., 2019b).

Beside pest and diseases, climate change and soil degradation threaten potato production. Rwanda like any other country is facing climate change challenges. It has been reported that between 2000 to 2050 rain fall will fall drastically from 400mm to 100mm. seasons will be shorter and yet with heavy rainfall. In addition to that, rising of temperatures will cause evapotranspiration contrary to potato which are produced in high elevation with low temperature. This can accelerated to produce varieties that will be adapted to low altitudes and to heat-tolerant. The greenhouse gases have risen and are quite challenging to agriculture production. It has been demonstrated that tuber crops including potato elevated carbon dioxide have physiological effects. In association with soil degradation for poor soil management including use of organic amendment limitation and crop rotation avoidance. Terracing slopes above 12% have led to the better use of nutrients in soils and lower terracing lead to conserve organic carbon and phosphorus levels and had promising better yields compared to upper slopes terracing. This strategic land management and soil will solve potato production in the face of ongoing climate variability (Shimira et al., 2020b).

2.4. Diseases associated with potato

2.4.1. Major viruses affecting potato and their impact on yield and quality

There are over 57 viruses which potato plants are susceptible worldwide (Kreuze et al., 2019) Bettoni et al., 2022). The six common viruses that have been shown to attack potato in Rwanda include: Potato virus Y (PVY genus Potyvirus), Potato leafroll virus (PLRV, Polerovirus), Potato Virus X (PVX, Potexvirus), Potato Virus S (PVS, Carlavirus), Potato Virus M (PVM, Carlavirus), and Potato Virus A (PVA). PVY, PVA, PLRV, and PVM are transmitted by aphids, PVX by contact, while PVS transmission is done by both, aphids and through contact mechanisms (Onditi et al., 2021). PVS, PVY and PLRV are major viruses identified and most damaging diseases in Rwanda (Nduwayezu et al., 2024). Production losses caused by PVY, PLRV and PVA can reach up to 90%, while PVX cause moderate loss estimated at 40% and losses caused by PVS are estimated at 20% (Okonya et al., 2021b).

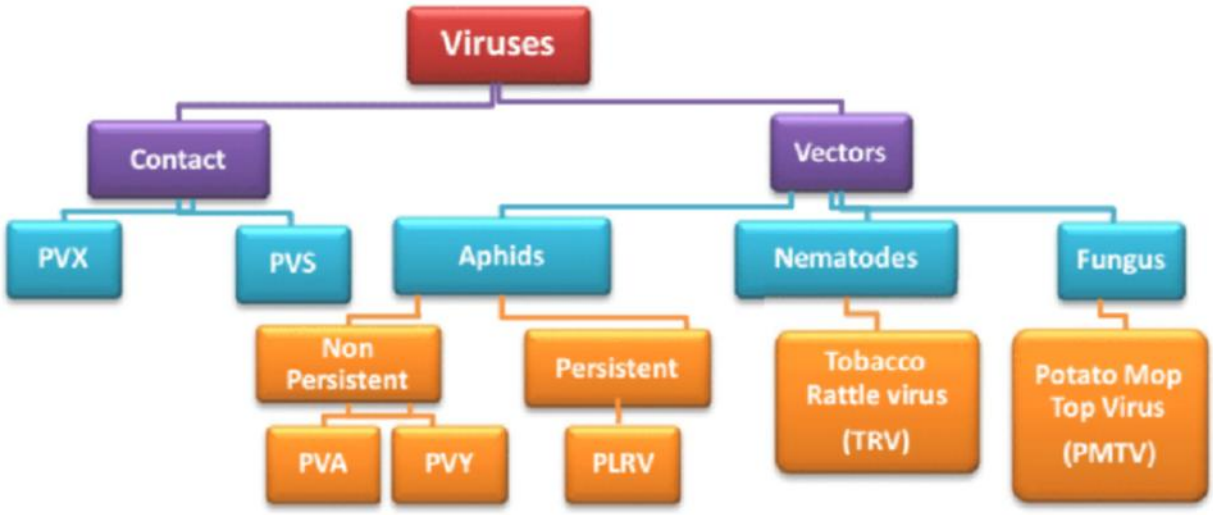

Figure 3: Viruses Transmission mode in Potato Production System, Bartle (2019).

2.4.1.1. Potato virus Y (PVY)

Potato virus Y affects a wide range of host in Solanaceae family and is distributed worldwide (Blanchard et al., 2008). Potato virus Y (PVY) of the potiviridae family and genus of potyvirus (Torrance & Talianksy, 2020), is a single stranded RNA virus of 10kb long genome (Blanchard et al., 2008) which encodes a wide range of polyprotein cleaved by three virus encoded proteases (P1, helper components proteinase, and nuclear inclusion body) into ten functional proteins P1, HCPro, P3, 6K1, CI, 6K2, VPg, Nia-Pro, Nib and CP P3N-PIPO extra protein (Verma et al., 2016). Moreover, research reports demonstrated the virus genome encodes a wide open reading frame (ORF), a viral genome linked protein (VPg) covalently attached to the 5'-RNA by a tyrosine, a poly adenine tail at the 3'-end of the genome and untranslated regions flanking this ORF and the second ORF which is PIPO dwells in between the previous ORF. The coat protein (CP) and the helper component protein (HC-Pro) carry out transmission of viral particles from plant to another, while HC-Pro alone carry out the RNA silencing suppressor , amplification of viral genome, systemic movement and symptom development. The CP, CI, Vg and PK_2 proteins carry out movement of viral particles within the host plant while the Vg alone carry out function in virus infection pathways by initiating viral replication, translation movements and plant modulation to resist from the viral infection. The PIPO protein carry out movement of replication and suppress systemic movement (Blanchard et al., 2008).

Phylogeny studies of PVY strains revealed to be in five definite biological classes PVY^{O}, PVY^{C}, PVY^{N}, PVY^{Z} and PVY^{Z} (Tran et al., 2022). Several of its strains have been discovered, PVY^{O} (common strain), PVY^{N} (tobacco vein necrosis strain) and PVY^{C} (stipple-streak strain), two recombinant from PVY^{O}, and PVY^{N} ($PVY^{O:N,}$ PVY^{NTN} -N-tuber necrotic and PVY^{N-wi} -N-Wilga) (Krüger & van der Waals, 2020) and PVY^{Z} and PVY^{E} (Blanchard et al., 2008). These main PVY strains are categorized based on their genome organization and their hypersensitivity reactions (HR) in N resistance genes. PVY^{O} strain excite Ny gene and induce a systemic mottle, while, PVY^{C} strain excite Nc gene and stipple streak; PVY^{Z} excites the Nz gene in the absence of Ny and Nc genes; PVY^{E} strain in the presence of Ny, Nc and Nz genes causes venal necrosis in the tuber (Verma et al., 2016). PVY^{O} and PVY^{C} cause leaf mottling and severe necrosis and leaf fall which lead plant to death; while PVY^{N} cause mild mosaic symptoms on leaves and necrotic lesion in potato tubers (Blanchard et al., 2008).

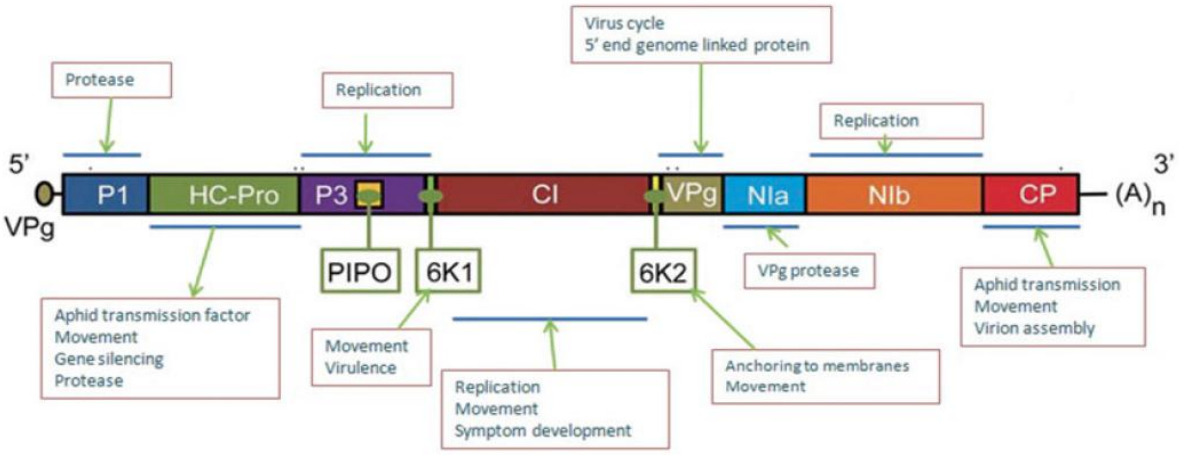

Figure 4: PVY genome organization described by Verma et al. (2016).

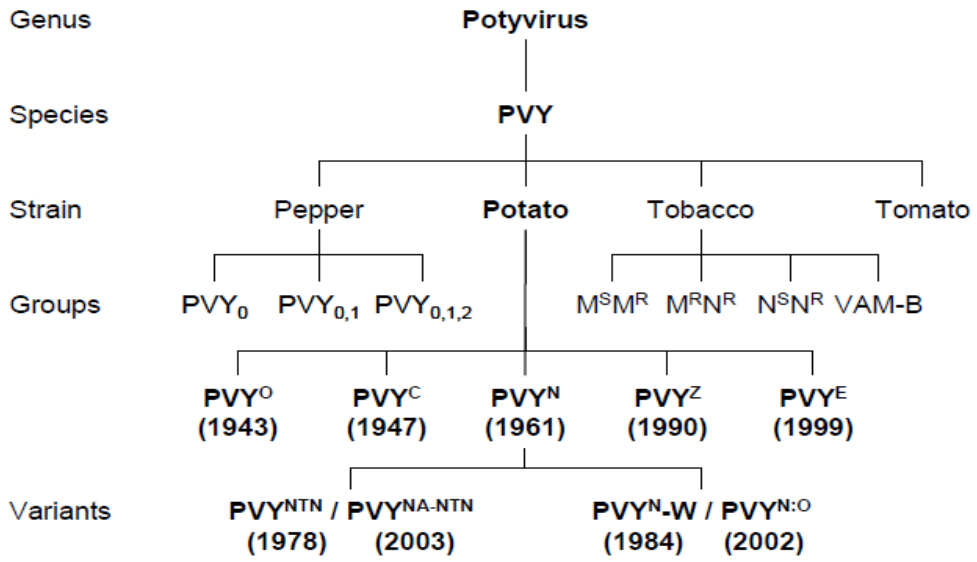

Figure 5: classification of PVY species and isolates in bracket affecting Solanaceae family.

Blanchard et al. (2008)

It has flexible, rod-shaped and non-enveloped particles of 984nm width when on purified preparation and 730nm and 11nm of width in leaf-dip preparations and its coat protein subunits are assembled in helical symmetry (Rizk et al., 2020). This is the most virus affecting potato worldwide and cause yield and quality degeneration (Lacomme et al., 2017), once spread in the field it cause the rejection of the certified seed and the loss is estimated between 10% to 80% (Rizk et al., 2020), depending upon the stage of the plant and type of strain the loss is estimated up to 100% (Naveed et al., 2017).

In potato, PVY is also called mosaic or rugose mosaic and is transmitted from plant to plant by aphids in non-persistent manner, by contact, by mechanical mechanisms (Tsedaley, 2015) or by grafting non-true seeds (Krüger & van der Waals, 2020). The virus does not replicate in aphid and has no latent period. It transmit the virus in potato while feeding on epidermal cells in short period. It loses ability to transmit while probing on the non-infected potato plants or virus non-host plants; contrary , they are infected when feeding on infected plants. (Krüger & van der Waals, 2020). PVY is also transmitted by vegetative propagation where potato tubers carry systemic infection once germinated transmit vertically the virus in the daughter tubers (Blanchard et al., 2008).

PVY foliar symptoms are characterized by mild severe mottle with leaf distortion on aerial parts while necrotic spots and vein necrosis are found on lower leaves. It has been observed that intermediate leaves fall apart and drop while attached to the stem. The infected plants are characterized by dwarfism, brittle associated with crinkled and puckered leaves and necrosis on tubers (Tsedaley, 2015). Phylogeny data showed that PVY recombinant strains transmit the potato tuber ringspot disease which cause tubers necrosis. These have caused serious economic losses to American and European potato industries decades back and nowadays progressively Asian and south American countries (Kreuze et al., 2019b).

2.4.1.2. Potato Virus A (PVA)

Potato virus A is a potyvirus member group that infect potatoes moderately and other members of the Solanaceae family crops. Historically, its nomenclature was given as PVA after 1914 and now is prevalent worldwide in potato productions (Zhang et al., 2021a). The yield lost globally as single infection is estimated up to 40% however, is beyond less prevalent than PVY, PVS and PLRV (Kreuze et al., 2019b). Generally, virus A on susceptible cultivars produce mild mottle symptoms without expressing symptoms (Maclachlan et al., 1954), when its infection is combined with PVX or PVY express severe mosaic symptoms which cause "potato crinkle disease" (Petrov et al., 2023). Mosaic symptoms varies depending upon the environmental conditions, type of potato variety and the type of PVA strain from mild mosaic to severe leaf necrosis. Different types of foliar symptoms have been used to categorize and to differentiate PVA strains symptoms from very mild, mild, moderate and severe (Zhang et al., 2021a).

Like most potyviruses group are of picornavirus supergroup plus RNA virus and are RNA-dependent RNA Polymerase (RdRp). They are filamentous type of 5'end C terminal viral genome linked protein (VPg) and 3'end terminal poly tail (A) (Hafrén, 2014).

PVA shares common characteristics of the potyvirus group and is a positive-sense single stranded RNA (ssRNA) of 9,565 nucleotides enveloped by one virus encoded coat protein. Its RNA is translated into a wide protein of 3,059 amino acids substantially processed into ten (10) functional proteins (Rajamäki et al., 1998). PVA genome encoded proteins includes P1 plays crucial role in virus adaptability to different host species due to its genome amplification activity. The C-terminal endorsal protein Nia, catalyze polyprotein cleavage, the Nib acts as RNA polymerase for viral replication activity (Zhang et al., 2021a). The nucleotides of PVA genome are composed of 3'poly-tail (A), virus encoded protein (VPg) and covalently attached to the 5'-end and each end has non-translated region (NTR)(Kekarainen et al., 2002). The short protein called PIPO the Potyviridae ORF was found out in the P3 protein frame (Zhang et al., 2021a).

PVA is transmitted in non-persistent manner by different species type of aphids (Bettoni et al., 2022b). PVA is transmitted through infected planting materials and by mechanical frictions (Zhang et al., 2021b).

Figure 6: A PVA genome organization including PIPO ORF described by (Hafrén, 2014)

Investigation studies conducted by Rajamäki et al. (1998) on the he CP genes of 20 PVA isolates originating from Hungary, Germany, Finland, The Netherlands, Scotland and the USA. They concluded that amino acid of CP gene sequence identities shared between these isolates was 92.9%. The amino acid sequence identities of the HC-pro gene, of ten of these isolates, shared 94.8% identity, and the 3' non-translated region (NTR) shared 93.4% sequence identity. The clusters that formed with phylogenetic analyses of the CP, HC-pro and the 3'NTR sequences, were only partially consistent with geographic origins.

2.4.1.3. Potato leafroll Virus (PLRV)

Globally, Potato leaf roll virus (PLRV) is more prevalent and most devastating pathogen which cause both quality and quantity yield losses (Olmedo-Velarde et al., 2023). PLRV is placed at second most prevalent disease in Africa, Asia, Europe and America and southern America (Farooq et al., 2022), rare in northern Europe (Petrov et al., 2023) and its estimated losses reaches 90% on susceptible varieties in Pakistan (Mubeen, 2020; REHMAN, 2020) and worldwide (R. R. Kumar et al., 2020). PVY is also called “potato phloem necrosis virus “ causes leaf roll, leaf curl and phloem necrosis as net necrosis. Diseased plants show small size tubers caused by thick, harsh, leathery upward rolling midrib leaflets (LP & HN, 2017). At primary infection, the plant remained dwarf and leaves are rolled and pale and late growing stage shows no symptoms and tubers are partially infected while at secondary infection the plant stands erect and smaller compared to non-diseased (M. F. Abbas et al., 2013). Mustansar et., al. 2020 reported that the virus symptoms on top leaves are rolled in primary infection and bottom leaves are rolled, junior buds turns purplish and yellow and the plant stands erect growth (fig.4).

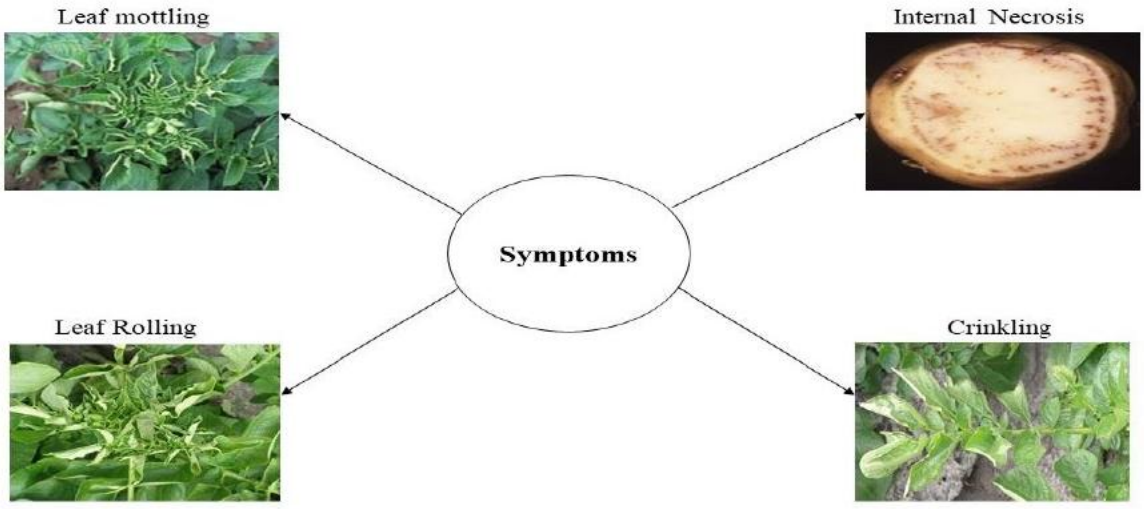

Figure 7: PLRV symptoms described by Mubeen et al. (2020)

Plant produced from diseased tubers are greenish, stunted and more upright than the healthy one mostly on bottom leaves. Diseased leaves characterized with thickness leather when touched, and when pressed in hands are cranked, brittle in texture rolling and curling straight at the margin . Symptoms seem to appear in the first month after planting. The diseased plants produce small and few tubers (LP & HN, 2017). In contrast, Rehman et., al, 2020 reported that PLRV symptoms on tubers lesion on the tubers, reduced size. Tubers have very thin skin and destroyable which cause not to be stored for very long time. They have also wrinkled shape, and its taste also change. It has been shown that it can be a source of infection.

The virus is only transmitted by several species of aphids including blackthorn aphid (Aphis nasturtii), Aphis fabae, Myzus ascalonicus, Potato aphid (Macrosiphum euphorbiae) (LP & HN, 2017) and green peach aphid is most reported (Petrov et al., 2023). Aphids transmit PLRV in persistent, circulating and non-propagative manner and experimentally by grafting (A. Abbas, 2016). Circulative and non-propagative manner consist of infecting tissues in the circulative phloem system of the crop thereby the aphid gut deposit the virus particles through its salivary glands (Olmedo-Velarde et al., 2023). Vector transmission of PLRV virions consist of acquisition, of the virus particles in the plant stylet with immediate absorption in haemolymph followed by latent period and inoculation of the virus in plant phloem circulation. From Tasheva et., al, 2010 studies on virus-vector relationship of PLRV and Myzus persicae Sulzer revealed 6 hours is the time required for green patch aphid to acquire the virus particles and minimal time is 3hours. 6

hours is the latent period of the virus in the vector and the inoculation time of PLRV by the green patch aphid is estimated between 30-120 minutes. Once the vector is infested by PLRV, it can infect the plant for its lifetime (Tasheva-Terzieva et al., 2010). The effective transmission reports revealed Myzus persicae and Myzus euphorbiae transmit PLRV 80% to 90%, aphis fabae 50% and aphis gossyppii 30% (Khaled et al., 2018). The PLRV tuber infections at secondary stage were estimated between 33%-50% and the loss is much observed when it is mixed with PVX or PVY (Loebenstein, 2001;Petrov et al., 2023).

The potato leaf roll virus is known to infect primarily potato, also it can infect other crops from Solanaceae family. Among Solanaceae commodities, it causes different symptoms such as interveinal necrosis in Datura stramonium; chlorosis, rolling and leatheriness of leaves in solanum villosum; stunting, chlorosis and rolling of leaves in physalis floridana and stunting, marginal yellowing, curling of leaflets and death of flowers in Lycopersicon esculantum (Loebenstein, 2001). Non Solanaceae species includes Amaranthus caudatus, A. graecizans, A. retroflexus, Celosia argentea, Gomphrena globosa and Nolana lanceolata. *Physalis floridana*, *P. angulata* and *Datura stramonium* are the most used for PLRV test. *Physalis floridana* and potato are the best used as the propagation hosts. *Physalis floridana* is a suitable indicator, test and propagation host to reproduce major symptoms as interveinal chlorosis, darkening of the veinal areas and slight cupping at the first two leaves, but *Datura stramonium* displays chlorosis in PLRV infected plants (LP & HN, 2017).

PLRV is regarded as the most pathogen causing heavy losses among other potato viruses. It is a member of Polerovirus genus and Luteoviridae family (Ashraf et al., 2020). The virus is positive sense single stranded RNA (ss^{+}RNA) of 5.8kb long that has viral protein (VPg) but without 5'end CAP or 3'end poly(A) tail (Talianksy et al., 2003). The PLRV genome consists of 7 open reading frames categorized in genomic and sub genomic RNAs (Farooq et al., 2022). These ORFs are separated by two genes (ORF 0,1and 2 – ORF 3,4 and 5) which are involved in in symptom development (ORF0), virus replication (ORF 2), encoding different proteins that helps aphid in PLRV transmission (ORF 3, 4 , 5). PLRV genome is organized such way that genomic RNA translates and two sub genomic RNA ORF0,1,2 and sgRNA1 around 2.3kb and sgRNA2 around 0.8kb. mRNA sgRNA 1 of ORF 3, ORF 3/5, and ORF 4 while sgRNA 2 translate two viral proteins of 7.1 kDa (ORF 6) and 14 kDa (ORF 7). The VPg sequence has been mapped to position 400–

431 of ORF 1, downstream of the putative protease domain and in front of the RNA-dependent RNA polymerase (Loebenstein & Gaba, 2012).

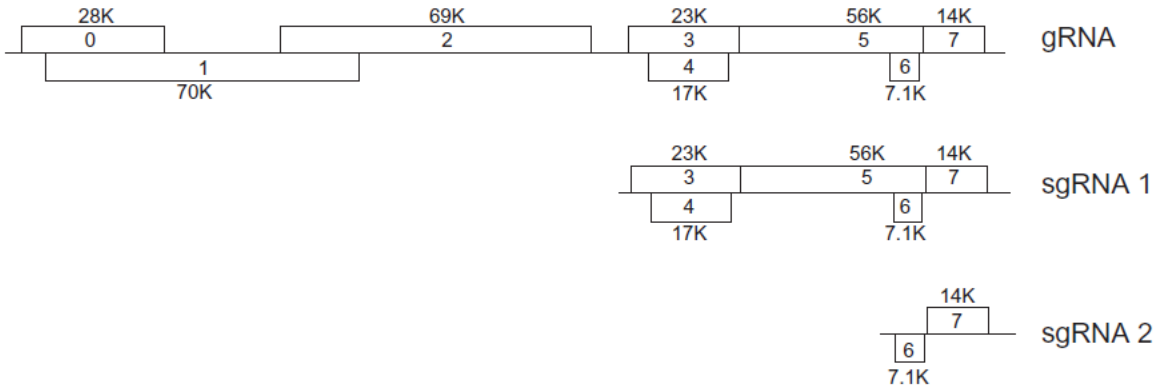

Figure 8: Potato leaf Roll virus genome organization. Loebenstein & Gaba (2012)

Farooq et., al 2022, reported that sgRNA1 translated in ORF3 to facilitate the virion formation, vector transmission and virus movement, the ribosome incorporates an amino acid to translate ORF5 into read through domain coat protein (CP-RTD) to connect ORF3 and ORF5. Moreover, ORF1 mRNA sgRNA1 expresses P3 for long movement of the infection and P4 from ORF4 to communicate cell to cell viral protein movement in the phloem while ORF2 mRNA sgRNA2 expresses P6 (ORF6) and P7 (ORF7) which is important in aphid enhancement fecundity. ORF0 encodes P0 protein which is important RNA silencing in viral suppression in connection with VPg which important in vector specificity.

2.4.1.4. Potato Virus M (PVM)

PVM is the most common virus affecting potato and is prevalent worldwide. The yield loss among potato cultivar caused by the virus is between 15% and 45%.(Ahmadvand et al., 2012). This virus is not popular as PVS in most countries to cause serious tuber yield damages except when is synergically combined with other potato viruses such as PVX or other viruses (Kreuze et al., 2019b). The virus is described as belong in the group of Carlavirus genus in the Betaflexiviridae family. PVM has flexuous filamentous virus particles (12-13nm width, 650nm long and single virion of 176S) that have positive sense single stranded RNA of 8.53kb (Salazar, 2001) that consists of six ORFs, 5'cap and 3'poly(A) tail structures (Tabasinejad et al., 2014). In contrast, Carlaviruses contain positive sense single stranded RNA of around 8.7kb with a six ORFs, 5'cap and 3'poly(A) tail structures. The ORF 1 has three domain motifs called helicase, methyl transferase and RNA-dependent RNA polymerase (RdRp) express proteins that play role in virus replication, while tree gene block (TGB) composed of ORF2, ORF3 and ORF4 express proteins (A. Kumar et al., 2023), 25, 12 and 7kDa proteins play role in membrane binding and virion cell to cell movement. ORF5 encodes a protein of 34kDA and ORF6 encodes a cysteine rich protein and 11kDa nucleic acid binding coat protein that binds DNA and RNA (Tabasinejad et al., 2014).

Like other Carlaviruses, PVM is non-persistently transmitted most important aphids including Myzus persicae and aphis nasturtii (Hafiz & Topkaya, 2023), and through mechanical inoculation experimentally sap inoculation in potato tender leaves (A. Kumar et al., 2023). Mostly, the primary root of PVM spread within potato field is seed cutting, machinery movements activities (Tabasinejad et al., 2014).

PVM virus symptoms vary from mild to severe depending upon the potato cultivar, virus strain type and environmental conditions (M. F. Abbas et al., 2013). The virus alone induces discreet symptoms in some potato cultivars (Plchova et al., 2015). The plants infected by the virus shows mosaic, twisting, curling and rolling of leaves; stunting of shoots and necrosis of stems in young plants and in older plants they show any symptoms (LP & HN, 2017). Reports revealed that they are similar to those of other potato infecting viruses such as PVX, PVS, and common strain of PVY (PVY^O strain) and their symptoms vary depending upon their combinations with PVM isolates and potato cultivars (Xu et al., 2010)(Yusubakhmedov et al., 2024)

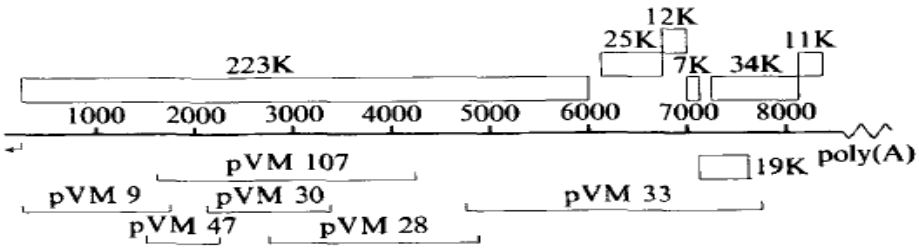

Figure 9: Genome organization of PVM. Zavriev et al. (1991)

Phylogeny studies conducted by Xu et.,al in 2010 determining genomic variability of PVM isolates, CP genes sequences categorized into two groups from seven Canadian isolates and eight international PVM isolates. Group I composed of PVM isolates from Italy, Germany, China, Poland and Russia and group II composed of Canadian and US isolates. Isolates in group I were divided into two sub-groups - Ia, and Ib. PVM isolates from China and Poland formed the group Ia and group Ib composed of isolates from Italy, Germany and Russia. Isolates in group II were divided into two sub-groups - IIa and IIb. The Nucleotides similarity between two groups were 75% and 95% amino acids. Isolates within the same group shared over 95% of amino acids and over 90% of nucleotide similarities.

2.4.1.5. Potato Virus X (PVX)

Potato virus X is one the most pathogen world widely prevalent (Petrov et al., 2023). PVX also called potato latent virus also known as potato latent mosaic, potato mottle or "**rivose masqell de la pomme de terre**" and "**mosaique legere**" in French (LP & HN, 2017) belongs to Potexvirus genus in Alphaflexiviridae family (Fuentes et al., 2021). The PVX genome is a single stranded RNA rod shaped virus with five ORFs, 5'cap and 3'end poly (A) tail ends (Hassan et al., 2021). The virus particles are flexuous and filamentous approximately of 470-580nm long with 1300 CP copies of left helical part. The CP N terminal phosphorylation by cellular enzymes induce the translation of RNA in RdRp to form sub genomic RNAs and its x-bodies are replicated to produce the nucleus (Verchot, 2022).

ORF 1 acts as replicase, encodes RdRp of 166 kDa, triple gene block (TGB) composed of ORF2, 3, 4 encode 25, 12 and 8 proteins and are involved in virus cell to cell movement. ORF5 encodes a 25kDa coat protein (Yu et al., 2008). The virus gene expression mechanisms initially employs RdRp to express its protein from genomic RNA and progressively the other part viral proteins are expressed from 3'coterminal sgRNAs. The TGB express proteins from two sgRNA which encodes virus circulation proteins and 5' and 3'UTRs regulate replication as well as gene expression from its cis regulatory regions (Verchot, 2022).

PVX is spread through mechanical inoculation by contact between infected plant and healthy plant or by instruments or an animal that have been in contact with infected plant. It can be spread also through contaminated tuber sprouts in contact with healthy tubers in storage (Loebenstein & Gaba, 2012;Fuentes et al., 2021). Potato production reports reveal the estimated yield reduction is between 10% to 40% (Hassan et al., 2021)and cause significant losses of tobacco and pepper (Shi et al., 2008). PVX mixed infection with PLRV causes production reduction between 40% to 70%, with PVY or PVA between 50% to 80% (Petrov et al., 2023) and PVX strains affected potato tubers around 40% (Dutta et al., 2024).

Various symptoms may be induced when PVX infect potato plants including severe mosaic, rugosity or leaves crinkling, dwarfing, streak necrosis and extensive leaves and tubers necrosis. However, these symptoms are distributed and vary depending upon the cultivar, virus strain and environmental conditions. Mild leaf mosaic and interveinal necrosis are observed in less virulent strains mostly between 16^{o}C to 22^{o}C of temperatures. PVX isolates cause mild symptoms in young

potato plant while distinctive symptoms like pale yellow veins and blot yellowing are observed in older potato plants. PXV affect also potato tubers in size reduction and number(Petrov et al., 2023). Mixed infections have been described by Fuentes et al., 2021, PVX mixed with PVY or PVA induces severe foliage disease, PVX mixed with PVA induces crinkles while mixed with PVY induces rugose mosaic and cause much yield tuber losses and mixed with PVS induces severe foliar symptoms (Fuentes et al., 2021).

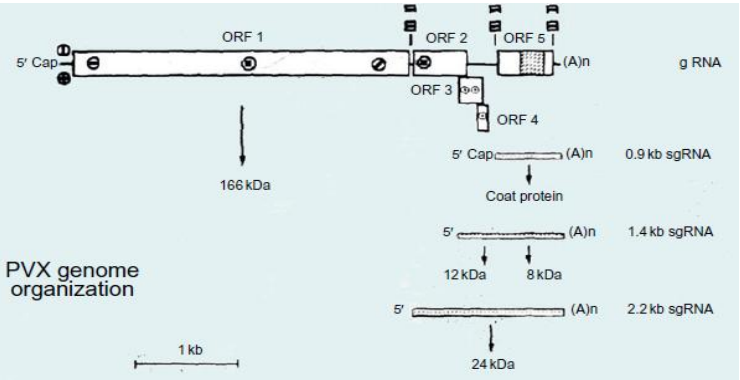

Figure 10: PVX genome organization. Loebenstein & Gaba (2012)

PVX strains are categorized based on genetic system which regulate pathogen behavior and the hypersensitive resistance genes interaction. However, the most relevant is the type of HR interaction. Nx and Nb mediated by a CP determine resistance sensitivity while Rx determine protein movement mediated resistance (Hajizadeh & Sokhandan-Bashir, 2017).

Phylogeny studies conducted by Hajizadeh et., 2017, Population genetic analysis, five PVX CP gene sequences from China, India, Iran, Japan, south America and UK were considered as one population and compared one hundred and fifty PVX CP sequences in the database. Phylogenetic analysis showed that PVX isolates from Asia were most common and the CP of all members found to be avirulent to Nx resistance gene potato cultivars which might have evolution and movement of the pathogen from one region to other as plausible explanation while American isolates showed also the same scenario. Those findings suggested a co-evolution of PVX with potato plants. PVX Populations were found near zero because there were no significant P value. In this study they concluded PVX isolates grouped into two lineages, but they lack correlation between phylogeny and geographical origin concluding that PVX isolates came from common ancestor, negative

selection and genetic drift may be the drivers of this evolution. This idea is fundament to utilize the PVX virus free planting material to prevent its dissemination.

2.4.1.6. Potato Virus S (PVS)

Potato virus is one the most important virus that infect potato discovered for the first time by the Netherlands and later on occurs worldwide (Lin et al., 2014). This virus belongs to Carlavirus genus and Betaflexiviridae family. Its genome is a positive sense single stranded RNA of 8.5kb composed of six ORFs, 5'end cap and 3'end poly(A) tail UTRs. (Song et al., 2017). ORFs encode different proteins includes NTP-binding helicase, methyltransferase and RdRp domains of 223kDa belong in ORF1, NTPase-helicase domain encoded from ORF2, proteins of 12 and 7kDa which help the virus cell to cell movement are encoded in ORF3 and ORF4; (ORF2,3 and 4 are TGB) while a CP of 34kDa is encoded in ORF5 and nucleotide binding protein , (a cysteine rich nucleic binding protein) of 11kDa encoded in ORF6 (Duan et al., 2018).

PVS attacks only Solanaceae and Chenopodiaceae members (Song et al., 2017) and is widespread by different type species of aphids in a non-persistent manner (Wang et al., 2016), by mechanical contact and vegetative propagation (Ristić et al., 2019) . Infected potato plant by PVS do not show obvious or the plant remains symptomless (Lambert et al., 2012) only mild rugose on leaves associated with vein deepening and bronzing (Song et al., 2017), mottling associated with early leaves loss and plant death later (Wang et al., 2016). Commonly PVS shows no symptoms on leaves and in some cases shows minor symptoms and production reduction. Only become severe when its strains presence and can cause tuber yield losses up to 20% and render them unmarketable (Topkaya et al., 2023). PVS is placed at fourth rank before PVY, PLRV and PVX in causing yield losses up 30% in Europe and when mixed with PVX, late blight, the yield losses are estimated between 40% to 75%(Petrov et al., 2023), while associated with PVM the reduction is estimated between 20% to 30% (Song et al., 2017), contrary to south America, when PVS present alone, reduce tuber size, and the yield loss is 20% (Santillan et al., 2018).

Biological and molecular studies showed that PVS strains are classified in two groups PVS^{A}(Andean) and PVS^{O}(Ordinary) (Wang et al., 2016) and are able to transmit systemic (PVS^{A}) and non-systemic (PVS^{O}) infections in Chenopodiaceae spp. (Chikh Ali et al., 2008). According to Duran et., al 2018, PVS^{A} is spread in non-persistent manner by aphids and triggers more severe

leaf symptoms on potato plants while PVS^{O} only triggers local lesions and cannot infect potato systemically (Duan et al., 2018).

Studies traced that PVS^{A} for the first time discovered in south America , then in Europe, USA and Asia. Biological attributes differences between PVS^{A} and PVS^{O} capability to infect Chenopodiaceae spp have been accommodated to differences of nucleotides binding proteins N-terminal ends of 11kDa and 7kDa of CP sequences of amino acids blocks were found (Santillan et al., 2018).

Studies have shown sequence variability of European PVS isolates by molecular probing, including those that invaded *C. quinoa* systemically, which they termed PVS^{CS} (CS = *Chenopodium* systemic) isolates and that isolates which were systemic in Chenopodium spp. but genetically related to PVS^{O} and distant from PVS^{A} should be named PVS^{O}-^{CS} (Ordinary–Chenopodium systemic). Deep molecular studies, comparing 53 complete CP nucleotide sequences of PVS, 13 of which originated from Australia and it has been revealed two clades associated with PVS^{O} and PVS^{A}, with seven subclades of PVS^{O} and three subclades of PVS^{A} (Lambert et al., 2012).

2.4.2. Other diseases associated with potato

2.4.2.1. Potato Bacteria Wilt

Bacteria wilt disease also known as potato brown rot is an important disease in potato transmitted by an aerobic gram negative called ralstonia solanacearum (Smith). Morphologically is a non-rod shaped, motile, which grows rapidly onto agar medium within 2 days (Van Der Wolf & De Boer, 2007). Ralstonia solanacearum species consist of several strains characterized by pathogenic races and biovars (Charkowski et al., 2019). Based on the different host range characteristics, it is grouped into five races and six biovars based on biochemistry and physiological characteristics. Potato bacteria wilt is transmitted by Rs biovar 1 to 4, race 1 and 4 while biovar 2 is mainly associated with soil borne infections. Biovar 1 is commonly distributed in south America and Africa and biovar 3 distribution is in Asia (Van Der Wolf & De Boer, 2007).

Rs infect species of Solanaceae family members including hot pepper (*Capsicum annum*), tomato (*Solanum lycopersicum*), potato (*S. tuberosum*), tobacco (*Nicotiana tabacum*), eggplant (*Solanum*

melongena) and other members of Musaceae family including triploid banana and plantains (Muthoni et al., 2012a).

Bacterial wilt, caused by the *Ralstonia solanacearum* species complex (RSSC), remains one of the most devastating plant diseases worldwide, particularly in tropical and subtropical regions where warm, humid conditions favor its persistence and spread. The pathogen's remarkable adaptability, extensive host range, and ability to survive latently in soils and plant tissues make it a formidable challenge for sustainable crop production. Recent studies in Rwanda (Uwamahoro et al., 2020;Mutimawurugo et al., 2019) and Kenya (Muthoni et al., 2012) have provided critical insights into the pathogen's genetic diversity, virulence mechanisms, and the limitations of current management strategies. These findings underscore the urgent need for integrated approaches that combine pathogen surveillance, host resistance breeding, and cultural practices tailored to local agroecosystems.

A key revelation from these studies is the genetic and phenotypic plasticity of *R. solanacearum*, which complicates disease management. While Uwamahoro et al. (2020) confirmed the dominance of phylotype II (R. solanacearum emend. Safni) in Rwanda, Mutimawurugo et al. (2019) identified race 1 biovar 3 isolates thriving in highland conditions contrary to the traditional association of race 3 biovar 2 with cooler climates. This divergence highlights the pathogen's capacity to adapt to atypical environments, potentially expanding its threat to new regions. Furthermore, the pathogen's ability to asymptomatically colonize non-solanaceous crops (e.g., via PCR-positive but symptomless tobacco and banana plants) suggests that latent reservoirs may perpetuate infection cycles unnoticed. Such findings challenge conventional crop rotation strategies and emphasize the need for molecular diagnostics to detect latent infections in seed tubers and alternative hosts.

The quest for durable host resistance remains fraught with obstacles, as evidenced by the susceptibility of all tested potato cultivars in Rwanda (Uwamahoro et al., 2020) and the instability of resistance traits under varying environmental conditions (Muthoni et al., 2012). While cultivars like Cruza 148 and CIP-58 exhibit relative tolerance, their susceptibility to latent infection risks inadvertently disseminating the pathogen. Muthoni et al. (2012b) argue that breeding programs must prioritize wild *Solanum* species (e.g., *S. phureja*) and recurrent selection to stabilize resistance, but this demands long-term investment and localized trailing. Meanwhile, smallholder farmers who dominate potato production in sub-Saharan Africa often lack access to clean seed or

the means to implement stringent phytosanitary measures. Thus, a multifaceted approach is imperative: combining resistant cultivars with community-based seed certification, farmer education on field sanitation, and innovative biocontrol agents to suppress soilborne inoculum. Without such integration, bacterial wilt will continue to undermine food security in vulnerable agro-ecologies.

2.4.2.2. Late Blight

The potato (Solanum tuberosum) is the fourth most essential food crop after rice, wheat, and maize worldwide. Its production recently extended to 1 million tons and 7.9 million tons, respectively, making it relevant staple for food security and livelihoods in Central and East Africa,. However, diseases like late blight and bacteriosis threaten productivity. Late blight alone cause degeneration of up to 75%, mostly in high-altitude regions with cool, humid climates. s (Vihabwa Katembo et al., 2024).

Potato late blight exhibits clear and often severe symptoms. The first visible signs are water-soaked spots on the leaves, which quickly enlarge in favorable conditions. These lesions are often bordered by a white, fuzzy growth made up of sporangia and sporangiophores that emerge through the leaf stomata. The sporangia can be spread by wind and rain, facilitating further infection. Tubers are also vulnerable infected ones develop a brown, rotting decay, making them unfit for sale and allowing the pathogen to survive between growing seasons. The disease advances swiftly, with new lesions forming in just three to four days under ideal conditions, emphasizing the need for prompt control measures. (Yuen et., 2021).

Phytophthora infestans primarily infects potato (*Solanum tuberosum*) but can also affect other solanaceous crops, such as tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), and bell pepper (*Capsicum annuum*) (Rhouma et al., 2024). The pathogen exists as a species complex, with closely related species such as *Phytophthora andina* and *Phytophthora betacei* infecting crops like *Solanum quitoense* (*naranjilla*) and *Solanum betaceum* (*tree tomato*) (Oliva et al., 2010); (Mideros et al., 2018). P. infestans exhibits high genetic diversity, with distinct clonal lineages found worldwide. In Europe and Africa, invasive European lineages predominate, whereas Latin America displays greater genetic diversity, likely due to sexual reproduction (Njoroge et al., 2019). The coexistence of A1 and A2 mating types in certain regions facilitates sexual reproduction,

producing long-lived oospores that persist in soil for years significantly complicating disease control (J. E. Yuen & Andersson, 2013).

P. infestans is an oomycete with a complex life cycle involving both asexual and sexual reproduction: Asexual phase produces sporangia that spread via wind or water. These can germinate directly or release motile zoospores, which infect plant tissues and sexual phase requires two compatible mating types (A1 and A2) to form oospores. These thick-walled spores persist in soil for years, serving as a long-term survival mechanism Their ggenetic rresistance mmechanisms encode NB-LRR proteins (e.g., *Rpi* and *RB* genes) that recognize pathogen effectors, triggering a hypersensitive response done by R Genes which constitute Qualitative resistance and Polygenic traits derived from wild relatives (e.g., *Solanum bulbocastanum*) provide broader, more durable resistance by Quantitative resistance (Vihabwa Katembo et al., 2024).

In Rwanda, potato late blight is a major constraint to production, particularly in regions like Musanze, where high humidity favors disease development. Farmers rely heavily on fungicides, such as mancozeb, often applying them excessively in a curative manner after symptoms appear (Muhinyuza et al., 2015). Research in Rwanda has highlighted the prevalence of clonal lineages of *P. infestans*, similar to those found in other parts of Africa (Njoroge et al., 2019).

2.5. Potato early generation seed production in Rwanda

Potato is a relevant crop in Rwandan agricultural sector which sustains food security sustainability and industrial food income generation and annual consumption is 125kg per person. The average production is between five (5) and twenty (20) tons per hectare which is still below compared to produce target in average at 30 tons per hectare annual yield (HAVUGIMANA et al., 2023b) in contrast in 2024 it has been reported that potato EGS production is increasingly high approximately around 60,000 hectares which covers 3.9% of total cultivated land per each season, 9.5% of total production and 145kg of annual consumption (RAB, 2024)

The potato EGS are micropropagated from disease free plantlets to produce the first generation (Early Generation Seeds) which now then transferred into screen houses for first generation seed tubers production (basic seeds). The basic seed is transferred into disease free fields for certification (certified seed) after four round multiplication (Fig.2). Despite these efforts, low virus-free and high yield planting materials hinder potato production in the country (Schulte-

Geldermann et al., 2022) and the major constraint is the limited access to sufficient early generation seeds provisions (Kigali, 2016).

This is demonstrated by the assessment reports showing public sector only covers 70% against private sector only produce between 7.5% to 12.5% of in vitro plantlets production due to high prizes of mini tubers which make screen houses inoperative and mini tubers unshaped market. In addition to that, small holder farmers depend on informal seed system due to little seed certified only 5% and low productivity of potato EGS value chain (Urinzwenimana Clement, 2024).

The country formal seed system depends on plant development varieties which are published in a regulated and sustainable system lead the public sector only generate and release 3% of potato planting material while the informal seed system which relies on quality declared seeds set aside by farmers mostly in private sector provides 97% of potato planting material from farmer groups and cooperatives. Nevertheless, the EGS demands are covered around three times from these potato seed systems (informal & formal) (USAID, 2016).

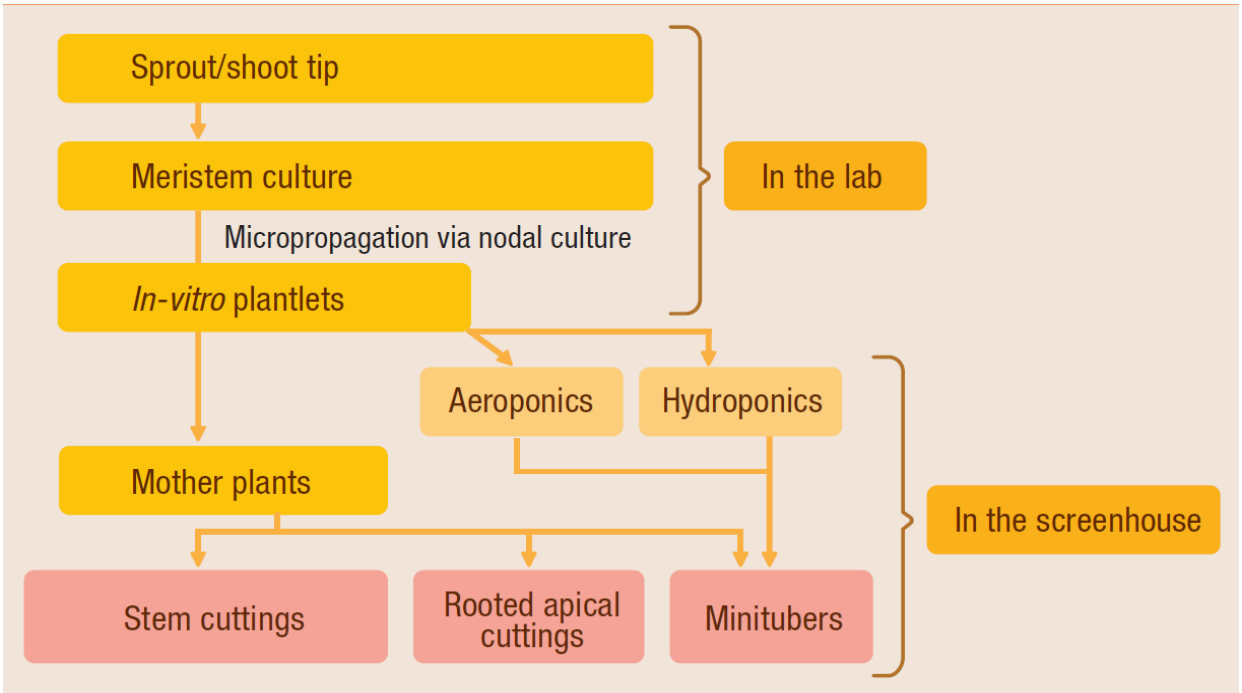

Figure 11: Rapid techniques used in the laboratory and screen houses-production scheme of potato EGS CIP (2021)

Programs have been designed to produce seeds for root multiplication trials including micropropagation of virus free internodes on Murashige and Skoog (MS) media containing macronutrients, micronutrients and necessary ions supplemented by vitamins under sterile condition to obtain plantlets. These cultures are exposed to photoperiod of 16 hours of light and 8 hours of darkness for at least 3 weeks and are grown into full plants which then hardened and acclimatized and planted into soil. Secondly, followed by production of mini tubers from plantlets. These are estimated between 100 to 200mg of mini tubers produced from one tissue culture container when planted in sterile and aphid free green house. The new innovative technology consists of producing soil less mini tubers in aeroponic system. This technology employs the healthy virus free in vitro plantlets to produce mini tubers. It helped to produce more mini tubers which complying to phytosanitary requirements. Moreover, the potato have been successfully utilized to propagate vegetatively following root apical cutting technology within seven days and 7-10 tubers weighing around 10g are produced from single cutting (Buckseth et al., 2022).

2.6. Diagnosis Methods of Plant viruses

Plant diseases continue to hamper major crop production and lead to economic losses in global agriculture industry. Economy globalization by free trade treaties and climate change instabilities have allowed the free movement of plant vectors and diseases across countries which made diagnosis of plant diseases and their vectors more relevant in today's world. Initially visual inspection of virus symptoms is a more crucial steps in identifying viral diseases symptoms because plant viral diseases produce similar physiological symptoms which may confuse whether they are from abiotic stresses, nutrient deficiency or other factors and certain viruses cause latent infection to plants.

The common market for free trade made possible germplasm circulation in which countries made quarantine services and prioritize detection of plant viruses more crucial to ensure the safe movement of potato germplasm across the border (Kumar Tiwari et al., 2019).

Viral symptom reproduction on specific host range susceptible host plant indicator has of great importance in both known and unknown viruses. Biological indexing using susceptible host plant indicator is traditional ways to diagnose and detect potato viruses by different researchers and seed inspectors for confirming pathogenicity tests, however, it is not enough for best results in virus identification. Host plant indicator is useful for reproducing viral symptoms and transmission

mechanisms studies. Moreover, host range studies are important for studying unknown viruses specific symptoms reproducibility and disclose different viral strains symptoms of the same virus.

Serological assays use polyclonal and or monoclonal antibodies in response to antigens. They are produced form animals by immunization, all have unique ability to bind coat proteins specific-epitopes to induce their synthesis. During serological assays, antibodies produced detect the specific pathogen's coat proteins to which they were raised against. The most commonly used serological detection method for plant viruses is the enzyme-linked immunosorbent assay (ELISA).

Nucleic acids methods are used for virus detection because of their high sensitivity and specificity. These methods use the short unique sequence called oligonucleotide with the genome unique to a given organism and is used to identify its presence. In a tube, a template acid nucleic extracted from a given tissue (Leaf, blood cells,…) combined with these unique synthesized nucleic acid fragments will bind specific sequences within the pathogen genome. With the help of Polymerase chain rection (PCR) these specific sequences bound by the synthetic nucleic acid fragments are replicated into multiple copies which are visualized on gel electrophoresis or by fluorescence. The best known test are conventional PCR, reverse transcriptase PCR (RT-PCR), multiplex PCR (mPCR), real time PCR (qPCR) and nucleic acid arrays. Immunocapture PCR (IC-PCR) combines both serological and nucleic acid methods. It consists of virus particles which are captured to a solid phase through their viral coat proteins with a specific antibody and then amplified in RT-PCR. This method is used to increase more sensitivity and specificity in plant viral detection (Raza et al., 2024).

Early and accurate plant viruses detection is an essential component for better study and effective management of crop systems. Plant viruses are mostly diagnosed by their symptoms, serological attributes and nucleic acids patterns.

2.6.1. Biological Indexing

Biological indexing is one the most traditional and epidemiological technique used in seed health, quarantine and seed certification programs. It used to produce viral symptoms on susceptible host plant indicator grown in vitro as experimentally to diagnose viruses (Kumar Tiwari et al., 2019). Host plant indicators in particular conditions are utilized to produce consistent main characteristics of symptoms when inoculated with a particular virus. However, host range knowledge for a specific virus and the corresponding symptoms it produces is very useful in virus and its strains studies and produce insights for unknown virus data (Gera & Marco, 2001).

Chenopodium Amarant color, C. annuum, Physalis floridana, C. album, Nicotiana glutinosa, N.rustica, N. tabacum cv. Samsun, N. benthamiana, Datura stramonium, D. metel, D. stramonium, Solanum demissum x, S.tuberosum Gomphrena globosa and Lycopersicon esculentum of herbaceous family have been found as susceptible plant to induce systemic infections, local lesions on mechanical transmission and their most importance is to reproduce variety of symptoms in response of the particular virus and its strains (Urooj et al., 2016).

Thesis diagnostic method of transmitting the virus in healthy tissue is very crucial technique for virus isolation to study viral symptoms and transmission mechanisms and is widely used both for detection and identification of some viruses and viroids. However, is not appropriate some viruses, it expresses different symptoms of some virus strains of the same virus and host and is not convenient for a large number of samples in short period (Gera & Marco, 2001).

2.6.2. Enzyme-Linked Immunosorbent Assays (ELISAs)

This method is relying on specific antibody interaction with specific targeted capsid viral protein (Kanapiya et al., 2024) and it can be polyclonal or monoclonal antibodies (Mehetre et al., 2021) . ELISA is a test on solid phase that utilizes specific antibody to bind to specific antigen with specific labelled antibody and at the end of the reaction when the revelation solution is added, the virus presence is measured visually by color development or by a spectrophotometer (ELISA microplate reader) (Urooj et al., 2016).

Based on binding of antibodies-antigen and sensitivity-specificity concept other types of ELISA have developed (Kanapiya et al., 2024). Direct ELISA methodology, the antibody is bound to microtiter plate well surface and bind the antigen (virus)in the sample. Then the antigen is detected

during incubation with the conjugate antibody labelled enzyme followed by addition of substrate for color development. Because of the virus is captured in between two antibodies, this method is called double antibody sandwich (DAS) ELISA (Alemu, 2015). DAS-ELISA is highly specific because it uses the detection antibody which must be conjugated to an enzyme (Yadav & Khurana, 2015).

Indirect methodologies, the primary antibody which is the virus specific antibody detects the presence or absence of the antigen. The antibodies of different animals of immobilizing the virus activity are use. In direct antibody coating (DAC), antigen-coated plate (ACP) or platetrapped antigen (PTA) method, the virus is directly added into wells to adsorb onto the plate surface without tapping layer; this step differs in DAS. The primary antibody (IgG) is added to bind the virus which after is conjugated with secondary antibody (IgG-AP); this antibody raises against animal IgG in which the primary antibody was produced and allow development of the color. while the triple antibody Sandwich (TAS), is identical to DAS ELISA, the monoclonal antibody from the animal added before to be detected by the antibody conjugate to the enzyme and the development of color is revealed after adding revelation substate solution. Finally, Protein-A-sandwich (PAS), the plate is primarily coated with protein A which binds Fc region of the antibodies; the trapping antibody is added and binds protein A and Fc region to increase the antigen binding sites to allow the binding of the antigen (virus particle from the samples); a sandwich is formed by adding trapping antibody bound to captured virus; then conjugate enzyme to protein A is added followed by color development when substate added (Alemu, 2015).

ELISA has been shown as the most appropriate and widely used technique in screening and detecting important viruses even though have some weakness of producing high throughput specific antibody which in other way require high cost (Kanapiya et al., 2024). however, it remains the method appreciated routinely employed for its sensitivity, accuracy, easy and rapid to use (Menghwar et al., 2016). However, serological methods cannot be used for potato viroids detection due lack of a coat protein, they are detected by polyacrylamide gels, molecular hybridization and DNA recombinant technologies (Raza et al., 2024).

Because of its adaptability, sensitivity and uses small amount of reagents, it is utilized in many experiments to test large number of samples in short period of time (Alemu, 2015). ELISA tests are strongly pacific and are accurate when the test reactions are duplicated or triplicated within

the same micro titer plate, and the use of monoclonal or polyclonal antibodies make them more precisive only if these antibodies are against the viral coat protein under investigation (Yadav & Khurana, 2015).

Immunoblotting is an immunoassay technique identical to ELISA whereby the plant sample is blotted onto nitrocellulose membrane and is used to detect the virus when interacted by the soluble substrate for color development (Alemu, 2015). This is also confirmed by Khurana, 2015; the tissue immunoblotting by the chromogenic labelled antibody is used to detect the virus onto the nitrocellulose membrane not on solid plate matrix. The Dot immunoblotting (DIBA) uses the soluble chromogenic substrate with radiolabeled probes and emit visible light detected by x-rays on the membrane; contrary, to tissue immunoblotting (TIBA), the sample (leaf, stem, roots or insect) is blotted onto the membrane and detection is done with labeled antibody(Alemu, 2015). TIBA has advantages in detection time, sensitivity, cost and convenience over ELISA, the specific antibody and large amounts of the virus concentrations aspects enabled the immunoblotting to get rid of false negative detection (Jeong et al., 2014).

2.6.3. Polymerase chain rection (PCR) technology

PCR is a scientific method used to amplify similar millions copies of DNA sequences in a genome within the tube (Jeong et al., 2014). It is a DNA based method used in detection plant viruses, however, for viruses with RNA genome, a complimentary copy of DNA (cDNA) by reverse transcription procedure is required to run PCR later on(Alemu, 2015). It is sensitive and specific in detecting a unique sequence or conserved sites of the viral genome using the degenerated primers to amplify them (Yadav & Khurana, 2015). The PCR technology is run in three steps including denaturation approximately 90^{o}C to 95^{o}C for double strand DNA(ds DNA) separation, annealing between 40^{o}C to 60^{o}C for the primers to bind targeted sequences and extension between 70^{o}C to 75^{o}C for stable DNA polymerase to extend new strands from primers. This is achieved between 20 to 40 cycles to complete new strands of DNA synthesized. The product amplicons are visualized by electrophoresis on agarose gel (Rubio et al., 2020).

Beside sensitivity, specificity and speed profits, limitations of sensitivity to inhibitors, contamination risks, complexity and cost, PCR have been modified to overcome these limitations (López et al.,2009), including reverse transcription PCR (RT-PCR), real time quantitative PCR (qPCR) nested PCR and multiplex PCR (Kanapiya et al., 2024).

RT-PCR is meant for RNA genome viruses and reverse transcriptase step is required before convention PCR runs. it is sensitive, specific in comparison with serological methods (ELISA). In Multiplex PCR, two and above DNA or RNA targets are amplified within the same sample with several primers the targets are detected at same time. This method is more sensitive and time saving compared to ELISA; the detectability range 16% against 10% of ELISA. But when compared to conventional PCR, it has been seen that conventional PCR is more used than multiplex PCR because of rection mixtures of many compatible primers and primer designing specific for each target. Nested PCR is used for its low amplicon product from virus concentration which is very low and invisible when electrophoresis is run. To increase sensitivity is coupled with immunocapture RT-PCR. Co-operational PCR is a nested PCR version which uses two or three primers. There are advantages associated with Co-PCR over conventional PCR including minimizing of contaminations, high sensitivity because of nested PCR and solve false positivity issues and it is more sensitive than RT-PCR. Real time qPCR employs detection less time and small quantities of the target for detection, it monitors the progress of the reaction while running and can quantify the target concentration (Jeong et al., 2014).

Immunocapture PCR employs the combination of serological benefits coupled with PCR to amplify using specific primers the virus particles captured by antibodies. The viruses are captured in a tube by specific antisera then amplified by RT-PCR. This method is employed in diagnostics of plant viruses with low concentration in infected host and or low concentration of the viral genome in the host to increase the sensitivity and accuracy during its detection (Yadav & Khurana, 2015). Virus purification step was omitted in this method and its sensitivity is enhanced 250 times than the conventional PCR. This method was regularly employed in PVY detection (Urooj et al., 2016).

Nested PCR (nPCR) employs amplification two or more and with primers can result in primer competition as the cycles increase and contamination rates goes high with two amplification rounds in different tubes. Differently with the conventional PCR cross contamination problems, the RT-PCR uses the modified primers that amplify the short amplicons and are labeled with a probe monitors and quantify the virus during the reaction in progress, it alleviate the cross contamination problems. M-PCR helps to generate multiple copies of several targets at the same time which is a core value in gaining time and cost, however, its primers need more

experimentation mostly in silico to check their compatibility. Moreover, regardless of Co-PCR advantages which are identical to nPCR and Reverse Transcriptase-PCR in terms of sensitivity, it has peculiar attribute of being associated with dot blot hybridization to characterize the nucleotide sequence and but it requires high through put RNA and increased amount of reagents to avoid inhibitors (Martinelli et al., 2015).

Conventional PCR efficiency is relies on the composition of its components including polymerase enzyme type, template DNA(dsDNA) from extraction, concentration and purity of dNTPs and cycles measurements (López et al., 2009). But it is affected by inhibitors and the quality of DNA (Fang & Ramasamy, 2015) and the DNA concentration must be 1-10^3cells/ml from plant extractions. Issues from sensitivity and specificity created by PCR and reverse transcriptase PCR are addressed by nested PCR with its simultaneous amplification and the sensitivity have been increased up to 10^2 cells/ml. nested PCR increases the contamination risks and small amount of reagents especially primers interference during second amplification and high quality RNA, Co-PCR coupled with nested PCR have been able to minimize the contamination and RNA associated colorimetric have resolved the sensitivity issue from 1-10cells/ml detection level. Amplification of DNA and RNA with two or more primers is more impactful to diagnose several viruses and bacteria at the same; only these compatible primers created competition with the reaction and made the detection invalid; multiplex nested PCR demonstrated to increase sensitivity and stability and facilitated DNA and RNA detection at the same time by alleviating self-priming and hairpin formation. At the time the sensitivity has increased 100times than conventional PCR (1cell/ml) and this confers multiplex associated with nested PCR have increased the sensitivity and accurate detection and findings interpretation. It has been demonstrated that Real time qPCR is very sensitive and accurate under specialized, stable and controlled conditions. It covers the wide range high through put technique in molecular biology because of its modernization which overtake the conventional PCR and multiplies possibilities in experiments due to its modified primers labelled with probes, pure reagents like dNTPS, low concentration of $MgCl_2$ different in conventional PCR and its chemistries (SYBER GREEN and Taq Man) and others best known are widely applied for pathogen detection by annealing the PCR amplicon internally. It has been shown that real time qPCR can be associated with multiplexing methods to characterize several species of microorganisms and viruses. This shows that real time PCR has overtaken conventional PCR's

place and took the lead in identification and control of pathogens in commodities for its accurate and fastest technology (López et al., 2009).

Restriction fragment length polymorphism (RFLP), a DNA fingerprint method that employs restriction enzymes to PCR product to identify single nucleotides (SNPs)and short tandem repeats (STR) or other polymorphic sequence to characterize an individual's number and size of sequence repeats (Martinelli et al., 2015). It distinguishes viruses or their strains through their restriction sites presence or absence (Alemu, 2015). First of all, the amplicons are produced from PCR, then after, restriction enzymes are used to digest the DNA which after run on agarose gel to differentiate the fragments. It has been used to characterize 18 PVY isolates(Yadav & Khurana, 2015).

Loop mediated isothermal amplification (LAMP) is an amplification method that employs about six different primers (FIP and BIP-internal primers; F3 and B3-external primers and loop primers LF and LB) targeting different sites with a given nucleic acid sequence that are involved in synthesizing the DNA in looping activity. This procedure uses the constant temperature between 60^{o}C and 65^{o}C without bunch of repeated cycles. The external primers binds to the target (DNA or RNA) then the internal primers form loops to the complementary sites and synthesis of new strand is initiated by producing D and stem loops structures. Finally the synthesis of new strand is shown by the exponential increase of the sequence targeted. This method demonstrated high sensitivity 1000times than conventional PCR. This confers it to conduct field diagnosis using crude extract of diseased plant on field for its ease use and easy sample preparation (Kanapiya et al., 2024). LAMP has been employed in plant viruses and viroids such as Tomato yellow leaf curl virus, Banana bunchy top virus (Yadav & Khurana, 2015).

Rolling cycle amplification (RCA) is a amplification method in which single stranded circular DNA amplified. RCA based amplified assays employs single independent amplification by phi29 polymerase at constant temperature and exo-resistant hexamer random primer. It has been discovered when cloning single stranded circular DNA of the begomoviruses. It has been employed to detect episomal banana badnavirus and have shown is more sensitive than PCR and serological methods (Yadav & Khurana, 2015). It has employed in study of genetic modified organisms (GMOs) and RT-LAMP have been designed in RNA viruses monitoring (e.g.: PVY, PLRV) (Jeong et al., 2014). It has been also succefully employed in geminiviruses genome entities

studies using restriction fragment length polymorphism (RFLP) analysis (Kumar Tiwari et al., 2019).

Nucleic acid sequence based amplification (NASBA) is an isothermal amplification method whereby RNA is transcribed with modified primers to integrate T7 RNA polymerase promoter sequence in the dsDNA intermediate by the reverse transcriptase PCR which makes the promoter functional and is amplified at 41^{o}C (Yadav & Khurana, 2015). This method is different from conventional PCR because it works with isothermal profiles between 40^{o}C-60^{o}C instead of thermo cycling and it highly sensitive and uses short time. RT-NASBA have been employed in Apple stem pitting virus (Jeong et al., 2014).

Polymerase chain reaction technology (PCR) a DNA method is robust, highly sensitive and specific to detect potato viruses and viroids. virus titer eradication, accuracy and efficiency determination rates are investigated through enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) techniques are used to identify specific variants (Raza et al., 2024).

2.6.4. Next generation sequencing (Omics) based Technologies

First generation sequencing disclosed by Frederick Sanger known sanger sequencing lead my many biology scientists develop their research areas because of their high through put technology relatively at cheapest price. As per today's world after next generation sequencing (NGS) introduction, sanger sequencing remained popular. NGS has facilitated the discoveries through the analysis of various cells molecules by metagenomics, genomics, proteomics and metabolomics disciplines called OMICs (Mehetre et al., 2021). First generation sequencing technology, 384 samples were sequenced per run and reads up to 1000 nucleotides. This technology has been kept changing give a way to second generation and third generation sequencings which referred as high through put sequencing (HTS) (Villamor et al., 2019).

NGS is an advanced technology that is used for quick and high through put sequencing of DNA or RNA to obtain deep information about virus genome constituents within accurate time (Kanapiya et al., 2024). NGS generate billions of sequences of the DNA between 25to 400 nt length which is smaller than from sanger sequencing which ranges between 300 to 700 nt (Yadav & Khurana, 2015). It used in discoveries of new viruses complete genomes and also in detection

of known viruses and its isolates to make sure that the diagnostic assays reproducibility to test across viral population (Kanapiya et al., 2024).

HTS platforms employs commonly ways of DNA fragmentation to create libraries, addition of DNA adapters to single fragments followed by fragment sequencing. , cDNA library is created by reverse transcription when RNA is the basic material to start with. These platforms also are classified based nucleotide sequences detection assay, nucleotide origin proximity and sequencing chemistry used (Villamor et al., 2019).

HTS is subdivided into two groups including second generation sequencing which consists of DNA library preparations and cDNA obtained from reverse transcription of RNA using oligonucleotides (OligodT). These simultaneously sequenced to generate up to 500nt and assembled by joining overlapping sequences reads based on nucleotide identity to be analyzed by bioinformatics (Rubio et al., 2020). Different Second generation sequencing platforms have been engineered by different companies, Roche 454 in 2005, uses pyrosequencing chemistry when nucleic acid binds beads then loaded into picotiter plate for emulsion PCR amplification. Illumina released by SOLEXA which implies a clonal amplification of DNA fragments ligated with adaptors on a flow cell using fluorescent labeled terminator dye and sequences are obtained by cyclic reversible termination process, at a time of base incorporation after washing, imaging and cleavage. This platform has narrow range of errors. SOLiD released in 2007 by applied biosystems. It employs sequence by ligation with DNA ligase method. Sequences are generated when nucleotides sequences are read two time by cycle repetitions of fluorescent labeled ligand probes followed by washing non ligated probes and imaging. This method is second after illumina the highest through put; however, it only cope with maximum read length of 75bp and 100bp for paired reads. Ion torrent uses pyrosequencing technology which is very similar to Roche 454 technology. Particularly, it employs sequences are generated after synthesis rection done for each bead in the picotiter plate and release of H ions when bases are incorporated. This platform generates 400bp read length and up to 80millions reads the latest version which is still lower than SOLiD and illumina systems (Villamor et al., 2019).

The third generation sequencing relies on single molecule sequencing without clonal amplification where by the DNA preparations time is short to give long reads of may kilobases. Long reads are more advantageous for genotyping, genome sequencing and recombination identification.

Nevertheless, it still limited because of its errors rate than in second generation sequencing (Rubio et al., 2020). Different platforms have been developed in this regard including Pac Bio-Illumina released in 2010, it is able to sequence single molecule by utilizing hairpin adaptors to generate a ssDNA template closed and is the only one platform uses second generation and third generation sequencing abilities. SMRT bell whereby its templates are under zeptoliter-sized chamber, the polymerase attached at the bottom of zero chamber waveguide and a fluorescent labeled nucleotide is added to detect a phosphate group. The platform can generate up to 370,000 reads with at least 20kb of read length but record higher error rate when accommodating base (Villamor et al., 2019). Oxford Nanopore (ONT) technology relies on single stranded DNA or RNA passage through tiny nanopores whereby the nucleotides in the sample are subjected to electrical current and the changes resulting from this current are computed to identify the nucleotide sequences. MiniIon ONT, a single molecule genome sequencing portable device developed by oxford, sequences short and long reads and detect modified bases in DNA or RNA within a short period of time. This technology is limited as far as whole genome sequencing is concerned but sanger sequencing's accuracy is more reliable and ONT technology provides quick and dynamic results within a short period of time during DNA or RNA sequencing on field. It has been used for several plant virus studies, for example, cucumber mosaic latent virus from Bulgaria, jasmine H virus from Ixora coccinea plant complete genome sequenced within 48hours. In comparison with other sequencing platforms, it perform sequencing the complete viral genomes without algorithm for assembly which contribute to minimize its errors and permits long read sequencing (Kanapiya et al., 2024).

2.6.5. CRISPR-CAS

CRISPR site was first discovered in bacteria genome in 1987 and Cas in 2002. Clustered regularly interspaced sequence short palindromic repeats (CRISPR) and CRISPR associated genes called CAS technology is used for genome editing in various fields including biology, medicine, microbiology worldwide(Li et al., 2023) and detection plant viruses (Kanapiya et al., 2024). It has been described as an adaptive immune response to viruses, plasmid and other genetic material intrusion. The bacteria responds by adaptation, expression and interference. (Li et al.; and Shukla et al., 2023).

From phylogeny studies to determine structural and functional attributes of Cas proteins are classified into two classes including class I of I,III,IV types and class II of II,V and IV types that have defined processes of RNA guide biogenesis which targets interference (Gosavi et al., 2020). The Cas system has been found to bacterial from adaptive system of RNA guide whereby Cas binds nucleic acid fragments from outside and incorporate them in its new space sequence CRISPR array into immune memory. When other nucleic acid from outside enters again the corresponding spacer sequences in the memory processes by transcribing them into mature crRNA by the help of CRISPR precursor RNA which as a conserved repeated sequences and a spacer sequence to interact that interact with Cas proteins to produce ribonucleoprotein complex (RNP). The concerned nucleic acid by crRNA is recognized by Cas and destroys the nucleic acid invaded (Li et al., 2023).

Specific high sensitivity enzymatic reporter unlocking (SHERLOCK), DNA endonuclease targeted CRISPR trans reporter (DETECTR) and one hour low cost multipurpose highly efficient system (HOLMES) ; these the three systems for nucleic acid detection (Cassedy et al., 2021).

SHERLOCK associated with isothermal amplification and CRISPR Cas 13a to detect RNA and plant viruses DNA sequences while DETECTR and HOLMES associated with Cas12a detect DNA plant viruses (Cassedy et al., 2021;Kanapiya et al., 2024).

Detection of pathogen nucleic acid by Cas associated proteins, the target must be amplified through recombinase polymerase amplification (RPA). The CRISPR Cas systems is enter with specific location on the DNA target and binds guide RNA to collaterally cleave the nucleic acid with fluorophore probes addition. The Cas 12a recognize s the DNA and emit fluorescence; within one

hour the cut product amplified and can be detected by fluorescence emission on the lateral flow strip (Cassedy et al., 2021).

It was reported that a CRISPR Cas system named Specific High- Sensitivity Enzymatic Reporter Unlocking (SHERLOCK) was developed for quick and sensitive nucleic acid detection using the CRISPR effector Cas13a combined with the isothermal amplification method named as report showed. Cas 12a endonuclease based detection assay named DETECTR was employed to guide sdDNA when inducing collateral cleavage of small ssDNA with a quencher and fluophore by crRNA to target recognition through emission of fluorescent signal upon binding to the target and induce reporter cleavage. HOLMES employs Cas 12a in association with LAMP to detect very quick the targeted DNA and RNA. In another way, a sample is amplified to increase the target DNA using RPA reaction or RT-RPA reactions when the target is RNA. The RPA product is then transcribed into RNA using a T7 RNA polymerase. The obtained transcripts are exposed to collateral cleavage with Cas12/13 in the presence of a quenchable reporter ssRNA, and fluorescence emitted quantified. SHERLOCK, DETECTR, and HOLMES very useful in detecting viruses, microorganism as well as transgenic genes because of their chemosensitivity (S. K. Sharma et al., 2021).

For sure the assay incorporate RT-RPA for viral amplification at stable temperature and employs few primers for it is very sensitive. CRISPR Cas 12a and Cas 13 have been employed to detect tobacco mosaic virus tobacco mosaic etch virus and PVX and Cas12a have been employed to detect four RNA viruses and viroids at the same time in apple. CRISPR Cas 12a coupled with RPA one step have been employed to detect PVY, PVX and tobacco mosaic virus (Kanapiya et al., 2024).

2.7. Control of viruses in potato

potato viruses are circulating from one tuber generation to the next by clonal propagation of sprouted planting tubers. Few viruses such as PLRV are controlled with chemical application control using insecticide, there are other viruses mostly transmitted in non-persistent way such as PVY in which using pesticides is insufficient and integrated virus management measures are needed. There several ways to control potato viruses but the most common are host plant resistance (genetic resistance), use of virus free seeds (Clean seed systems), cultural practices (Kreuze et al., 2019).

2.7.1. Host Plant resistance

Host plant resistance is one of the major ways to control the potato viruses effectively. With the introduction of resistant genes which confers the resistance for example PVY, PLRV, and PVX by potato breeders have helped to reduce crop yield caused by these viruses. This came from with the introduction of potato degeneration after its introduction in Europe from south America (Andean spp.) and modern solanum tuberosum spp. several years ago which seriously weakened the crop. The discovery of resistant genes of PVX and potyviruses helped to control these viruses. Resistant genes confers to the plant hypersensitive resistance (HR) and extreme resistance (ER) limits virus infection spread and reduce in between fields. These type of resistance have been found to be durable and easy to perform in controlling PVX and these are monogenic and contains one recessive gene S which is important to resist against also PVS while other dominant are resistant to PVM. ER are important in controlling several strains (various R_x and R_y genes from S. tuberosum ssp. *andigena*, S. *acaule* and S. *stoloniferum)* and is more beneficial than HR in breeding purposes. HR have used for PLRV infection resistance by its introduction polygenically in the field in quantitative resistance and have been proven too long last than major resistance genes. However, HR and ER have be found more useful to confer a long-lasting protection against viruses(Solomon-Blackburn & Barker, 2000). This was elucidated by Valkonen et.al, 2015, most viruses like PVY are transmitted by aphid in non-persistent manner, pesticides can prevent their spread because the viruses are affected by the effect of insecticide after it gain phloem's plant and alternative oils and mulching measures limits and variable success. With the increase of virus resistant cultivars still are the most dependable pathways for potato virus control. Nevertheless, long-lasting resistance is challenged by the evolvement of new viral strains, as in the PVY and PMTV which most damaging diseases at large scale. HR from wild potatoes confers strain specific immunity but can be disrupted by recombinant virus strains mostly in high temperatures, differently, with ER dominant R genes which inhibit virus replication and confers immunity to the plant. Advanced molecular genetics gene for gene resistance model highlighted R genes as maker assisted selection in breeding to enable more efficiently transfer of resistance gene to potato varieties. Molecular markers and genetic transformation can accelerate breeding schemes durable and broad-spectrum resistance (Valkonen, 2015).

Deeply than resistant varieties, control of potato viruses through potato transgenic variety mechanism. This consists of expressing the protein constitutively in potato to alter the virion replication to confer the protection against the virus (Khurana, 2004). The most transformed potato varieties were produced from tobacco genes which encoded protein capsid, replicase and proteinases which then, introduced into potato genome to produce resistance against viruses (Palukaitis, 2012).

2.7.2. Clean seed systems

Seed production systems are widely growing and it is very recommended to use different innovative technologies available to enhance the quality of seed and build a robust seed system certification and control of quality assurance of the seed produced and marketed by private seed multipliers. To achieve this, quite number of technologies are employed as driving model to acquire quality seed.

Tissue culture technique is employed to produce virus free planting materials. These planting materials are produced by culturing meristematic tissues, different parts of the plant organs like leaves, internodes, petioles, roots and shoot tips to alleviate the traditional means of seed production associated issues (Buckseth et al., 2022).

Meristem culture combining with thermotherapy, chemotherapy are used to obtain virus free planting materials (Khurana, 2004). The use thermotherapy, chemotherapy and cryotherapy were employed alone and in combination to eliminated single and mixed potato viruses (PVS, PVA, and PVM) from potato cultivars. Different treatments were designed of chemotherapy, 100 mg L−1 ribavirin on in vitro shoots up to 30 days, thermotherapy, in vitro shoots exposed on photoperiod of 16 hours darkness at 40^{o}C of the days interchangeably at 28^{o}C for the night. Vitrification in cryotherapy, the grown shoot tips were subjected to liquid nitrogen and other with no liquid nitrogen for PVS elimination treatments. after three months of recovery and three months of post regeneration in green house, the virus were tested within micro tuber produced in vitro virus free and virus infected batches. As the results, the viruses were successfully eliminated by combining chemotherapy with cryotherapy, or by consecutive chemotherapy, combined chemotherapy and thermotherapy, then cryotherapy treatments irrespective of cultivar (Bettoni et al., 2022).

2.7.3. Cultural practices

Effective cultural practices are essential for managing potato viruses sustainably. Key strategies include sanitation (removing infected plant debris and disinfecting tools) and roguing (early removal of infected plants) to reduce virus reservoirs. Certified virus-free seed tubers are critical, as infected seed perpetuates viruses like PVY and PLRV. Crop rotation with non-host plants (e.g., cereals) minimizes soil-borne inoculum, while intercropping with barrier crops (e.g., maize) disrupts aphid vectors. Adjusting planting dates to avoid peak aphid activity and maintaining weed-free fields (to eliminate alternate hosts) further reduce transmission risks. Additional practices focus on environmental modification. Soil solarization with transparent polyethylene sheets reduces soil-borne pathogens by raising soil temperatures. Optimal plant spacing improves airflow, lowering humidity that favors virus spread, while deep summer plowing exposes and desiccates pathogen resting structures. Irrigation management (e.g., avoiding excess moisture) prevents conditions conducive to virus-carrying nematodes. Integrating these methods with vector control (e.g., reflective mulches to deter aphids) and farmer education on symptom recognition enhances their efficacy. Together, these cultural practices form a sustainable foundation for potato virus management, reducing reliance on chemical inputs while safeguarding yield and seed quality (Niwas et al., 2021).

3. MATERIALS AND METHODS

3.1. Sampling

3.1.1. Screen houses and plant tissue culture seed stock

Samples were collected from the plantlets grown in different screen houses located in the districts: Musanze, Gicumbi, Burera, Rubavu, Nyamagabe, Nyaruguru (**Fig.12**) and from plant tissue culture laboratories in RAB Musanze plant tissue culture, and Agri seed Africa laboratories. The composite leaf samples were randomly taken from each screen house at two months after planting (2MAP) with symptoms and without symptoms. Composite samples from tissue culture laboratories were removed from jars and transferred to well labelled paper bags. Five tender plantlets were used as one sample for virus detection from each jar. A total of one hundred fifty samples were collected including 90 samples collected from the screen houses (**Table 1**.), whereas 60 samples were collected from the tissue culture laboratory (**Table 2**.) respectively.

For screen houses, a composite sample was formed by leaves taken from ten plants, while for tissue culture laboratory, it was formed by ten stem ends from the tray. The sampling was done considering varieties in farmers' screen houses and tissue cultures laboratories.

The sampling was done in screen houses and tissue culture laboratories as it is shown in the tables below.

Table 1: considered screen houses and number of samples per variety

District	Farmer	Variety	Number of samples	Total per farmer
Musanze	Horizon	Kirundo	4	12
		Gikungu	4	
		Kinigi	4	
	Nzabarinda Isaac	Kirundo	3	6
		Gikungu	3	
	RAB	Kirundo	3	6
		Gikungu	3	
	SPF	Kinigi	3	15
		Kirundo	3	
		Ndamira	3	
		Gikungu	3	
		Kazeneza	3	
Burera	Semarembo	Kinigi	3	3

Gicumbi	Uwase	Kirundo	3	6
		Ndamira	3	
Rubavu	IABU/Busasamana	Kirundo	3	5
		Kazeneza	2	
Nyamagabe	F Grapaz	Kinigi	3	6
		Cruza	3	
	Abatubura	Kirundo	3	8
		Gikungu	3	
		Kinigi	2	
	RAB	Twihaze	8	15
		Cyerecyezo	7	
	Nkurikiyimfura	Kinigi	3	3
Nyaruguru	ADENYA	Cruza	3	5
		Kinigi	2	
Total of samples				***90***

Table 2: Sampled varieties and number of samples in tissue culture seed derived stock

Tissue culture laboratory	**Type of variety**	**Number of samples**
RAB Musanze	Kirundo	13
	Gikungu	10
	Kinigi	10
	Cruza	6
	Ndamira	4
	Kazeneza	2
	Cyerecyezo	2
	Twihaze	1
S/Total		***48***
Agri Seed Company Musanze	Kirundo	3
	Gikungu	2
	Kinigi	3
	Cruza	2
	Ndamira	1
	Cyerecyezo	1
s/total		**12**
G/Total		**60**

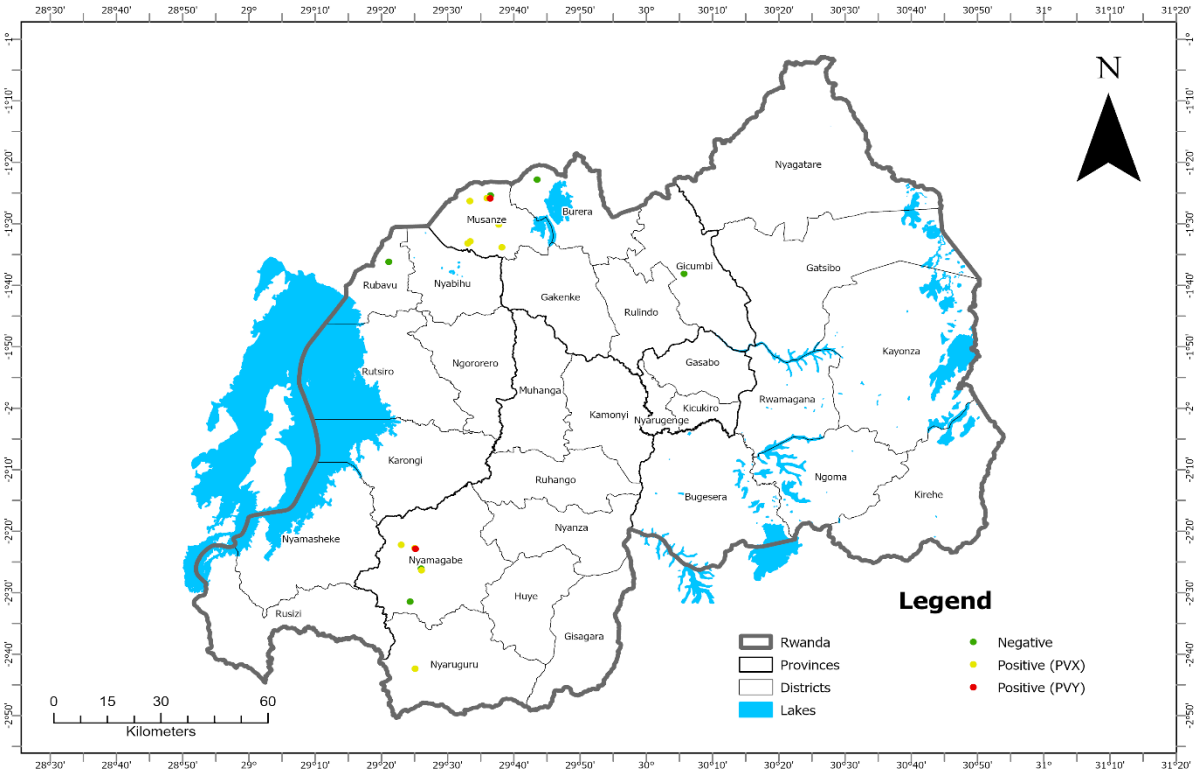

Figure 12: Screen houses location, Sampling and potato viruses distribution

3.1.2. Seed multipliers sampling

Composite leaf samples collected from seed multipliers in different districts where grown potato: Rubavu, Musanze, Nyabihu, Burera, Gicumbi, Karongi and Nyamagabe. Incidence and severity were assessed in each field.

3.1.3. Field Disease Assessment

Five rectangle shape plots of 50 plants (each square of 10 plants x 5 lines) marked in two diagonal lines (in X shape) (Fig.12) across the field (DELP, 1986). In each rectangle plot, visual symptoms were examined and incidence index evaluated by calculating percentage of affected plants over total number of plants (JF Brown, 1997). GPS coordinates (Altitude, latitude and longitude coordinates) were recorded from the field where leaf samples were collected.

3.1.4. Sampling

A total of hundred and thirty six composite samples were collected from each symptomatic field and two samples each field (**Fig.13**). Each field, potato plant severity was rated using scale from zero (0) to four (4).

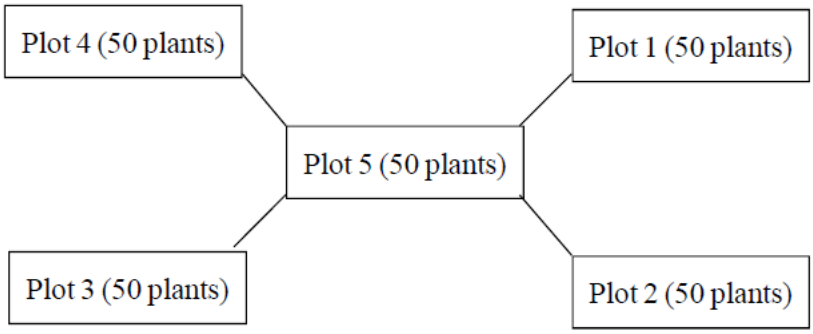

Figure 13: Sampling diagram design used in the potato diseases field study in Rwanda

Leaf samples were collected and transferred into well labeled plastic bags under cool box under ice packs. All Samples from plant tissue culture laboratories, screen houses and seed multipliers were kept on cold at minus 20 Celsius degrees.

Table 3: Samples collected from seed multiplier fields

seed category	Seed Multiplier field	field incidence	type of variety	Total No of samples
Pre-basic	2	20	Kirundo	28
	16	4.8	Kirundo	
	30	6.8	Cruza	
	31	54.8	Kinigi	
	36	12.8	Gikungu	
	42	0	Nkunganire	
	43	0.8	Kinigi	
	48	0.8	Nkunganire	
	50	3.2	Kinigi	
	52	0	Gikungu	
	53	0	Gikungu	
	54	0	Kirundo	

	55	0	Kirundo	
	56	0	Kazeneza	
Basic	1	3.2	Cruza	68
	3	0	Cruza	
	4	11.6	Gikungu	
	5	11.2	Kirundo	
	20	10.4	Gikungu	
	21	80	Kinigi	
	22	37.2	Gikungu	
	23	6	Kazeneza	
	25	23.6	Gikungu	
	27	10.4	Ndamira	
	29	20.8	Gikungu	
	32	24.4	Kinigi	
	37	22	Cyerekezo	
	38	10.8	Kazeneza	
	39	5.5	Kinigi	
	40	1.2	Kinigi	
	41	2.8	Ndamira	
	44	0.8	Gikungu	
	45	0	Gikungu	
	46	3.2	Kazeneza	
	47	0.4	Cyerekezo	
	51	0	Gisubizo	
	57	0	Cyerekezo	
	58	2	Gikungu	
	59	1.6	Ndamira	
	60	3.2	Gikungu	
	61	0	Nkunganire	
	62	0	Gikungu	
	63	0	Gikungu	
	64	0.8	Kinigi	
	65	0.8	Nkunganire	
	66	0	Gisubizo	
	67	0.8	Kazeneza	
	68	8	Cyerekezo	
certified	6	14.4	Gikungu	38
	7	18.8	Nkunganire	
	8	10.4	Cruza	
	9	13.2	Kinigi	

	10	12.4	Ndamira	
	11	12	Kinigi	
	12	9.2	Nkunganire	
	13	6.4	Gikungu	
	14	5.8	Kinigi	
	15	6	Kinigi	
	17	4.4	Nkunganire	
	18	3.2	Kinigi	
	19	6.8	Kirundo	
	26	18.8	Gikungu	
	28	17.6	Ndamira	
	33	13.6	Cruza	
	34	6	Cyerekezo	
	35	25.6	Gikungu	

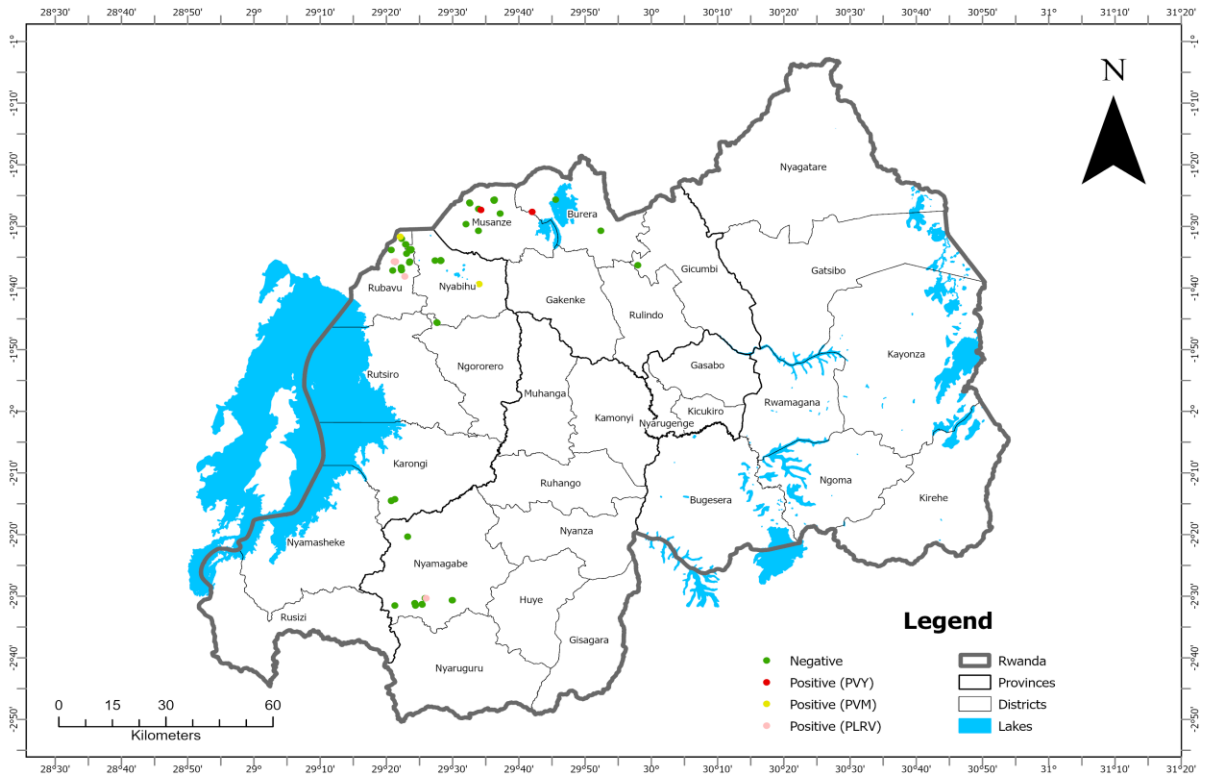

Figure 14: Seed multiplication fields location, sampling and potato viruses distribution

3.5. Laboratory analysis

To identify the six main varieties which are PLRV, PVY, PVX, PVM and PVA, the composite samples were processed using DAS-ELSA (Double Antibody Sandwich – Enzyme Linked Sandwich Antibody) test. The test was performed in Musanze RAB Plant Pathology Laboratory.

Leaf samples were analyzed from PLRV, PVA, PVM, PVS, PVX and PVY using standard protocol developed by CIP's double antibody sandwich Enzyme-Linked Immunosorbent Assay (DAS ELISA) kit instruction manual (Clark and Adams, 1977). The assay was successfully ensured by visual observation to check the color by examining the revelation and calculating absorbance using microplate ELISA reader ELx808 TM model.

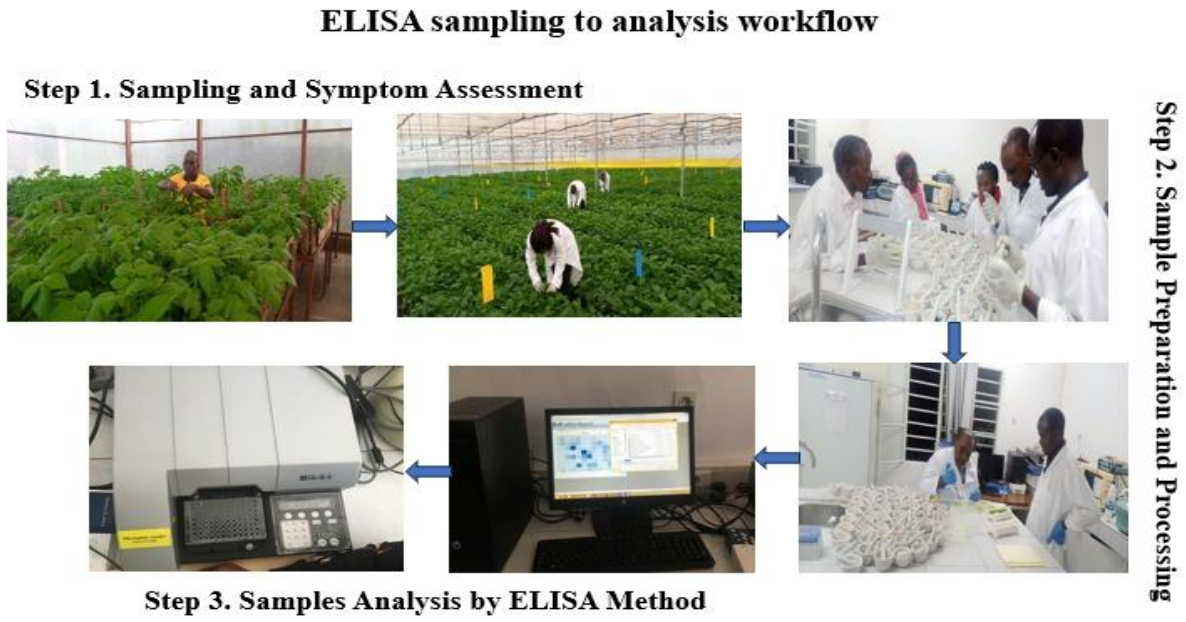

Figure 15: ELISA test workflow Analysis

Figure 16: Potato Varieties symptom description: A-Potato symptomatic leaves; B- Healthy potato leaves; C-D- potato plantlets in Jars

3.6. Data Analysis

Data analysis and visualization were performed using Microsoft Excel (version 2016) and the OmicShare free online platform (https://www.omicshare.com/tools). Heatmaps and bar plots were generated using R 4.1 (R Development Core Team, 2015) and the OmicShare platform. Descriptive statistics were computed to compare groups and potato infections, and statistical significance was set at $p < 0.05$. The sampling map was created using QGIS (version 2.18).

4. RESULTS AND DISCUSSION

4.1. Overview

This chapter presents the results from the accurate distribution of potato viruses surveys conducted on various early generation seeds categories ranging from tissue culture seed stocks to certified seeds. The analysis was grounded on field observations and confirmation based on laboratory analysis. Research findings are categorized by seed categories to elucidate patterns in disease prevalence among different seed categories. Representative reports generated from the data referenced and all quantitative results are systematically described following scientific reporting standards for plant pathology and disease epidemiology.

4.2. Sampling and Analytical framework

A total of 284 composite samples were systematically collected across respective seed categories including tissue culture laboratories derived-seed stocks (60 samples), screen houses (90 samples), seed multiplication plots (134 samples). Each represented category of seeds (pre-basic, basic and certified seeds) in potato seed system, the phytosanitary control continue declining when they are exposed to different viruses in the environment as the generation passes by.

4.3. Results

4.3.1. Plant Tissue culture seed stock

Sixty (60) composite samples derived from tissue-cultured pre-basic seed stock were collected from eight potato varieties, Kinigi, Gikungu, Ndamira, Kirundo, Kazeneza, Cyerekezo, Cruza, and Twihaze, and tested using a double-antibody sandwich ELISA (DAS-ELISA) kit (CIP-Lima). Most of the tested samples were virus-free; however, PVX and PVY were detected in some varieties (**Fig.17a**). Among the positive samples, PVX accounted for 10% (6samples) of

infections, while PVY was less prevalent, at 0.6% (1sample) ($p<0.0001$) . Notably, all infected samples originated from tissue-cultured plantlets. Three potato varieties, Kinigi, Gikungu, and Kirundo, showed higher virus susceptibility compared to Ndamira, Kazeneza, Cyerekezo, Cruza, and Twihaze. Variability in detection rates (**Fig.17a**) may reflect differences in sample sizes, yet the bar plots highlight significant infection disparities among varieties.

4.3.2. Screen houses

A total of 90 composite leaf samples were systematically collected from screenhouses in six districts: Musanze, Rubavu, Burera, Gicumbi, Nyamagabe, and Nyaruguru. The samples represented eight potato varieties, Kinigi, Kirundo, Gikungu, Cruza, Cyerekezo, Ndamira, Twihaze, and Kazeneza, and were screened for six potato viruses: Potato virus X (PVX), Potato virus Y (PVY), Potato virus S (PVS), Potato virus A (PVA), Potato virus M (PVM), and Potato leafroll virus (PLRV). The pre-basic mini tuber production stage serves as an intermediate phase in the early-generation seed value chain and forms the foundation for certified seed potato production.

As shown in **Figure 17b**, virus-free samples were more prevalent than infected ones. Among the positive samples, PVX was detected at 32.4% (36 samples) of cases, followed by PVY at 6.3% (7 samples) which is significantly important virus in the screen house ($p<0.0001$). Geographically, Musanze recorded the highest virus prevalence, while Nyamagabe had the lowest infection rates ($p=0.0028$) while other regions showed minimal viral presence. Among the eight varieties, older cultivars (Kinigi, Gikungu, Kirundo, Cruza) and newly released varieties (Ndamira, Twihaze, Kazeneza) exhibited higher virus incidence compared to Cyerekezo. Notably, Twihaze was found to be co-infected with both PVX and PVY, highlighting the risk of multiple viral infections in susceptible genotypes. Finally, farmers, specifically SPF KIGEGA and Sopyrwa are the most affected by PVX followed by RAB Musanze and Deboke ($p=0.0008$).

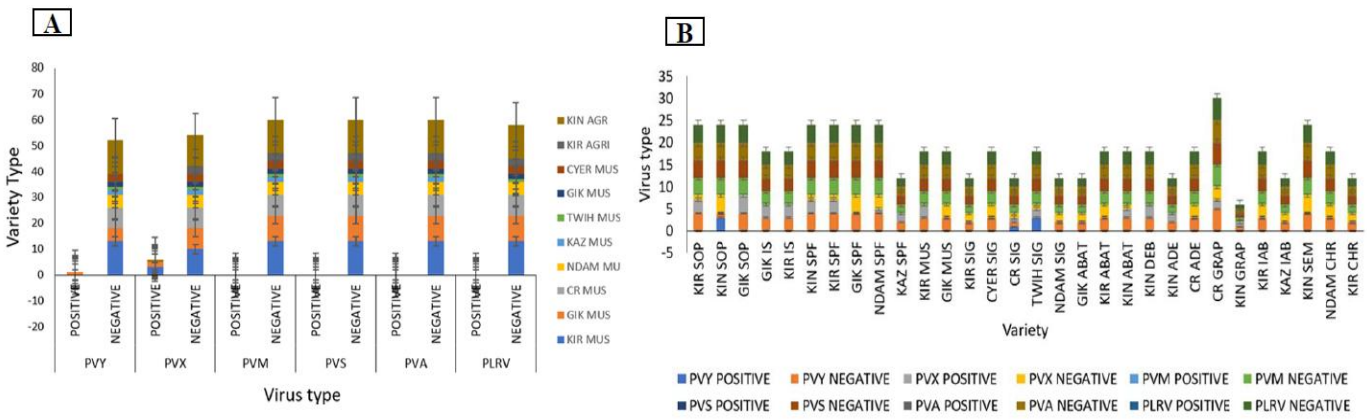

Figure 17: Tissue Culture (A) and Screen House (B): Virus infection Vs Potato Varieties.

4.3.3. Seed Multiplication fields

A total of 134 composite potato leaf samples were screened for six major potato viruses, PVX, PVY, PVS, PVA, PVM, and PLRV, across pre-basic, basic, and certified seed multiplication fields (**Fig. 17c**). Results indicated that most samples tested negative for viral infections; however, low infection rates were detected: PVY (5.36%, 4samples), PLRV (6.7%, 5samples), and PVM (2.68%, 2samples). Infected varieties included Kinigi, Gikungu, Gisubizo, Cyerekezo, Nkunganire, and Kazeneza. The basic seed category showed higher virus prevalence compared to pre-basic and certified seed categories, reflecting inconsistencies in seed quality management among multipliers. Kinigi exhibited the highest infection rate, followed by Gikungu. Uniform sample sizes across seed categories minimized variability in detection (see error bars, **Fig. 18c**).

We evaluated nine early-generation potato varieties for six major potato viruses, PVY, PLRV, PVA, PVX, PVS, and PVM, using DAS-ELISA. Each variety was classified as positive (P) or negative (N) for viral infection, and total virus incidence (IC) was calculated per variety (**Figure 18d**). The results revealed striking varietal differences in susceptibility. Three varieties: Gikungu (GKB3), Kazeneza (KAZPB3), and Kinigi (KINPB2) exhibited near 100% infection rates, predominantly with PVY and PLRV. In contrast, Cruza (CB1), Cyerekezo (CYB2), Ndamira (NDAB3), and Nkunganire (NKC2) showed minimal or no viral detection, suggesting either inherent resistance or limited exposure to inoculum.

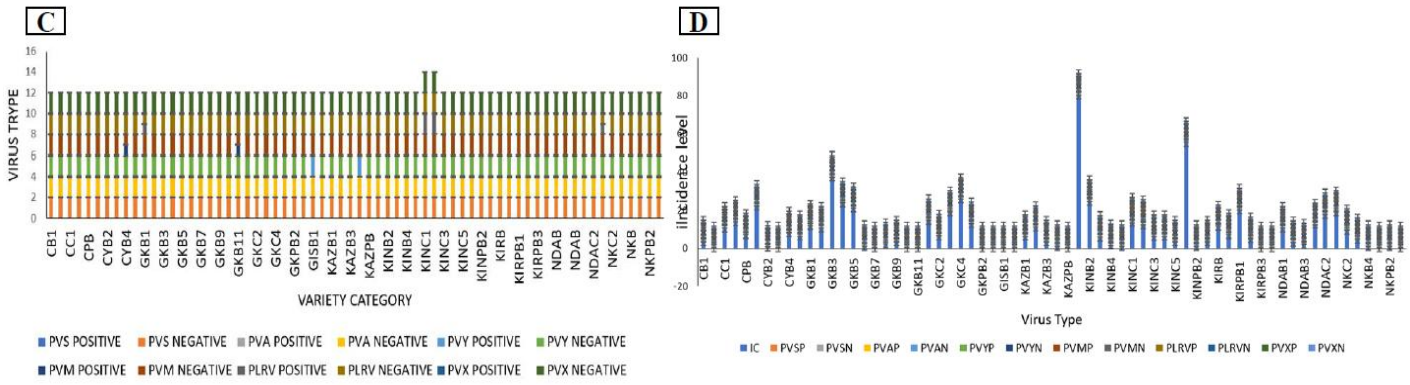

Figure 18: Seed category vs Potato Viruses (C) and incidence vs Potato Viruses vs Variety (D)

The relationship between potato varieties and viral infections was analyzed using hierarchical clustering to visualize patterns of virus incidence across varieties through a heatmap-based correlation matrix (**Fig.19**). The results revealed that PVY with 5.36% (p= 0.0002) and PLRV 6.7% (p= 0.042) which were predominantly detected in Kinigi and Nkunganire which clustered together as the most heavily infected varieties while the remaining varieties were largely virus-free.

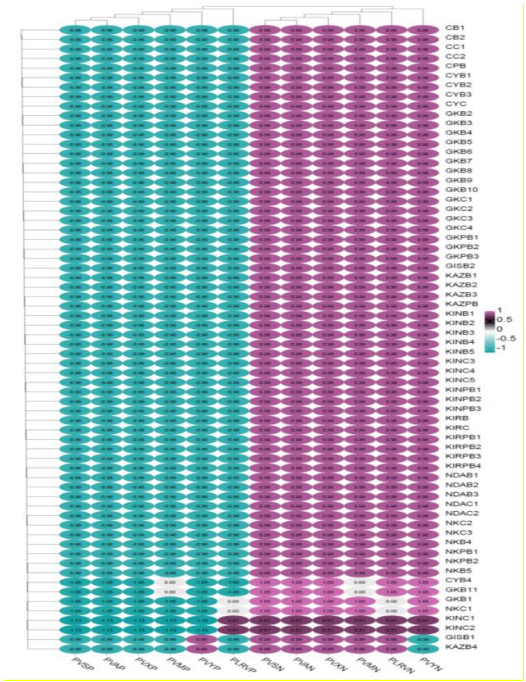

Figure 19: Variety vs virus type

4.4. Discussion

This study investigated the prevalence of major viruses across potato early generation seeds (EGS) production value chain in Rwanda from in vitro tissue culture to screen houses and seed multiplication fields. The detection of PVX and PVY in tissue culture plantlets and in screen houses constitute a significant and concerning finding, as these stages are expected to provide the cleanest starting material.

The presence of these viruses in the foundational material suggests the contamination during in vitro multiplication phases since no visible symptoms were recorded. This finding aligns with documented challenges faced by the tissue culture facilities in Rwanda. As noted by (Urinzwenimana Clement, 2024), limited production capacity creates high demand pressure for basic seed. This pressure can lead to the perpetuation of a cycle where virus-infected material is inadvertently used for propagation, facilitating virus accumulation within the EGS value chain (Tessema et al., 2023).

The higher prevalence of PVX is particularly indicative of issues in laboratory aseptic conditions, as infected plantlets often remain asymptomatic (Loebenstein & Gaba, 2012). PVX is primarily transmitted through mechanical means via contaminated tools, hands, or direct plant contact. Its presence points to a need for stricter sanitation protocols during micropropagation. While PVX alone can cause tuber yield and quality reductions of 10-40% (Sindarovska & Kuchuk, 2021;Hassan et al., 2021), its economic impact is severely exacerbated in co-infections with PVY, where losses can reach 50-80% (Petrov et al., 2023).

In contrast, the detection of PVY is alarming due to its seed-borne nature and multiple transmission routes, including aphid vectors, mechanical contact, and vegetative propagation, which leads to the systemic infection of daughter tubers (Blanchard et al., 2008; Tsedaley, 2015). The significant ($p<0.0001$) presence of PVY in screenhouses implicates agricultural practices related to vector control. As Tessema et al., 2024 suggest, factors such as variety susceptibility, vector abundance, and climate dynamics contribute to the establishment and accumulation of mixed infections. The consistent detection of PVY from tissue culture to field multiplication sites indicates the continual propagation of initially infected material, leading to a progressive increase in viral load across generations (Temfack Deloko et al., 2021).

The susceptibility of Rwandan predominant potato varieties (Kinigi, Gikungu and Kirundo) to both PVX and PVY heightens the risks (Borus, 2025). These varieties are preferred for their high dry matter content and processing suitability (Rukundo et al., 2019), making their vulnerability a critical threat to the national potato value chain and underscoring the urgent need for integrated phytosanitary strategies.

Furthermore, the detection of PVY (p= 0.0002) and *Potato leafroll virus* (PLRV) (p= 0.042) in open seed multiplication fields signals a degradation of seed quality. These aphid-borne viruses are responsible for substantial economic losses; PLRV can cause yield reductions of 33–50% (Loebenstein, 2001), escalating to over 90% in co-infection with PVY (Okonya et al., 2021), while PVY alone can cause losses ranging from 10% to 80% (Rizk et al., 2020). Their persistence is a clear indicator of inadequate aphid management, potentially due to insufficient or ill-timed insecticide applications, and the use of infected seed stock(Khurana, 2004). The basic seed category is at heightened risk because aphid pressure intensifies post-planting and is notoriously difficult to manage, especially when pesticide regimes are inconsistent (Loebenstein & Gaba, 2012). This is compounded by recent reports of significant virus infections in Rwandan potato fields (Nduwayezu et al., 2024).

Previous surveys reveal significant variations in the prevalence of key potato viruses (PVX, PVY, PLRV) across different production stages and regions in Africa. In Ethiopia, viruses are detectable even in tissue culture plantlets, with screen house facilities showing alarmingly high infection rates: PVX and PVY each exceed 50%, while PLRV reaches over 80%. This problem intensifies in open fields, where early-generation seed crops were found to be severely affected, with PLRV reaching 100% incidence (Tessema et al., 2024).

The dominant virus differs by country. In Kenya, PVX was the most prevalent in open fields at 54.8%, significantly higher than PVY (8.2%) and PLRV (6.8%) (Onditi et al., 2021b). Conversely, in Uganda, PLRV (26.4%) and PVY (17.8%) were more common than PVX (15.7%) (Byarugaba, 2021). Similarly, in Cameroon, PVY was the most dominant virus in farmers' fields with an overall infection rate of 42.3%, followed by PLRV (21.3%) and PVX (5.3%); infections frequently occurred as single, double, and triple combinations (Temfack Deloko et al., 2021).

A critical finding across studies is the inaccuracy of visual diagnosis. In Cameroon, 17.5% of asymptomatic samples tested positive for viruses, while 38.7% of symptomatic samples tested negative, underscoring the need for laboratory-based detection (Temfack Deloko et al., 2021). Furthermore, a strong association between altitude and virus incidence was confirmed. In Cameroon, virus prevalence was higher at low altitudes (59.3%) than at high altitudes (41.5%). This aligns with findings from Rwanda, where the prevalence of PVX and PVY was highest at low altitudes and decreased with increasing elevation. For instance, PVY was present in 57.5% of fields at low altitude but only 15% at high altitude (Nduwayezu et al., 2024). This altitudinal gradient was consistent with farmer perceptions, who identified viruses as a major constraint, particularly in high-altitude areas.

4. CONCLUSION AND RECOMMENDATION

This study aimed to (1) determine the precise prevalence of potato viruses across early-generation seed (EGS) production stages (tissue culture to certified seeds) and (2) quantify infection rates at each stage. To address this issue, surveys and laboratory experiment were employed to highlight virus accumulation throughout EGS production chain system. It has been found that the in vitro tissue culture is largely virus free but PVX and PVY infection were identified. The virus prevalence increased in screen houses and seed multiplication fields especially PVY and PLRV. It has been found that Kinigi, Gikungu and Kirundo were largely affected the viruses as the result of weakness in aseptic protocol implementation, virus testing and vector management which gave room to viral build up.

The findings indicate that PVX and PVY poise minimum risks to seed potato health in tissue culture and screen houses under current protocols. However, significant vulnerabilities were identified notably at Musanze RAB tissue culture facility where operational lapses such as contamination of tools and infected source materials led to elevated PVX incidence. District-level analysis highlighted Musanze, Nyamagabe and Nyaruguru as high-risk zones for PVX inoculum build-up with seed multipliers such as SPF and Sopyrwa showing the highest virus prevalence. Additionally, varieties Gisubizo and Kazeneza demonstrated genotype-dependent varieties susceptibility to PLRV, with certified seed category exhibiting PLRV incidence indicating risks of post-production transmission. These results emphasize systemic fragmentation within Rwanda's seed potato value chain where regional disparities, facility-specific operational issues and genetic susceptibility jointly drive viral transmission.

To mitigate PVX spread, I recommend to implement strict decontamination protocols including removal contaminated potato plantlets batches and sterilization of materials and tools used in micropropagation to maintain aseptic conditions. Regular virus testing and adoption of virus clean up techniques such as Thermotherapy and meristem culture are advise for high-value potato varieties like Kinigi. Furthermore, establishing mandatory virus testing at critical EGS production stages which will ensure phytosanitary integrity. Surveillance of virus incidence in screen houses should be strengthened alongside subsidized clean seed management interventions.

Based on these findings, more evidence-based recommendations to mitigate the viruses infections are proposed to strengthen potato early generation seed systems in Rwanda:

1. **Scientific/technical recommendations:**
    i. **Enhancing diagnostic capacities** by introducing serological (ELISA), molecular Biology (PCR, RT-PCR, RT-qPCR) for highly sensitive techniques of low virus titer and latent infections and next generation sequencing machines;
    ii. **Establishment of virus indexed mother stocks** to maintain central repository of virus indexed mother plants for all commercially released potato varieties.
    iii. **Integration of host plant resistance and breeding programs**: national program which emphasize on developing and deploying varieties which have resistant/tolerant to viruses especially PVY and PLRV in order to minimize the dependency of on phytosanitary measures alone.
2. **Policy recommendations:**
    i. **Empowering regularity bodies**: seed certification standards should be revised to put mandatory virus testing across early generation seed production pathway;
    ii. **Fostering public-private partnership**: private companies should be encouraged to get more screen houses and laboratories, cost sharing agreement should be created and facilitation of getting foundational clean seed stock from public institution.
    iii. **Capacity building in using field tools for diagnostics**: seed inspectors and extension agents should be trained and have access to affordable tools for field diagnostics like lateral flows devises to diagnose major viruses at farm level in order to facilitate quick decision making.
3. **Farmer level recommendation:**
    i. **Strengthen farmer field school (FFS):** including theory practical sessions on virus transmissions, benefits of using certified seeds in long term and how identify and alleviate viruses in the field;
    ii. **Improve accessibility of certified seeds**: putting in place credit schemes to buy certified seeds in order to overcome cost barriers and avoid reuse of infected seed tubers.

For future research, I propose investigating the dynamics of vector-mediated transmission to inform strategies that inhibit and limit the PVX spread through mechanical means alongside with

studies on PLRV transmission via aphids. These efforts will enhance monitoring and control viral infections in early generation seeds.

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APPENDICES

Appendix 1: Statistical analysis outputs

Table 1: Tissue Culture and Screen houses comparison with potato viruses data

	Tissue Culture Data						Green house Data					
	PVY	PVX	PVM	PVS	PVA	PLRV	PVY	PVX	PVM	PVS	PVA	PLRV
Negative	59	54	60	60	60	60	83	53	90	90	90	90
Positive	1	6	0	0	0	0	7	37	0	0	0	0
Total	60	60	60	60	60	60	90	90	90	90	90	90
% Negative	98.33	90.00	100.00	100.00	100.00	100.00	92.222	58.889	100.00	100.00	100.00	100.00
p-value for χ^2 test of goodness of fit	<0.0001	<0.0001	-	-	-	-	<0.0001	0.092	-	-	-	-

Table 2: Tissue Culture seed derived stock comparison with potato viruses data

	PVY		PVX		PVM		PVS		PVA		PLRV	
	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE
AGRISEED_AFRICAN	12	0	12	0	12	0	12	0	12	0	12	0
RAB_MUSANZE	47	1	42	6	48	0	48	0	48	0	48	0
Total	59	1	54	6	60	0	60	0	60	0	60	0
% Negative	98.333	1.667	90.000	10.00	100.00	0.000	100.00	0.000	100.00	0.000	100.00	0.000
p-value for χ^2 test of independence	0.97		0.33		-		-		-		-	

SCREEN HOUSE DATA

Table 3: Districts Screen houses comparison with Potato viruses data

	PVY		PVX		PVM		PVS		PVA		PLRV	
District	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE
BURERA	4	0	4	0	4	0	4	0	4	0	4	0
GICUMBI	5	0	5	0	5	0	5	0	5	0	5	0
MUSANZE	39	3	17	25	42	0	42	0	42	0	42	0
NYAMAGABE	25	4	19	10	29	0	29	0	29	0	29	0
NYARUGURU	5	0	3	2	5	0	5	0	5	0	5	0
RUBAVU	5	0	5	0	5	0	5	0	5	0	5	0
Total	83	7	53	37	90	0	90	0	90	0	90	0
%	92.222	7.778	58.889	41.111	100.000	0.000	100.000	0.000	100.000	0.000	100.000	0.000
p-value for χ^2 test of	0.91		0.0028		-		-		-		-	

independence						

Table 4: Screen houses owners comparison with potato viruses data

	PVY		PVX		PVM		PVS		PVA		PLRV	
OWNER	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE
ABATUBURAMBUTO	8	0	6	2	8	0	8	0	8	0	8	0
ADENYA	5	0	3	2	5	0	5	0	5	0	5	0
CHRISTELLE	5	0	5	0	5	0	5	0	5	0	5	0
DEBOKE	3	0	0	3	3	0	3	0	3	0	3	0
F_GRAPAZ	6	0	3	3	6	0	6	0	6	0	6	0
IABU	5	0	5	0	5	0	5	0	5	0	5	0
ISAAC	6	0	0	6	6	0	6	0	6	0	6	0
RAB_MUSANZE	6	0	3	3	6	0	6	0	6	0	6	0
RAB_SIGIRA	8	4	10	2	12	0	12	0	12	0	12	0
SEMAREMBO	4	0	4	0	4	0	4	0	4	0	4	0
SOPYRWA	9	3	5	7	12	0	12	0	12	0	12	0
SPF	18	0	9	9	18	0	18	0	18	0	18	
Total	83	7	53	37	90	0	90	0	90	0	90	0
%	92.22	7.78	58.89	41.11	100.00	0.000	100.00	0.000	100.00	0.000	100.00	0.000
p-value for χ^2 test of independence	0.074		0.0008		-		-		-		-	

SEED MULTIPLIERS DATA

Table 5: Seed Multiplication fields potato varieties with potato viruses data

	PVY		PVX		PVM		PVS		PVA		PLRV	
	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	POSITIVE
Cruza	10	0	10	0	10	0	10	0	10	0	10	0
Cyerekezo	10	0	10	0	9	1	10	0	10	0	10	0
Gikungu	36	0	36	0	35	1	36	0	36	0	35	1
Gisubizo	2	2	4	0	4	0	4	0	4	0	4	0
Kazeneza	8	2	10	0	10	0	10	0	10	0	10	0
Kinigi	26	0	26	0	26	0	26	0	26	0	23	3
Kirundo	12	0	12	0	12	0	12	0	12	0	12	0
Ndamira	10	0	10	0	10	0	10	0	10	0	10	0
Nkunganire	14	0	14	0	14	0	14	0	14	0	13	1
Total	128	4	132	0	130	2	132	0	132	0	127	5
%	96.97	3.03	100.00	0	98.48	1.52	100	0	100	0	96.21	3.79
p-value for χ^2 test of independence	0.0002		-		0.669		-		-		0.732	

Table 6: Seed categories comparison with potato viruses data

	PVY		PVX		PVM		PVS		PVA		PLRV	
	NEGATIVE	**POSITIVE**	**NEGATIVE**	**POSITIVE**	**NEGATIVE**	**POSITIVE**	**NEGATIVE**	**POSITIVE**	**NEGATIVE**	**POSITIVE**	**NEGATIVE**	**POSITIVE**
Basic	64	4	68	0	66	2	68	0	68	0	67	1
Certified_1	36	0	36	0	36	0	36	0	36	0	32	4
Prebasic	28	0	28	0	28	0	28	0	28	0	28	0
Total	128	4	132	0	130	2	132	0	132	0	127	5
%	96.97	3.03	100.00	0	98.49	1.52	100	0	100	0	96.21	3.79
p-value for $\chi 2$ test of independence	0.179		-		0.717		-		-		0.042	