MOLECULAR CHARACTERIZATION OF POTATO VIRUS Y AND EVALUATION OF NOVEL POTATO CLONES FOR EARLINESS AND RESISTANCE IN KENYA.

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A Thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Crop Protection of Masinde Muliro University of Science and Technology.

JULY, 2025

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ACKNOWLEDGEMENT

I acknowledge Masinde Muliro University of Science and Technology, my esteemed institution of choice, for the assistance it gave me to achieve this milestone. I appreciate my able supervisors Prof. Were Hassan, Dr. Okoth Patrick, and Dr. Lung'aiya Henry, for their patience and guidance during this journey. I appreciate the efforts of Prof. Torrance Lesley and Graham Cowan from James Hutton Institute - Scotland, for the great support they have offered throughout this journey. I acknowledge the assistance offered by Mariam Nyongesa Were and Denis Olekete from Masinde Muliro University of Science and Technology to make this work successful. To my extended family, you have been great pillars of encouragement. Thank you.

ABSTRACT

The potato (*Solanum tuberosum*), is the second most important food crop in Kenya after maize and the fourth most important crop in the world after rice, wheat, and maize. Numerous pests and a broad spectrum of bacterial, viral, and fungal diseases can affect the potato crop. Potato virus Y (PVY) is one such disease that can result in up to 100% crop loss and substantial production reductions. New variations with distinct biological characteristics that affect potato production worldwide are produced as a result of PVY strain evolution. There is still little and inadequate documentation regarding its phylogeography in East Africa in comparison to the global distribution of PVY. To investigate this, a survey was conducted to assess the distribution of PVY infection in Kenya's major potato-growing areas: Kiambu, Nyandarua, Meru, Nakuru, Bomet, West Pokot, Kisii, and Bungoma. Positively identified PVY samples were subjected to sequencing to unveil the level of genetic diversity within the Kenyan potato gene pool. A randomized complete block design was used in four trial sites for potato populations from Scotland to test for earliness and PVY resistance in KALRO-Kakamega, Alupe, and Sang'alo. Seed bulking was done in Kapsokwony. Data was recorded at 40, 70, and, 90 days post-emergence to capture data for earliness and test for PVY infection in the potato cultivars. Bioinformatics tools were used for the *De novo* assembly and mapping, genome alignment, recombination, and phylogenetic analysis. The overall prevalence of the strains in the eight counties stood at 36.62%. PVY was more prevalent in Meru (100% affected), followed by Kiambu (61%), Bomet (40%), and Nyandarua (26.7%). Bungoma, Nakuru, Kisii, and Elgeyo Marakwet recorded 0% of PVY infection. Molecular characterization of seven potato virus Y-positive strains and a whole genome sequencing of the PVY Kenyan isolate revealed the molecular footprint of PVY in Kenya. The N-type (PVY^{N}) and recombinant PVY strain type (PVY $^{N:O)}$ were prevalent in the areas of study sampled. Partial sequences of the seven potato virus Y strains identified from farmer fields deposited in the NCBI database were OR571473, OR571477, OR571474, OR571476, OR571478, OR571479, OR571475, and a complete genome of Kenyan PVY isolate, accession number PP069009. The evolutionary relationship of the Kenyan isolate PP069009 was unveiled, demonstrating a strong 92% probability of clustering with a sequence from the United States of America [JQ924287]. Population genetics analysis revealed a calculated nucleotide diversity (π) of 0.00354881, while Analysis of Molecular Variance (AMOVA) had an øST value of 0.45224. The extensive haplotype network depicted diverse PVY strains across continents. Potato population screening from Scotland was accessed at 40, 70, and 90 days post-emergence. Tests of Between-Subjects Effects combining genotypes and three field sites were significant (F = 54.43.435, DF= (61-2), P <0.05) with Sang'alo displaying the best mean phenotype of 2.6675. 12 of the 60 genotypes were selected for further processing in KEPHIS and KALRO. I, therefore, present the first molecular footprint of the Kenyan PVY whole genome (PP069009), illustratively describing its' phylogenetic positioning, historical transmission patterns across continents, and the adaptive selection pressures, emphasizing its potential role in PVY's adaptive evolutionary process. However, I stress the need for further investigations into the specific evolutionary dynamics shaping PVY populations in distinct geographical regions, especially Africa, as the virus is continually evolving. Upon adoption, climate-smart cultivars will support potato productivity among small-scale farmers. Information obtained from this study will help in policy development on managing PVY infections in Kenya.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADC-	Agricultural Development Corporation
Bp-	Base pair
cDNA -	Complimentary DNA
CP-	Coat protein
DAS- ELISA –	Double antibody sandwich
ddH20 –	double distilled water
dNTP -	deoxynucleotide triphosphate
Dot-iba –	Dot immunobinding assay
ELISA-	Enzyme-linked immunosorbent assay
ER-	Extreme resistance
FAO-	Food Agricultural Organisation
FAOSTAT –	Food Agricultural Organization statistics database
GLM-	General linear model
GPS –	Global positioning system
HR-	Hypersensitive reaction
IC- PCR-	Immuno capture PCR
ICTV -	International Committee on taxonomy of viruses
IgM-	Immunoglobulin M
KALRO-	Kenya Agricultural and Livestock Research Organization
MABs-	Monoclonal antibodies
ORF-	Open reading frame
PPB-	Participatory Plant Breeding
PCR-	Polymerase chain reaction
PVY-	Potato virus Y
pNPP-	Para- nitrophenyl– phosphate substrate
PTNRD-	Potato tuber necrotic ring disease
PVY ^{O} –	Potato virus Y ordinary
RNA-	Ribonucleic acid
ROS –	Reactive Oxygen species
RT-PCR-	Reverse transcription PCR
SA-	Salicylic acid
SDGs –	Sustainable development goals
TAE-	Tris-acetate – EDTA

VIB –	Vlaams Instituut Voor Biotechnologie
Vpg-	Viral protein genome
WUE-	Water use efficiency
USDA –	United States Department of Agriculture
UNESE-	United Nations Economic Commission for Europe

DEFINITION OF TERMS

Agroecological zone - A land resource mapping unit which is characterized by its climate, landform, soils, and/or land cover. It has a specific range of potentials and constraints for land use.

Clone - A clone is a group of genetically identical individuals that originate from a single parent organism, typically through asexual reproduction. In cloning, no genetic mixing occurs as in sexual reproduction — the offspring (clones) are exact genetic copies of the parent. E.g Potatoes are commonly propagated by tuber cuttings, Commercial bananas are propagated through suckers or tissue culture, not seeds.

Haplotype - A set of closely linked genetic markers or DNA variations on a chromosome that tend to be inherited together.

Molecular characterization - A broad term that refers to the use of molecular markers, such as DNA, RNA, and proteins, to identify the genetic characteristics of cells or tissues.

Pathogenicity- This is the ability of an organism to cause disease and specifically to harm the host. This ability represents a genetic component of the pathogen and the overt damage done to the host is a property of the host-pathogen interactions.

Plant Variety - This is a specific, identifiable group of plants within a species that has distinct, uniform, and stable characteristics which distinguish it from other varieties of the same species. These characteristics may include features like plant height, leaf shape, flower color, fruit size, disease resistance, or yield performance.

Phylogenetic tree - A phylogenetic tree is a graphical representation that illustrates the evolutionary relationships between biological entities, such as sequences or species. it shows how entities are connected through common ancestry, with the branching order (topology) indicating relationships and branch lengths representing the amount of evolutionary change between nodes.

Potato virus Y – This is a plant pathogenic virus belonging to the Potyviridae family and is one of the most significant plant viruses impacting potato production.

Recombinant strain - An organism with chromosomes that contain a stable set of recombination events between chromosomes inherited from two or more inbred strains.

RNA-Seq - is a technique that uses next-generation sequencing to reveal the presence and quantity of RNA molecules in a biological sample, providing a snapshot of gene expression in the sample, also known as transcriptome.

Sanger sequencing - a technique for DNA sequencing based upon the selective incorporation of chain-terminating dideoxynucleotides (ddNTPs) by DNA polymerase during in vitro DNA replication.

Serology - The scientific study or diagnostic examination particularly concerning the immune system's response to pathogens or introduced substances. Phytoserology focuses on plants and plant products, particularly in terms of identifying, determining relationships, and studying plant viruses.

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CHAPTER ONE

INTRODUCTION

1.0 Background of the Study

1.1 Importance of the Potato Plant

Potato (*Solanum tuberosum*) is a cool weather, herbaceous annual plant that grows up to 40 inches tall and produces tubers. Its compound leaves manufacture starch that is transferred to the stolons. It is the fourth most important food crop in the world in terms of production after maize, rice, and wheat (FAOSTAT 2023), and plays an important role in contributing positively to 13th Sustainable Development Goals (SDGs) of climate change (United Nations, 2022) and addressing Aspiration 1 of Agenda 2063 of “A prosperous Africa based on inclusive growth and sustainable development”. It is the second most significant food crop in Kenya after maize and is valuable in developing countries facing the challenge of food security (Kimathi *et al.,* 2021; Scott, 2021, Muthoni *et al.,* 2023).

Potatoes offer a better substitution for food alternatives in many households as they produce more calories per farmed area than any other crop utilized for local consumption (Beales 2019). For small-scale farmers, it serves as a direct or indirect source of high income (Mengui & Lee, 2019; CIP, 2020). The potato farming industry, therefore, needs to address the sufficiency of its sustainability in production, in efforts to feed the increasing world population (Wasilewska-Nascimento *et al.,* 2020).

The 2024 Global Report on Food Crises states that in 2023, almost 282 million individuals across 59 countries and territories faced severe hunger, an increase of 24 million from the previous year. This rise is partly due to the report's broader scope in identifying food crises. However, it also indicates a significant decline in food security, particularly in developing countries.

1.2 Geographic Distribution of Potato Production

The total world potato production currently is estimated at 376 million tonnes in 2023, with China (95.5 million tonnes) and India (56 million tonnes) being the top producers (FAOSTAT, 2023). Until the 1900s, Europe, and North America were the largest consumers of potatoes in all of their forms and applications. Climate change and extreme weather events, reduced agricultural output, and lower nutrient intake for low-income populations affected their nutritional health, but because of the adaptability of the potato crop, demand for potatoes has increased across Asia, Africa, and Latin America.

There has been a significant increase in productivity. The most important component in improving potato yield is the area. Asia, Africa, and Latin America account for more than 20 million hectares, accounting for approximately 65% of the worlds' area under potato cultivation. From 2020 to 2030, the worldwide potato area has an expansion potential of more than 14.5 million hectares, with Asia, Africa, and Latin America accounting for over 9 million hectares. To provide nutritious and sustainable potato food to an expanding population, the global, regional, and national potato agrifood system must greatly improve to provide decent employment and livelihood for producers and each partner in the food chain, and provide nutritious potato products to consumers.

Total potato production in Latin America, Asia, and Africa increased from 32 million tons in 1961 to 226 million tons in 2020, a more than sixfold increase with an average annual growth rate of 3.37% (FAOSTAT 2023). In Kenya, the level of importance of potato farming is consumption and income generation. The country's production stands at an average of between 6–10 tons/ha, much below the potential of over 20 tons/ha (Vlaams Instituut Voor Biotechnologie [VIB], 2019). The highest record was in 2012 at 2,915,067 tonnes, and the lowest was recorded in 2016 at 1,335,883 metric tonnes (FAOSTAT 2023).

1.3 Constraints to Potato Production

Potato production faces multiple challenges that significantly impact both yield and sustainability. This includes low yields arising from a shortage of good quality seed potatoes where many farmers struggle to access these, leading to lower productivity; adoption of improved varieties, where despite the development of improved potato varieties that are more resilient and productive, many farmers continue to use older, less effective varieties. This is often due to a lack of awareness, availability, or financial means to invest in new seeds; lack of good agricultural practices due to inadequate knowledge or implementation of it, such as proper soil management, irrigation, and pest control, further limiting potential yields; high cost of production inputs; pathogenic organisms such as fungi, bacteria, and viruses; over-reliance on pesticides and slow multiplication rate and seed degeneration over a short period after implementation.

The introduction of climate-smart potato varieties is likely to help improve the production trajectory upwards. Vision 2030 aims at transforming Kenya into a middle-income generating country and by generously improving Irish potato production, a significant contribution towards achieving this national vision can be realized by improving multiple channels of income generation through the versatility of potato farming, enhancing food security, and improving its' nutritional value through value addition to other existent food types (Devaux *et al.,* 2020).

Domesticated potato cultivars can be crossed with several wild *Solanum* species (Kondrak *et al.,* 2020), and desirable and beneficial traits can be transferred to improve the survival of the cultivatable varieties. The global seed potato industry is confronted with major challenges, as only a relatively small percentage of potato growers worldwide strictly adhere to using high-quality potato seeds (Kimathi *et al.*, 2021). The ideal potato takes approximately three to four months to mature, and in regions with little land but plenty of labor, it can produce roughly 50 tonnes per hectare (Tadesse *et al.,* 2019), but marker assisted breeding has helped reduced the maturation time to less than 90 days. Local potato cultivars require specific ecological conditions (Table 1.1) to thrive and perform well. The ability of different host potatoes to survive through different biotic and abiotic stresses depends on the inherent characteristics of the host plant.

Table 1.1: *Optimal ecological requirements for potato production in Kenya*

Ecological requirements	**Optimal ecological ranges**
Altitude	1500 – 2800 Metres above sea level
Rainfall	Well-distributed rainfall of 850 – 1200 mm of rainfall
Temperature	Potatoes perform very well in cool climatic conditions of 15 – 20 º Celsius. Temperarute optimum for tuberisation is 15 º celcius
Soils	Free-draining medium loam soils that allow for maximum tuber growth. pH range of 5.5 to 7.5

Potato is affectected by various diseases including alternaria, black dot, black leg and bacterial soft rot, black scarf and stem canker, common scab, late blight, latent and mild mosaic viruses, pink rot, potato lesaf roll virus, potato virus Y, powdery scab, ring rot, silver scurf, potato mop top virus, tobacco rattle virus wart disease and ring rot. However, Potato virus Y has the most economic impact in potato production.

The top producers of certified seed potatoes are ADC, Kisima Farm, GTIL, Stock Man Rozen, and KALRO (NPCK 2022) but due to an interplay of many factors, they account for less than five percent of the seeds farmers use (Kaguongo *et al.,* 2014). Certified potato seeds currently in use include Kenya Faulu, Kenya Karibu, Kenya mavuno, Kenya Mpya, Kenya Sifa, Kenya Sherekea and purple gold just to mention a few. All the varieties are high yielding but later maturers (121 – >130 days).

1.4 Problem Statement

The potato crop is vital for food security and poverty alleviation in Africa. However, there is a significant knowledge gap regarding the comprehensive genomic status of Potato Virus Y (PVY) in Africa, particularly in Kenya. The understanding of the distribution of PVY strains affecting potato production in Kenya is outdated, and is further complicated by insufficient data on mutations and recombination events within the Kenyan PVY genome. The scientific community of breeders together with farmers lack essential information about the specific pathotypes responsible for crop losses caused by various PVY strains, highlighting the urgent need for more research.

While seed certification methods have proven effective in reducing other viral infections to manageable levels, the spontaneous evolution of new PVY strains makes the re-emergence of the disease a recurring issue that escapes certification. The current highland potato crop supply is inadequate to meet the needs of the growing population. Kenyan farmers face challenges in seed production techniques, pest and disease management, market access and storage facilities, and limited policy implementation regarding norms and regulations along the value chain. Food insecurity is escalating due to population growth and shrinking agricultural lands. Poor crop performance, resistance breakdown of PVY-resistant cultivars already in circulation through the seed systems, and virus mutation through random recombination events all have contributed to reduced potato productivity.

1.5 Justification

Existing scientific evidence and simulation models recommend that plant disease pressures will significantly increase as climate change intensifies. This will negatively impact food safety and the sustainability of natural ecosystems as the extent and mechanisms of these impacts remain largely ambiguous. Effective monitoring and management of potato virus Y should be one of the highest priorities, which when synchronised will minimize the disease effects on potato crop survival, ensure food safety and environmental sustainability, promoting better socio-economic outcomes. In Kenya, this is made difficult by scientific knowledge gaps around the evolutionary genealogy of PVY, widening host range, transmission, and the emergence of new viral strains from random recombination events and mutations under the increasing intensity of extreme weather events. International trade adds to the emergence of admixtures from different parental origins, increasing the challenges faced by solanaceous crops to resist PVY infections. Potato Virus Y stands out as a significant and economically impactful threat. The emergence of numerous PVY strains with increased virulence continues to cause havoc in potato production, and for this reason, Its detrimental impact makes it a focal point for further research. A clear understanding of the genetic admixtures of potato virus Y is crucial for developing effective management strategies. PVY resourceful strategies should entail the identification and clear assessment of both the target environment, the kind of PVY strains in operation, and farmer demands to guarantee that the demand-supply cycle is not stressed. It is necessary to increase the area under potato cultivation by producing climate-smart varieties that are adaptable to a wider agroecological zone than the highlands. With an expanding population, shrinking lands, and limited potato production area in the highlands, new potato cultivars that can flourish in the midlands and are resistant to PVY would benefit potato seed systems in Kenya and improve efficiency in seed supply to farmers. Adequate screening for resistant or tolerant potato plants (with clearly visible symptoms) is ideal to maximize PVY-free seeds in the field. This study provides valuable insights into the genetic diversity and distribution of PVY strains globally, highlighting the importance of understanding evolutionary dynamics for effective management and control strategies of

PVY on a global scale. In terms of yield increase, evaluating the stability of the resistance gene among the potato populations tested in this study from Scotland is extremely beneficial to the potato seed system in Kenya. The presence of potato varieties whose resistance to PVY is consistent has the potential to add onto the countrys potato production systems as an effective strategy to offset losses caused by potato virus Y-infected tubers.

1.6 Research Objectives

General Objective :

To evaluate the molecular diversity of Potato Virus Y (PVY) in Kenya’s major potato-growing regions and assess the earliness and resistance to PVY of novel potato clones in selected midland agro-ecological zones.

1.6.1 Specific Objectives:

1. To determine the distribution of PVY in the main potato-growing areas of Kenya.
2. To characterize the Kenyan PVY whole genome using molecular tools.
3. To screen a potato population for earliness and resistance to Potato Virus Y

1.6.2 Null Hypotheses

1. **H_0**: No new PVY strains exist in Kenya's major potato-growing areas.
2. **H_0**: There exists no genetic and evolutionary variation within the Kenyan PVY genome in comparison to the world’s collection of PVY genomes
3. **H_0**: No difference exists in resistance to PVY and display of earliness from screened potato population compared to local potato cultivars.

CHAPTER TWO

LITERATURE REVIEW

2.1 Potato virus Y

Potato Virus Y is a highly diverse, genetically variable virus (Miroslav *et al.,* 2021) that affects Irish potatoes worldwide, belonging to the genus Potyvirus, one of the largest groups of plant viruses, containing over 160 species (ICTV). It stands out as a significant and economically impactful biotic threat (Xu *et al.,* 2022) in Solanaceous species, such as potatoes, pepper, and tobacco (Gray *et al.*, 2010, Scholthof *et al*., 2011, Quienouille *et al*., 2013, Green *et al*., 2017), as well as weeds of the Amaranthaceae, Leguminosae, Chenopodiaceae, and Compositae families (Kerlan *et al*., 2006). Sustainable solutions are needed to combat various biotic stresses specifically Potato virus Y and abiotic stresses like heat stress that affect its production. These solutions are essential for ensuring a stable food supply for the growing population (Kimathi *et al*., 2021, Scott *et al.,* 2021).

Potyviruses are frequently found in mixed infections worldwide, and since their genetic variability results from mutations and recombination, they cause more severe symptoms (Pacheco *et al.,* 2012). The severity of the infection is subject to variables such as the amount of virus inoculum at the seed level or growth stage after emergence, the host plant's potential to fend off viral attacks, the vector's pressure in the form of aphid populations on a particular plant, and the prevailing conditions of weather while the crop is in the field. It is known to have various genetic strains or "admixtures," which can differ in their capability to infect and cause disease in potato plants and lead to variations in the severity and symptoms of the disease, as well as the level of resistance displayed by different potato cultivars. A greater understanding of these is vital for developing operative management strategies worldwide and offers better ideas to be used for breeding resistant potato varieties. Furthermore, the genetic admixtures of potato virus Y can also contribute to the emergence of new strains with increased aggressiveness and capability to overcome resistance genes in potato plants, posing a continuous threat to potato production worldwide.

Climate-smart agriculture advocates for the production of crop varieties that can adapt to climatic changes. Quantitative Trait Locus (QTL) analysis enables the pinpointing of particular genomic regions, empowering breeders with insights into the genetic regulation of traits and thereby aiding in making informed choices within their breeding initiatives, as highlighted by Torrance *et al.,* (2020).

Heat stress is one of the major abiotic stresses affecting potato growth and productivity in semi-arid and different zones of different plants. Optimal tuber yield occurs within a range of average daytime temperatures between 14 and 22 °C. However, exceeding these temperatures can potentially hinder or entirely impede potato tuber production, resulting in adverse consequences for both producers and consumers and impairs a variety of physiological and biochemical processes (Sallam *et al.,* 2021). It accelerates the production of reactive oxygen species (ROS) in plants, such as singlet oxygen, superoxide anion, hydrogen peroxide, and hydroxyl radical, leading to oxidative stress (Hasanuzzaman *et al.,* 2020).

A study by Prashar *et al.,* 2014 did a QTL analysis on the 06H1 parental and progeny clones, utilizing yield data collected from trials spanning 2009 to 2011 that pinpointed genomic regions responsible for the variability in yield among the potato clones. Leveraging the linkage map and genotype information derived from the Illumina Infinium 8k Potato SNP Array, the study successfully mapped QTL associated with yield traits in both the 06H1 parental and progeny clones. Within one of the three QTL intervals, there was a correlation observed with increased yield in a *Phureja–Tuberosum* hybrid diploid potato population (06H1). This correlation was associated with a candidate gene encoding HSc70, as noted in studies by Torrance *et al.*, (2020) and (Campbell *et al.*, 2022). It is from this previous study that bore the selection of the 60 genotypes from the 06H1 population used in the current study, originally from Scotland for testing in Africa (Kenya and Malawi). This was guided by their resistance to PVY and ability to withstand slightly higher temperatures in the field. The process of selecting genotypes with PVY resistance involved assessing both resistance data and the presence of a specific SNP marker (c2_22749), as outlined by Torrance *et al.,* (2020).

2.2 Origin and Spread of Potato Virus Y

The Incas were the first farmers in South America to embrace potato growing. It was domesticated from the wild potato species in the Andean region (Hawkes 1990). Intermittent cropping at higher elevations suppressed viral vector propagation and reduced viral disease buildup in potato crops (Bertschinger 1992). Potato was later introduced into North America and Asia from South America in the second half of the 15th and 16th centuries during an exchange with Colombia. It has since spread to other nations thereafter (Glendinning, 1983; Nunn and Qian, 2010).

Viral infections in Potato are more than fifty different varieties, with the most important viruses raising concern over huge crop yield loss, tuber damage, and an increased disruption to the certified potato seed scheme (Kreuze *et al.,* 2020, Stevenson *et al.*, 2001). Of the 50 viral types that infect potatoes that are in existence, at least 20 remain confined in the Andes (Kreuze, 2020). Among the challenges faced by solanaceous crops, Potato Virus Y (PVY) stands out as a significant and economically impactful threat (Xu *et al.*, 2022). Stringent policies governing the movement of seed nationally and internationally need to be put in place to control the transfer of viruses from one region to another. PVY variety is highest in these continents compared to the rest of the world where potato domestication is practiced (Gibbs and Ohshima, 2010; Quenouille *et al.,* 2013). PVY^C (Salaman, 1930) was the first PVY strain group to be found and defined, followed by PVY^O (Smith, 1931).

Potato Virus Y affects other Solanaceous plants, and the N-strain was discovered in 1935 from tobacco plants growing in the vicinity. Positive PVY^N strains were also found in potato cultivars from Peru and Bolivia in 1941 and 1942 (Nobrega and Silberschmidt, 1944;). PVY^c was the main strain group in potato agriculture between 1930 and 1950, causing plant loss. PVY^O isolates expanded most rapidly in the 1950s and 1960s, followed by PVY^N isolates in the 1970s and 1980s.

The first link between Potato tuber necrotic ring spot disease (PTNRD) and PVY infection (PVY^{NTN}) was discovered in 1982 in Hungary (Beczner *et al.,* 1984), 1987 in Bosnia (Buturovie and Kus, 1989), 1988 in Czechoslovakia (Dedic *et al.,* 1989), 1989 in Austria (Schiessendoppler, 1990), 1992 in Denmark and Great Britain (Wright, 1992). Slovenian seed potato system collapsed in the 1980s as a result of severe crop loss owing to PVY^{NTN} infection (Weilguny and others). PVY^{NTN} isolates were discovered in America and Asia in the 1990s (McDonald and Kristjansson 1993; McDonald and Singh 1996; Oshima *et al.*, 2000). In 1984, the first PVYN-Wi isolate was discovered in Poland (Chrzanowska, 1991). Cross-border contamination from seed imports has led to a rise in the proliferation of several strains. For example, in Brazil (Galvino-Costa *et al.,* 2012), the PVY^C, PVY^N, and PVY^O strain groups have since been changed. In Australia, isolated occurrences have been documented (Kehoe and Jones, 2016).

2.3 Potato Virus Y Symptoms and Interactions

Potato Virus Y, genus Potyvirus; family Potyviridae, has a global range, is spread by aphids, and has a significant economic impact on potato production (Scholthof *et al.,* 2011). In industrialized nations, seed production is done in hydroponic systems, pots, or screen houses using clean pathogen-free micro-plants cultivated in tissue cultures and sown to generate mini tubers (Were *et.al.,* 2013). Crop degeneration is caused by increased pathogen proliferation, which has a detrimental influence on the state of potato seed tubers, resulting in reduced potato output in developing nations once contaminated plant seed material is employed (Gildemacher *et al.,* 2009). Virus infection in a plant begins with a single cell of a vulnerable plant. The inoculum diffused throughout the plant system, causing a response that culminates in the production of suppressor proteins whose role is to interfere with and inhibit RNA silencing (Dunoyer and Vionnet, 2005; Alvarado and Scholthof *et al.,* 2009). The symptoms vary depending on the strain, the plant variety and environmental conditions. They include chlorotic mosaic pattern in the leaves appearing as green and yellow variation. The leaves amy be deformed with reduced leaflet size, may be crinkled or wavy, with dry corky areas or necrotic streaks visible on an infected plant.

Plant-virus interactions are classified as immune (non-host plants) or infectible (host plants). A virus may lack the pathogenicity to infect a certain plant. Plants that have an immunological response to virus infection do not sustain virus replication, even when the cells are directly inoculated with the virus (Hull, 2001). As a result, the virus-host plant relationship might be either compatible or incompatible (Hinrich-Berger *et al.,* 1999).

Potato plants that are vulnerable to viral attack might be tolerant or resistant to PVY infection, depending on the host defense system. Disease signs are visible in sensitive potato plants, but none to moderate symptoms are seen in plants that are tolerant or resistant to the virus, regardless of viral amount (Ravnikar 2005). When plants can resist viral attack by restricting virus invasion and reproduction, an incompatible interaction occurs, which can be reacted to either extreme resistance (ER) or a hypersensitive response (HR).

Extreme resistance is manifested when viral effects are minimal and no sign of viral infection is seen on the plant. Some susceptible tolerant cultivars have minimal necrosis in the form of dot-like lesions. Virus proliferation is controlled in infected cells, preventing the virus from spreading from cell to cell (Solomon-Blackburn and Barker 2001; Tian and Valkonen 2015). In hypersensitive resistance response, the mechanism responsible for preventing viral transfer from the source of infection to the phloem of the plant remains unclear. Because many of the infected cells eventually die, limited necrotic damage at the site of infection is obvious (Valkonen 2015).

2.4 Aetiology of the PVY Disease

Viruses can circumvent plant viral defenses and transmit disease throughout the plant from the source of infection (Carr *et.al.,* 2019). Horizontal disease transmission by insect vectors from one host to another has evolved due to the vectors' re-invention of infection tactics (Lefeuvre *et al.,* 2019, Tenllado *et.al.,* 2020). Mutation (Tromas and Elena 2010), recombination (Chare and Holmes 2006), natural selection (Cuevas *et al.,* 2012), and genetic drift all contribute to the genetic unpredictability and evolutionary processes at work in PVY (Moya *et al.,* 2004).

It possesses a single-stranded positive-sense RNA genome of around 10000bp (Jakab *et al.,* 1997; Chare and Holmes, 2006) that codes for mature viral proteins, including the protein P3N-PIPO, which is essential for the virus's plant infectivity (Adams *et al.,* 2012, Wylie *et al.,* 2017, Kelsie *et al.,* 2018). PVYs' genome encodes an RNA-dependent RNA polymerase (RdRp), which lacks checkpoint activity during fresh RNA strand synthesis (Friedberg *et al.,* 1995). As a result, spontaneous mutations occur at a high rate during viral replication. This is caused by untreated replication mistakes and recombination events (Domingo and Holland 1997, Gago *et al.*, 2009).

The viral genome's 5' end is covalently connected to the viral protein genome-linked encoded protein (VPg). At the 3' -end, there is a poly-A sequence. The PVY genome contains a single Open Reading Frame (ORF). P1, HC-Pro, P3, VPg, 6k1, CI, 6k2, NIa, NIb, and CP are the ten functional proteins produced by three viral proteases (P1, HC-Pro, and Nib) (Figure 2.1). These proteins have several roles in the viral cycle. The peptide P3N-PIPO was discovered and documented as a result of an overlapping ORF +2 shifting of the P3 cistron (Chung *et al.,* 2008).

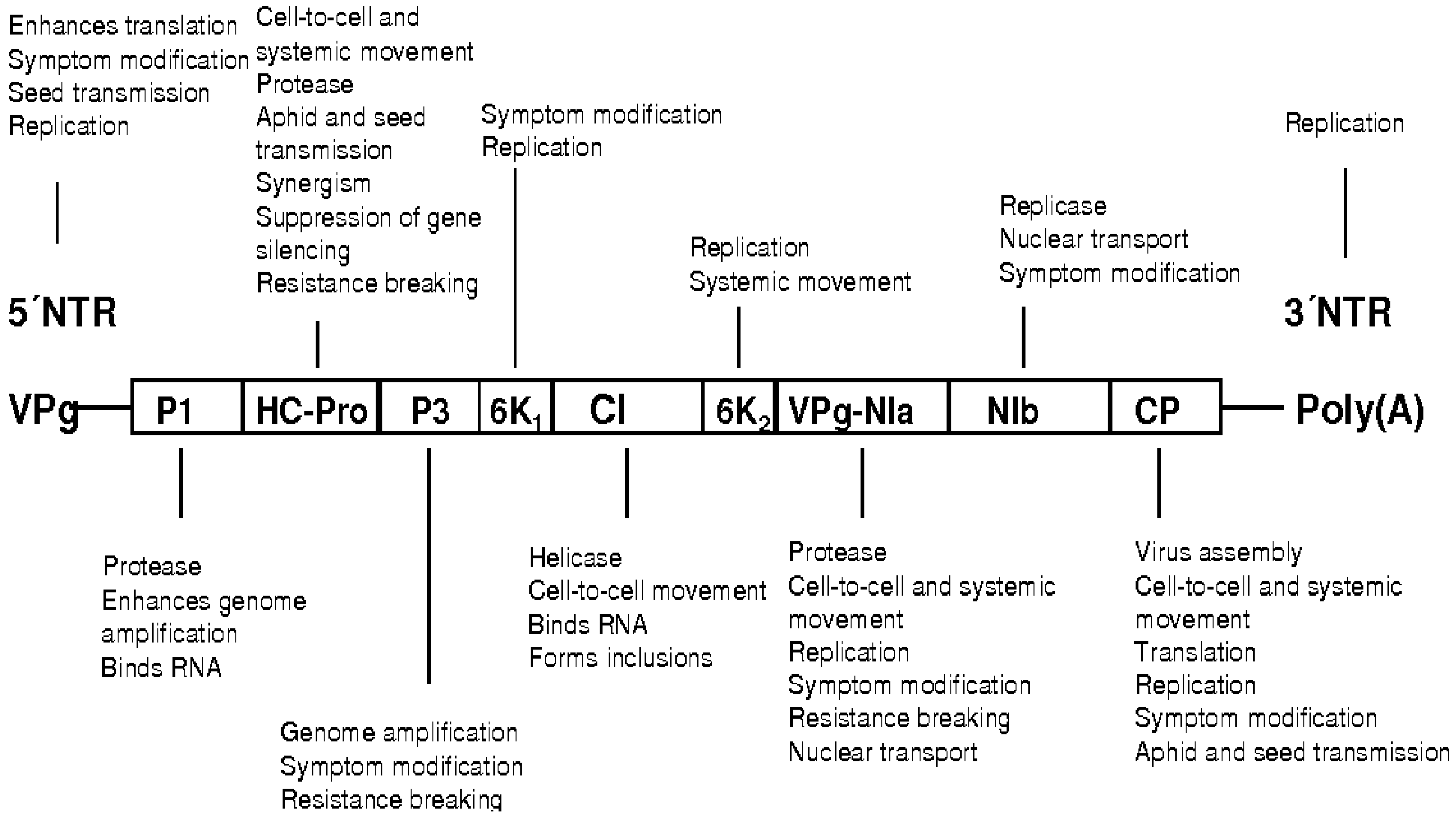

Fig 2.1: PVY genome in the 5' to 3' direction **Source:** Maki -Valkama, 2000

Pathogenicity super-silences are determined by HC-Pro. When the study of viral infections was in its early phases, viral abnormalities seen on plants were labeled as "mosaic," "crinkle," and "rugosity" (Smith 1931; MacLeod 1962). Depending on the host, several disease signs might be seen on the leaves and tubers of potato plants (Karasev and Gray 2013a). The earliest signal of the causative virus might manifest distinct symptoms in plants, serving as a guide for identifying the likely causal virus of a disease. One or more viruses and strains, as well as a variety of different biotic and abiotic variables, can cause the manifestation of one or more symptoms (Le Hingrat 2011).

2.5 Control and Management of the Virus

The rate of recurrence of PVY infections in farmer fields is a concern in the fast-paced attempts to control and manage the disease threat. Aphids often require relatively little time to pick up the virion and convey it to the host plant for new infections, which presents a hurdle (Fereres and Moreno 2009; Robert *et al.,* 2000; Bragard *et al.*, 2013). Without the use of poisonous pesticides, strategies for preventing aphids from settling on new plants remain a murky area. To retain acceptable cultivar seed qualities, seed systems generate the same seed to meet local market demands (Frost *et al.*, 2013). This causes the virus to accumulate inoculum across generations. It asks for thorough seed quality monitoring to reduce multiplication levels to a minimum. To tackle the PVY issue in farms, cultural, chemical, and a combination of these treatments have been used.

2.5.1 Cultural Approaches

Prophylactic approaches recommend using virus-free seed potatoes. This would ensure that PVY infections caused by the usage of filthy tubers are extremely rare, reducing the inoculum level caused within a host crop (Steinger *et al*., 2014). The use of virus-resistant cultivars improves potato crop output in locations where aphid populations are high. The geographical location puts the hazards of PVY transmission by aphids in jeopardy. Locations with minimal aphid infestation reduce the pace at which vector disease spreads throughout fields (Robert *et al.,* 2000). Klueken (2009) focused on aphid flight behaviors based on temperature changes and discovered that flight movement was linked favorably with temperature rise.

The physiological status of a plant influences PVY transmission by aphids since plant sensitivity to new infections tends to diminish with age (Gibson 1991; Robert *et al*., 2000; Sigvald 1985; Dupuis 2016). The majority of susceptibility is high 25 days after emergence, with resistance growing at a 10% rate each week. Planting and haulm destruction time might help minimize PVY spread risk by destroying pre-sprouting seed potatoes, but this must be done with optimal aphid flight timing to limit PVY infections (Saucke and Doring 2004; Basky 2003; Steinger *et al*., 2014).

PVY management requires the eradication of all sources of inoculum, which might include plants from contaminated seed tubers, weeds, or volunteer plants (Jones *et al*., 1996). Excessive plant removal from land surfaces, which results in bare patches, may increase the spread of PVY by aphids (Döring *et al*., 2014).

The use of crop borders in the field to reduce viral inoculum to a host crop (Boiteau *et al*., 2009; Difonzo *et al.,* 1996) can be an effective cultural approach to reduce PVY transmission. This works as a barrier to the crop of interest or as a viral reservoir collection, diverting inoculum from the host plant (Boiteau *et al.,* 2009). Straw mulching is also beneficial in attempts to control the spread of the potato virus Y (Heimbach *et al*., 2004; Saucke and Doring 2004; Kirchner *et al*., 2014; Dupuis *et al.,* 2010) since straw is thought to alter an aphid's optical acuity of a host crop (Döring *et al.*, 2014).

2.5.2 Chemical Approaches

To assist lessen the impact of PVY infections on the potato crop, oil, pesticides, and elicitors have been used. Spraying refined mineral oil on potato plants has been shown to reduce the number of PVY infections (Dupuis *et al.,* 2014, Hansen and Nielsen 2012, Kirchner *et al.,* 2014, Steinger, 2014). According to Steinger (2014), oil treatments reduced the average PVY incidence by 39% over 4 years. To avoid the phytotoxicity of the host plant, use caution while dealing with mineral and vegetable oils (Kirchner *et al*., 2014). The necessity for a moderate usage of mineral oil to reduce PVY is highlighted by reports of tuber loss for specific cultivars after being subjected to the treatment (Kirchner *et al.,* 2014). Delays in host plant stem damage could help make up for the apparent production drop (Dawson *et al*., 2015).

Treatments with insecticides are successful, but because PVY may be acquired and inoculated in such a short amount of time, it is essential to use an insecticide that can swiftly kill or disable the vector to prevent plant infection (Bragard *et al.,* 2013). By effectively eliminating aphids, lambda-cyhalothrin, dimethoate, and pymetrozine reduce the likelihood that the vector will become infected (Boquel *et al.,* 2014; Margaritopoulos *et al.,* 2010).

Insecticide-infused synthetic pheromones are becoming more popular as a technique of managing the aphid population to help stop the development of PVY. Due to the intensive usage of pesticides, aphid populations have evolved several coping techniques. The number of vectors displaying various degrees of pesticide resistance has increased noticeably in response to efforts to chemically control the vectors that carry PVY. *Aphis nasturtii* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas), Myzus persicae (Sulzer), and *Sitobion avenae* (Fabricius) are a few of them (Foster *et al.,* 2014; 2002). This was accomplished by being able to modify different biochemical and molecular mechanisms within the vector for survival, such as increased carboxylesterase production and mutations in the acetylcholinesterase enzyme, voltage-gated sodium channel, nicotinic acetylcholine receptor, and pyridine resistance (Bass *et al*., 2014).

According to Kessmann *et al.,* 1994), it is possible to successfully induce systemic resistance in plants against infections by applying salicylic acid, for example (Vasyukova and Ozeretskovskaya 2007). Treatments with the functional equivalent of SA, acibenzolar-S-methyl (Bion®), on tomato plants infected with PVY indicated induced resistance in a decrease of PVY transmission (Petrov and Andonova 2012). (Dupuis *et al*., 2014). To ensure that seed providers presenting their products on the market comply with quality standards for seed potatoes, a certification program for potatoes is essential. Concerning the minimum commercial quality standards for the certification of high-quality seed meant for international marketing, UNECE (United Nations Economic Commission for Europe) has created an international standard for the certification and marketing of seed potatoes (UNECE 2015).

2.6 Characterisation of PVY Strains

Potato Virus Y strain groups can be differentiated by biological, serological, and molecular methods.

2.6.1 Biological identification of PVY Strains

Potato Virus Y infects Solanaceous and non-Solanaceous species. In the past, the distinction between the hosts for PVY was based on the plant species from which the samples were collected. On this basis, four PVY strains were identified as potato, tobacco, tomato, and pepper strains. Over time this perception lost meaning due to the ability of some strains to infect more than one host species. It has been recorded that some PVY strains are infectious to both tobacco and pepper cultivars (McDonald and Kristjansson, 1993). The effective characterization is achieved by evaluating the ability of an isolate to initiate the expression of symptoms on one host e.g. tobacco causes necrosis in another e.g. potato, using the same isolate.

In the major potato-growing areas across the world, there are at least nine different strain families of PVY. This contains the non-recombinant parental strains PVY^O, PVY^C, and PVYN (Chikh-Alietal 2013; Kehoe and Jones 2016). Several studies have been conducted to highlight the various processes by which different strains induce infections in different hosts (Verbeek *et al.,* 2010, Mondal *et al.,* 2016, Mondal and Gray 2017).

2.6.1.1 PVYo and PVYc

Systemic mottle or mosaic is caused by the most prevalent strain, PVY^O, in several potato varieties (Lorenzen *et al.,* 2006, Crosslin, 2013). On potato leaves infected by the PVY^O and PVY^C strain families, similar symptoms are seen (de Bokx and Huttinga 1981; Beemster and de Bokx 1987). PVY^C causes the mottle stripe sign of a mild infection to appear on the leaves and stems of susceptible potato varieties. In cultivars containing the Nctbr gene, a hypersensitive reaction (HR) is triggered (Cockerham 1970; de Bokx and Huttinga 1981). In potato cultivars afflicted by PVY^{O}, symptoms of severe infection include leaf necrosis, leaf rugosity, crinkling, and overall stunting of the diseased plant. When present in cultivars, the Nctbr resistance gene causes a hypersensitive reaction rather than suppressing PVY^{O}'s effects (Jones 1990). (Szajko *et al.,* 2014). According to Karasev (2011), certain PVY$^{O\text{-}O5}$ isolates from North America cause more severe necrosis and elicit a hypersensitive reaction comparable to that of PVY^{O} isolates (Karasev *et al.,* 2011). In greenhouses, several PVY^{O} isolates cause the plant disease Potato Tuber Necrotic Ring Spot Disease (PTNRD) in vulnerable potato cultivars (Glais *et al.,* 2015). (Gray *et al.,* 2010; Davie 2014).

2.6.1.2 PVY ^{N}

Because PVY^{N} causes extremely modest mosaic among potato cultivars, mottling, and vein necrosis in tobacco, it is known as the "tobacco venial necrosis strain" (Riggotti and Gruerli, 2007). The hypersensitive resistance genes Nytbr, Nctbr, and Nztbr can be overcome by PVY^{N}, but not in individuals bearing the Ny-1 and Ny-2 genes (Szajko *et al*., 2014). PVY cases have been documented in North America, Africa, and New Zealand (Crosslin et al., 2002; Ibaba 2009, 2012). In field settings, PVYN often results in PTNRD in tubers, although it may also be triggered in an ideal setting, such as a greenhouse (Kerlan and Tribodet 1996).

2.6.1.3 PVY^{NTN}

Potato Tuber Necrotic Ringspot Disease is brought on by PVY^{NTN} (Beczner *et al.,* 1984). Elevated external tuber rings that induce internal tuber symptoms are its defining feature. Typically, the tubers are harmed, costing the producers money (Crosslin, 2013). It causes vein necrosis and is resistant to the potato resistance genes Nytbr and Nctbr. It causes HR in hosts with the Ny-1 and Ny-2 resistance genes in potatoes. It has a wide range of kinds, including $\text{PVY}^{\text{-AST}}$, PVY^{NTN}-H, PVY^{NTN}-NIB, 423.3, and N4 (Kerlan *et al.,* 2011, Galvino-Costa *et al.,* 2012). In hosts bearing the Nytbr, Nctbr, and Nztbr resistance genes, HR is activated (Chikh Ali *et al.,* 2014).

2.6.1.4 PVY $^{\text{N-Wilga}}$

The strain type PVY $^{\text{N-Wilga}}$ was discovered in the cultivar "Wilga" and was originally characterized in Europe (Crosslin, 2013). Vein necrosis and the PVY^{O} strain CP sequence were the symptoms seen in tobacco. It displays signs resembling those of isolates of PVYN on tobacco and potatoes. An indicator plant called *Solanum brachycarpum* can detect several PVYN-^{wi} symptoms.

2.6.1.5 PVY^{Z} and PVY^{E} Strains

There are two odd strain groups: PVY^{Z} and PVY^{E} (Jones, 1990; Kerlan, 2011). They have symptoms that are similar to PVY^{NTN} isolates. They are capable of overcoming the resistance genes for Nytbr and Nctbr. These isolates cause tobacco leaves to exhibit mosaic symptoms. The sole classified isolate within the PVY^{E} strain category is PVY-^{MON} from Brazil, which can inhibit the Nztbr HR gene (Galvino-Costa *et al.,* 2012).

2.6.1.6 $\text{PVY}^{\text{N:O}}$

The strain type $\text{PVY}^{\text{N:O}}$ displays properties of PVY^{O} and PVY^{N} strains, and it was documented in North America (Crosslin *et al.,* 2005; Piche *et al*., 2004; Singh *et al.,* 2003).

2.6.1.7 PVY^U

According to Janzac (2015), PVY isolates collected from several Brazil-based sampling sites belonged to the PVY^U serogroup, which had just been discovered. This came about as a result of the discovery of certain amino acid residues in the PVY^{UCP} isolates' N-terminal region (Janzac *et al.,* 2015). There are reported biological differences between the PVY^U and well-known PVY potato strains (Janzac *et al.,* 2015).

2.6.2 Serological Characterization of PVY Isolates

The antigenic traits of different PVY strain groups have become more significant in attempts to supplement biological categorization. The identification of PVY isolates has been easier because of the development of monoclonal antibodies, and the coat protein sequence has demonstrated the existence of several PVY strains (CP). To prepare a serological diagnosis, increasing antibody titers or Imunoglobin M antibody detection are used. The focus of viral detection using serological methods is on the interaction between viral antigens and their particular antibodies (Hsu, 1996). The ELISA test, also known as an enzyme-linked immunosorbent assay, is quick and affordable. The lack of specificity of the Antisera of a mixed strain combination group and their limited availability, particularly in underdeveloped countries, present a difficulty for serological characterization. The above-mentioned shortcomings can be quickly addressed by adding more sophisticated diagnostic methods, such as RT-PCR. Utilizing ELISA, Immunocapture (IC) -PCR aids in the capture of many viruses. Dot-immuno binding assays, often known as Dot-Ibas, use dotting antibodies or antigens directly onto materials like nitrocellulose membranes. It prefers labs with fewer laboratory resources (Sumi *et al.,* 2009).

Analysis of several monoclonal antibodies has shown that they have specificity to various PVY strains. The C-terminal region of PVY carries unique antigenic characteristics (Ranki *et al.,* 2008; Nikolaeva *et al*. 2012; Tian *et al.,* 2014). PVY^{O} Monoclonal Antibodies Recognize PVY^{O}, PVY^{C}, and $PVY^{N\text{-}Wi}$ Strain Groups (Crosslin *et al.,* 2006).

Monoclonal antibody 1F5, which is thought to be specific for PVY^{N}, identified IF5 ($PVY^{O\text{-}O5}$ isolate) (Karasev *et al.,* 2010). PVY^{Z} and PVY^{E} isolates are serotype-N (Kerlan *et al.,* 2011; Galvino-Costa *et al*., 2012), with the bulk of PVY^{NTN} isolates being serotype-N and serotype-O (Hu *et al.,* 2009; Chikh Ali *et al*., 2010).

Robaglia (1989) was the first to finish a PVY isolate's RNA sequencing. Reverse transcriptase-polymerase chain reaction (RT-PCR) has enabled the genetic characterization of PVY isolates globally (Lorenzen *et al.,* 2006). PVY isolates with P1, HC-Pro, CP, or the whole genome (approx. 9700 kb) have undergone partial isolation. To distinguish distinct PVY strains, assays have been developed (Crosslin *et al.,* 2002; Crosslin *et al.,* 2005; Nie and Singh, 2002). The PVY Coat protein area has been investigated sparsely in various parts of the world (Blanco-Urgoiti *et al.,* 1996; 1998; Boonham *et al.,* 2002, Ellis *et al.,*1996 Singh *et al.,*1996).

2.6.3 Molecular Characterization of PVY Isolates

Next-generation sequencing (NGS) has enabled researchers to reach new heights in their understanding of pathogen genetic makeup in general (Studholme *et al.,* 2011). In contrast to previous attempts in bacteria and eukaryotes, viral features have made developing marker genes difficult. The genetic characterization of PVY isolates has been achieved using whole RNA sequencing (Kehoe and Jones 2016) and purified virion RNA (Fabre *et al.,* 2012; Kutnjak *et al.,* 2015).

2.7 Next-generation Sequencing application in Crop improvement

Potato virus Y is incurable under normal field conditions. The known prophylactic measures used in Kenya are focused on preventing or slowing down the viral spread in fields using resistant varieties (Dupuis *et al.,* 2019). Virus-free propagating material has been achieved using molecular procedures. For proper control strategies to be deployed, there is a need to have molecular-level data on the specific viral strains because they may symptomatically reveal interesting epidemiological properties that may help in the proper diagnosis of the viral disease (da Silva *et al.,* 2020). Midway through the year 2000 advances in next-generation sequencing technology assisted in transforming scientific searches, increasing the production of sequence data that was helpful to people (Mardis, 2013). Studies using next-generation sequencing need reliable and efficient techniques for sample preparation. Massive parallel sequencing of individual DNA molecules is now possible because of nanotechnology concepts and advances. NGS is distinguished by its high throughput and single-molecule DNA sequencing characteristics (Taishan *et al.,* 2021).

Short-read sequencing procedures involve clonal amplification and sequencing (Levy *et al.,* 2016, Goodwill *et al.,* 2016). Following synthesis, DNA fragments can be amplified into multiple pieces of spatially separated template fragments using Ion Torrent or Illumina. Clonal amplification is carried out using a bead-based technique using Ion Sphere particles in a micro-well using an emulsion Polymerase chain reaction. Ligation of adapter sequences to DNA fragments is aided and later captured in a water-in-oil emulsion droplet with a bead coated in complementary adapters, deoxynucleotides (dNTPs), primers, and DNA polymerase. Loading micelles into microwells on a semiconductor device and flooding with unmodified A, T, G, or C nucleotides sequentially during sequencing to ensure effective amplification (Taishan *et al.,* 2021).

Illumina's NGS method is based on synthesis sequencing with a fluorescent-labeled reversible terminator. Paired-end sequencing is a significant advancement in NGS technology in which both ends of DNA fragments are sequenced into a library and forward and reverse reads are aligned as read pairs (Nakazato 2013). When compared to single-read data, read alignment improves accuracy and increases the capacity to detect indels. The examination of differential read-pair organization reduces polymerase chain reaction duplicates that occur during the amplification phase (Illumina, 2014). After Sanger sequencing, second-generation sequencing is a more sophisticated sequencing method. The production of libraries, sequencing, and data analysis are necessary steps in the next-generation sequencing workflow. First-discovery templates that correspond to molecules of interest for sequencing can be found in a high-quality library. The two next-generation sequencing methods are DNA sequencing (DNA-Seq) and RNA sequencing (RNA-Seq) (Light Body *et al.,* 2019).

2.7.1. Library Preparation for DNA- seq

Whole genome sequencing, whole exome sequencing, epigenome sequencing, and targeted sequencing are all examples of DNA-Seq (Rizzo *et al.,* 2012). When preparing templates for DNA-seq, polymerase chain reaction, and hybridization techniques are taken into consideration. Target sequencing and whole exome sequencing depend on template preparation via hybridization capture (Liang *et al.,* 2018). The fundamental processes of fragmentation, end-repair, adaptor ligation, and size selection go into the creation of a DNA-Seq library (Podnar *et al.,* 2014).

2.7.2. Library Preparation for RNA-seq

RNA-Seq is the greatest analytical tool for functional genomics studies such as differential gene expression, alternative splicing, and variant findings. mRNA sequencing (mRNA-Seq), short RNA sequencing (smRNA-Seq), and whole transcriptome sequencing (WTS) (Taishan *et al.,* 2021). Total RNA isolation, target RNA enrichment, and reverse transcription of RNA into complementary DNA are the three processes required to complete it (cDNA). RNA stabilization, RNA separation, enrichment, library creation, library controls, barcoding, and RNA sequencing are the main components of RNA sequencing processes.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Survey In Major Potato Growing Areas in Kenya

A survey was conducted in Kenya's main potato growing counties namely; Kiambu, Meru, Nakuru, Bomet, Uasin ngishu, West Pokot, Transnzoia, and Bungoma. It was done during the long rainy season (2018 March - June rains). Potato fields were selected randomly across several administrative levels: two sub-county per county, and two to four wards in each sub-county, dependent on the availability of the potato crop on the farm. A global positioning system (GPS-Garmin Inc. Kansas, USA) was used for geo-referencing purposes for all potato samples collected from the farmer fields.

3.1.1 Sampling of PVY Infected Crop From Farmer Potato Fields

The survey was conducted by walking across potato fields and physically evaluating potato plants for the presence of common viral disease signs. Ten potato plants were selected and analyzed in each farmsite along two longitudinal transects. In smaller farms (0.2 to 1.5 ha) all of the plants along the diagonals were examined. The proportion of plants displaying PVY symptoms to the total number of plants observed in a field was used to assess disease incidence. In each field, at least five leaf samples with viral symptoms and five without were collected and placed in sampling bags, labeled, and stored in a cold box to preserve them for eventual virus testing and analysis.

3.1.2 Serological Characterization of PVY Samples from Farmer Fields

For serological detection and differenction, the collected virus free and infected potato samples were initially tested by double-antibody sandwich ELISA (DAS - ELISA) for the presence of PVY following the manufacturers' instructions, with a few modifications where needed after troubleshooting. The monoclonal $PVY^{O/C}$, $PVY^{C,}$ and PVY^{N} antibodies were used for differentiating the PVY strains. All antibodies were diluted (1:1000), dispensed (200µl) into the wells of an ELISA plate, covered tightly in a humid box then incubated for four hours at 30° C. The washing buffer, extraction buffer, and coating buffer were prepared according to the manufacturer's instructions. The plates were washed three to four times with washing buffer and blotted on paper towels. While incubating the ELISA plates, potato leaf samples were weighed. Four milliliters of extraction buffer were added per gram of sample and ground using a mortar and pestle. Two hundred microliters of the plant extraction were added onto each well, plate covered tightly, and incubated overnight at 4°C. After washing with washing buffer and dried using a bloating paper, the enzyme conjugate was diluted in 1:1000 with a conjugate buffer and 200µl loaded into test wells, incubated for 5hours at 30°C. This was followed by washing and blotting drying.

Freshly prepared para-nitrophenyl-phosphate substrate (pNPP) was dissolved at one gram/ml in substrate buffer then added into test wells and incubated at room temperature (20-25°) in the dark, done with several replicates. The results were recorded visually (color changing). Virus-free plant samples of potato were used as negative controls and a previously confirmed PVY-infected potato sample obtained from the field was used as a positive control.

3.2 Molecular Detection of PVY Strains

3.2.1. RNA Extraction and Reverse Transcription for Kenyan PVY Strains

A spectrum plant total RNA kit was used to extract RNA from young leaves of virus-free and infected plants (Sigma-Aldrich). Total RNA was extracted as instructed by the manufacturer with slight modifications. After adding liquid nitrogen to a sterile mortar, frozen potato leaves were thoroughly ground using a sterile pestle. The tissue was transferred to RNase–free micro centrifuge that had been cooled on ice. lysis buffer was added to each sample (1.5 ml per 0.25g of tissue powder) and the lysate was homogenized using a vortex to dispense the sample and then incubated for 3 minutes at room temperature. A volume of 350 µl was transferred to a clean homogenization tube, and centrifuged at 13,000 round per minute for 5 minutes. One volume of 70 % ethanol was added to each homogenate. Samples were vortexed then transferred to a spin cartridge, centrifuged at 13000rpm for 15 seconds, flow through discarded and the spin cartridge reinstated into the same collection tube. A repeat was done using buffer II, and a final spin at 13000rounds per minute for 2 minutes to dry the membrane with bound RNA. Purified RNA was stored at -20 °C.

The quality of the extracted RNA was checked by running one µl of the RNA extract on a 1.2% agarose gel, and the RNA concentration was measured using a NanoDrop spectrophotometer. Reverse Transcription (RT) was synthesized in a 20 µl reaction using M-MLV reverse transcriptase (Thermo Scientific). A volume of 10 µl purified RNA was mixed with 1µl of initiating primer M4T (5'- GTT TTC CCA GTC ACG AC (T) 15- 3') and denatured at 95° C for 5 minutes then chilled on ice. The RT reaction was incubated at 37°C for two hours by adding 4µl of M-MVL buffer X 5, 0.5µl of mM dNTPs, 2µl of 10 mM DDT, 1µl of reverse transcriptase (M-MVL 200 U/ µl) and 1.5µl of ddH_2O.

3.2.2 Cloning and Sequencing of PCR Fragments

High-fidelity polymerases cloned for sequencing were used to create multiplex-PCR fragments. Thermo Fisher Scientific's GeneJET PCR purification kit was used to purify PCR products, and the cDNA content was determined using a NanoDrop spectrophotometer. Furthermore, 5 µl of purified cDNA was run on an agarose gel

(1.2%) to ensure purity and concentration. Using the Clone JET PCR cloning kit (Thermo Scientific), the purified PCR product was ligated into the pGEM T cloning vector and then transformed into commercial (Life Technology) or lab-made competent cells of *Escherichia coli* strain DH5 according to the manufacturer's instructions. Plasmid DNA was extracted from overnight cultures of chosen *E. coli* colonies using the Thermo Scientific GeneJET plasmid miniprep kit and digested with Fast Digest BglII (Thermo Scientific). Agarose gel electrophoresis was used to examine the digests. At the James Hutton Institute, clones with predicted insert sizes were chosen for sequencing using the Sanger sequencing technique (Scotland). Each sample and PCR product had one to three clones sequenced.

3.2.3 PCR Conditions for Coat Protein and P1 Gene Amplification

PCR was utilized to amplify the virus's 3' terminal genomic region using primers that were designed (Table 3.1). the master mix comprised of 2.5µl cDNA template, 2µl of each primer, 0.5µl of 10mM dNTP, 10µl of 10X taq polymerase buffer, 0.2µl of Taq DNA polymerase (5U/l), 1.5µl of $MgCl_2$, and 31.5µl double distilled H_2O. The initial molecular categorization was performed using uniplex PCR results (Glais *et al.,* 2015; Chikh Ali *et al.,* 2010b; 2013).

Table 3.1: *Primers names and sequence in the Sequence in 5′ – 3′*

Name of Primer for CP	Sequence in 5′ – 3′	Name of Primers for P1	Sequence in 5′ – 3′
YoCP2FOR	AGAGCAAGGCAGCATCCAGT	P1oFOR	CATGGCAACCTACATGTCAACAATC
YoCP2REV	TGCACCGAACCATAAGCCCA	P1REV	AAAATGCATCATTGAGTAACCTTGGAAC
		P1nFOR	CATGGCAACTTACACATCAACAATCC
		P1REV	AAAATGCATCATTGAGTAACCTTGGAAC

The PCR cycle was configured, to begin with a 2-min denaturation at 94°C, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 47°C for 1 min, extension at 72°C for 2 min, and final extension of 10 min at 72°C. The results of amplification were separated on 1.2% agarose gel in 1X TAE buffer and stained with 0.75 µl Gel red (Biotium, USA).

3.3 Next-Generation Sequencing of Kenyan PVY Whole Genome

3.3.1 Sample Collection

Symptomatic potato leaf samples for Next Generation Sequencing were gathered from fields at KALRO Tigoni (Kenya) and immediately deposited in well-labeled falcon tubes containing RNAlater® (RNA stabilizing solution). The samples were maintained cold in the field and subsequently moved to the Biosciences Eastern and Central Africa (Bec-A) Hub at International Livestock Research Institute, Nairobi (ILRI) at 40°C for further testing.

3.3.2 Sample Preparation and RNA Extraction

Total RNA was extracted using the RNeasy plant mini kit for RNA extraction – Qiagen, with slight modifications to the manufacturers instructions. Using a mortar and pestle, the entire leaf was crushed in liquid nitrogen. One hundred milligram of the resultant powder was placed in a liquid nitrogen-free 2ml microcentrifuge tube. The RNA Quantity and Quality were tested using a nanodrop to ensure the quality and quantity.

3.3.3 Library Preparation

Library preparation followed the Illumina TruSeq low sample preparation procedure with slight modifications from the manufacturer's instructions. Fifteen microliters of Elute Prime Fragment mix containing random hexamers for RT priming and first strand cDNA synthesis buffer was gently pipetted up and down six times to thoroughly mix before being placed in a pre-programmed thermocycler at 94° C for eight minutes and held at 4° C to elute, fragment, and prime the RNA before immediately proceeding to synthesize a first strand of DNA.

Fifty microlitres of the SuperScriptII was added to the entire stock (one microlitre SuperScriptII for every nine microliters first strand master mix) to the first strand master mix tube, mixed gently but thoroughly, and centrifuged at maximum speed for thirty seconds. Eight microlitres of the first strand mix containing SuperScriptII was added to seventeen microlitres of the primed mix and gently pipetted up and down six times, spun down, and placed in a pre-programmed thermo cycler at 25°C for 10 min, 42°C for 50 min, 70° C for 15 min, held at 4° C with pre-heated lid set to 100° C. This immediately proceeded to synthesize the second strand.

The thawed second strand Master Mix was centrifuged at 600xg for 5 seconds. Twenty-five microlitres were added to the products of the first strand cDNA synthesis, the entire volume pipetted, mixed thoroughly six times up and down, spun down then incubated in a pre-heated thermo cycler for one hour.

To purify the ds cDNA, 90µl of well-mixed AMPure XP beads were added to the PCR tubes containing 50µl of ds cDNA at room temperature, and to thoroughly mix it pipetted ten times up and down and then transferred to a sterile 1.5ml labeled microcentrifuge tubes and incubated at room temperature for 15 mins. The microcentrifuge tubes and their contents were placed on a magnetic stand at room temperature for 5 min to bind al the beads to the side of the tubes. The supernatant was carefully removed from each well and discarded leaving the microcentrifuge tubes on the magnetic stand. Without disturbing the pellet, a hundred microliters of freshly prepared 80% ethyl alcohol were added and incubated at room temperature for 30 seconds. The 80% ethyl alcohol washes were repeated twice.

The microcentrifuge tube containing the pellet was allowed to stand at room temperature to dry. Thawed centrifuged resuspension buffer (RSB) was added to each tube after its removal from the magnetic stand and the entire volume was gently pipetted ten times (up and down) to resuspend the pellet, then incubated at room temperature for 2 min. The microcentrifuge tube was placed in a magnetic stand for five minutes with the cap open. Fifty microliters of supernatant (ds cDNA) were removed from each microcentrifuge tube and transferred to clean empty PCR tubes. Ten microliters of resuspension buffer was added to each tube followed by forty microliters of End Repair Mix. The tubes were placed in a pre-heated thermal cycler at 30°C for thirty minutes. A hundred microliters of End Repair Mix was transferred to 1.5µl microcentrifuge then 160µl of well-vortexed AMPure XP beads were added and the entire volume gently pipetted 10 times up and down to mix thoroughly followed by 15 min of incubation at room temperature.

The tube was transferred to a magnetic stand at room temperature for 5 min until the liquid was clear. From the tube, 127.5µl of the supernatant was removed and discarded. Into the tubes on the rack 200µl freshly prepared 80% EtOH was added over the pellet carefully without disturbing it and incubated at room temperature for 30 seconds. The supernatant was carefully removed and discarded without disturbing the pellet then the wash step was repeated once. Carefully, the tubes were air-dried at room temperature for 15 min on the magnetic stand.

The tubes were removed from the magnetic stand, 20 µl of RSB were added and pipetted gently up and down the entire volume 10 times to mix thoroughly then incubated at room temperature for two minutes. The tubes were placed back on the magnetic stands at room temperature until the liquid was clear. Twenty microliters of supernatant were transferred to a new sterile PCR tube and 2.5µl RSB was added followed by 12.5µl thawed A-tailing mix then the entire volume was gently pipetted up and down 10 times to obtain a homogenous mix. It was spun down and placed on a pre-programmed thermocycler at 37°C for thirty minutes, 70°C for five minutes, and a hold temperature of 4°C with the pre-heat lid option.

The libraries were quantified using Qubit High Sensitivity Kit and quality was checked on 2% agarose gel and Agilent Technologies tape station with sensitivity D 1000 screen tape for selection. The libraries were diluted to a suitable concentration in re-suspension buffer then diluted to 10nM then 4nM depending on calculations obtained from concentration to nM. The 4 nM libraries were pooled into two pools. A sample sheet was generated for each pool in the Illumina experiment manager. The pools were diluted further to 7 pM final concentration then loaded to an Illumina mini-seq system for sequencing.

3.4 Screening of Potato Populations for Earliness and Resistance to PVY

3.4.1 Establishment of Field Trials

A random complete block design was used to set up the screening sites for the 06H1 potato population used in this study. It entailed categorizing comparable experimental units as replicates or blocks. The experimental unit's blocks were designed to be as uniform as feasible. Random numbers were used to randomize the potato mini tubers for planting within the blocks. The treatments were duplicated in the same way. The number of blocks represents the number of replications for each accession. The treatments are the several accessions of each variety, which are distributed at random among blocks of neighboring subjects, one accession per block. Four test sites were identified and settled on (Table 3.2).

Table 3.2: *Trial Sites for Potato Mini Tubers*

Site	Location	Elevation M (a.s.l.)	Average temp. (°C)	Precipitation (mm)	Previous crop
KALRO Kakamega	Kakamega Lat. N0.017° Long. E034.047°	1580	19.6 Min 21.9; Max	1600-2000	Sweet potato
KALRO Alupe	Busia Lat. N0.029° Long. E034.007°	1100	23	1000-1400	Sweet potato
Sang'alo Institute of Science and Technology	Bungoma Lat. N0.51667° Long. E034.6°	1920	18.5	1400-1800	Maize
Kapsokwony (site for seed bulking)	Bungoma Lat. N0.8507° Long.E34.702°	1952	14; Min 26.3;Max	1450- 1800	Sweet potato/ Maize

3.4.2 Evaluation of Potato Populations for PVY Infection

The trials were monitored closely for symptoms above the ground during the crop growth period and symptoms in the tubers after harvest. Symptoms on shoots were recorded on each potato plant. A severity score of 1-5 was adopted where; 1: no necrotic ring spots on leaves or tubers, no light/dark green mosaic patterns on leaves, veins, or shoots, no stunted growth, no death tubers, 2: slight necrotic ring spots on leaves or tubers, slight light/dark green mosaic patterns on leaves, veins or shoots, slightly stunted growth, slight death of growing points ;3: moderate necrotic ring spots on leaves or tubers, moderate light/dark green mosaic patterns on leaves, veins or shoots, moderately stunted growth, moderate death of growing points; 4: severe mosaic distribution of all leaves with a general reduction of leaf size severe necrotic ring spots on leaves or tubers, severe light/dark green mosaic patterns on leaves, veins or shoots, severely stunted growth, severe death of growing points; and; 5: complete chlorosis, necrotic ring spots on leaves or tubers, dark green mosaic patterns on leaves, veins or shoots, complete stunted growth, complete death of growing points. A mean shoot severity score was calculated per genotype based on all individual plant scores at 40 days after emergence, 70 DAE and 90 DAE. A record of the number of stolon present, tuber number, flowering present was recorded.

To characterize PVY isolates, symptomatic potato leaf samples with a variety of name tags were collected from the survey locations as well as the three trial sites in Kenya. They were placed in refrigerated boxes and stored in bags. The systemic foliar symptoms of the gathered potato samples were assessed visually.

3.5 Data Analysis for the Study

Appropriate biostatistics and bioinformatics tools were employed for data analysis of the field and nucleotide sequences obtained from the study.

3.5.1 Field Data Analysis

Version 9 of SPSS was used to appropriately analyze data collected from the field survey and field trials of the potato mini tubers.

3.5.2 Molecular Data Analysis

The sequence data received following Sanger and next-generation sequencing was analyzed using bioinformatics techniques.

3.5.2.1 De-novo Assembly and Mapping

Raw readings were trimmed first with the 9.0 version of CLC Genomics Workbench, and quality scores were assigned. Contigs were constructed using the de novo assembly function CLCGW, with the following parameters recorded: automated word and bubble size, minimum cost length, mismatch cost, insertion cost, deletion cost, and similarity percentage. A BLAST search was performed on the generated Contigs to look for matched virus sequences in the Genebank. All contigs that were positively matched to the reference in the gene bank were imported into the most recent version of Geneious for mapping.

3.5.2.2 Genome Alignment

A minimum of twenty previously published whole PVY genome references were acquired from the Gene bank and integrated into Genius 9.0. The nucleotide alignments from these analyses were translated into proteins using the MAFFT translate align option, which was then verified visually.

3.5.3 Phylogenetic Analysis

To delineate the evolutionary relationships and contextualize the genetic divergence of the Potato Virus Y (PVY) sample isolated from Kenya, a comprehensive phylogenetic analysis was undertaken. Utilizing the NCBI Nucleotide Blast, a dataset comprising the PVY_Kenya genome and a selection of sequences representing diverse countries and continents within the first 100 sequences was compiled including a sequence of most appropriate virus as an outgroup for comparative analysis. Employing the MAFFT alignment tool (Katoh *et al.,* 2002) followed by Bayesian phylogenetic inference through MrBayes (Huelsenbeck & Ronquist 2001) within the SATO v0.1.4 pipeline (Wekesa *et al.,* 2021), a phylogenetic tree was constructed.

3.5.4 Recombination Analysis

To examine the role of recombination in PVY evolution, recombination analyses were employed using two different approaches to identify potential recombination events in PVY sequences concerning the Kenyan isolate. Potential recombinant and parental sequences were identified using seven different algorithms, including RDP, GENECONV, BOOTSCAN, MAXCHI, CHIMAERA, SISCAN, and 3SEQ, implemented in the OpenRDP (https://github.com/PoonLab/OpenRDP) packages. Default settings were used throughout.

3.5.5 Selection Pressure

Branch selection: In this investigation of selection pressures within the Potato Virus Y (PVY) phylogeny, a comprehensive analysis utilizing the aBSREL (Smith *et al.* 2015) and RELAX (Wertheim *et al.,* 2015) methods aimed to discern episodic diversifying and relaxed selection, respectively, focusing particularly on the PVY_Kenya branches in comparison to other phylogenetic groups. In the investigation of pervasive positive/diversifying and negative/purifying selection within the Potato Virus Y (PVY) genome using the FUBAR (Murrell *et al.,* 2013), compelling evidence emerged regarding the distribution and nature of selective pressures across multiple sites.

CHAPTER FOUR

RESULTS

4.0 Introduction

The analysis and reporting of the findings from this study are presented based on the objectives of the study. Descriptive statistics of objective one presented the prevalence of the PVY virus in the main potato-growing areas in Kenya. The field trials were carried out in 8 counties namely, Kiambu, Nyandarua, Meru, Bungoma, Elgeyo Marakwet, Kisii, Bomet, and Nakuru.

4.1. The Distribution of PVY in Main Potato Growing Areas in Kenya

This study sought to determine the distribution of PVY in eight main potato-growing counties in Kenya, namely Kiambu, Nyandarua, Nakuru, Meru, Bomet, Kisii, Elgeyo Marakwet, and Bungoma (Fig 4.1). A total of 71 farms across these counties were surveyed to collect data on the distribution and prevalence of Potato Virus Y. The PVY prevalence was determined by calculating the rate of the severity of PVY in each county and expressing it as a percentage of the total area where the experiment was conducted mathematically expressed as: Prevalence = (severity rate /Areas where the experiment was carried out per county) * 100%.

Analysis of data from farmers' fields revealed a varied distribution of PVY in the main potato-growing varying from one agroecological zone to the other (Table 4.1).

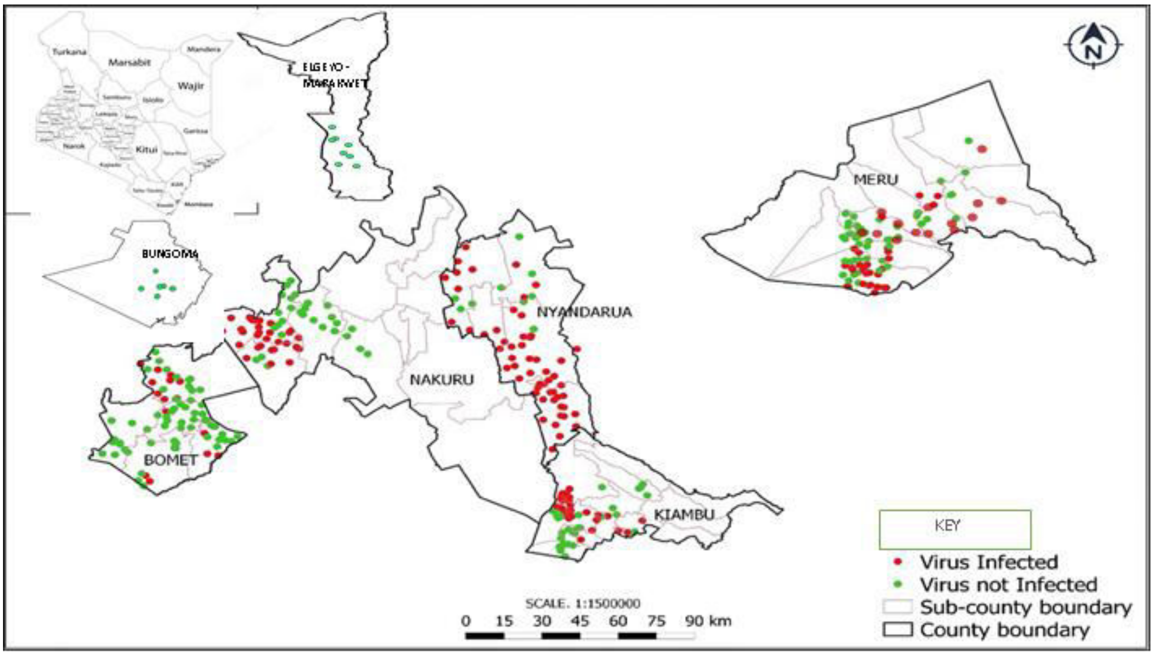

Figure 4.1: Map of the Study Area

Table 4.1: *PVY Prevalence in Main Potato Growing Areas*

County	Total Farm Visited	Areas Affected	PVY Distribution in %
Kiambu	13	8	61
Nyandarua	15	4	26.7
Nakuru	12	0	0
Meru	10	10	100
Bomet	10	4	40
Kisii	5	0	0
Elgeyo Marakwet	4	0	0
Bungoma	2	0	0

Source: Survey data 2018 - 2019

Results from this study revealed that PVY was most prevalent in Meru with the entire areas under experiment being totally 100% affected. Kiambu County had a PVY distribution of 61%, Bomet at 40%, and Nyandarua at 26.7%. Four counties, Bungoma, Nakuru, Kisii, and Elgeyo Marakwet recorded 0% on PVY infections. The general distribution of PVY in main potato growing areas revealed a non-prevalence of 63.38% and a PVY prevalence of 36.62% (Figure 4.2). However in the areas that has PVY infection in the famrs, the severity of the virus differed from one region to another due to probable reason of different PVY strain types, different weather conditions, potato variety type/ potato cultivar of choice, and innate plant response to Potato v irus Y infection.

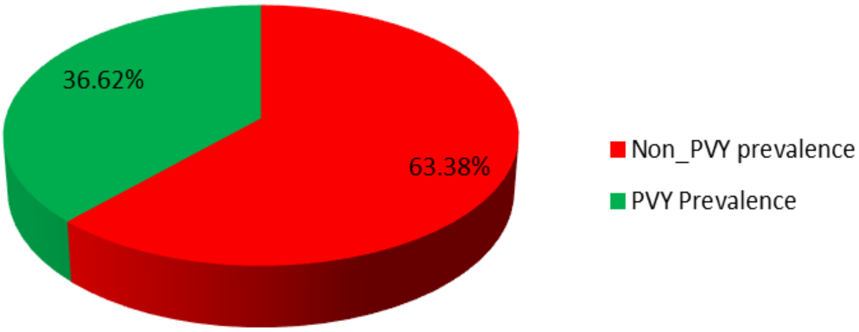

Figure 4.2: *Prevalence and non – preverelance of potato virus Y infection in the farmer fields surveyed*

Rugosity of the leaves, Light yellow/green mosaic pattern on veins, and stunted growth mosaic pattern on leaves were the guiding symptoms on the leaves during plant assessment (Fig. 4.3). A sample of these leaves representated were collected for further serological tests to ascertain the presence or absence of the virus, from Frm in Kimbo (Meru), and Bomet.

Figure 4.3: *PVY Infected potato leaves from farmer fields with PVY infection (mosaic patterns on leaves, crinkling of leaves, vein necrosis on the leaves)*

Different patterns of symptoms (Fig. 4.4) have been recorded by different authors after verification through serological tests. This is not limited to chlorotic mottling, severe rugose wrinkling, moderate to severe dwarfing, and premature death in severe infection. Further studies have been done on the potato tuber where the virus affects the general outlook of the tuber flesh.

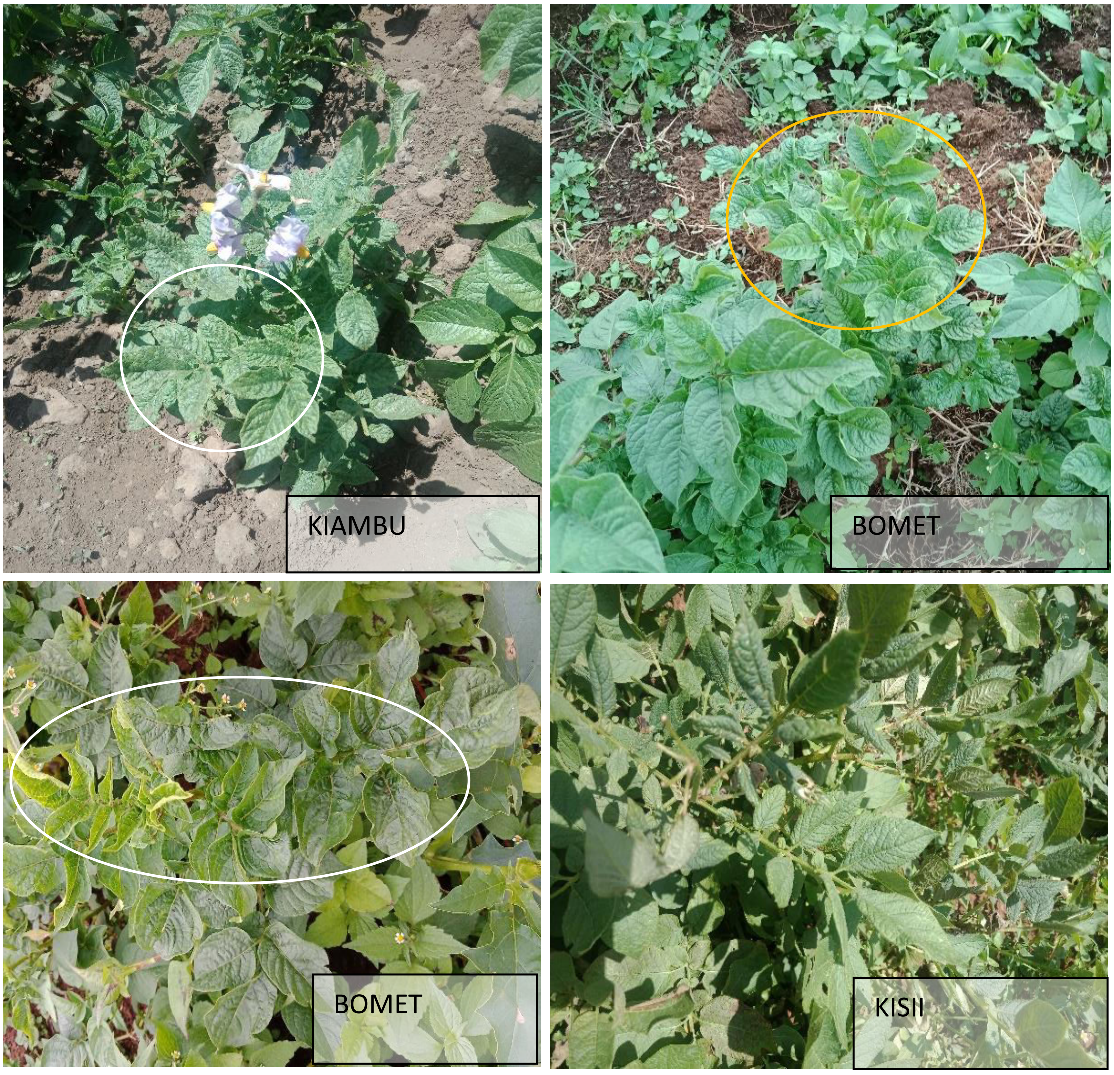

Figure 4.4: *PVY–Infected potato leaves (crinkling of leaves, vein necrosis on the leaves) from farms in Bomet and Kiambu, healthy plant from Kisii.*

During the survey, the symptomatic local vegetable *Solanum nigrum* was noted to present phenotypic symptoms of stunted growth, light yellow venation with yellow mosaic patterns, and deep green pigmentation on the leaf blade that appeared crinkled (Fig 4.5). other solanaceous plants that can host PVY include woody nightshade (*Solanum dulcamara*), buffalo bur (*Solanum rostratum*), and eggplant (*Solanum melongena).*

Figure 4.5: *Black nightshade (Solanum nigrum) infected with PVY*

The symptoms of Potato virus Y can range from very slight symptoms, mild light rings of darker brown in patterns to moderate necrotic ring spots and even loss of crop in severe infections. A test between subjects was calculated to assess the severity of PVY in the areas surveyed. The prevalence of PVY indicated possible recombinant strains was confirmed further by the general linear model inferential statistical test involving analysis of variance for severity as the dependent variable and counties as the independent variable (Table 4.2) The corrected model was statistically significant with a p-value < 0.05. Furthermore, the results showed that there exist differences in the severity between the eight counties, which constitute the main potato-growing areas in Kenya. The variation in severity of the PVY infecion in these counties was found to be significant with p-value = 0.000, less than the significance level of 0.05.

Table 4.2: *Test between subjects to assess severity of PVY in the area studied*

Dependent Variable: Severity of potato PVY virus in the surveyed area

Source	**Type III Sum of Squares**	**Df**	**Mean Square**	**F**	**Sig.**
Corrected Model	22.367[a]	7	3.195	5.393	.000
Intercept	14.241	1	14.241	24.038	.000
County	22.367	7	3.195	5.393	.000
Error	37.324	63	.592		
Total	90.540	71			
Corrected Total	59.692	70			

R Squared = 0.775 (Adjusted R Squared = 0.705)

The post hoc test to identify exactly which groups differ from each other indicated the exact severity numbers in each county (Table 4.3.) that revealed a possibility of the PVY infection being more severe in Meru at 1.70, Kiambu at 1.06, Nyandarua at 0.67, and Bomet at 0.5.

Table 4.3: *Severity of potato virus Y in the major potato growing areas*

The county where the survey was conducted	**N**	**Subset**		
		1	**2**	**3**
Nakuru	12	0.00		
Kisii	5	0.00		
Elgeyo marakwet	4	0.00		
Bungoma	2	0.00		
Bomet	10	0.50	0.50	
Nyandarua	15	0.67	0.67	
Kiambu	13		1.06	1.06
Meru	10			1.70
Sig.		0.204	0.164	0.052

Nakuru, Elgeyo Marakwet, Bungoma, and Kisii did not show any symptoms of the PVY virus (Fig 4.5).

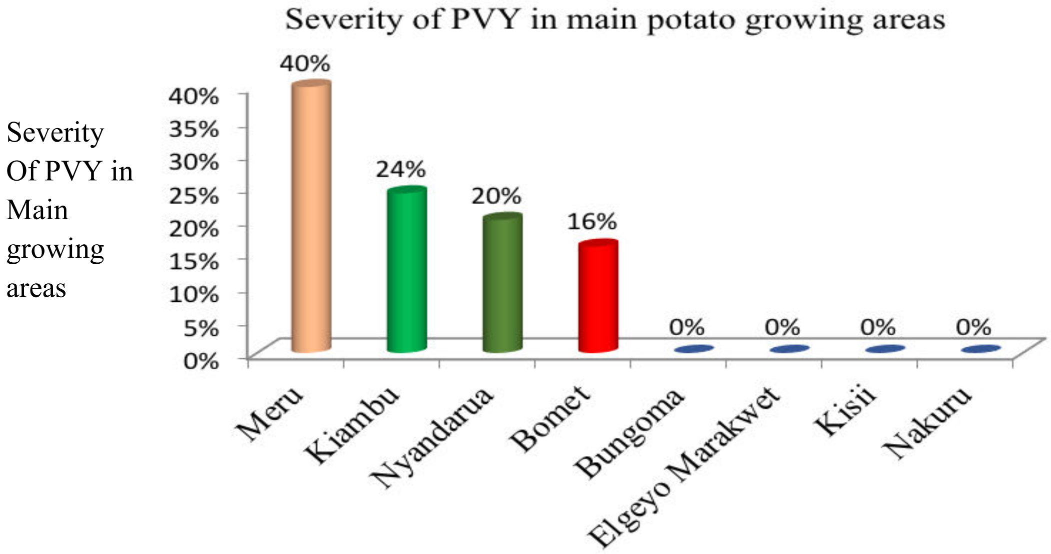

Figure 4.6: *Severity of PVY infections in main potato growing areas surveyed.*

There was a possibility of new strain recombinations of PVY in potato-growing areas of Kenya due to varying disease symptoms expression in the field (Table 4.1, 4.2, and 4.3; Figure 4.2 through to Figure 4.4). Further, the findings reveal varying symptoms on the potato plant; slight light/dark green mosaic patterns on leaves, veins, and shoots. However, the plants still showed signs of growth. In some survey areas, there were slight necrotic ring spots on leaves (score of 1), and the plants equally showed slight death (Score of 1) of growing points. In other experimental survey sites, there were moderate necrotic ring spots on leaves, moderate light/dark green mosaic patterns on leaf veins, or shoots with evidence of moderate death of the growing points. In other experimental fields, some plants revealed no sign of phenotypic presentation of the disease but tested positive for PVY. This can be concluded to mean that the virus is evolving at gene level, requiring verification of this findings using molecular tools. The statistical significance of the results was confirmed through the general linear model Univariate test with (F = 5.393, DF (7-1), $P < 0.05$).

This could be interpreted as there being possibilities of new or recombinant strains in the potato growing areas and their severity differing per county as revealed by the post hoc test.

4.2 Characterization of the Kenyan of PVY

4.2.1 Detection of PVY Strains using Sanger and RNA-seq

To further confirm combination events and mutations responsible for the changes of potato virus Y at the gene level, potato plants of different cultivars, collected across major potato-producing counties were subjected to serological and molecular testing revealing different strains existing in the Kenyan farmer fields. PVY samples from farmer fields collected during the survey were subjected to sanger sequencing that yielded data to discriminate the existing strain types. One PVY Kenyan isolate from Tigoni Potato research farm was subjected to Whole genome sequencing using RNA-seq.

4.2.2. PVY Strains Sample Processing

For the analysis of P1 and CP sequences, RNA extractions were performed on serologically confirmed PVY positive plants collected during the survey, and their concentrations were estimated using a Nanodrop spectrophotometer (Table 4.4). Samples with inadequate RNA concentrations were cloned into pGEM-T vectors to obtain complete P1 sequence data. From the list, 7 samples were settled upon. Before sequencing, PCR products were visualized through gel electrophoresis (Figure 4.7).

PVY accessions listed in Hu *et al.,* 2009 were utilized for the initial identification of PVY strains, with the caveat that additional sequence data is necessary for definitive assignment.

Table 4.4: *Quantification and quality of RNA for RT-PCR*

Serial No.	Potato variety	Sample ID	RNA Quality	Plant RNA Concentration (ng/µl)
1	Dutch	K1	2.02	239
2	Kenya Mpya	K4	2.07	481
3	Kenya Mpya	K6	1.94	187.1
4	Screen house variety	K12	1.95	72
5	Dutch	K16	1.99	176.5
6	Dutch F2	K20	2.04	307.5
7	Shangi	K23	2.05	375.9
8	Kenya Mpya	K27	1.88	212
9	Dutch	K30	2.03	724.6
10	Dutch	K33	1.77	210.9
11	Dutch	K36	2.09	236.5
12	Shangi	K37	2.00	437.3
13	Dutch	K38	2.09	519.3
14	Dutch	K40	2.07	216.1
15	Dutch	K69	2.08	204.8
16	Dutch	K74	2.09	229
17	Dutch	K76	1.97	141.3
18	Dutch	K78	1.96	76.1
19	Dutch	K80	2.06	271.6
20	Sherekea	K82	1.79	42

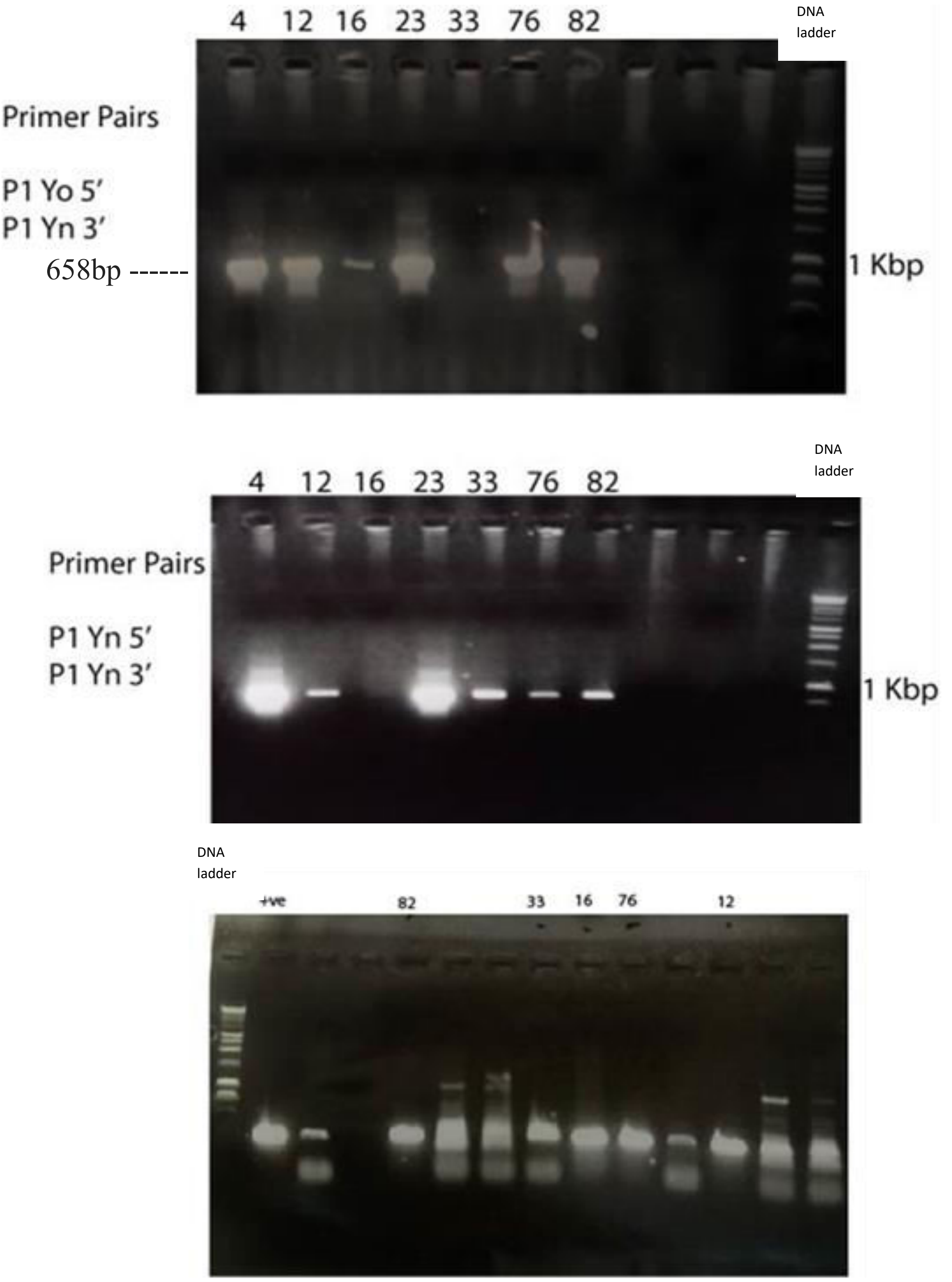

Figure 4.7: *Gel electrophoresis of PCR products of Potato Virus Y (+ve) samples collected from farmer fields.*

4.2.3 Phylogenetic Analyses of Different Potato Virus Y isolates

Different clades of the tree comprised different strains of PVY from different parts of the world. Distinctively, four monophyletic groups were observed from the original speciation event. The Kenyan isolate K4 (PVY$^{n:o}$ recombinant type) shared the oldest common ancestor with samples from MN539908.1, MN380536.1, KY863549.1 (collectively from Egypt), MK639789.1 (Kazakhstan), and KU757290 (Brazil), illustrating maximum support by 100% probability, suggesting a possible close revolutionary relationship in comparison to the rest of the isolates included in this analysis (Figure 4.8). The second clade of isolate K12, K16 K23, K32 and K76 (All Kenyan PVY strain isolates with partial sequences) shared the most recent common ancestor and are portrayed to have possibly undergone the most recent speciation event lately in comparison to the rest included in the study, while still displaying a maximum support probability of 91 %. This also portrayed a possible evolutionary relationship with the samples KY847990.1, JQ954357.1, EF027900.1 (Britain), MN539909.1, MN539905.1 (Egypt) several isolates from France (MN414588.1, MN414583.1, MN414582.1) and EF027900.1 (United Kingdom) where they shared the second common ancestor from the root node, still supported with a good probability of 91%. However, there is a possibility of a difference in time of evolution indicated by the lengths of the branch extensions from the oldest common ancestral node. This clade comprised majorly of PVY strain samples belonging to the ^{N,} or $^{O\text{ -}N}$ recombinant type. The last clade comprising KJ746449.1 (Poland), OR133700.1 (China), KY847997.1, and Sherekea (Kenyan PVY recombinant strain) shared the most recent common ancestor and distinctively had a lower similarity index supported by a 56 % probability from the rest of the clades, a possible indication of an evolutionary relationship that was divergent in comparison to the other clades revealed in the study.

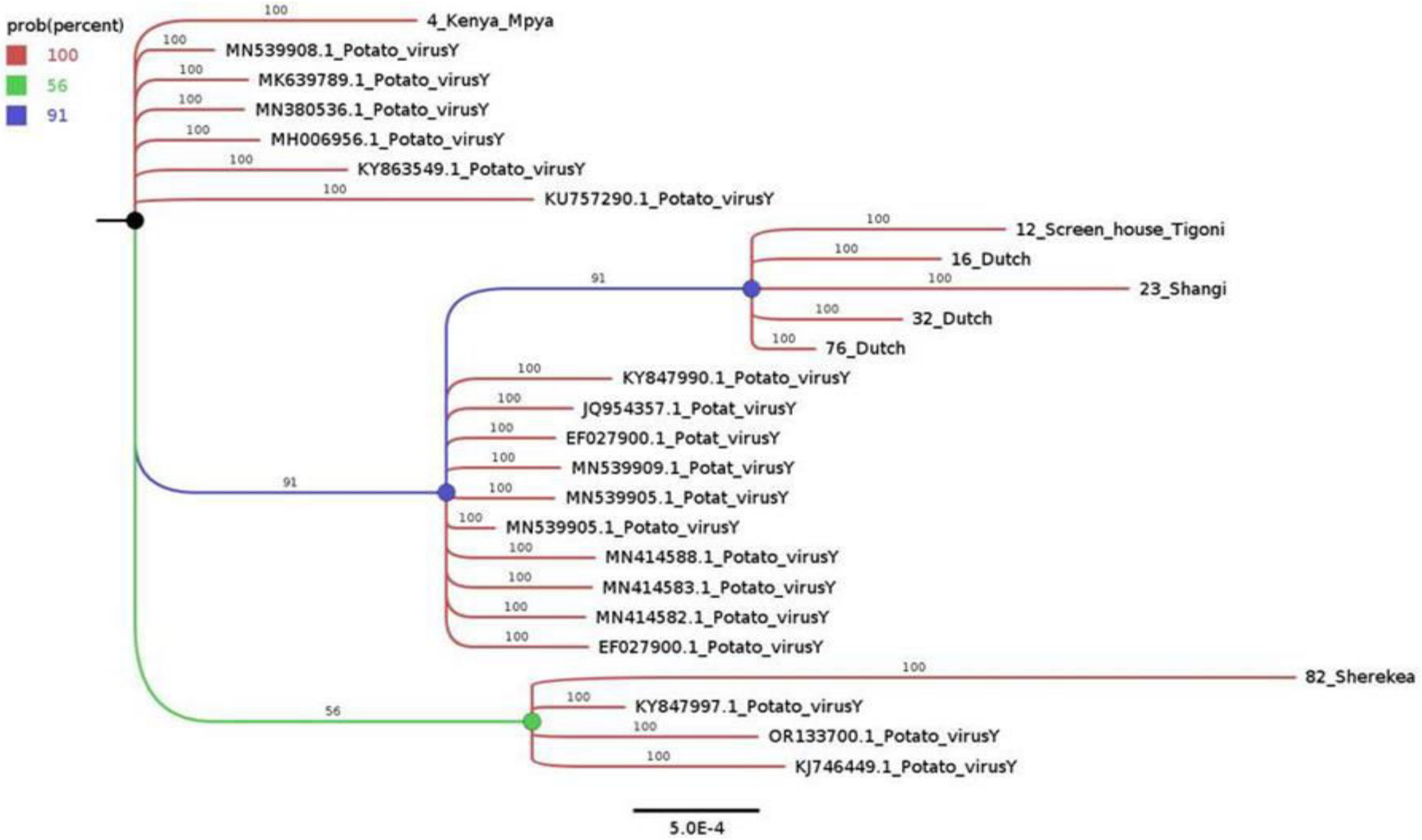

Figure 4.8: *Phylogenetic analysis of potato virus Y strains detected from Kenya based on the protein coat gene sequences (nt) generated using the MrBayes program, visualized by figtree*

The sequence reads obtained after subjecting the 7 samples to Sanger sequencing were adequate to distinguish the strain types existing in the farmer fields. Recombinant PVY strain type PVY^{N} and PVY $^{N:O}$ (Table 4.5) were found prevalent in a representative data set in the area of study sampled.

Table 4.5: *PVY strain type found among sampled potato accessions from farmer fields*

Sample ID	Natural host	P1- N type	P1 O-N recombinant	symptoms on leaves	Year of sampling	PVY type	Accession number
K4	Potato	✓		-mild mosaic -stunted growth	2018	N or recombinant	OR571473
K33	Potato	✓		- stunted short-growth Yellowing specks on leaves	2018	N or recombinant	OR571477
K12	Potato		✓	- Deepening venation -mild mosaic pattern	2018	Recombinant	OR571474
K23	Potato		✓	-moderate mosaic pattern -stunted growth	2018	Recombinant	OR571476
K76	Potato		✓	- crinkling of leaves - rugose mosaic	2018	Recombinant	OR571478
K82	Potato		✓	- mosaic pattern - deep venation -small leave sizes	2018	Recombinant	OR571479
K16	Potato	✓	✓	- deep green pigmentation - deep yellow specs -small leave size, crinkled	2018	N and Recombinant	OR571475

4.3 Phylogenetic Analysis of the Whole Genome of Potato Virus Y Kenyan Isolate.

To delineate the evolutionary relationships and contextualize the genetic divergence of the Potato Virus Y (PVY) sample isolated from Kenya, a comprehensive phylogenetic analysis was undertaken and revealed (Figure 4.9 a and b).

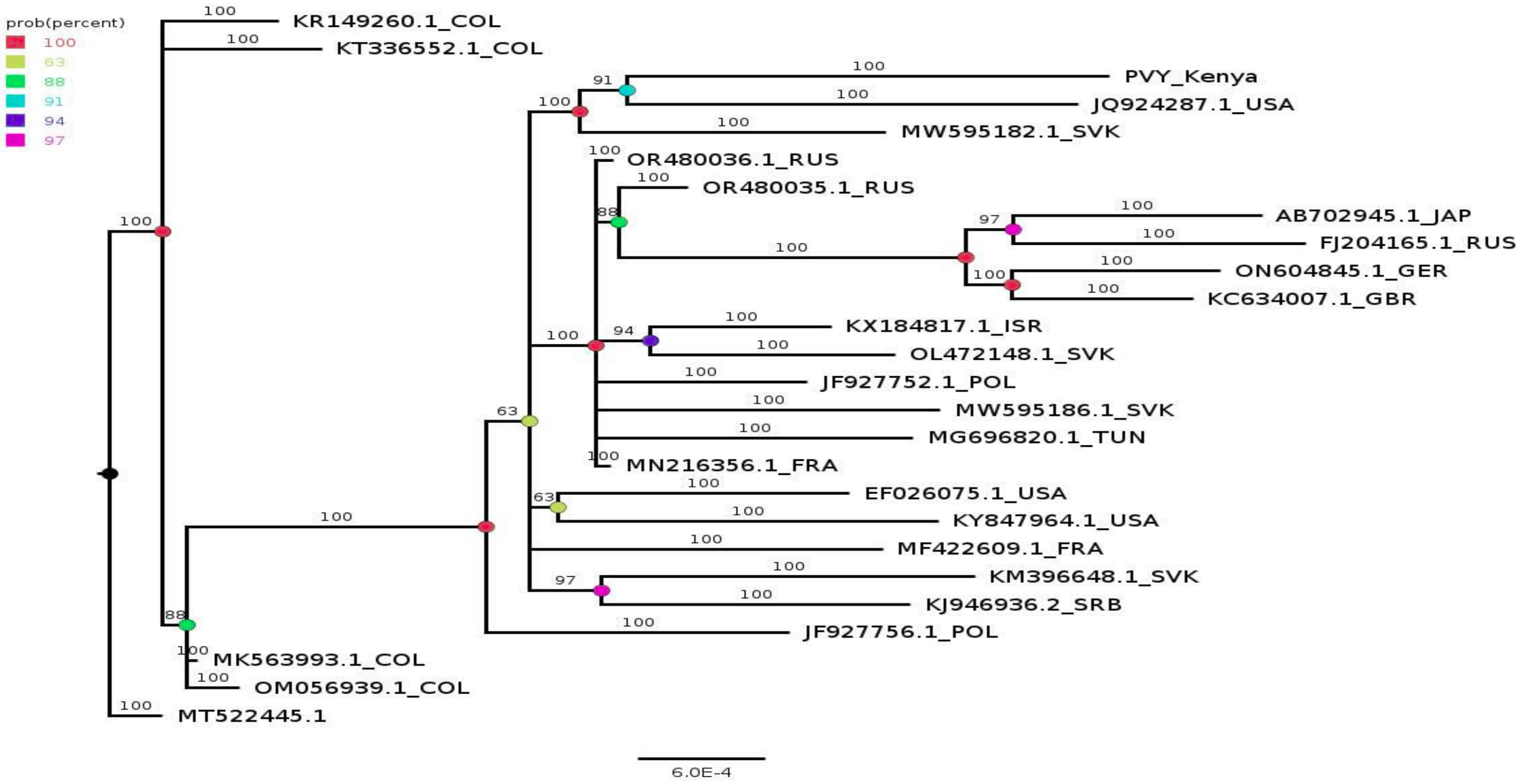

Figure 4.9a: *Phylogenetic analysis revealing the genetic relatedness and evolutionary connections of the PVY using the MrBayes program, visualized with the fig-tree program, using MT52245.1 as an outgroup*

In efforts to further delineate the evolutionary relationships and contextualize the genetic divergence of the Potato Virus Y sample isolated from Kenya, about full genome sequences of Potato virus Y obtained from the NCBI, a comprehensive phylogenetic analysis was undertaken to include more PVY accessions from Europe, Asia, Australia, and Africa, to demonstrate the genealogy of the virus on a larger world scale level using MrBayes program (Figure 4.9b), with a Pepper motile virus, accession number M11598 as the root. Accession number PP256240 from Australia shared the oldest common ancestor at the second node with the rest of the clades with other accessions included in this phylogenetic analysis. Nevertheless, with relevance to accessions from Africa, MF382053 and MF602675 from Zimbabwe were depicted to be more related to the PVY sample from South Africa KF770835 and ON604844 from Rwanda, as they shared the most recent common ancestor in contrast to the Kenyan isolate PP069009 used in this study, which showed the highest probability of having undergone the most recent speciation event. PVY_Kenya isolate (GenBank accession number PP069009) and isolates from the United States of America (JQ924287), with maximal support at 92% probability. It is worth noting that this analysis revealed a possible wider genetic distance existing Potato virus Y samples obtained from Africa, with samples obtained from the south of Africa (MF382053 and MF602675 from Zimbabwe, KF770835 from south Africa) being divergently different in comparison to the our representative from East Africa (PP069009). The phylogenetic analysis revealed a closer association of the Kenyan isolate having shared the most recent common ancestor with a representative from North Africa (MG696820 from Tunisia), in comparison to the rest of the accessions from Africa. This indicates a possible evolution arising from gene flow and /or different environmental conditions affecting the genome resulting in recombination events and unlimited mutations.

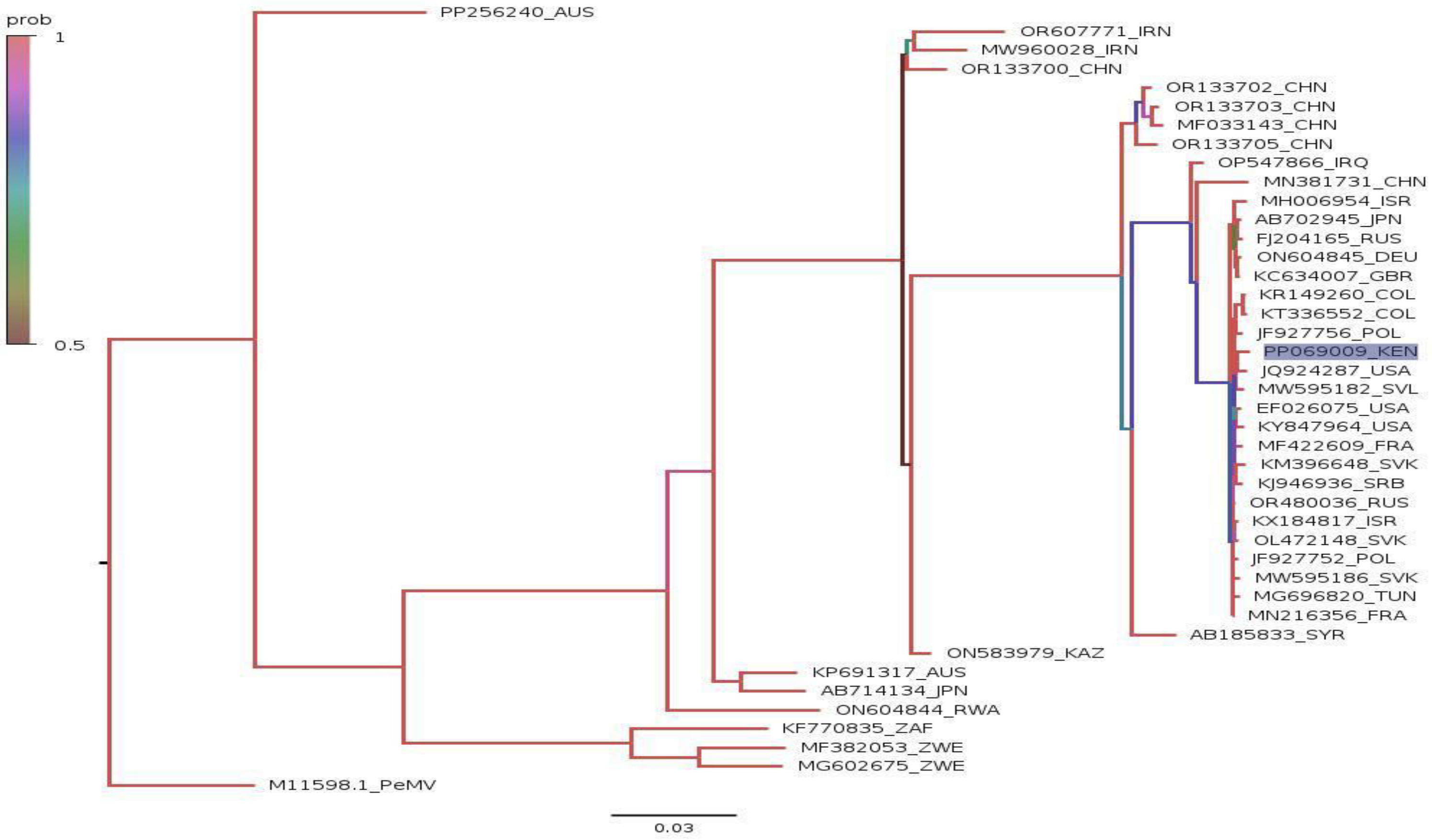

Figure 4.9b: *Genetic relatedness and evolutionary connections of the isolate PP069009 within the broader context of global PVY diversity using the MrBayes program, visualized with the fig-tree program with Pepper motile virus M11598. as the outgroup*

4.4 Recombination Analysis

The recombinant analysis conducted for the phylogeny of potato virus Y (PVY) revealed a mosaic-like genetic structure within the PVY Kenya isolate. Employing the GENECONV method, multiple instances of gene recombination events were identified across the genome, denoted by the specific gene segments (Start-End) involved. Notably, a significant number of these events, spanning various regions, yielded a p-value of zero, indicating an exceptionally high statistical significance. Furthermore, while most events exhibited a p-value of zero, a few instances displayed slightly higher yet still relatively low p-values, suggesting potential recombination events with slightly reduced statistical significance. The analysis highlights the complex nature of genetic exchanges within the PVY_Kenya genome, underscoring substantial genetic diversity arising from recombination events with multiple parental sequences. Notably, specific genomic regions exhibited varied strengths of evidence for recombination, further emphasizing the intricate mosaic pattern characterizing the genetic makeup of the PVY_Kenya isolate.

4.5 Selection Pressure

4.5.1 Branch Selection

Selective pressures are the factors that influence the survival and reproductive success of an organism in its environment. These pressures are crucial in shaping a population's genetic makeup through natural selection. However, contrary to expectations, neither aBSREL nor RELAX detected statistically significant evidence of episodic diversifying selection or relaxed selection within the PVY_Kenya branches or among the other groups in the phylogeny. This underscores the complexity of selective forces shaping PVY evolution and highlights the need for further nuanced investigations into the dynamics of selection within viral populations.

4.5.2 Site Selection Test

In the exploration of episodic positive/diversifying selection within the Potato Virus Y (PVY) genome, the MEME (Murrell *et al.,* 2012) method was employed, revealing evidence of selective pressure at a specific site, 2066, with statistical significance set at a p-value threshold of 0.05, as illustrated in Figure 4.10. Specifically, MEME identified a key parameter, β+, as pivotal in distinguishing between null and alternative models. In the null model, both β+ and β− are constrained, whereas in the alternative model, β+ remains unrestricted. Positive selection at individual sites is inferred when the β+ parameter exceeds α+ and is further validated as significant through the likelihood ratio test. This signifies that site 2066 exhibited characteristics indicative of positive selection, highlighting its potential importance in the adaptive evolutionary process of PVY.

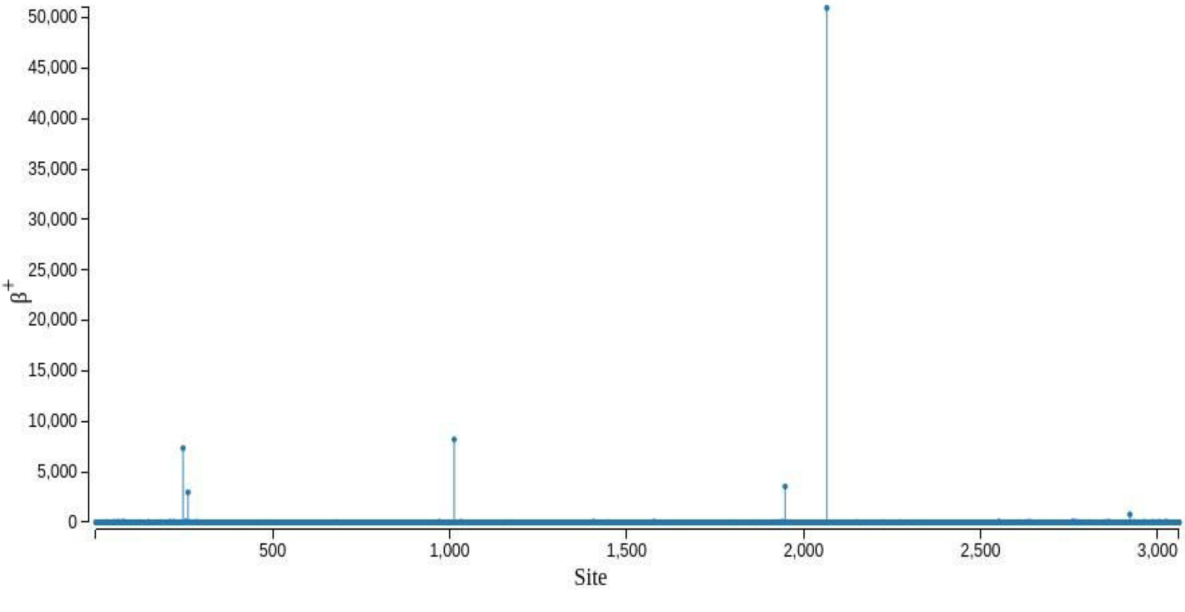

Figure 4.10: *Demonstration of episodic positive/diversifying selection within the Potato Virus Y PVY genome , selective pressure at 2066 position, at a (p < 0.05)*

The analysis was conducted under the General Time Reversible (GTR) model, complemented by the specific model fitting (AICc = 31645.59, log L = -15721.77). FUBAR detected pervasive positive/diversifying selection at five distinct sites and pervasive negative/purifying selection at 267 sites, each with a posterior probability (Prob [α > β]) exceeding 0.9. Notably, positive selection sites clustered predominantly towards the end of the alignment, while purifying selection was concentrated at the beginning, as depicted in Figure 4. 11.

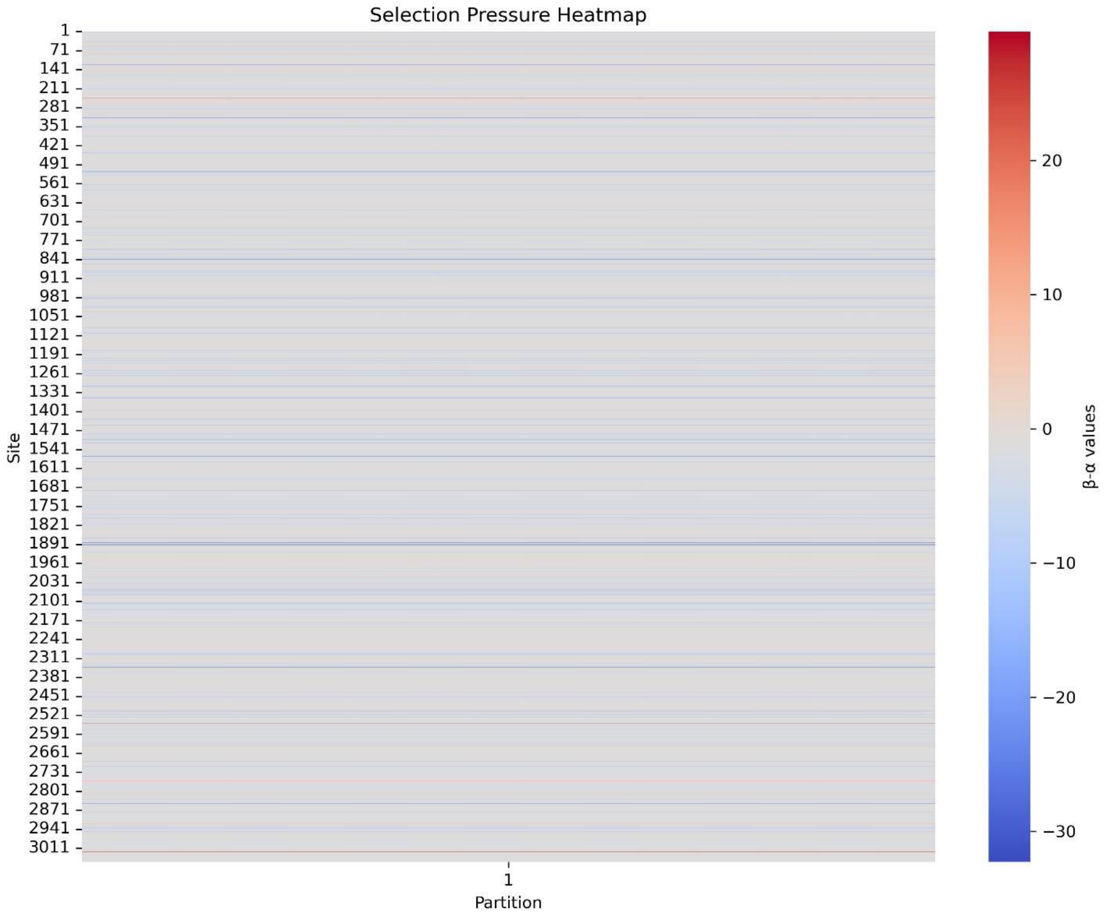

Figure 4.11: *Pervasive positive/diversifying and negative/purifying selection within the PVY genome at 267 sites*

The identification of pervasive positive/diversifying selection at five specific sites and pervasive negative/purifying selection at 267 sites within the Potato Virus Y (PVY) genome, each supported by a robust posterior probability (Prob [α > β] > 0.9), unveils the complex interplay of selective forces shaping the genetic landscape of this viral population. The notable clustering of sites under positive selection towards the terminal regions of the alignment and the concentration of sites experiencing purifying selection at the beginning suggests a spatially distinct pattern of evolutionary pressures along the PVY genome. This spatial distribution implies a differential functional significance across the viral genome, where regions towards the end potentially experience more adaptive changes, likely associated with factors such as host interactions, immune evasion, or adaptation to specific environmental conditions. Conversely, the regions exhibiting purifying selection at the start encompasses critical genetic elements vital for the virus's replication, structural integrity, or conserved functional domains essential for its life cycle. Such divergent selective pressures across the genome signify a delicate balance between adaptive evolution, where beneficial mutations confer advantages, and the preservation of essential genomic elements to maintain viral fitness. These findings underscore the dynamic nature of PVY evolution, emphasizing the pivotal role of selective pressures in shaping the genetic diversity and adaptability of this virus, thereby providing valuable insights for understanding its evolutionary strategies and potential implications for disease management and control.

4.6 Population Genetics Analysis of Potato Virus Y (PVY) Phylogeny

An alignment of Potato Virus Y (PVY) sequences was generated using the Muscle program v5 (Edgar *et al.,* 2004) and subsequently analyzed for haplotype with DnaSP v6 (Rosaz *et al.,* 2017). The program allows the thorough characterization of the levels and designs of DNA sequence disparity at diverse time scales, using polymorphic variants (intraspecific data), divergence data (interspecific or interpopulation data), or a combination of both (Rozsaz *et al.,* 2017). It also allows for the analysis of different recreations under a wide array of demographic circumstances.

Analyzing PVY samples from distinct continents—America, Africa, Asia, and Europe—revealed a complex genetic landscape reflective of the virus's worldwide distribution and diversity. The calculated nucleotide diversity (π) of 0.00354881 emphasized a relatively low overall genetic diversity within the PVY population, despite its multi-continental origins. The identification of 290 segregating sites, with 53 being parsimony-informative, highlighted substantial variations across the viral genome, showcasing the diverse genetic makeup of PVY strains originating from various countries within each continent. Tajima's D statistic further illuminated the population dynamics, indicating a value of -2.26205, signifying potential departures from neutral evolution (Table 4.6).

Table 4.6: *Haplotype diversity*

Haplotype Analysis	
Nucleotide diversity	pi = 0.00354881
No segregating sites	290
No parsimony-informative sites	53
Tajima's D statistic	D = -2.26205
Analysis of Molecular Variance (AMOVA)	
Genetic differentiation	$ø_{ST}$= 0.45224 ($p<0.001$)
Fixation indices	Phi_ST: 0.45224 ($P < 0.001$)
	Phi_SC: 0.39131 ($p = 0.006$)
	Phi_CT: 0.10010 ($p = 0.161$)

This finding implies various evolutionary pressures acting upon PVY populations across continents, potentially leading to an excess of low-frequency polymorphisms or recent population growth within specific geographical regions. Subsequent analysis using the Analysis of Molecular Variance (AMOVA) unveiled a structured genetic landscape within the PVY population across continents. The $ø_{ST}$ value of 0.45224, indicative of genetic differentiation among continents, portrayed a moderate yet discernible level of differentiation between the PVY strains originating from America, Africa, Asia, and Europe. The fixation indices (Phi_ST, Phi_SC, Phi_CT) provided a comprehensive understanding of genetic variance across different hierarchical levels. Phi_ST (0.45224) demonstrated substantial genetic differentiation among continents, while Phi_SC (0.39131) indicated genetic variation among populations within continents, albeit to a lesser extent. However, Phi_CT (0.10010) did not achieve significance ($p = 0.161$), implying limited variation among continents relative to the total genetic diversity observed. Significance testing of these indices reaffirmed the observed genetic structure, with both Phi_ST ($p < 0.001$) and Phi_SC ($p = 0.006$) showing significant values, indicating tangible genetic structure among continents and populations. These findings underscore a structured genetic landscape within the PVY population across continents, characterized by moderate differentiation among continents, low nucleotide diversity, and potential departures from neutrality. This multi-continental analysis emphasizes the need for further investigations into the specific evolutionary dynamics and environmental pressures shaping PVY populations within distinct geographical regions, essential for developing tailored management and control strategies on a global scale.

4.6.1 Haplotype Diversity

Haplotype networks are used in the analysis of population genetic data to visualize genealogical relationships at the intraspecific level, as well as to make inferences about biogeography and history of populations (Leigh *et al.,* 2015). Haplotype networks have been defined (Manolopoulou *et al.,* 2011) but popArt embraces the least spanning, median-joining, and TCS network methods as well as AMOVA (Excoffier *et al.,* 2005) and Tajima's D statistic (Tajimas *et al.,* 1989). With the primary function of POPART being to infer and visualize genetic relationships among intraspecific sequences, an extensive haplotype list was generated from the DnaSP v6 and further analyzed with PopArt 1.7.2 (Leigh *et al.*, 2015) program to generate haplotype networks and maps. The resulting list depicted a diverse spectrum of haplotypes, each characterized by a unique combination of sequences identified by accession numbers and country codes, denoting their geographical origins. The frequencies associated with these haplotypes provided valuable insights into the genetic diversity and distribution of PVY strains across different continents—America, Africa, Asia, and Europe. The diverse array of haplotypes signifies the potential evolutionary divergence or recombination events within the PVY population. This information served as crucial input for constructing phylogenetic or haplotype networks (Figure 4.12a) and maps (Figure 4.12b) using the PopArt program, enabling visual representations that illustrate the genetic relationships among PVY types from various geographic locations. Overall, 23 haplotypes were identified based on the PVY sequences drawn from different parts of the world (Figure 4.12a). Haplotype 1 is comprised of the Kenyan whole genome sequence PP069009. PVY sequences from Russia and France were found in haplotype 2. Haplotypes 9,11,15 and 19 were from Colombia. Haplotypes 5 and 7 were traced back to Poland. Haplotypes 6, 10, and 16 were from Slovakia. These networks and maps are expected to offer comprehensive insights into the evolutionary history, migration patterns, and sources of genetic diversity within the global PVY population, contributing significantly to my understanding of the virus dynamics and adaptation across diverse environments.

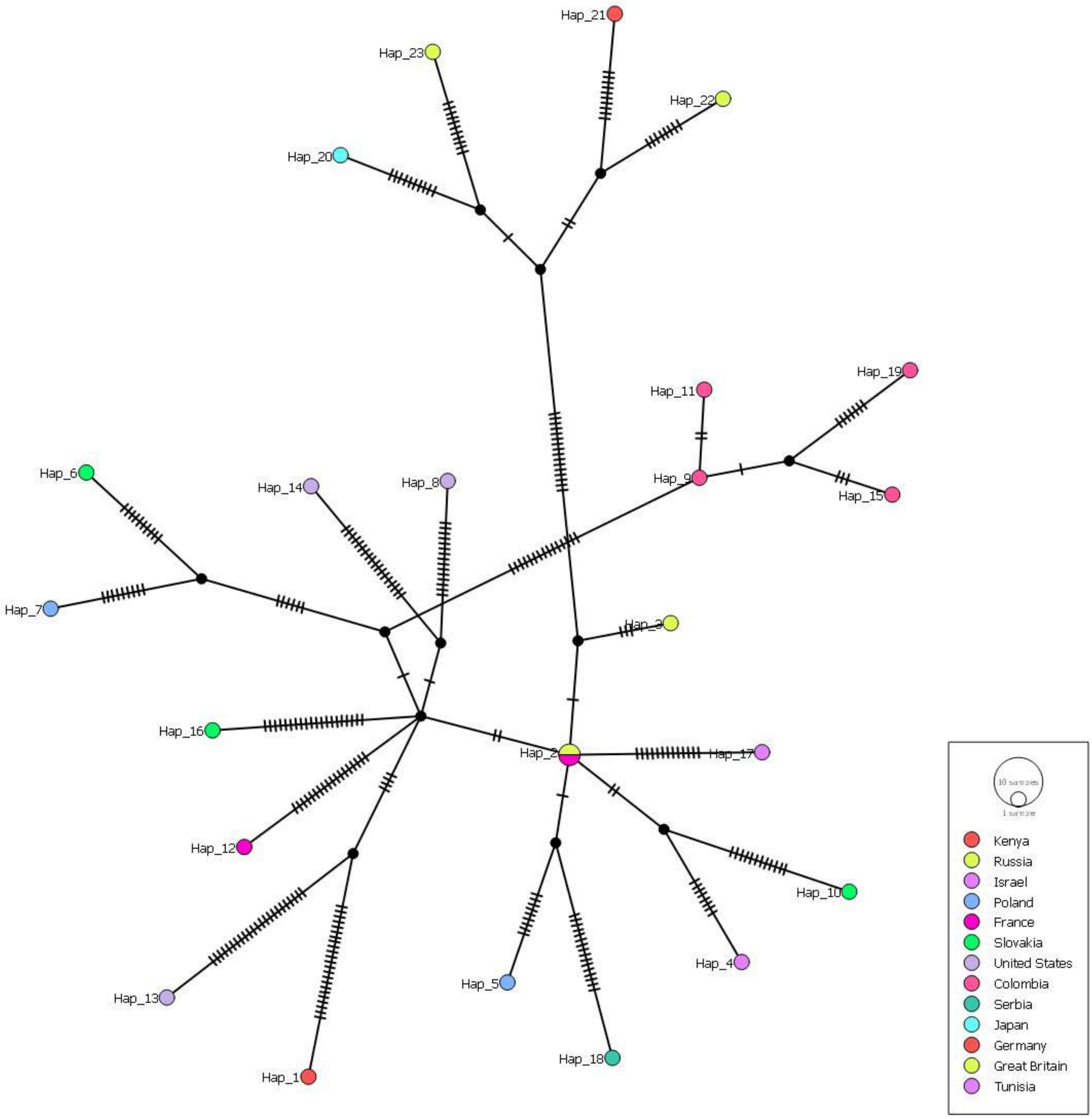

Figure 4.12a: *Haplotype networks of selected potato virus Y genome sequences revealing their geographical origins around the World.*

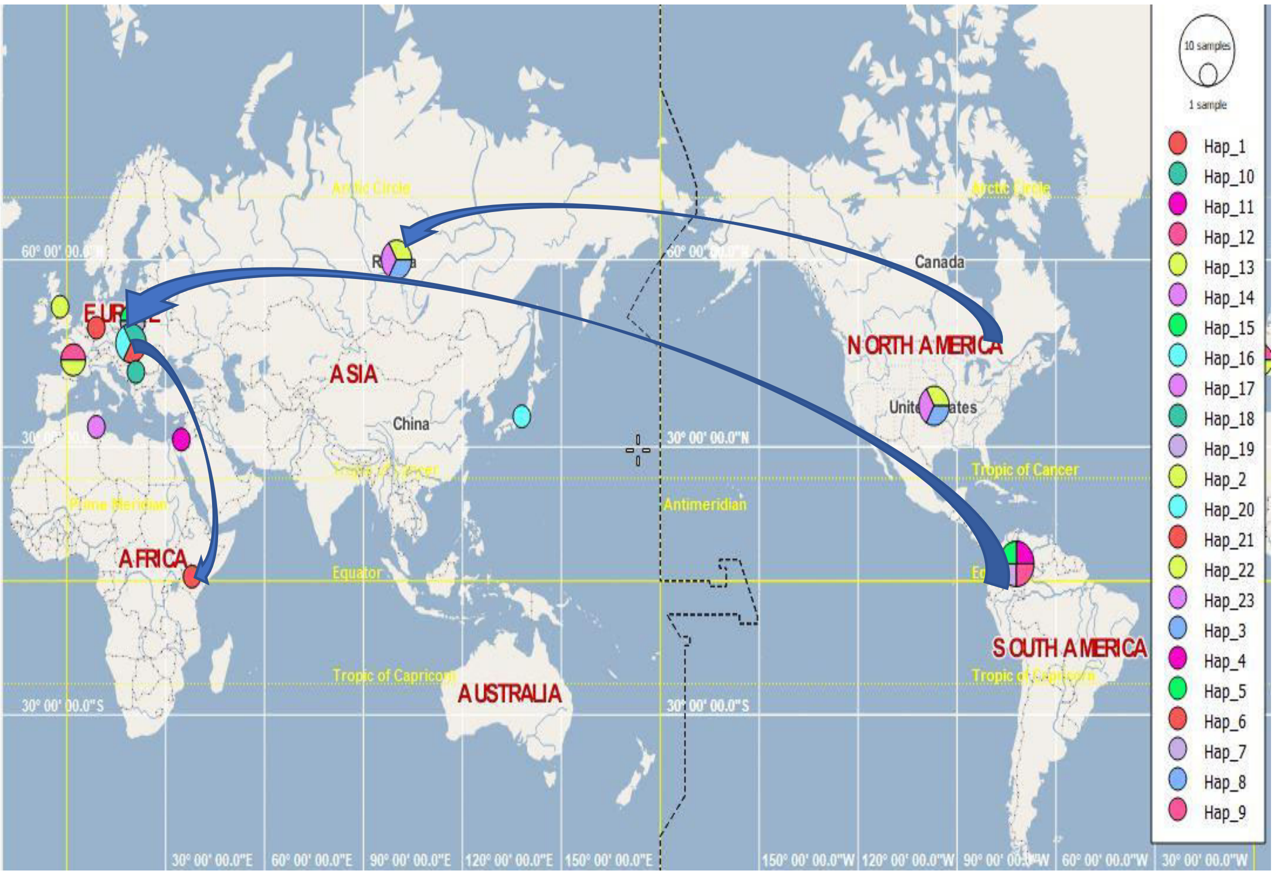

Figure 4.12b: *Haplotype maps providing insights into the genetic diversity and distribution of PVY strains across different continents.*

4.7 Screening Potato Populations for Resistance to PVY and Earliness

The experiment was carried out in three field experimental areas Kakamega, Sang'alo, and Alupe (Figure 4.13). Each field trial comprised four replicated blocks (1, 2, 3, and 4), and each block had a randomized plot of genotypes (Appendix 2). Further, each genotype plot was planted with three tubers spaced 0.5 m apart with a 1 m path to separate the plots. The trial used 60 genotypes from the 06H1 population to assess tuber initiation, at days 40, 70, and 90. One plant in each plot was dug up and phenotypic characteristics (growing well, stunted, wilted virus symptoms, severe death of plant, and complete death of growing plant) were assessed and scored visually for virus infection. The results for this objective are presented per region or experimental area and a final combination of the three field experimental areas.

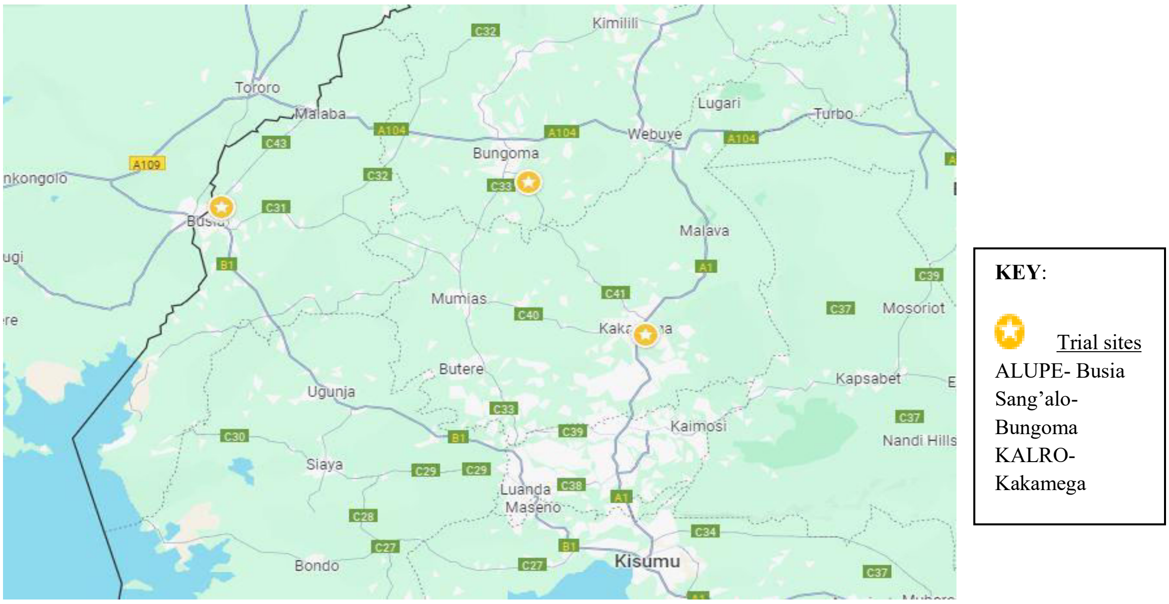

Figure 4.13. *Section of the Kenyan map field trial sites for mini tubers evaluation in Kakamega, Busia, and Bungoma.*

4.7.1 Screening Potato Populations for Resistance to PVY and Earliness in Kakamega at Day 40.

The screening of the potato population for resistance of PVY and earliness in the Kakamega region at day 40 post-emergence harvest was annalysed using a Univariate general linear model, where the genotypes (treatment) were the independent variable and the phenotypes were the dependent variable (Table 4.7). Blocking created homogeneity of other factors such as temperature, soil texture, soil fertility, rainfall, etc.), such that the main effect on the results was only due to the treatment. Through randomization, each genotype (treatment) was replicated once per block.

Table 4.7: *Tests of Between-Subjects Effects in Kakamega at day 40*

Dependent Variable: Phenotypes

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	416.746[a]	59	7.063	6.076	.000
Intercept	3519.004	1	3519.004	3027.100	.000
Genotype	416.746	59	7.063	6.076	.000
Error	209.250	180	1.163		
Total	4145.000	240			
Corrected Total	625.996	239			

a. R Squared = .666 (Adjusted R Squared = .556)

The results of the tests between different genotypes revealed that there was an observable difference in earliness in Kakamega at day 40 post-emergence harvest. The variation was statistically significant with (F = 6.076, DF (59-1), $p < 0.05$). The adequacy of the corrected general linear model was validated by R Squared = .666, which shows that 66.6% of the variations in the phenotypes are explained by the genotypes (treatments).

Post hoc analysis showed the average phenotype for each genotype in the Kakamega trial area at day 40 had a mean difference between the genotypes (treatments) in terms of the outcome of the experiment. 57 Genotypes, an equivalent of 95%, either revealed the symptoms of the potato plants wilting, severe death of the growing points, or the complete death of the growing points. Most genotypes had no symptoms of PVY infection. Genotypes 2, 9, 13, 26, 32, 38, 44, and 57 started wilting but were still surviving or showing signs of recovering. Upon further testing, PVY infection was ruled out.

4.7.2 Screening Potato Populations for Resistance to PVY and Earliness in Kakamega on Day 70

The screening of the potato population for resistance of PVY and earliness in the Kakamega region at day 70 post-emergence harvest was generated using a univariate general linear model (Table 4.8), where the genotypes (treatment) were the independent variable and the phenotypes were the dependent variable.

Table 4.8: *Tests of Between-Subjects Effects in Kakamega at day 70*

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	202.933[a]	59	3.440	3.918	.000
Intercept	4301.067	1	4301.067	4899.949	.000
Genotype	202.933	59	3.440	3.918	.000
Error	158.000	180	.878		
Total	4662.000	240			
Corrected Total	360.933	239			

a. R Squared = .562 (Adjusted R Squared = .419)

Results of the tests between different genotypes revealed a difference in potato population for earliness in Kakamega at day 70 post-emergence harvest. The variation was statistically significant with (F = 3.918, DF (59-1), $p < 0.05$). The adequacy of the corrected general linear model was validated by R Squared = .562, which shows that 56.2% of the variations in the phenotypes are explained by the genotypes (treatments).

However, the post hoc results show that at day 70, there was not much distinction between the genotypes on resistance to the infection affecting the potato crop and earliness in Kakamega. There was no PVY disease phenotypic presentation on the potato plant.

Figure 4.14: *Kakamega trial site on routine field assessment.*

4.7.3 Combined Average Screening for Resistance to PVY and Earliness at Days 40 and 70 Kakamega Site

The average for each genotype for both days 40 and 70 was derived by summing the phenotype scores for all the blocks and dividing by four. The data was then analyzed for resistance of PVY and earliness. The tests between different genotypes for combined effect at days 40 and 70 (Table 4.9) revealed a difference in potato population earliness in Kakamega for the derived data.

Table 4. 9: *Tests of Between-Subjects Effects in Kakamega at days 40 and 70*

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	198.828[a]	59	3.370	6.306	.000
Intercept	3900.234	1	3900.234	7298.684	.000
Genotype	198.828	59	3.370	6.306	.000
Error	96.188	180	.534		
Total	4195.250	240			
Corrected Total	295.016	239			

a. R Squared = .674 (Adjusted R Squared = .567)

The difference was statistically significant with (F = 6.306, DF (59-1), $p < 0.05$). The adequacy of the corrected general linear model was validated by R Squared = .674, which shows that 67.4% of the variations in the phenotypes are explained by the genotypes (treatments).

However, the post hoc results showed that at days 40 and 70 combined, there was not much distinction between the genotypes on resistance to PVY and earliness in Kakamega. This showed that there were other factors affecting crop performance in the field because serological tests ruled out the possibility of potato virus Y affecting crop field performance.

4.7.4 Screening for Resistance to PVY and Earliness in Alupe Site at Day 40

Of the three sites included in this study for field crop performance testing i.e. Alupe, KALRO Kakamega, and Sang''alo Institute of Science and Technology, Alupe had the highest daily temperature recording and received the least amount of rainfall. The screening of the potato population for resistance of PVY and earliness in the Alupe region at day 40 post-emergence harvest revealed that there was a difference in potato population in earliness (Table 4.10) at day 40 post-emergence harvest.

Table 4.10: *Tests of Between-Subjects Effects in Alupe at day 40*

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	176.046[a]	59	2.984	1.612	.009
Intercept	1139.704	1	1139.704	615.594	.000
Genotype	176.046	59	2.984	1.612	.009
Error	333.250	180	1.851		
Total	1649.000	240			
Corrected Total	509.296	239			

a. R Squared = .546 (Adjusted R Squared = .511)

The variation was statistically significant with (F = 1.612, DF (59-1), $p < 0.05$). The adequacy of the corrected general linear model was validated by R Squared = .546, which shows that 54.6% of the variations in the phenotypes are explained by the genotypes (treatments).

A post hoc analysis showing the average phenotype for each genotype in the Alupe trial area at day 40 revealed a mean difference between the genotypes (treatments) in terms of the outcome of the experiment. 42 genotypes an equivalent of 70%, either revealed the signs of the potato plants wilting, severe death of the growing points, or the complete death of the growing points. However, 12 genotypes 2, 3, 11, 13, 14, 17, 35, 40, 43, 45, and 51 had a severity of 1, meaning there were no necrotic ring spots on leaves/tubers, no light/dark green mosaic patterns on leaves, veins or shoots, no stunted growth, and grew well. Therefore, at day 40 post-emergence harvest, genotypes 2, 3, 11, 13, 14, 17, 35, 40, 43, 45, and 51 showed remarkable survival ability and earliness despite the high temperatures recorded in Alupe at the time of the field trials.

The results of tuber yields in Alupe at day 40 post-emergence harvest are shown in Table 4.11

Table 4.11: *Selected tuber yield at Alupe trial site 40 days post-emergence*

Genotype	Tuber number	Average Tuber weight (g)
14	6	95
17	1	5
40	4	28.75
43	1	0.25
2	2	40
3	4	75
11	1	5
13	1	7
35	4	23.75
44	1	0.75
45	2	1.5
51	6	92.5

4.7.3 Screening of Potato Populations for Resistance to PVY and Earliness in Alupe Site at Day 70

The screening of potato population for resistance to PVY and earliness in the Kakamega region at day 70 post-emergence harvest revealed a difference in potato population resistance to PVY and earliness in Alupe at day 70 post-emergence harvest

(Table 4.12). The variation was statistically significant with (F = 2.529, DF (59-1), $p < 0.05$).

Table 4.12: *Tests of Between-Subjects Effects in Alupe at day 70*

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	149.183^{a}	59	2.529	2.529	.000
Intercept	3952.817	1	3952.817	3952.817	.000
Genotype	149.183	59	2.529	2.529	.000
Error	180.000	180	1.000		
Total	4282.000	240			
Corrected Total	329.183	239			

R Squared = .453 (Adjusted R Squared = .424)

The adequacy of the corrected general linear model was validated by R Squared = .453, which shows that 45.3% of the variations in the phenotypes were explained by the genotypes (treatments). The R-squared value of 45.3% was too small; meaning much of the variation (55%) in the phenotypes could not be explained by the treatment (genotypes) and its effect.

However, the post hoc results (Appendix 13) show that at day 70, there was not much distinction between the genotypes on resistance to PVY and earliness in Alupe.

4.7.4 Combined Average Screening of Potato Populations for Resistance to PVY and Earliness in Alupe at Day 40 and 70

The average for each genotype for days 40 and 70 was derived by summing the phenotype scores for all the blocks and dividing by four. The outcome of the tests between different genotypes for combined effect revealed a difference in potato population earliness in Alupe (Table 4.13). The difference was statistically significant with (F = 2.259, DF (59-1), $p < 0.05$.

Table 4.13: *Tests of Between-Subjects Effects for Alupe day 40 and 70 combined*

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	102.053[a]	59	1.730	2.259	.000
Intercept	2334.384	1	2334.384	3048.992	.000
Genotype	102.053	59	1.730	2.259	.000
Error	137.813	180	.766		
Total	2574.250	240			
Corrected Total	239.866	239			

R Squared = .525 (Adjusted R Squared = .517)

The adequacy of the corrected general linear model was validated by R Squared = .525, which shows that 52.5% of the variations in the phenotypes are explained by the genotypes (treatments).

Consequently, days 40 and 70 combined revealed not much distinction between the genotypes on earliness in Alupe. The severity of the crop loss increased towards day 70, when all potato populations wilted, stunted, or completely died. Additionally, the extremely high temperatures beyond what the crop could survive reduced the chances of crop growth beyond 40 days. All the genotypes showed either signs of wilting, stunted growth, or severe or complete death.

4.7.5 Screening Potato Populations for Resistance to PVY and Earliness in Sang'alo at Day 40

The screening of the potato population for resistance of PVY and earliness in the Sangalo region at day 40 post-emergence harvest between different genotypes (Table 4.14) revealed a difference in potato earliness. Notably the variation was statistically significant with (F = 5.245, DF (59-1), $p < 0.05$).

Table 4.14: *Tests of Between-Subjects Effects in Sang'alo at day 40*

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	424.246[a]	59	7.191	5.245	0.000
Intercept	2166.004	1	2166.004	1580.064	0.000
Genotype	424.246	59	7.191	5.245	0.000
Error	246.750	180	1.371		
Total	2837.000	240			
Corrected Total	670.996	239			

a. R Squared = 0.732 (Adjusted R Squared = 0.682)

The adequacy of the corrected general linear model was validated by R Squared = .682, which showed that 73.2% of the variations in the phenotypes are explained by the genotypes (treatments). A Post hoc analysis (Appendix 15) showed a difference between the genotypes (treatments) in terms of the outcome of the experiment the average phenotype for each genotype in the Sang'alo trial area at day 40, there was. 47 genotypes an equivalent of 78%, either revealed the signs of the potato plants wilting, stunned, severe death of the growing points, or the complete death of the growing points. 13 genotypes 18, 24, 28, 34, 36, 37, 38, 43, 44, 45, 46, 51, and 56 had a severity of 1, meaning there were no necrotic ring spots on leaves/tubers, no light/dark green mosaic patterns on leaves, veins or shoots, no stunted growth, and grew well. Therefore genotypes 18, 24, 28, 34, 36, 37, 38, 43, 44, 45, 46, 51, and 56 showed remarkable earliness in the Sang'alo trial area. Serological tests revealed no presence of PVY infection. The survival rate of the potato experimental crop was better and more promising in the Sangalo test site in comparison to Kakamega and Alupe. Nevertheless, 47 genotypes were stunted, severely dying, or dead which indicated the possibility of having an external factor influencing the crop performance e.g. unpredictable weather conditions, status of the soil fertility, etc. Randomly selected tuber number and weight (Table 4.15) in Sangalo at day 40 post-emergence harvest revealed great potential in crop performance.

Table 4.15 *Tuber number and average tuber weight in (g) on selected genotypes at 40 days post-emergence*

Genotype	Tuber number	Average Tuber weight (g)
18	3	21
24	3	16.25
28	5	18
38	2	10.25
43	1	2
44	3	15
452	2	5.25
46	2	11
56	1	3
51	5	19

4.7.6 Screening Potato Populations for Resistance to PVY and Earliness in Sang'alo at Day 70

The screening of the potato population for resistance of PVY and earliness in the Sang'alo region at day 70 post-emergence revealed a difference in potato earliness in Sang'alo, 70 post-emergence harvest (Table 4.16). The variation was statistically significant with (F = 4.322, DF (59-1), $p < 0.05$).

Table 4.16:*Tests of Between-Subjects Effects in Sangalo at Day 70*

Dependent Variable: Phenotypes

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	158.912[a]	59	2.693	1.322	.004
Intercept	1701.337	1	1701.337	835.012	.000
Genotype	158.913	59	2.693	4.322	.004
Error	366.750	180	2.038		
Total	2227.000	240			
Corrected Total	525.662	239			

a. R Squared = .630 (Adjusted R Squared = .604)

The adequacy of the corrected general linear model was validated by R Squared = .604, which shows that 63.0% of the variations in the phenotypes are explained by the genotypes (treatments). Post hoc analysis Appendix 16) showed the average phenotype for each genotype in the Sang'alo trial area at day 70. Based on these findings, there was a difference between the genotypes (treatments) in terms of the outcome of the experiment.

4.7.7 Screening Potato Populations for Resistance to PVY and Earliness in Sang'alo at Day 90

The screening of potato population for resistance of PVY and earliness in the Sang'alo region at day 90 post-emergence harvest (Table 4.17) revealed a difference in the potato population earliness in Sang'alo at day 90 post-emergence harvest. The variation was statistically significant with ($F = 3.424$, DF (59-1), $p > 0.05$).

Table 4.17: *Tests of Between-Subjects Effects in Sang'alo at day 90*

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	386.052[a]	3	128.684	3.424	.736
Intercept	201284.619	1	201284.619	663.508	.000
Phenotypes 90	386.052	3	128.684	.424	.002
Error	71593.948	236	303.364		
Total	295240.000	240			
Corrected Total	71980.000	239			

a. R Squared = .751 (Adjusted R Squared = .712)

The adequacy of the corrected general linear model was high with a value of 0.751, which shows that 75.1% of the variations in the phenotypes are explained by the genotypes (treatments) at day 90. This means that much of the variation (75.1%) in the phenotypes was explained by the treatment (genotypes) and its effect in Sang'alo at day 90 post-emergence harvest.

A post hoc analysis (Appendix 17) showed the average phenotype for each genotype, revealing a difference between the genotypes (treatments) in terms of the outcome of the experiment. 56 genotypes an equivalent of 93.3%, either revealed mild signs of the potato plants wilting. Genotypes 18, 28, 38, and 43 had a severity of 1, meaning there were no necrotic ring spots on leaves/tubers, no light/dark green mosaic patterns on leaves, veins, or shoots, no stunted growth, and grew well. Thus, at day 90 post-emergence harvest, most crop performance was above average.

4.7.8 Combined Average Screening of Potato Populations for Resistance to PVY and Earliness in Sang'alo at Day 40, 70, and 90

The average for each genotype for both days 40 and 70 was derived by summing the phenotype scores for all the blocks and dividing by four. Results of the tests between different genotypes for combined effect at day 40, 70, and 90 revealed a difference in potato population resistance to PVY and earliness in Kakamega for the derived data (average of day 40, 70, and 90 post-emergence harvest). The difference was statistically significant (Table 4.18) with (F = 3.733, DF (59-1), $p < 0.05$).

Table 4.18: *Tests of Between-Subjects Effects in Sangalo at days 40, 70, and 90 combined*

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	4066.705[a]	12	338.892	1.133	.334
Intercept	96389.323	1	96389.323	322.181	.000
Sphenotypes	4066.705	12	338.892	3.733	.014
Error	67913.295	227	299.178		
Total	295240.000	240			
Corrected Total	71980.000	239			

a. R Squared = .616 (Adjusted R Squared = .587)

The adequacy of the corrected general linear model was validated by R Squared = .616, which shows that 61.6% of the variations in the phenotypes are explained by the genotypes (treatments).

A Post hoc analysis (Appendix 18) showed the average phenotype for each genotype in the Sang'alo trial area at days 40, 70, and 90 combined. Based on these findings, there was a difference between the genotypes (treatments) in terms of the outcome of the experiment. 53 genotypes an equivalent of 88%, either revealed mild signs of the potato plants wilting, with no PVY infection was recorded. Only seven genotypes 18, 24, 28, 34, 36, 37, 38, and 43 had a severity of 1, meaning there were no necrotic ring spots on leaves/tubers, no light/dark green mosaic patterns on leaves, veins, or shoots, no stunted growth, and grew well. Therefore, at day 40, 70, and 90 combined post-emergence harvest, genotypes 18, 24, 28, 34, 36, 37, 38, and 43 showed remarkable growth. All the genotypes were resistant to Potato Virus Y in the Sang'alo field trial site.

4.7.9 Screening Potato Populations for Resistance to PVY and Earliness Combining Kakamega, Alupe, and Sang'alo

In the three trial sites, all genotypes that showed symptoms of PVY tested negative for PVY infection. In Alupe, the temperatures were too high for the survival of the crop, and almost all the genotypes wilted or died at day 70 post-emergence harvest and could not get to day 90. Sang'alo, conditions were prime, so much more data was collected, however, close to or at day 90 post-emergence harvest nearly all the crops in the blocks were dying but were mature for harvesting. Therefore, the data used for this testing summed all the phenotype test scores for each genotype per experimental area at days 40 and 70 and derived their average. This created three sets of variables namely genotypes and regions as independent variables and the mean of phenotypes per genotype per region as the response variable.

A univariate general linear model test that combined genotypes and regions as independent variables and phenotypes (mean per genotypes per region) was run (Table 4.19) revealing a difference in potato populations' resistance to PVY and earliness.

Table 4.19: *Tests of Between-Subjects Effects combining genotypes and regions*

Dependent Variable: Av phenotypes

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	112.895[a]	61	1.851	3.483	.000
Intercept	1923.545	1	1923.545	3619.817	.000
Genotype	55.042	59	.933	3.756	.005
Region	57.853	2	28.927	54.435	.000
Error	62.704	118	.531		
Total	2099.144	180			
Corrected Total	175.599	179			

a. R Squared = .643 (Adjusted R Squared = .608)

The variation was statistically significant with (F = 3.756, (DF=(61-2), P < .05). An indication that different screened potato populations showed varied levels of earliness. Equally, the echelons of earliness differed per region, which was significant at (F = 54.43.435, (DF=(61-2), P < .05). Thus, the experimental region was a determining factor supporting the genotypes' performance in the test sites. The adequacy of the corrected model was affirmed by R Squared of .643; showing that 64.3% of phenotypes in each region were explained by the genotypes.

A Post hoc analysis (Appendix 19) showed the average phenotype for each genotype in the three regions combined and it revealed a difference between the genotypes (treatments) in terms of the outcome of the experiment. 57 genotypes an equivalent of 95% revealed the signs of the potato plants' mild wilting, stunted growth of the potato crop. Only three genotypes 28, 43, and 45 had a severity of 1, meaning there were no necrotic ring spots on leaves/tubers, no light/dark green mosaic patterns on leaves, veins, or shoots, and no stunted growth, and grew well.

Consequently, a post hoc analysis (Appendix 19) revealed the average phenotype for each region. Based on these findings, there was a difference between the experimental areas (treatments) in terms of the outcome of the experiment (Table 4.20). Sang'alo area had the best mean phenotype of 2.6675, which could be translated to mean that all the potato plants generally survived and the climatic conditions were conducive for maximum growth of the crop. Alupe region on average had nearly all its' potato crops stunted, mainly due to very high temperatures and minimal soil water content that limited the crop field performance. On the other hand, screened potato populations in Kakamega on average had slightly severe crop performance due to external biotic factors, including infection by potato virus y which was proven to not infect the test potato crop.

Table 4.20: *Average phenotypes per region (Kakamega, Alupe and Sang'alo field trial sites)*

Experimental regions	N	Subset		
		1	2	3
Sang'alo	60	2.6675		
Alupe	60		3.1107	
Kakamega	60			4.0288
Sig.		1.000	1.000	1.000

4.8 Bulking of 06H1 Potato Seed at Kapsokwony Sampled from the Three Test Sites

Seed bulking took place on a farm in Kapsokwony, located in Mt Elgon, Bungoma county, with coordinates of Latitude 00050.331' and Longitude 034041.706' at an altitude of 1869 meters. The area experiences an average annual rainfall ranging between 1500 mm and 1800 mm, and moderate temperatures between 15 - 26°C. The soil is red volcanic loam, well-drained. The selected genotypes for bulking were chosen based on their performance, exceeding the average at the Sang'alo trial site. Before planting, the land in Kapsokwony had the maize crop cultivated for two seasons followed by potatoes. Land preparation involved clearing the maize stalks and initial plowing. This was followed by a second plowing done manually after two weeks of decomposing weeds and plant material, to create suitable soil conditions for proper tuber germination. A total of 12 potato genotypes were prepared, with four genotypes having reached the second generation and eight genotypes progressing to the third generation. These were stored in a ware- room for sprouting, each genotype distinguished by assigned numbers during the first growth phase. Planting took place on March 31st, 2021, with the plants relying solely on rainwater for moisture supply.

After 23 days, the first germination data was recorded, showing an impressive 85% germination rate across all 12 genotypes tested. Subsequent inspections were conducted to closely monitor progress and identify any issues before the eventual harvest. Ninety days after planting, the plants were ready for harvesting, with genotype 54 standing out as particularly noteworthy for its exceptional field performance. Randomly selected tubers from each plant in this genotype weighed over one kilogram. The initial planting took place on 31/03/2021, with the potato genotypes numbered as follows: 6, 7, 14, 18, 20, 34, 35, 36, 50, 52, 54, and 56. genotypes 6, 7, 20, 34, 35, 52, 54, and 56 had been cultivated up to the third generation. Ridges were constructed across the slope, raised about 30 cm above ground level. Rows were spaced 1 meter apart, with approximately 30 cm between individual plants. The soil was carefully tilled to a fine tilth to facilitate smooth germination and root development. Planting holes were dug to a depth of about 10 cm, and the soil was thoroughly mixed for optimal growth conditions with Mavuno planting fertilizer. The sprouted tubers were then planted on the mixed soil and covered lightly with soil by hand for genotypes 6, 7,14,18, 20, 34,35, 36, 50, 52, 54, and 56. 90% germination percentage was recorded and 405 plants were recorded. The plants that had germinated had good vigor and uniform growth.

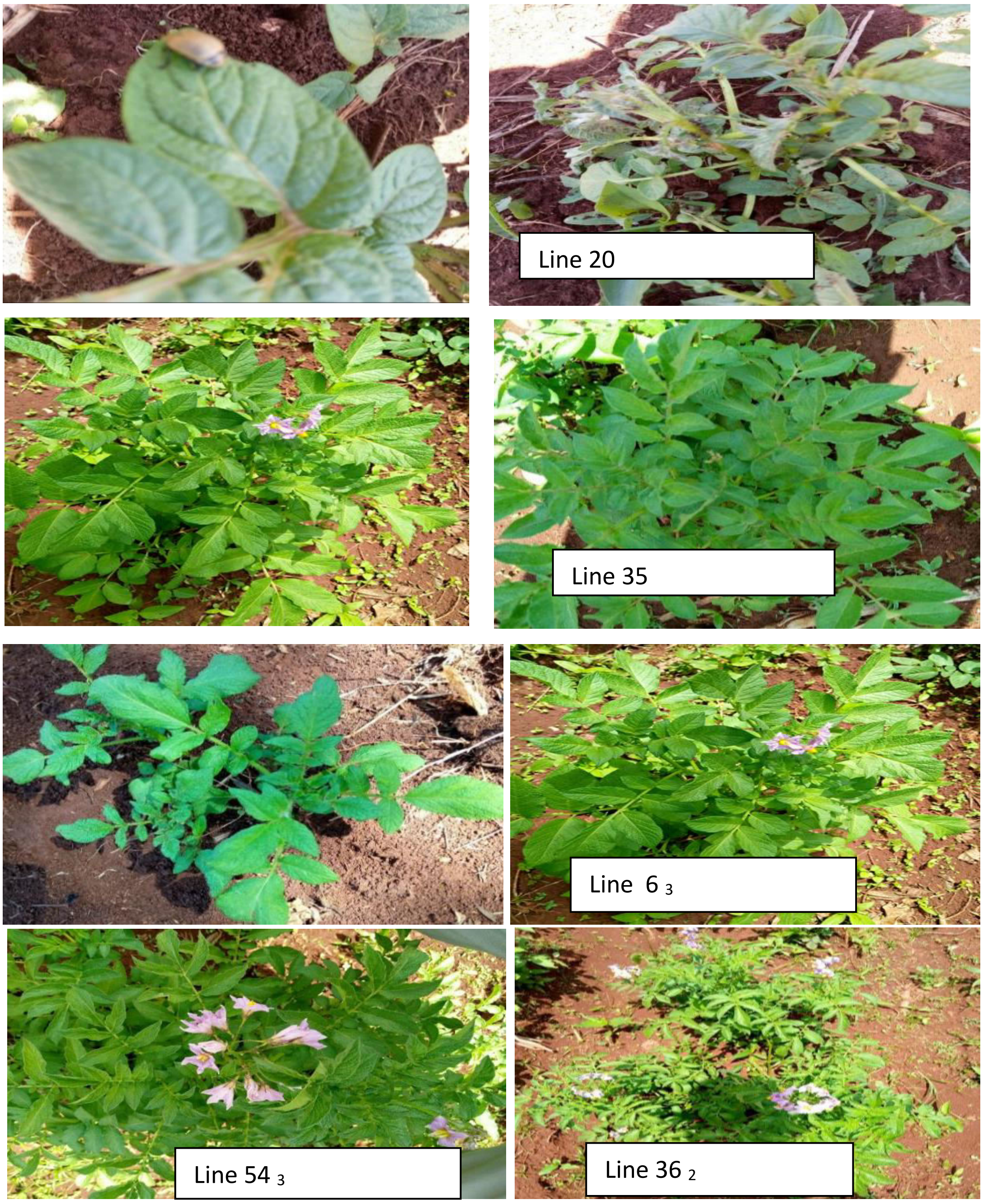

Figure 4.15: *Sample genotypes of potato lines at the Kapsokwony site for seed bulking from the field trials n Sang'alo field trials*

The second generation of genotype 6 had an average of 12 tubers per plant, with plants having tubers ranging between 9 and 23. The average weight recorded for the randomly picked 10 potato plants was 524g. The tubers for the randomly picked plants were graded as small (<20g), medium (20 – 70g), and big (> 70g) sized tubers.

Figure 4.16: *Tuber grading for genotype 6 (Clone 95) generation 2; small tubers (<20g), medium tubers (20 – 70g), and big tubers (> 70g)*

360 potato crops from genotype 6 generation 2 were planted. From this, 60 plants that were showing signs of virus infection were tested against 6 potato viruses namely: Potato Virus Y, Potato Virus X, Potato Virus S, Potato Virus A Potato Leaf roll Virus, and Potato Virus V by ELISA. All results were negative for the above-mentioned viruses.

The third generation of genotype 6 potato plant had 10 and 18 tubers per plant, most of the tubers being medium to large, with the average tuber weight per plant recorded being 464g.

Figure 4.17: *Potato tuber harvest of selected genotypes after seed bulking*

The third generation of genotype 54 produced oval-shaped potato tubers suitable for chips. Averagely each potato plant had thirteen tubers (Table 4.21).

Table 4.21: *Tuber weight per plant of Clone 299 during seed bulking*

Serial no	Tuber no./plant	Tuber weight
1	14	1720g
2	15	1705g
3	13	1245g
4	19	1218g
5	7	1075g
6	8	1065g
Average:	**13**	**1338g**

Most of the plants had tuber weight of over one kilogram, with an average weight of 1338g per plant (Figure 4.13). Big-sized tubers were the majority.

Figure 4.18: *Tuber grading for Clone 299 generation 3; small tubers (<1030g), medium tubers (1065-1075g) and big tubers (>1500g)*

CHAPTER FIVE

DISCUSSION

5.1 Distribution of PVY in Main Potato Growing Areas

A survey was conducted in 71 farms across the main potato growing areas of Kenya and it is edvident that climate erraticism is a threat to agricultural production systems and mitigation of food insecurity worldwide. Uncertainties in climatic factors like wind and the sun's radiation cause an increase in temperatures beyond optimal, which affects the moisture content, and increases the atmospheric CO_2. This negatively impacts crop performance every season (Aggarwal *et al.,* 2018). The potato crop which was introduced to Africa by Christian missionaries at the end of the seventeenth century through the establishment of small plantations (International Plant Biotechnology Outreach [IPBO] 2019) has gained importance as a food crop, attracting immense scientific research.

This study sought to undertake a molecular and serological typing of Potato Y isolates from the different agroecological zones of Kenya. To achieve this, data was collected from various farmer fields across the main potato growing areas and KALRO sites in Nyandarua, Kiambu, Meru, Nakuru, Bomet, Kisii and Elgeyo marakwet counties. Small scale farmers who practice the majority of potato farming in Kenya have farm sizes is less than five acres (MoALF, 2016), mainly cultivating crops for subsistence. This study documents that PVY was more prevalent in Meru with the entire region under study having different levels of PVY infections, followed by Kiambu at 61%, Bomet at 40%, and Nyandarua at 26.7%. The presence of PVY infections in farmer fields practising potato farming can be supported by a study by Chepkoech (2022), where there is a possible deficit in farmers embracing certified seeds currently available in the country's seed systems where they recycle their seed, stretching it over several crop generations, which may be prone to infection by PVY and various biotic factors.

The virus is continually evolving thus there is a need to keep the farmers updated on the cause of action to mitigate new variants. In a recent survey, Kenyan farmers were found with limited knowledge of the concept of viruses and virus control and they engage in practices that promote potato virus transmission (Onditi 2020). According to a study by Sastry and Zitter (2014), ineffective management of the virus including precise diagnosis and monitoring in efforts to understand its life circle, sources of virus infection, and disease control can lead to PVY prevalence. An interesting finding in this study to support the fact that the virus is evolving was documented PVY phenotypic symptoms were absent in some potato plants in the field but positively identified as having the virus infection through confirmatory serological procedures. This evidence is also supported by a study by (Onditi 2020, 2021) where asymptomatic PVY infections were detected in different local cultivars embraced by farmers like Shangi, Tigoni, Kenya Baraka, and Asante for various preferred traits. The counties of Bungoma, Nakuru, Kisii, and Elgeyo Marakwet recorded a (0%) prevalence of PVY. This can be attributed various possible factors; the possibility of farmers in these regions are adopting the use of clean, certified potato seeds, the climatic condition not favourable for the development of the disease, or absence of the vector due to use of insectides. Additionally, the practice of intermittent farming in the Midlands and the absence of environmental conditions necessary for the disease's development may have played a role, as confirmed by a study by Bourne *et al*. (2021). The Kenyan government, through county governments, NGOs, and private sectors, is utilizing farmer groups to maximize efficiency in agricultural production. This strategy involves dispersing newly developed technologies to farmers, providing training, and efficiently disseminating information through extension services.

Eidt *et al*., (2020) recommend the unity of farmers working in such organizational groups at the community level since it eases effort placed to disseminate impactful measures between the government and respective groups instead of working with individual farmers.

5.2 Molecular Footprints of Potato Virus Y Isolate Infecting Potatoes in Kenya

Pathogen adaptability is inevitable for better survival to the advanced resistance of hosts through mutations, recombination, or gene flow (Zhan *et al.,* 2015). Loss of genetic purity, gene erosion, and accumulation of pathogens in the seed potato have contributed to fluctuations observed in the global scales of potato production (IPPC Secretariat *et al.,* 2021; Kreuze *et al.,* 2020). Accurate analysis of the evolutionary course of pathogens, population dynamics, and population genetic structure, work together towards achieving plant populations that are resistant or tolerable to different biotic and abiotic factors, without causing crop loss which has repercussions to world population. Phylogenetic annayses revealed interesting molecular foot prints in the Kenyan PVY isolate in comparison to the worlds'documeted PVY sequences.

The most cost-effective way of combating PVY infections is by using resistant plant material. However, potato seeding systems in Kenya are challenged in providing seed potatoes with broad resistance or tolerance to all PVY strains. In contrast, developed countries consistently supply PVY-resistant seeds, reducing potential crop losses. Marker-assisted breeding, which selects desirable traits controlled by major genes or quantitative trait loci, has proven effective for breeding PVY resistance. Extreme resistance (ER) in potatoes, conferred by the Rysto gene, prevents viral replication without causing cell death by closely interacting with the PVY coat protein. However, over-expression of the PVY coat protein can induce a visible hypersensitive reaction in plants like tobacco (Marta Grech *et al*., 2022). domain. Each deletion that affects the Coat Protein core region impairs the ability of Rysto to trigger defense (Marta Grech *et al.,* 2022).

The molecular mechanism behind Ry_{sto}-triggered recognition and immunity remains poorly unstated. Understanding these mechanisms is crucial for enhancing plant disease resistance and developing effective crop protection strategies. Rysto directly associates with PVY coat protein in plants, conditioned by the presence of a central 149 amino acid sequence in the coat protein. High-throughput sequencing has opened new possibilities for understanding the diversity and complexity of mixed viral infections. Different potato cultivars respond variably to (PVY) infection, showing tolerance (no visible symptoms but potential yield loss), resistance (no symptoms), or susceptibility (symptoms appear). Plants recognize virus infections, including PVY, through RNA silencing, a universal defense mechanism that interferes with viral replication. HVPro was identified as the first PVY protein suppressor of RNA silencing. The P1 protein, which precedes HCPro in the viral polyprotein, may stabilize HCPro and enhance its ability to suppress RNA silencing (Fernandez *et al.,* 2013). The relative efficiency of antiviral RNA silencing in a potato plant, along with the virus's ability to suppress this silencing, determines the level of virus accumulation in infected tissues.

PVY sequences in GenBank span all continents except Antarctica (Fuentes *et al*., 2019), yet evidence on the molecular footprints of PVY in Africa is scarce. This study sought to do a comprehensive phylogenetic analysis of Potato virus Y strain types, utilizing the NCBI Nucleotide Blast of curated sequence dataset comprising the partial sequences of the Kenyan PVY, that saw the amplification of the P1 (full sequence) and Coat protein region of isolates from the farmer fields and the whole genome of PVY_Kenya. 7 potato plants of different cultivars, collected across the major potato-producing counties that tested positive for PVY revealing phenotypic characteristics of disease presentation like foliar mosaic, leaf ruffling, and mottle were collected in farms and were sequenced to reveal the existence of different strains within the Kenyan farms. Seven different algorithms were used in this study and we identified potential recombinant and parental sequences (Figure 4.8). Crucial insights are revealed among specific plant virus genera, species of interest, and phylotypes there within through phylogenetic analysis into the evolutionary relationships existing amongst them. It also divulges the diversity of geographical isolates and reveals their origin and evolution as supported by studies done by Hull *et al.,* (2002) and Rashid *et al*., (2020).

Haplotype networks offer a global pictorial possibility of the route of potato domestication as it is recorded that the Incas being the first South American farmers, initiated potato farming, and the domestication of arable Irish potatoes originated from random breeding activities between the wild potato species crossed with the domesticated varieties in the Andean region (Hawk *et al.,* 1990) that eventually spread to other nations in the world as supported by studies done by Glendinning *et al.,* (1983) and Nun *et al.,* (2010).

Data from the NCBI GenBank database illustrate how viral genomes change over time, adapting to different host ranges and environments, despite the efforts placed to ensure effective seed certification methods to minimize viral infections. Historic PVY strain groups PVY^{C} and PVY^{Z} have been faced over time by the continuous progression of the virus. The spontaneous emergence of new PVY strains complicates certification and farm management efforts, highlighting concerns in potato production. The findings of this study of the predominant strain being the recombinant type PVY $^{N:O}$ and PVY ^{N} are coherent with a study done by (Onditi *et al.,* 2022) on prevalent strains currently present in Tigoni research center. There is a continuous evolutionary shift detected over the years resulting from the genome recombination of the parental strains (PVY^{O} and PVY^{N}) to new recombinant strains, principally PVY^{NTN} and PVY$^{N-Wi/N:O}$ in diverse parts of the ecosphere supported by studies done by Kehoe *et al.,* (2016); Funke *et al.,* (2017) and Tran *et al.,* (2022). A similar study conducted in Kashmir, India, by (Wani *et al*., 2021) agrees with the findings of this study on the most prevalent PVY strain types currently are PVY ^{N} and PVY ^{NTN} recombinant type.

The Kenyan isolate K4 (PVY$^{n:o}$ recombinant type) shared the oldest common ancestor with samples from MN539908.1, MN380536.1, KY863549.1(collectively from Egypt), MK639789.1 (Kazakhstan), and KU757290 (Brazil), illustrating maximum support by 100% probability, suggesting a possible close revolutionary relationship in comparison to the rest of the isolates included in this analysis. However, there is a limitation in the number of positively identified samples used in this study. The choice of sequencing (Sanger sequencing) used was adequate to delineate the PVY recombinant strains existing in the major potato-growing areas in Kenya. However, we recommend most recent molecular tools RNA interference (RNAi), trans-grafting, cisgenesis/intragenesis, and genome editing tools, like zinc-finger be embraced in future research to further unveil the finer details at the gene level and precisely identify and differentiate PVY strains beyond the four main strains of PVY such as those described byTran *et al*., (2022).

Phylogenetic analysis of sequences from several parts of the world retrieved from the GenBank vis a vis our Kenyan whole genome isolate GenBank accession number PP069009 and a U.S.A counterpart JQ924287 revealed a shared common last ancestor node with isolates from United States of America (Figure 4.9a; Figure 4.9b) This displayed maximal support at 92% probability. This phylogenetic positioning suggests a close evolutionary relationship between the PVY_Kenya isolate and its counterpart from the United States, potentially tracing back to a common ancestral lineage rooted in Slovakia. Notably, the MT522445.1 sequence of the Potato Yellow Vein Virus was included as an outgroup for comparative analysis. Employing the MAFFT tool (Katoh *et al.,* 2002) followed by Bayesian phylogenetic inference through MrBayes (Huelsenbeck *et al.,* 2001) within the SATO v 0.1.4 pipeline (Wekesa *et al.*, 2023) a phylogenetic tree was constructed (Figure 4.10). We established a strong provision for a migration trail from South America to Europe (Figure 4.12b). This can be explained by a probable situation in which PVY was first introduced into Europe from South America, through gene flow from the global trade of potatoes and further possibly spread to Africa.

A mosaic-like genetic structure within the PVY Kenya isolate was evident, with prominent multiple illustrations of gene recombination events. A study done by Gibbs *et al.,* 2017 revealed the five distinct phylogroups that all PVY isolates for which complete genome sequences were then known were as a result of recombination from distinct parent phylogroups. Studies done by (Glais *et al.,* 2017; Karazev *et al*., 2013) agree with this study that recombination is a significant driving force in the evolution and divergence of many plant viruses. This is also supported by the findings of the study done by Hasiow- Jaroszewka (2016) that support continual recombinations within the PVY genome as demonstrated in figures 4.12a and 4.12b where acumens of genetic diversity and distribution of PVY strains across different continents (America, Africa, Asia, and Europe) have been illustrated.

The diverse array of haplotypes signifies the potential evolutionary divergence or recombination events within the PVY population. The findings of this study are similarly consistent with that of a study by Mao *et al.,* (2019), which observed that genetic variations of this pathogen have geographically driven adaptation as a significant factor of the diversity of Potato virus Y. Previous studies on PVY in different parts of the world share parental history with where it was first domesticated in the Andean region of South America and spread to other regions through similar historical events (Torrance *et al.,* 2020; Fuentes *et al.,* 2019) which has a shared evolutionary background with potato virus V according to a study by Fuentes *et al*., (2022). Studies from (Fribourg *et al.*, 2019) and (Gibbs *et al.,* 2010) support most of the viruses, (example wild potato mosaic virus, sunflower ring blotch virus, potato virus V, Peru tomato virus, and Biden's mosaic virus) all of which are from the PVY lineage were isolated from plants native to South America.

According to a study by Glasa *et.al*.,(2021) RNA viruses show exciting evolutionary dynamic forces due to huge populace sizes, rapid replication rates, and high mutation rates, evidenced in this piece of molecular study on the PVY whole genome; that hypothesized that the PVY_Kenya isolate derives genetic material from multiple parental sequences, as evidenced by the reference sequences obtained from the NCBI database. This helps the virus’ adaptability to different geographical positions. Plant pathogens often undergo strong selective pressures that rapidly change depending not only on the vagaries of the ecosystems they inhabit but also on direct inputs from humans through participation in cross border transactions in international trade and research (Derbyshire *et al.,* 2020).

High mutation frequencies determined by mutation and fitness produce quasispecies, which is very common among RNA viral species (Sanjuan *et al.,* 2008). The direction and strength of selection can be calculated and categorized as purifying selection, neutral evolution, or positive selection, depending on values obtained from a study. RNA viruses can quickly adapt to changing selective pressures and new hosts. This study revealed Pervasive positive/diversifying selection at five specific sites and pervasive negative/purifying selection at 267 sites within the Potato Virus Y (PVY) genome, each supported by a robust posterior probability (Prob $[\alpha > \beta] > 0.9$), unveiling a complex interplay of selective forces shaping the genetic landscape of this viral population. Here, the selection pressures within PVY_Kenya branches suggest a more intricate evolutionary history or potentially different, undetectable types of selective pressures acting on PVY evolution and a relatively low overall genetic diversity within the PVY population, despite its multi-continental origins. This finding agrees with a similar study done by Gao 2020 on the evolutionary history and global spatio-temporal dynamics of potato virus Y in Russia, suggesting South America was a hub for the domestication of potatoes which later was spread to the rest of the world (Figure 4.12a &Figure 4.12b). Europe has also played a major role in the spread of many potato viruses. However, due to the rate of recombination scenarios within the PVY, further investigations need to be employed to offer a complete understanding of the evolution of PVY on a global scale.

5.3 Screening Potato Populations for Resistance to PVY and Earliness

Climate-smart agriculture aids in mitigating challenges facing crop production by introducing genotypes that can cope with the unpredictability of climate while being able to meet the world's food demands. Climate-smart agriculture ensures there is increased crop productivity and improved adaptation of crops as efforts aimed at improving food security (Totin *et al.,* 2018). Introgression of resistant genes from wild potato varieties into commercial cultivars has been successful (Gao *et al.,* 2020; Quenouille *et al.,* 2013) even though over time there is resistance breakdown noted from the cultivars, resulting in new PVY infection. Kenyan farmers have been changing potato cultivars over time due to reasons of preference determined by changing weather atterns and resistance beakdown of certain potato cultivars over time. The potato farming industry has noted a shift from cultivars Nyayo and Desiree which were the major cultivars grown in the 1980s and early 1990s (Crissman *et al*., 1993) to Tigoni, Nyayo, Dutch Robyjn, Asante, and Shangi varieties being more embraced n efforts to combat climate change (MoA/GTZ/PSDA 2009, Onditi *et al.,* 2020).

Undomesticated Irish potato relatives are latent sources of required characters whose genes can be introgressed into cultivatable varieties. Having undergone evolution in an extensive range of agroecological niches, differential responses of tuber produce in higher temperatures have been documented (Guedes *et al.,* 2019, Torrance *et al*., 2020, Campbell *et al*., 2022). Factors responsible for a specific influence on potato yield in diverse surroundings are not properly known, nonetheless, upholding crop productivity under stress environments is one of the uppermost urgencies in modern-day agronomy. Domesticated potato cultivars can be crossed with several wild *Solanum* species (Kondrak *et al.,* 2020), and desirable and beneficial traits can be transferred.

The Global Seed Potato Industry is confronted with major challenges, as only a relatively small percentage of potato growers worldwide strictly adhere to using high-quality potato seeds (Kimathi *et al.*, 2021). In today's rapidly changing world, the accuracy of weather forecasts plays a crucial role in various sectors, including agriculture, transportation, and disaster management. Moreover, as climate change continues to impact weather patterns, the ability to predict and prepare for extreme weather events becomes even more vital. To this end, accurate weather forecasting is of great importance for agriculture, particularly in planning day-to-day activities and optimizing crop production. It enables farmers to make informed decisions about the optimal times for planting, irrigating, and harvesting their crops. Agronomic research targeting the improvement of food security in Africa (Scott *et al.,* 2021) should address gaps existing that are limiting an increase in yields. To hasten the time-consuming crossbreeding process, various advanced techniques are being integrated into the plant breeding programs. These include a genomic-led approach to predict breeding ideologies, gene expurgation, high-throughput phenomics for trait assessment, and environmental manipulation to understand and breed for target environments. Improvements in polymerase chain reaction (PCR), the user-friendliness of high-quality enzymes for DNA alteration, and the introduction of fluorescent automated sequencing have facilitated the sequencing of diverse plant species (Pervez *et al*., 2022). These developments are rooted in molecular principles related to the manipulation and application of genetic variations that influence plant traits (Cortés and Du, 2023). Sequencing methodologies have given rise to numerous next-generation sequencing (NGS) techniques, offering innovative avenues to investigate plant genomes and construct genome assemblies.

Quantitative Trait Locus (QTL) analysis stands as a potent method employed in genetics and plant breeding to investigate the underlying genetic architecture of intricate traits. This approach enables the pinpointing of particular genomic regions, empowering breeders with insights into the genetic regulation of traits and thereby aiding in making informed choices within their breeding initiatives, as highlighted by Platten *et al*. 2019. Prashar *et al.* 2014 conducted QTL analysis on the 06H1 parental and progeny clones, utilizing yield data collected from trials spanning 2009 to 2011. This analysis pinpointed genomic regions responsible for the variability in yield among the potato clones. Leveraging the linkage map and genotype information derived from the Illumina Infinium 8k Potato SNP Array, the researchers successfully mapped QTL associated with yield traits in both the 06H1 parental and progeny clones.

Ideal tuber yield occurs within a range of average daytime temperatures (14 and 22 °C). However, exceeding the stated temperature range can potentially hinder or entirely impede potato tuber production, resulting in adverse consequences for both producers and consumers. Within one of the three QTL intervals, there was a correlation observed with increased yield in a Phureja–Tuberosum hybrid diploid potato population (06H1). This correlation was associated with a candidate gene encoding HSc70, as noted in studies by (Torrance *et al.* 2020 and Campbell *et al.* 2022).

The results from the univariate general linear model tests (one-way ANOVA) both at days 40 and 70 for Kakamega and Alupe regions; Sang'alo at days 40 70, and 90, and a combination of the three areas revealed a significant difference in the display of earliness from the screened potato populations in all the three regions. All genotypes were resistant to PVY.

The results from this study revealed that there exixts a difference in resistance to PVY and display of earliness from screened potato populations in comparison to local potato cultivars. Furthermore, the findings revealed that the resistance to PVY and display of earliness differed according to various genotypes. In Kakamega genotypes 18, 31and 45 did well at day 40 post-emergence harvest; however, only genotype 18 grew well at day 70 post-emergence harvest in the same area. Despite the signs that the cultivars were showing resistance to PVY and earliness in Kakamega, bacterial infection in many plots contributed to the death of many plants beyond 40 days.

For the Alupe region, genotypes 14, 17, 40, 43, 2, 3, 11, 13, 35, 44, 45, and 51 showed a good level of resistance to PVY and earliness. On the other hand at day 70 post-emergence harvests in Alupe, only genotype 17 survived. The major abiotic stress that negatively affects the potato crop is heat stress. This is majorly evident in semi-arid zones, impairing a variety of physiological and biochemical processes (Sallam *et al.,* 2021). It accelerates reactive oxygen species production (ROS) in plants, such as singlet oxygen, superoxide anion, hydrogen peroxide, and hydroxyl radical, which collectively lead to oxidative stress (Hasanuzzaman *et al.,* 2020). This hurts the general crop performance. While there is a significant disparity in how diverse potato cultivars react to traumatic surroundings, unraveling the inherent basis of their responses to abiotic stresses remains a complex task in potatoes and many other crop species. Nevertheless, recent advancements in genetic and genomic methodologies have facilitated some headway, particularly through the utilization of diploid potatoes to streamline genetic scrutiny, as highlighted by (Marand *et al.*

2019). Most importantly, this region had higher temperatures throughout the experiment period and that contributed to the death of many potato plants in various plots beyond 40 days. On the other hand, in Sang'alo, there was a remarkable growth for 18, 24, 28, 34, 37, 38, 43, 44, 45, 46, 56, 36, and 51 genotypes at day 40 post-emergence harvest. At day 70 the genotypes 18, 28, and 43 survived; while at day 90 in this region only 18 and 28 genotypes did well. In Sang'alo most of the plants in different plots died beyond day 40 due to fungal infection.

In the three regions at day 40, a good number of potato plants showed close to none to mild symptoms, which revealed that they are either tolerant or resistant to the PVY virus. This is supported by some studies, for example, according to studies done by Ravnikar (2005), PVY symptoms are palpable in sensitive potato plants, while none to mild signs are clear in plants that display tolerance or resistance to the virus, the quantity of the virus notwithstanding.

When plants resist viral attack by limiting virus invasion to cells and its' replication, there exists an incompatible interaction, which can be responded to with extreme resistance (ER) or a hypersensitive response (Ravnikar 2005). Extreme resistance is expressed when the viral symptoms are the bare minimum and no symptom of viral infection is notable on the plant. On the other hand, susceptible tolerant cultivars express limited necrosis in the form of dot-like lesions in some genotypes (Valkonen *et al.,* 1996). The mechanism responsible for the prevention of viral translocation to the rest of the plant parts from the point of infection to the phloem of the diseased plant remains unknown in hypersensitive resistance response. However, virus multiplication is restricted in the infected cells hindering the viral movement from one cell to another (Solomon-Blackburn and Barker 2001; Tian and Valkonen 2015). Other studies emphasize the fact that in the past, the distinction between the hosts for

PVY was based on the plant species from which the samples were collected. On this basis, four PVY strains were identified as potato, tobacco, tomato, and pepper strains. Over time this perception lost meaning due to the ability of some strains to infect more than one host species. It has been recorded that some PVY strains are infectious to both tobacco and pepper cultivars (McDonald and Kristjansson, 1993;). However, PVY^{O} is the most dominant strain worldwide and is accountable for systemic mottle or mosaic in many potato cultivars (Lorenzen *et al.,* 2006, Crosslin, 2013). Similar symptoms are expressed on potato leaves infected with PVY^{O} and PVY^{C} strain groups (de Bokx and Huttinga 1981; Beemster and de Bokx 1987).

From this field experiment, the nature and interplay of multiple components, including host vulnerability, pathogen attack, and the effect of different environmental conditions on 60 genotype performance, an extension study to Africa from a study by Torrance *et al.*, (2020) was revealed. The host innate characteristics contribute to disease resistance pest resistance, supported by a study done by Singh *et al,* (2023). Disease symptoms manifest only when all three components are present, allowing for disease management by targeting and eliminating any one of these components. Abiotic stresses of heat and drought cause noteworthy challenges to the agronomic performance of the potato, with breeding for resistance proving difficult due to the intricate range of tolerance mechanisms and the variability of stress conditions. (Seleiman *et al.,* 2021). The results from the univariate general linear model tests (one-way ANOVA) both at days 40 and 70 for Kakamega and Alupe regions; Sang'alo at days 40 70, and 90, and a combination of the three areas revealed that the difference was significant in the display of earliness from screened potato populations in all the three regions with there beibg a varied display of earliness among the 60 genoypes tested. No PVY infection was present in the three trial sites, with all the genotypes tested rto be confirmed as resistant to the virus. The use of disease-resistant varieties is the most effective and natural method of disease and pest control in potatoes. Drought, heightened salt concentrations, increased temperature beyond optimum, and pathogenic bacteria invasion, collectively pose significant challenges to the agronomic performance of plants (Habti *et al.,* 2020). However, the genetic dissection of abiotic stress responses in potatoes and other crop species has been challenging, despite the significant diversity in how potato genotypes respond to stress conditions, especially heat from a study done by Campbell *et al.,* (2022). Current developments in genetic and genomic tools have provided prospects to overcome these challenges (Shelake *et al.,* 2022). These advancements have allowed researchers to make progress in understanding abiotic stress responses, particularly through the use of diploid potatoes for streamlined genetic analysis (Karetnikov *et al.*, 2023; Torrance *et al.,* 2020). The importance of accurate weather forecasts cannot be overstated in today's rapidly changing world. In addition, the identification of important functional and regulatory genes involved in abiotic stress resistance is crucial for developing new high-yielding crop cultivars.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The presence of Potato Virus Y in Irish potatoes has been a global concern due to its genetic admixtures that have been found to contribute to the spread and severity of the disease, as well as negatively impact potato yield and quality. The rise of new and recombinant PVY strains is attributed to several interrelated factors. The widespread cultivation of genetically similar potato varieties increases the chances of cross-infection and viral recombination. Global trade and the movement of agricultural products facilitate the spread of different PVY strains, leading to new genetic combinations. The use of infected seed potatoes and the lack of stringent quarantine measures contribute to the dissemination of the virus. Additionally, aphid vectors, which are the primary mode of PVY transmission, thrive in diverse climates and regions, further promoting viral spread and genetic mixing. Agricultural practices such as monocropping and the use of pesticides that affect aphid predators can inadvertently worsen PVY outbreaks, enhancing the opportunities for genetic admixture. Together, these factors create a dynamic environment where PVY can continually evolve and adapt, posing ongoing challenges for the cultivation of healthy Irish potatoes worldwide.

From this study, to understand the distribution of PVY in the main potato-growing agroecological zones, a field survey was carried out in eight main potato-growing counties in Kenya. The results revealed that PVY was most prevalent in Meru with the entire areas under experiment being totally 100% affected followed by Kiambu County at 61%, Bomet at 40%, and Nyandarua at 26.7% as at the time the survey was conducted. Four counties, Bungoma, Nakuru, Kisii, and Elgeyo Marakwet recorded

0% on PVY strains. The general distribution of PVY in main potato growing areas revealed a non-prevalence of 63.38% and a PVY prevalence of 36.62% as of the time the survey was done. Technology and Research play a crucial role in providing new and improved diagnostic tools that quickly identify viral infections in plants. Research Collaboration between research institutions and farmers aids in to development and dissemination of virus-resistant potato varieties.

This study has also delineated the phylogeography of PVY of Kenya, clearly unveiling the dynamic phylogeny of PVY, the first molecular footprint of the Kenyan PVY recombinant strains and the whole PVY genome PP069009 extrapolated in detail. It contributes to scientific knowledge by unraveling the molecular phylogeography and evolutionary connections of the PVY_Kenya isolate. Placed in the broader context of global PVY diversity, this study sheds light on phylogenetic positioning, historical transmission patterns across continents, and adaptive selection pressures, emphasizing its potential role in PVY's adaptive evolutionary process. Phylogenetic analysis of the different PVY strains present in the farmers' field exudes the possibilities of continuous recombination events and mutations in the gene pool of PVY, thus explaining why symptoms expressed per plant vary depending on the strain type infecting the potato plant, with some hosts showing no phenotypic symptoms common to the already published. suggesting continuous recombination activities within the genome that may give rise to new strains. Molecular tools provided a glimpse into the genomic dynamics of viruses, offering valuable insights into PVY's genomic diversity, particularly within the economically significant virus genus.

The field performance evaluation of 60 genotypes from the 06H1 population, originally from The James Hutton Institute in Scotland yielded varied results in three test sites (Kakamega, Alupe, and Sang’alo). All the genotypes were resistant to Potato

Virus Y. The Univariate general linear model tests both at days 40 and 70 for Kakamega and Alupe regions, Sang'alo at days 40 70, and 90, and a combination of the three areas revealed that there was a significant difference in display of earliness from screened potato populations in all the three regions. The evaluation of potato populations in the three sites revealed no presence of Potato virus Y among different genotypes. A combined analysis of days 40 and 70 showed minimal variation in PVY resistance and early growth among genotypes. The Sang'alo field site had the best mean phenotype of 2.6675, which can be translated as few potato plants wilted but a large proportion survived. In conclusion, advancements in genetic and genomic tools have played a crucial role in improving our understanding of heat stress responses in these potato cultivars. The option of PVY control using certified seed tubers in Kenya has not been successful because the quantity of certified potato seed available (5%) is very low. These advancements from the James Hutton Institute will have more cultivars introduced to Kenyan farmers that are resistant to PVY and mature earlier than the local cultivars. The data obtained at day 90 post-emergence also revealed a significant number of genotypes (12) that can be monitored and once certified, released to the farmers (Clone 95, 304, 264, 268, 165, 258, 276, 239, 14, 299, and clone 143). Clone 299 has been accepted for commercialisation by Winfield Africa. This is an optimistic contribution to the potato farming industry as we advocate for the practice of using clean potato seed by farmers to help in the mitigation of the losses incurred by the use of unclean seed. The shorter duration taken to reach maturity (90 days) will help improve food availability, achieved through the use of seed with a recorded maturity period wait in comparison to the local varieties in Kenya which take 100 to 130 days to mature.

6.2 Recommendation

The virus is evolving and thus asymptomatic virus infections go unnoticed until it's very late. To mitigate this, periodic virus surveys can be considered as a way to recognize regions and cultivars infected with the virus and come up with strategies for how to manage the spread of the virus. To aid farmers in overcoming barriers to information and utilization on the spread and management of PVY, it is essential to strengthen the countrys' plant health clinic services. This includes increasing the occurrence of training, ensuring the significance of these pieces of training is appropriate and delivered timely, and diversifying the types of training received to address the farmers' needs. Such improvements will enhance farmers' knowledge and skills, thereby boosting potato production and managing PVY in the farms. More research collaboration between research institutions and farmers should be encouraged as it aids in the development and distribution of climate-smart potato varieties that can cope with the different biotic and abiotic factors affecting potato production.

There is need to continually study the PVY viral genome, particularly in East Africa, to illuminate more light on its' phylogeny and evolutionary connection within Africa and the rest of the world since there is a paucity of this data. We also recommend the study of weeds as potential hosts to PVY to understand the scope of host preference by the pathogen and make tailored decisions toward the management of the spread of disease.

We recommend that the remaining 11 clones selected based on their agronomic performance in the field Clone 95,304,264,268,165, 258, 276, 239, 14, and Clone 143) undergoing necessary approval stages for release to potato seed systems in

Kenya, should be subjected to further field evaluation over several years to monitor the stability of the resistant gene to PVY over several years.

6.3 Limitations of the Study

This study had potential limitations. The sampling of the fields depended on the availability of the crop in the fields and this may introduce some biases in accessing the distribution of potato virus Y in the different agroecological zones, especially farms accessed in Bungoma county as the rainy season was coming to an end. My observation was that there may be other compounding factors that may have played a role in explaining why Bungoma, Elgeyo- marakwet; Kisii, and Naruku did not have any PVY present in the field e.g. use of pesticides that could have killed the aphids, therefore, lacking a vector to spread the disease, or the climatic conditions could have not been favorable for the development of the disease. I only studied the Irish potato plants and disease presentation on the plant above ground. Futher research can be done on different soil samples across the surveyed areas to unveil soil properties that could be influencing the potato crop performance in relation to elevated disease presentation. Changes in weather patterns can be included in similar studies to assess if they contribute to the presentation of the disease in susceptible plants across the different agroecological zones. Weeds can be studied as potential alternative hosts of PVY and their effects analysed to understand the pathogenicity of the virus and its effects to potato farming.

There is paucity of information on molecular studies done on the whole genome of PVY in Africa and more so Kenya, which presents a literature gap. There is need for further development in this area of study. Only one potato virus Y isolate was used in this study. I recommend that similar studies in future include more isolates from

Kenyas' different agroecological zones for whole genome sequencing to give a more comprehensive phylogeographic analysis.

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APPENDICES

APPENDIX 1: BUFFERS TO BE USED FOR ELISA

1. Coating buffer (pH 9.6)

1.59 g sodium carbonate (Na_2CO_3)

2.93 g sodium bicarbonate ($NaHCO_3$)

0.20 g sodium azide (NaN_3) Dissolve in 900 ml H2O, adjust pH to 9.6 with HCl, and make up 1 liter.

2. PBS (pH 7.4) phosphate-buffered saline

8.0 g sodium chloride (NaCl)

0.2 g monobasic potassium phosphate (KH_2PO_4)

1.15 g dibasic sodium phosphate (Na_2HPO_4)

0.2 g potassium chloride (KCl)

0.2 g sodium azide (NaN_3) Dissolve in 900 ml H_2O, adjust pH to 7.4 with NaOH or HCl, and make up to 1 liter.

3. PBS-Tween (PBST)

PBS + 0.5 ml Tween 20 per liter

4. Sample extraction buffer (pH 7.4)

PBST + 2% PVP (e.g. Serva PVP-15 polyvinyl pyrrolidone)

5. Sample extraction buffer (pH 8.5)

0.05 M Tris containing 0.06 M sodium sulfite, pH 8.5

6. Conjugate buffer

PBST + 2% PVP + 0.2% egg albumin (e.g. Sigma A-5253)

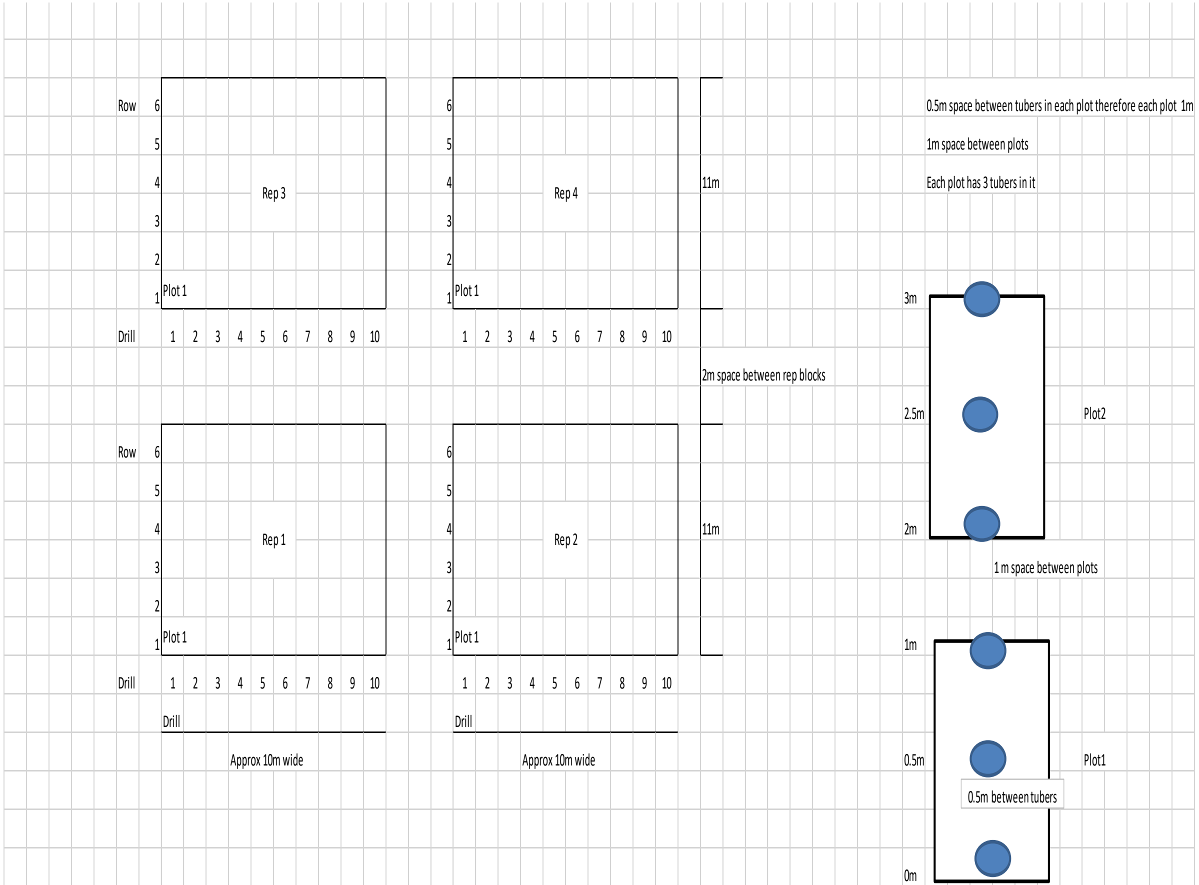
7. Substrate buffer

97 ml diethanolamine

600 ml H_2O

0.2 g sodium azide (NaN_3) Adjust to pH 9.8 with HCl and make to 1 liter with H_2

APPENDIX 2: PLANTING PLAN IN THE THREE SITES



Kakamega trial site Plot

Table of numbers assigned to the 60 clones of minitubers for trials in Kenya

Genotype number (assigned)	Clone
1	58
2	199
3	255
4	179
5	185
6	95
7	304
8	88
9	174
10	166
11	247
12	256
13	180
14	264
15	152
16	267
17	187
18	268
19	295
20	165
21	260
22	61
23	253
24	269
25	87
26	57
27	83
28	79
29	21
30	80
31	129
32	78
33	200
34	258
35	278
36	276
37	113
38	261
39	246
40	15

41	29
42	69
43	279
44	28
45	222
46	27
47	229
48	291
49	155
50	239
51	32
52	14
53	13
54	299
55	220
56	143
57	288
58	121
59	297
60	184

APPENDIX 3: KAKAMEGA SITE; SORTING OF MINI TUBERS FOR PLANTING

APPENDIX 4: SANG'ALO SITE INSPECTION WITH PROF. LESLEY TORRANCE AND MARIAM WERE

APPENDIX 5: PARTIAL SEQUENCE FOR ACCESSION   OR571473

```
LOCUS       OR571473               310 bp ds-RNA     linear   VRL 23-OCT-2023
DEFINITION  Potato virus Y isolates 4_Kenya_Mpya capsid protein gene, partial
            cds.
ACCESSION   OR571473
VERSION     OR571473.1
KEYWORDS.
SOURCE      Potato virus Y
  ORGANISM  Potato virus Y
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Stelpaviricetes;
            Patatavirales; Potyviridae; Potyvirus.
REFERENCE   1  (bases 1 to 310)
  AUTHORS   Kithinji, N., Graham, C.H., Wekesa, C., Were, H. and Okoth, P.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-SEP-2023) Biological Science Department, Masinde
            Muliro University of Science and Technology, Kakamega - Webuye
            Road, Kakamega, Western 50100, Kenya
COMMENT     ##Assembly-Data-START##
            Sequencing Technology:: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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                     /db_xref="taxon:12216"
                     /country="Kenya"
                     /collection_date="Jun-2022"
     CDS             <1..>310
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                     /codon_start=3
                     /product=" capsid protein"
                     /protein_id="WOD55104.1"

/translation="EQGSIQSNPNKGKDKDVNAGTSGTHTVPRIKAITSKMRMPTSKG

ATVLNLEHLLEYAPQQIDISNTRATQSQFDTWYEAVRMAYDIGETEMPTVMN
GLMVRC
                     "
ORIGIN
        1 tagagcaagg cagcatccag tcaaacccga acaaaggaaa agataaggat gtgaatgctg
       61 gtacatctgg gacacatact gtgccgagaa tcaaggctat cacgtccaaa atgagaatgc
      121 ccacaagcaa gggagcaacc gtgctaaact tagaacactt gcttgagtat gctccacaac
      181 aaattgatat ttcaaatact cgggcaactc aatcacagtt tgatacgtgg tatgaggcag
      241 tgcggatggc atacgacata ggagaaactg agatgccaac tgtgatgaat gggcttatgg
      301 ttcggtgcaa
```

APPENDIX 6: PARTIAL SEQUENCE FOR ACCESSION OR571474

```
LOCUS       OR571474               310 bp ds-RNA     linear   VRL 23-OCT-2023
DEFINITION  Potato virus Y isolates 12_Screen_house_Tigoni capsid protein gene,
            partial cds.
ACCESSION   OR571474
VERSION     OR571474.1
KEYWORDS.
SOURCE      Potato virus Y
  ORGANISM  Potato virus Y
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Stelpaviricetes;
            Patatavirales; Potyviridae; Potyvirus.
REFERENCE   1  (bases 1 to 310)
  AUTHORS   Kithinji, N., Graham, C.H., Wekesa, C., Were, H. and Okoth, P.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-SEP-2023) Biological Science Department, Masinde
            Muliro University of Science and Technology, Kakamega - Webuye
            Road, Kakamega, Western 50100, Kenya
COMMENT     ##Assembly-Data-START##
            Sequencing Technology:: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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GLMVRC
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      121 ccaaaagcaa gggagcaacc gtgctaaact tagaacactt gcttgagtat gctccacaac
      181 aaattgatat ttcaaatact cgggcaactc aatcacagtt tgatacgtgg tatgaggcag
      241 tgcggatggc atacgacata ggagaaactg agatgccaac tgtgatgaat gggcttatgg
      301 ttcggtgcaa
```

APPENDIX 7: PARTIAL SEQUENCE FOR ACCESSION OR571475

```
LOCUS       OR571475              309 bp ds-RNA     linear   VRL 23-OCT-2023
DEFINITION  Potato virus Y isolates 16_Dutch capsid protein gene, partial cds.
ACCESSION   OR571475
VERSION     OR571475.1
KEYWORDS.
SOURCE      Potato virus Y
  ORGANISM  Potato virus Y
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Stelpaviricetes;
            Patatavirales; Potyviridae; Potyvirus.
REFERENCE   1  (bases 1 to 309)
  AUTHORS   Kithinji, N., Graham, C.H., Wekesa, C., Were, H. and Okoth, P.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-SEP-2023) Biological Science Department, Masinde
            Muliro University of Science and Technology, Kakamega - Webuye
            Road, Kakamega, Western 50100, Kenya
COMMENT     ##Assembly-Data-START##
            Sequencing Technology:: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:12216"
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GLMVRC
                     "
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      121 caaaagcaag ggagcaaccg tgctaaactt agaacacttg cttgagtatg ctccacaaca
      181 aattgatatt tcaaatactc gggcaactca atcacagttt gatacgtggt atgaggcagt
      241 gcggatggca tacgacatag gagaaactga gatgccaact gtgatgaatg ggcttatggt
      301 tcggtgcaa
//
```

APPENDIX 8:PARTIAL SEQUENCE FOR ACCESSION OR571476

```
LOCUS       OR571476              309 bp ds-RNA     linear   VRL 23-OCT-2023
DEFINITION  Potato virus Y isolates 23_Shangi capsid protein gene, partial cds.
ACCESSION   OR571476
VERSION     OR571476.1
KEYWORDS.
SOURCE      Potato virus Y
  ORGANISM  Potato virus Y
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Stelpaviricetes;
            Patatavirales; Potyviridae; Potyvirus.
REFERENCE   1  (bases 1 to 309)
  AUTHORS   Kithinji, N., Graham, C.H., Wekesa, C., Were, H. and Okoth, P.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-SEP-2023) Biological Science Department, Masinde
            Muliro University of Science and Technology, Kakamega - Webuye
            Road, Kakamega, Western 50100, Kenya
COMMENT     ##Assembly-Data-START##
            Sequencing Technology:: Sanger dideoxy sequencing
            ##Assembly-Data-END##
FEATURES             Location/Qualifiers
     source          1..309
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      181 aaattgatat ttcaaatact cgggcaactc aatcacagtt tgatacgtgg tatgaggcag
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      301 ttcggtgca
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```

APPENDIX 9: PARTIAL SEQUENCE FOR ACCESSION OR571477

```
LOCUS       OR571477              309 bp ds-RNA     linear   VRL 23-OCT-2023
DEFINITION  Potato virus Y isolates 32_Dutch capsid protein gene, partial cds.
ACCESSION   OR571477
VERSION     OR571477.1
KEYWORDS.
SOURCE      Potato virus Y
  ORGANISM  Potato virus Y
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Stelpaviricetes;
            Patatavirales; Potyviridae; Potyvirus.
REFERENCE   1  (bases 1 to 309)
  AUTHORS   Kithinji, N., Graham, C.H., Wekesa, C., Were, H. and Okoth, P.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-SEP-2023) Biological Science Department, Masinde
            Muliro University of Science and Technology, Kakamega - Webuye
            Road, Kakamega, Western 50100, Kenya
COMMENT     ##Assembly-Data-START##
            Sequencing Technology:: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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      181 aattgatatt tcaaatactc gggcaactca atcacagttt gatacgtggt atgaggcagt
      241 gcggatggca tacgacatag gagaaactga gatgccaact gtgatgaatg ggcttatggt
      301 tcggtgcaa
//
```

APPENDIX 10: PARTIAL SEQUENCE FOR ACCESSION OR571478

```
LOCUS       OR571478              309 bp ds-RNA     linear   VRL 23-OCT-2023
DEFINITION  Potato virus Y isolates 76_Dutch capsid protein gene, partial cds.
ACCESSION   OR571478
VERSION     OR571478.1
KEYWORDS.
SOURCE      Potato virus Y
  ORGANISM  Potato virus Y
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Stelpaviricetes;
            Patatavirales; Potyviridae; Potyvirus.
REFERENCE   1  (bases 1 to 309)
  AUTHORS   Kithinji, N., Graham, C.H., Wekesa, C., Were, H. and Okoth, P.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-SEP-2023) Biological Science Department, Masinde
            Muliro University of Science and Technology, Kakamega - Webuye
            Road, Kakamega, Western 50100, Kenya
COMMENT     ##Assembly-Data-START##
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            ##Assembly-Data-END##
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      181 aattgatatt tcaaatactc gggcaactca atcacagttt gatacgtggt atgaggcagt
      241 gcggatggca tacgacatag gagaaactga gatgccaact gtgatgaatg ggcttatggt
      301 tcggtgcaa
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APPENDIX 11: PARTIAL SEQUENCE FOR ACCESSION   OR571479

```
LOCUS       OR571479               309 bp ds-RNA linear   VRL 23-OCT-
2023DEFINITION  Potato virus Y isolate 82_Sherekea capsid protein gene, partial
cds. ACCESSION   OR571479
VERSION     OR571479.1
KEYWORDS.
SOURCE      Potato virus Y
  ORGANISM  Potato virus Y
            Viruses; Riboviria; Orthornavirae; Pisuviricota; Stelpaviricetes;
            Patatavirales; Potyviridae; Potyvirus.
REFERENCE   1  (bases 1 to 309)
  AUTHORS   Kithinji, N., Graham, C.H., Wekesa, C., Were, H. and Okoth, P.
  TITLE     Direct Submission
  JOURNAL   Submitted (16-SEP-2023) Biological Science Department, Masinde
            Muliro University of Science and Technology, Kakamega - Webuye
            Road, Kakamega, Western 50100, Kenya
COMMENT     ##Assembly-Data-START##
            Sequencing Technology:: Sanger dideoxy sequencing
            ##Assembly-Data-END##
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      301 tcggtgcaa
/
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```
LOCUS       PP069009                9668 bp    RNA     linear   VRL
Potato virus Y isolate HPV_Kenya, complete genome
GenBank: PP069009.1

FASTA Graphics
Go to:
LOCUS       PP069009                9668 bp    RNA     linear   VRL
23-JAN-2024
DEFINITION  Potato virus Y isolate HPV_Kenya, complete genome.
ACCESSION   PP069009
VERSION     PP069009.1
KEYWORDS    .
SOURCE      Potato virus Y
  ORGANISM  Potato virus Y
            Viruses; Riboviria; Orthornavirae; Pisuviricota;
Stelpaviricetes;
            Patatavirales; Potyviridae; Potyvirus.
REFERENCE   1  (bases 1 to 9668)
  AUTHORS   Kithinji,N.M., Graham,C.H., Wekesa,C.S., Were,H. and
Okoth,P.K.
  TITLE     Molecular Evolutionary Analysis of Potato Virus Y
Infecting Kenyan
            Potato
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 9668)
  AUTHORS   Kithinji,N.M., Graham,C.H., Wekesa,C.S., Were,H. and
Okoth,P.K.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-DEC-2023) Biological Science Department,
Masinde
            Muliro University of Science and Technology, Webuye Road,
Kakamega,
            Western 50100, Kenya
COMMENT     ##Assembly-Data-START##
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            Coverage              :: 3000x
            Sequencing Technology :: Illumina
            ##Assembly-Data-END##
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gtaagttatt
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     4381 gtacgtgtcg agctacaatg aagttgacac cttggctaag ctcctaacag
acaagaatat
     4441 gatggtcaca aaggttgatg gcagaacaat gaagcacggt tgcctagaaa
ttgtcacaaa
     4501 agggaccagt gcgagaccac attttgttgt agcaaccaac ataattgaga
atggagtgac
     4561 tttggacata gacgtggttg tagattttgg gttgaaagtc tcaccgttct
tggacattga
     4621 caataggagc attgcttaca ataaggtgag tgttagctat ggtgagagaa
ttcaaaggct
     4681 gggtcgtgtt ggacgcttca agaaaggagt agcattgcgc attggacaca
ctgagaaggg
     4741 aattattgaa attccaagca tgatcgctac agaggcggct cttgcttgct
ttgcatataa
     4801 cttgccagtg atgacaggag gcgtctcaac tagtctgatt ggcaattgca
ctgtgcgcca
     4861 agttaaaaca atgcagcaat ttgaattgag tcccttcttt atccagaatt
tcgttgccca
     4921 cgatggatca atgcatcctg tcatacatga cattcttaaa aagtataaac
ttcgagattg
     4981 tatgacacct ttgtgcgatc agtctatacc atacagggca tcgagcactt
ggttatcggt
     5041 tagtgaatat gagcgacttg gagtggcctt agaaattcca aagcaagtca
aaattgcatt
     5101 ccatatcaaa gagatccctc ctaagctcca cgaaatgctg tgggaaacgg
ttgtcaagta
     5161 caaagacgtt tgtttatttc caagcattcg agcatcgtcc atcagcaaaa
tcgcatacac
     5221 attgcgtaca gacctcttcg ccatcccaag aactctaata ttggtggaga
gactgcttga
     5281 agaggagcga gtgaagcaga gccaattcag aagtctcatc gacgaaggat
gctcaagcat
     5341 gttttcaatt gtcaatttga caaacactct cagagctaga tatgcaaaag
attacaccgc
     5401 agagaatata caaaaacttg agaaagtgag aagtcaattg aaagaattct
caaatttgga
     5461 tggttctgca tgtgaggaaa atttaataaa gaggtatgag tctttgcagt
tcgttcatca
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     5521 ccaagctgcg acgtcacttg caaaggatct caagttgaag gggacttgga agaagtcatt
     5581 agtggccaaa gacttgatca tagcaggcgc tgttgcaatt ggtggaatag gactcatata
     5641 tagttggttc acacaatcag ttgagactgt gtctcaccaa gggaaaaata aatccaaaag
     5701 aattcaagcc ttgaagtttc gccatgctcg tgacaaaagg gctggctttg aaattgacaa
     5761 caatgatgac acaatagagg aattctttgg atctgcatac aggaagaagg gaaaaggtaa
     5821 aggcaccact gttggtatgg gcaagtcaag caggaggttt gttaatatgt atggatttga
     5881 cccaacagaa tattcattca tccagttcgt tgatccgctc actggagctc aaattgaaga
     5941 gaacgtctat gctgatatta gaaacatcca agatcgcttt agtgatgtcc gcaagaaaat
     6001 ggtagaggat gatgaaatcg aattgcaagc attgggcagc aacacaacca ttcatgctta
     6061 cttcaggaaa gattggtctg acaaggctct aaaaattgat ttgatgccac acaacccact
     6121 caaaatctgt gataaatcga atggcattgc taagtttcct gaaagagaac ttgagttgag
     6181 gcaaactggg ccagcaatag aggttgatgt gaaagacatt ccaaaacagg aagtggagca
     6241 tgaagccaaa tcactcatga gaggtttaag ggatttcaat ccaattgctc aaacagtttg
     6301 cagagtaaaa gtgtctgttg aatatggaac gtctgaaatg tatggggtcg gttttggtgc
     6361 gtatattata gtaaaccacc atctattcaa gagtttcaat ggatccatgg aagtgcgatc
     6421 aatgcatgga acattcagag tgaagaattt gcatagcttg agcgttttgc cgatcaaagg
     6481 cagagacatt atcatcataa agatgccaaa ggatttccct gttttcccac aaaaactgca
     6541 ctttcgagct ccagtgcaga atgagaggat ttgtttggtt ggaactaatt ttcaagaaaa
     6601 acatgcatca tcaatcatca cagaaacgag tactacatac aatgtaccgg gcagcacttt
     6661 ttggaagcat tggattgaaa caaatgatgg gcattgtgga ttaccagtag tgagtacagc
     6721 tgatggatgt ctagttggaa tacacagctt ggcgaataat gtgcaaacca cgaattatta
     6781 ttcagccttt gatgaggatt ttgaaagtaa gtatctccga actaatgagc ataatgagtg
     6841 gaccaaatcg tgggtatata acccagatac tgtgttgtgg ggtccattga agctcaagga
     6901 gagtacccct aaaggcctgt ttaagacaac aaaacttgta caggatttaa tcgatcatga
     6961 tgttgttgta gagcaagcta aacattctgc gtggatgtat gaggctctaa cagggaattt
     7021 gcaagctgtg gcgacaatga agagtcagct ggtgacaaag cacgtggtca aaggggagtg
     7081 tcggcacttc aaagagttct taactgtgga ttcggaagca gaagctttct tcaggccttt
     7141 gatggatgct tatgggaaga gcttgttaaa tagagaagca tatataaagg acataatgaa
     7201 atactcaaag cctattgatg ttggaacagt agactgtgat gcttttgaag aggctatcaa
     7261 tagggttatc atttatctgc aagtgcatgg cttccagaaa tgcaattaca tcaccgatga
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     7321 gcaggaaatt ttcaaagctc tcaatatgaa agctgctgtc ggagctatgt atggaggcaa
     7381 gaagaaagac tacttcgagc attttactga ggcggataaa gaggaaattg ttatgcaaag
     7441 ttgccttcga ttgtacaagg gctcgcttgg catatggaat ggatcattga aagcagaact
     7501 tcggtgcaaa gagaagatac ttgcaaataa gacaaggaca ttcactgctg cacctttaga
     7561 tactctactg ggtggaaagg tgtgcgttga tgattttaat aatcaattct actcgaagaa
     7621 cattgaatgc tgctggactg ttggaatgac taagttttat ggaggttggg acagattgct
     7681 tcggcgtcta cctgaaaatt gggtgtactg cgatgccgat ggttcacaat tcgatagttc
     7741 actcacccca tacctaatta atgctgttct catcatcaga agcacataca tggaagattg
     7801 ggacttgggg ttgcaaatgt tgcgcaattt gtacacagaa ataatttaca caccaatctc
     7861 aactccagat ggaacaattg tcaagaagtt tagaggtaat aatagcggtc aaccttctac
     7921 cgttgtggat aattctctca tggttgtcct tgctatgcat tacgctctca ttaaggagtg
     7981 cgttgagttt gaagaaatcg acagcacgtg tgtattcttt gttaatggtg atgacttatt
     8041 gattgctgtg aatccggaga gagagagcat tctcgataga atgtcacaac atttctcaga
     8101 tcttggtttg aactatgatt tttcgtcgag aacaagaagg aaggaggaat tgtggttcat
     8161 gtcccataga ggcctgctaa tcgagggtat gtacgtgcca aagcttgaag aagagagaat
     8221 tgtatccatt ttgcaatggg atagagctga tctgccagag cacagattag aagcgatttg
     8281 tgcagcaatg atagaatcct ggggttattt tgagttaacg caccaaatca ggagattcta
     8341 ctcatggttg ttgcaacagc aaccttttc aacgatagca caggaaggaa aagctccata
     8401 catagcgagc atggcattga agaagctgta catgaatagg acagtagatg aggaggaact
     8461 gaaggctttc actgaaatga tggttgcctt ggatgatgaa cttgagtgcg atacttatga
     8521 agtgcaccat caaggaaatg acacaatcga tgcaggagga agcactaaga aggatgcaaa
     8581 acaagagcaa ggtagcattc aaccaaatct caacaaggaa aaggaaaagg acgtgaatgt
     8641 tggaacatct ggaactcata ctgtgccacg aattaaagct atcacgtcca aaatgagaat
     8701 gcccaagagt aaaggtgcaa ctgtactaaa tttggaacac ttactcgagt atgctccaca
     8761 gcaaattgac atctcaaata ctcgagcaac tcaatcacag tttgatacgt ggtatgaagc
     8821 ggtacaactt gcatacgaca taggagaaac tgaaatgcca actgtgatga atgggcttat
     8881 ggtttggtgc attgaaaatg gaacctcgcc aaacatcaac ggagtttggg ttatgatgga
     8941 tggagatgaa caagtcgaat acccactgaa accaatcgtt gagaatgcaa aaccaacact
     9001 aaggcaaatc atggcacatt tctcagatgt tgcagaagcg tatatagaaa tgcgcaacaa
     9061 aaaggaacca tatatgccac gatatggttt agttcgtaat ctgcgcgatg gaagtttggc
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     9121 tcgctatgct tttgactttt atgaggtcac atcacgaaca ccagtgaggg
ctagggaagc
     9181 gcacattcaa atgaaggccg cagcattgaa atcagcccaa tctcgacttt
tcgggttgga
     9241 cggtggcatc agtacacaag aggagaacac agagaggcac accaccgagg
atgtctctcc
     9301 aagtatgcat actctacttg gagtcaagaa catgtgatgt agtgtctctc
cggacgatat
     9361 ataagtattt acatatgcag taagtatttt ggcttttcct gtactacttt
tatcataatt
     9421 aataatcagt ttgaatatta ctaatagata gaggtggcag ggtgatttcg
tcattgtggt
     9481 gactctatct tttaattccg cattattaag tcttagataa aagtgccggg
ttgtcgttgt
     9541 tgtggatgat tcatcgatta ggtgatgttg cgattctgtc gtagcagtga
ctatgtctgg
     9601 atctatctac ttgggtggtg ttgtgatttc gtcataacag tgaccgtaaa
cttcaatcag
     9661 gagacagg
//
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APPENDIX 12:PHENOTYPES MEAN PER GENOTYPE IN KAKAMEGA AT DAY 70

Potato varieties	N	Subset						
		1	2	3	4	5	6	7
18	4	1.50						
44	4	2.00	2.00					
37	4	2.25	2.25	2.25				
43	4	2.25	2.25	2.25				
45	4	2.25	2.25	2.25				
40	4	2.50	2.50	2.50	2.50			
2	4	2.75	2.75	2.75	2.75	2.75		
13	4	2.75	2.75	2.75	2.75	2.75		
5	4		3.25	3.25	3.25	3.25	3.25	
25	4		3.25	3.25	3.25	3.25	3.25	
41	4		3.25	3.25	3.25	3.25	3.25	
38	4		3.50	3.50	3.50	3.50	3.50	3.50
22	4			3.75	3.75	3.75	3.75	3.75
24	4			3.75	3.75	3.75	3.75	3.75
15	4				4.00	4.00	4.00	4.00
17	4				4.00	4.00	4.00	4.00
21	4				4.00	4.00	4.00	4.00
31	4				4.00	4.00	4.00	4.00
47	4				4.00	4.00	4.00	4.00
53	4				4.00	4.00	4.00	4.00
16	4					4.25	4.25	4.25
20	4					4.25	4.25	4.25
27	4					4.25	4.25	4.25
33	4					4.25	4.25	4.25
51	4					4.25	4.25	4.25
58	4					4.25	4.25	4.25
1	4						4.50	4.50
9	4						4.50	4.50
19	4						4.50	4.50
57	4						4.50	4.50
59	4						4.50	4.50
14	4						4.75	4.75
30	4						4.75	4.75
32	4						4.75	4.75
35	4						4.75	4.75
39	4						4.75	4.75
46	4						4.75	4.75

50	4						4.75	4.75
52	4						4.75	4.75
3	4							5.00
4	4							5.00
6	4							5.00
7	4							5.00
8	4							5.00
10	4							5.00
11	4							5.00
12	4							5.00
23	4							5.00
26	4							5.00
28	4							5.00
29	4							5.00
34	4							5.00
36	4							5.00
42	4							5.00
48	4							5.00
49	4							5.00
54	4							5.00
55	4							5.00
56	4							5.00
60	4							5.00
Sig.		.111	.060	.062	.066	.072	.078	.082

APENDIX 13: AVERAGE PHENOTYPE PER GENOTYPE IN KAKAMEGA

Potato varieties	N	Subset											
		1	2	3	4	5	6	7	8	9	10	11	12
45	4	1.6250											
44	4	1.7500	1.7500										
18	4	2.0000	2.0000	2.0000									
2	4	2.2500	2.2500	2.2500	2.2500								
13	4	2.5000	2.5000	2.5000	2.5000	2.5000							
31	4	2.5000	2.5000	2.5000	2.5000	2.5000							
27	4	2.6250	2.6250	2.6250	2.6250	2.6250	2.6250						
38	4		2.8750	2.8750	2.8750	2.8750	2.8750	2.8750					
43	4		2.8750	2.8750	2.8750	2.8750	2.8750	2.8750					
57	4			3.0000	3.0000	3.0000	3.0000	3.0000	3.0000				
5	4				3.2500	3.2500	3.2500	3.2500	3.2500	3.2500			
9	4				3.2500	3.2500	3.2500	3.2500	3.2500	3.2500			
40	4				3.2500	3.2500	3.2500	3.2500	3.2500	3.2500			
26	4					3.5000	3.5000	3.5000	3.5000	3.5000	3.5000		
32	4					3.5000	3.5000	3.5000	3.5000	3.5000	3.5000		
21	4					3.6250	3.6250	3.6250	3.6250	3.6250	3.6250	3.6250	
37	4					3.6250	3.6250	3.6250	3.6250	3.6250	3.6250	3.6250	
39	4					3.6250	3.6250	3.6250	3.6250	3.6250	3.6250	3.6250	
20	4					3.7500	3.7500	3.7500	3.7500	3.7500	3.7500	3.7500	3.7500
30	4					3.7500	3.7500	3.7500	3.7500	3.7500	3.7500	3.7500	3.7500
34	4					3.7500	3.7500	3.7500	3.7500	3.7500	3.7500	3.7500	3.7500
12	4						3.8750	3.8750	3.8750	3.8750	3.8750	3.8750	3.8750
47	4						3.8750	3.8750	3.8750	3.8750	3.8750	3.8750	3.8750
48	4						3.8750	3.8750	3.8750	3.8750	3.8750	3.8750	3.8750
35	4							4.0000	4.0000	4.0000	4.0000	4.0000	4.0000
41	4							4.0000	4.0000	4.0000	4.0000	4.0000	4.0000
49	4							4.0000	4.0000	4.0000	4.0000	4.0000	4.0000
23	4							4.1250	4.1250	4.1250	4.1250	4.1250	4.1250
25	4							4.1250	4.1250	4.1250	4.1250	4.1250	4.1250
50	4							4.1250	4.1250	4.1250	4.1250	4.1250	4.1250
51	4								4.2500	4.2500	4.2500	4.2500	4.2500
22	4									4.3750	4.3750	4.3750	4.3750
24	4									4.3750	4.3750	4.3750	4.3750
15	4									4.5000	4.5000	4.5000	4.5000
17	4									4.5000	4.5000	4.5000	4.5000
53	4									4.5000	4.5000	4.5000	4.5000
4	4										4.6250	4.6250	4.6250
7	4										4.6250	4.6250	4.6250
11	4										4.6250	4.6250	4.6250
16	4										4.6250	4.6250	4.6250
33	4										4.6250	4.6250	4.6250
58	4										4.6250	4.6250	4.6250
1	4										4.7500	4.7500	4.7500
19	4										4.7500	4.7500	4.7500

59	4										4.7500	4.7500	4.7500
14	4											4.8750	4.8750
42	4											4.8750	4.8750
46	4											4.8750	4.8750
52	4											4.8750	4.8750
3	4												5.0000
6	4												5.0000
8	4												5.0000
10	4												5.0000
28	4												5.0000
29	4												5.0000
36	4												5.0000
54	4												5.0000
55	4												5.0000
56	4												5.0000
60	4												5.0000
Sig.		.098	.064	.102	.109	.051	.052	.055	.055	.057	.059	.060	.062

APPENDIX 14: PHENOTYPES MEAN PER GENOTYPE IN ALUPE AT DAY 70

Genotype	N	Subset				
		1	2	3	4	5
17	4	1.75				
2	4	2.25	2.25			
40	4	2.75	2.75	2.75		
43	4	2.75	2.75	2.75		
45	4	2.75	2.75	2.75		
46	4	2.75	2.75	2.75		
5	4	3.00	3.00	3.00	3.00	
28	4	3.00	3.00	3.00	3.00	
59	4	3.00	3.00	3.00	3.00	
3	4	3.25	3.25	3.25	3.25	3.25
9	4	3.25	3.25	3.25	3.25	3.25
16	4	3.25	3.25	3.25	3.25	3.25
36	4	3.25	3.25	3.25	3.25	3.25
18	4		3.50	3.50	3.50	3.50
37	4		3.50	3.50	3.50	3.50
56	4		3.50	3.50	3.50	3.50
14	4		3.75	3.75	3.75	3.75
20	4		3.75	3.75	3.75	3.75
30	4		3.75	3.75	3.75	3.75
31	4		3.75	3.75	3.75	3.75
54	4		3.75	3.75	3.75	3.75
1	4		4.00	4.00	4.00	4.00
6	4		4.00	4.00	4.00	4.00
7	4		4.00	4.00	4.00	4.00
13	4		4.00	4.00	4.00	4.00
27	4		4.00	4.00	4.00	4.00
44	4		4.00	4.00	4.00	4.00
57	4		4.00	4.00	4.00	4.00
11	4			4.25	4.25	4.25
22	4			4.25	4.25	4.25
26	4			4.25	4.25	4.25
32	4			4.25	4.25	4.25
35	4			4.25	4.25	4.25
41	4			4.25	4.25	4.25
49	4			4.25	4.25	4.25
4	4			4.50	4.50	4.50
10	4			4.50	4.50	4.50
15	4			4.50	4.50	4.50
23	4			4.50	4.50	4.50
24	4			4.50	4.50	4.50
42	4			4.50	4.50	4.50
53	4			4.50	4.50	4.50

58	4			4.50	4.50	4.50
25	4				4.75	4.75
39	4				4.75	4.75
50	4				4.75	4.75
52	4				4.75	4.75
55	4				4.75	4.75
60	4				4.75	4.75
8	4					5.00
12	4					5.00
19	4					5.00
21	4					5.00
29	4					5.00
33	4					5.00
34	4					5.00
38	4					5.00
47	4					5.00
48	4					5.00
51	4					5.00
Sig.		.084	.051	.056	.056	.057

APPENDIX 15: AVERAGE PHENOTYPE PER GENOTYPE IN VALUE

Genotype	N	Subset								
		1	2	3	4	5	6	7	8	9
2	4	1.7500								
16	4	1.7500								
40	4	1.8750	1.8750							
43	4	1.8750	1.8750							
45	4	2.0000	2.0000	2.0000						
17	4	2.1250	2.1250	2.1250	2.1250					
46	4	2.1250	2.1250	2.1250	2.1250					
3	4	2.2500	2.2500	2.2500	2.2500	2.2500				
5	4	2.3750	2.3750	2.3750	2.3750	2.3750	2.3750			
14	4	2.3750	2.3750	2.3750	2.3750	2.3750	2.3750			
9	4	2.6250	2.6250	2.6250	2.6250	2.6250	2.6250	2.6250		
13	4	2.6250	2.6250	2.6250	2.6250	2.6250	2.6250	2.6250		
44	4	2.6250	2.6250	2.6250	2.6250	2.6250	2.6250	2.6250		
1	4	2.7500	2.7500	2.7500	2.7500	2.7500	2.7500	2.7500		
6	4	2.7500	2.7500	2.7500	2.7500	2.7500	2.7500	2.7500		
11	4	2.7500	2.7500	2.7500	2.7500	2.7500	2.7500	2.7500		
30	4	2.7500	2.7500	2.7500	2.7500	2.7500	2.7500	2.7500		
35	4	2.7500	2.7500	2.7500	2.7500	2.7500	2.7500	2.7500		
57	4	2.7500	2.7500	2.7500	2.7500	2.7500	2.7500	2.7500		
18	4	2.8750	2.8750	2.8750	2.8750	2.8750	2.8750	2.8750		
27	4	2.8750	2.8750	2.8750	2.8750	2.8750	2.8750	2.8750		
59	4	2.8750	2.8750	2.8750	2.8750	2.8750	2.8750	2.8750		
4	4	3.0000	3.0000	3.0000	3.0000	3.0000	3.0000	3.0000		
28	4	3.0000	3.0000	3.0000	3.0000	3.0000	3.0000	3.0000		
37	4	3.0000	3.0000	3.0000	3.0000	3.0000	3.0000	3.0000		
56	4	3.0000	3.0000	3.0000	3.0000	3.0000	3.0000	3.0000		
20	4	3.1250	3.1250	3.1250	3.1250	3.1250	3.1250	3.1250	3.1250	
51	4	3.1250	3.1250	3.1250	3.1250	3.1250	3.1250	3.1250	3.1250	
7	4	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500
10	4	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500
12	4	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500
24	4	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500
31	4	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500
34	4	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500
41	4	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500
55	4	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500
23	4		3.3750	3.3750	3.3750	3.3750	3.3750	3.3750	3.3750	3.3750
33	4		3.3750	3.3750	3.3750	3.3750	3.3750	3.3750	3.3750	3.3750
36	4		3.3750	3.3750	3.3750	3.3750	3.3750	3.3750	3.3750	3.3750
50	4		3.3750	3.3750	3.3750	3.3750	3.3750	3.3750	3.3750	3.3750
21	4			3.5000	3.5000	3.5000	3.5000	3.5000	3.5000	3.5000
26	4			3.5000	3.5000	3.5000	3.5000	3.5000	3.5000	3.5000
38	4			3.5000	3.5000	3.5000	3.5000	3.5000	3.5000	3.5000
49	4			3.5000	3.5000	3.5000	3.5000	3.5000	3.5000	3.5000
53	4			3.5000	3.5000	3.5000	3.5000	3.5000	3.5000	3.5000

58	4			3.5000	3.5000	3.5000	3.5000	3.5000	3.5000	3.5000
8	4				3.6250	3.6250	3.6250	3.6250	3.6250	3.6250
22	4				3.6250	3.6250	3.6250	3.6250	3.6250	3.6250
15	4					3.7500	3.7500	3.7500	3.7500	3.7500
25	4					3.7500	3.7500	3.7500	3.7500	3.7500
29	4					3.7500	3.7500	3.7500	3.7500	3.7500
39	4					3.7500	3.7500	3.7500	3.7500	3.7500
47	4					3.7500	3.7500	3.7500	3.7500	3.7500
54	4					3.7500	3.7500	3.7500	3.7500	3.7500
48	4						3.8750	3.8750	3.8750	3.8750
60	4						3.8750	3.8750	3.8750	3.8750
19	4							4.0000	4.0000	4.0000
42	4							4.0000	4.0000	4.0000
32	4								4.6250	4.6250
52	4									4.7500
Sig.		.060	.060	.061	.061	.062	.062	.088	.059	.059

APPENDIX 16: PHENOTYPES PER GENOTYPE IN SANGALO AT DAY 70

Different varieties of screened potato cultivars’	N	Subset			
		1	2	3	4
18	4	1.00			
28	4	1.25			
2	4	1.50	1.50		
14	4	1.50	1.50		
21	4	1.50	1.50		
35	4	1.50	1.50		
1	4	1.75	1.75	1.75	
20	4	1.75	1.75	1.75	
36	4	1.75	1.75	1.75	
44	4	1.75	1.75	1.75	
45	4	1.75	1.75	1.75	
50	4	1.75	1.75	1.75	
56	4	1.75	1.75	1.75	
5	4	2.00	2.00	2.00	2.00
9	4	2.00	2.00	2.00	2.00
41	4	2.00	2.00	2.00	2.00
42	4	2.00	2.00	2.00	2.00
46	4	2.00	2.00	2.00	2.00
4	4	2.25	2.25	2.25	2.25
13	4	2.25	2.25	2.25	2.25
39	4	2.25	2.25	2.25	2.25
57	4	2.25	2.25	2.25	2.25
58	4	2.25	2.25	2.25	2.25
3	4	2.50	2.50	2.50	2.50
16	4	2.50	2.50	2.50	2.50
19	4	2.50	2.50	2.50	2.50
51	4	2.50	2.50	2.50	2.50
7	4	2.75	2.75	2.75	2.75
8	4	2.75	2.75	2.75	2.75
17	4	2.75	2.75	2.75	2.75
22	4	2.75	2.75	2.75	2.75
27	4	2.75	2.75	2.75	2.75
31	4	2.75	2.75	2.75	2.75
47	4	2.75	2.75	2.75	2.75
11	4	3.00	3.00	3.00	3.00
12	4	3.00	3.00	3.00	3.00
15	4	3.00	3.00	3.00	3.00
30	4	3.00	3.00	3.00	3.00
32	4	3.00	3.00	3.00	3.00
37	4	3.00	3.00	3.00	3.00
48	4	3.00	3.00	3.00	3.00
52	4	3.00	3.00	3.00	3.00
55	4	3.00	3.00	3.00	3.00
10	4	3.25	3.25	3.25	3.25
24	4	3.25	3.25	3.25	3.25

29	4	3.25	3.25	3.25	3.25
33	4	3.25	3.25	3.25	3.25
53	4	3.25	3.25	3.25	3.25
54	4	3.25	3.25	3.25	3.25
59	4	3.25	3.25	3.25	3.25
60	4	3.25	3.25	3.25	3.25
6	4	3.50	3.50	3.50	3.50
26	4	3.50	3.50	3.50	3.50
40	4	3.50	3.50	3.50	3.50
25	4		4.00	4.00	4.00
34	4		4.00	4.00	4.00
49	4		4.00	4.00	4.00
43	4			4.25	4.25
23	4				4.50
38	4				4.50
Sig.		.057	.057	.057	.056

APPENDIX 17: PHENOTYPES PER GENOTYPE IN SANGALO AT DAY 90

Different varieties of screened potato cultivars'	N	Subset			
		1	2	3	4
18	4	1.00			
28	4	1.00			
38	4	1.00			
43	4	1.00			
21	4	1.50	1.50		
35	4	1.50	1.50		
1	4	1.75	1.75	1.75	
20	4	1.75	1.75	1.75	
36	4	1.75	1.75	1.75	
44	4	1.75	1.75	1.75	
45	4	1.75	1.75	1.75	
50	4	1.75	1.75	1.75	
56	4	1.75	1.75	1.75	
5	4	2.00	2.00	2.00	2.00
9	4	2.00	2.00	2.00	2.00
41	4	2.00	2.00	2.00	2.00
42	4	2.00	2.00	2.00	2.00
46	4	2.00	2.00	2.00	2.00
4	4	2.25	2.25	2.25	2.25
13	4	2.25	2.25	2.25	2.25
39	4	2.25	2.25	2.25	2.25
57	4	2.25	2.25	2.25	2.25
58	4	2.25	2.25	2.25	2.25
3	4	2.50	2.50	2.50	2.50
16	4	2.50	2.50	2.50	2.50
19	4	2.50	2.50	2.50	2.50
51	4	2.50	2.50	2.50	2.50
7	4	2.75	2.75	2.75	2.75
8	4	2.75	2.75	2.75	2.75
17	4	2.75	2.75	2.75	2.75
22	4	2.75	2.75	2.75	2.75
27	4	2.75	2.75	2.75	2.75
31	4	2.75	2.75	2.75	2.75
47	4	2.75	2.75	2.75	2.75
11	4	3.00	3.00	3.00	3.00
12	4	3.00	3.00	3.00	3.00
15	4	3.00	3.00	3.00	3.00
30	4	3.00	3.00	3.00	3.00
32	4	3.00	3.00	3.00	3.00
37	4	3.00	3.00	3.00	3.00
48	4	3.00	3.00	3.00	3.00
52	4	3.00	3.00	3.00	3.00
55	4	3.00	3.00	3.00	3.00

10	4	3.25	3.25	3.25	3.25
24	4	3.25	3.25	3.25	3.25
29	4	3.25	3.25	3.25	3.25
33	4	3.25	3.25	3.25	3.25
53	4	3.25	3.25	3.25	3.25
54	4	3.25	3.25	3.25	3.25
59	4	3.25	3.25	3.25	3.25
60	4	3.25	3.25	3.25	3.25
6	4	3.50	3.50	3.50	3.50
26	4	3.50	3.50	3.50	3.50
40	4	3.50	3.50	3.50	3.50
25	4		4.00	4.00	4.00
34	4		4.00	4.00	4.00
49	4		4.00	4.00	4.00
14	4			4.25	4.25
23	4				4.50
2	4				4.50
Sig.		.057	.057	.057	.056

APPENDIX 18: AVERAGE PHENOTYPE PER GENOTYPE IN SANG'ALO A DAY, 40, 70, AND 90

Different varieties of screened potato cultivars	N	Subset							
		1	2	3	4	5	6	7	8
18	4	1.00							
24	4	1.00							
28	4	1.00							
34	4	1.00							
37	4	1.00							
38	4	1.00							
43	4	1.00							
44	4	1.50							
45	4	1.50							
46	4	1.75							
56	4	1.75							
36	4	1.75	1.75						
51	4	1.75	1.75						
13	4	1.50	2.00	2.00					
31	4	2.00	2.00	2.00	2.00				
39	4	2.00	2.00	2.00	2.00				
5	4	2.25	2.25	2.25	2.25	2.25			
9	4	2.25	2.25	2.25	2.25	2.25			
35	4	2.25	2.25	2.25	2.25	2.25			
54	4	2.25	2.25	2.25	2.25	2.25			
57	4	2.50	2.50	2.50	2.50	2.50	2.50		
6	4	2.75	2.75	2.75	2.75	2.75	2.75	2.75	
25	4	2.75	2.75	2.75	2.75	2.75	2.75	2.75	
42	4	2.75	2.75	2.75	2.75	2.75	2.75	2.75	
49	4	2.75	2.75	2.75	2.75	2.75	2.75	2.75	
16	4	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
21	4	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
22	4	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
23	4	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
30	4	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
33	4	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
2	4		3.25	3.25	3.25	3.25	3.25	3.25	3.25
41	4		3.25	3.25	3.25	3.25	3.25	3.25	3.25
50	4		3.25	3.25	3.25	3.25	3.25	3.25	3.25
52	4		3.25	3.25	3.25	3.25	3.25	3.25	3.25

1	4			3.50	3.50	3.50	3.50	3.50	3.50
32	4			3.50	3.50	3.50	3.50	3.50	3.50
4	4				3.75	3.75	3.75	3.75	3.75
17	4				3.75	3.75	3.75	3.75	3.75
19	4				3.75	3.75	3.75	3.75	3.75
47	4				3.75	3.75	3.75	3.75	3.75
14	4				4.00	4.00	4.00	4.00	4.00
40	4				4.00	4.00	4.00	4.00	4.00
58	4				4.00	4.00	4.00	4.00	4.00
59	4				4.00	4.00	4.00	4.00	4.00
7	4					4.25	4.25	4.25	4.25
10	4					4.25	4.25	4.25	4.25
29	4					4.25	4.25	4.25	4.25
11	4						4.50	4.50	4.50
26	4						4.50	4.50	4.50
48	4						4.50	4.50	4.50
53	4						4.50	4.50	4.50
8	4							4.75	4.75
12	4							4.75	4.75
15	4							4.75	4.75
20	4							4.75	4.75
3	4								5.00
27	4								5.00
55	4								5.00
60	4								5.00
Sig.		.059	.056	.056	.059	.060	.060	.060	.060

APPENDIX 19: AVERAGE PHENOTYPES PER GENOTYPE IN THE THREE FIELD TRIAL SITES COMBINED

Potato varieties	N	Subset							
		1	2	3	4	5	6	7	8
45	3	1.000							
28	3	1.2500	1.2500						
43	3	1.2500	1.2500	1.2500					
2	3	2.1267	2.1267	2.1267	2.1267				
13	3	2.3767	2.3767	2.3767	2.3767	2.3767			
44	3	2.4200	2.4200	2.4200	2.4200	2.4200	2.4200		
5	3	2.6267	2.6267	2.6267	2.6267	2.6267	2.6267	2.6267	
57	3	2.6667	2.6667	2.6667	2.6667	2.6667	2.6667	2.6667	
46	3	2.7100	2.7100	2.7100	2.7100	2.7100	2.7100	2.7100	
9	3	2.7933	2.7933	2.7933	2.7933	2.7933	2.7933	2.7933	2.7933
31	3	2.8333	2.8333	2.8333	2.8333	2.8333	2.8333	2.8333	2.8333
35	3	2.8333	2.8333	2.8333	2.8333	2.8333	2.8333	2.8333	2.8333
16	3	2.8767	2.8767	2.8767	2.8767	2.8767	2.8767	2.8767	2.8767
40	3	2.8767	2.8767	2.8767	2.8767	2.8767	2.8767	2.8767	2.8767
29	3	3.0000	3.0000	3.0000	3.0000	3.0000	3.0000	3.0000	3.0000
27	3	3.0033	3.0033	3.0033	3.0033	3.0033	3.0033	3.0033	3.0033
21	3	3.0433	3.0433	3.0433	3.0433	3.0433	3.0433	3.0433	3.0433
37	3	3.0433	3.0433	3.0433	3.0433	3.0433	3.0433	3.0433	3.0433
1	3	3.0833	3.0833	3.0833	3.0833	3.0833	3.0833	3.0833	3.0833
36	3	3.1267	3.1267	3.1267	3.1267	3.1267	3.1267	3.1267	3.1267
38	3	3.1267	3.1267	3.1267	3.1267	3.1267	3.1267	3.1267	3.1267
34	3	3.1667	3.1667	3.1667	3.1667	3.1667	3.1667	3.1667	3.1667
41	3	3.1667	3.1667	3.1667	3.1667	3.1667	3.1667	3.1667	3.1667
14	3	3.1700	3.1700	3.1700	3.1700	3.1700	3.1700	3.1700	3.1700
51	3	3.1833	3.1833	3.1833	3.1833	3.1833	3.1833	3.1833	3.1833
17	3	3.2100	3.2100	3.2100	3.2100	3.2100	3.2100	3.2100	3.2100
20	3	3.2100	3.2100	3.2100	3.2100	3.2100	3.2100	3.2100	3.2100
39	3	3.2100	3.2100	3.2100	3.2100	3.2100	3.2100	3.2100	3.2100
18	3		3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500
30	3		3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500
56	3		3.2500	3.2500	3.2500	3.2500	3.2500	3.2500	3.2500
50	3		3.2533	3.2533	3.2533	3.2533	3.2533	3.2533	3.2533
24	3		3.2933	3.2933	3.2933	3.2933	3.2933	3.2933	3.2933
49	3		3.3333	3.3333	3.3333	3.3333	3.3333	3.3333	3.3333
4	3			3.4600	3.4600	3.4600	3.4600	3.4600	3.4600
12	3			3.4600	3.4600	3.4600	3.4600	3.4600	3.4600
3	3				3.5000	3.5000	3.5000	3.5000	3.5000
11	3				3.5433	3.5433	3.5433	3.5433	3.5433
22	3				3.5867	3.5867	3.5867	3.5867	3.5867
25	3				3.6267	3.6267	3.6267	3.6267	3.6267
32	3				3.6267	3.6267	3.6267	3.6267	3.6267
47	3				3.6267	3.6267	3.6267	3.6267	3.6267
59	3				3.6267	3.6267	3.6267	3.6267	3.6267
26	3					3.6667	3.6667	3.6667	3.6667

33	3					3.6700	3.6700	3.6700	3.6700
7	3					3.7100	3.7100	3.7100	3.7100
48	3					3.7100	3.7100	3.7100	3.7100
58	3					3.7100	3.7100	3.7100	3.7100
6	3					3.7500	3.7500	3.7500	3.7500
42	3					3.7500	3.7500	3.7500	3.7500
10	3					3.8333	3.8333	3.8333	3.8333
54	3					3.8333	3.8333	3.8333	3.8333
15	3						3.9167	3.9167	3.9167
19	3						3.9167	3.9167	3.9167
53	3						3.9167	3.9167	3.9167
23	3							3.9700	3.9700
8	3							4.0433	4.0433
55	3							4.0833	4.0833
60	3							4.1267	4.1267
52	3								4.2500
Sig.		.054	.072	.055	.050	.058	.052	.052	.058