SEROLOGICAL AND MOLECULAR CHARACTERIZATION OF PHASEY BEAN MILD YELLOWS VIRUS AND CUCURBIT APHID-BORNE YELLOWS VIRUS INFECTING GROUNDNUT (*Arachis hypogaea* L.) IN WESTERN KENYA

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A Thesis Submitted in Partial Fulfilment of the Requirements for the Award of the Degree of Doctor of Philosophy in Sustainable Agricultural Systems of Masinde Muliro University of Science and Technology.

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DECLARATION

This Thesis is my original work prepared with no other than the indicated sources and support and has not been presented elsewhere for a degree or any other award.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a thesis entitled '**Serological and molecular characterization of Phasey bean mild yellows virus and Cucurbit aphid-borne yellows virus infecting groundnut (*Arachis hypogaea* L.) in western Kenya'**

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DEDICATION

This Thesis is dedicated to my wife Miriam Naliaka and my sons Jermaine Phinehas Mabele and Niall Nathans Mabele for their patience, encouragement and support during this research.

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ABSTRACT

Groundnut or Peanut (*Arachis hypogaea* L.) is an important oilseed legume crop in Kenya, but yields are declining due to pests and diseases. Plant viruses cause serious disease of crop plants reducing both quality and quantity of the final produce. New viruses previously not known to infect groundnuts, are being detected in groundnuts in the advent of Next Generation Sequencing (NGS) technologies. Phasey bean mild yellows virus (PBMYV, genus: *Polerovirus*; family: *Luteoviridae*) and Cucurbit aphid-borne yellows virus (CABYV, genus *Polerovirus*, family *Luteoviridae*) causes Phasey bean mild yellows disease (PBMYD) and Cucurbit aphid-borne yellows disease (CABYD) in cultivated crops and wild hosts respectively. PBMYV and CABYV poleroviruses were detected through NGS in groundnut samples from Kenya. The discovery of these new viruses with interveinal leaf necrosis, chlorosis, mottling, curling, deformation and puckering symptoms, highlights the underestimated diversity within the genus, which could be related to virus-like diseases with yet unidentified causal agents or the emergence of new diseases in cultivated crops. The viruses are new in Kenya and on groundnuts as a host. The serological and molecular characteristics of PBMYV and CABYV detected were not well known in Kenya. This would hinder their precise diagnosis and management in Kenya and on the new host. This study determined the occurrence, distribution, biological, serological and molecular characterization of PBMYV and CABYV detected in Kenya. Two disease diagnostic surveys were conducted during the short and long rain seasons of 2020 and 2021 in Bungoma, Busia, Kakamega and Siaya Counties of western Kenya. Socio-economic data of the groundnut farmers was captured in a questionnaire. A total of 345 farms were surveyed (245 in long rains and 100 during short rains). A total of 673 leaf samples were collected. Biological characterization of CABYV was done through mechanical and vector inoculation on popularly grown groundnut varieties, leguminous and solanaceous hosts. Disease incidence and severity was scored on the disease diagnostic score sheet. The survey data collected was subjected to analysis of variance using R Statistical Software Package version 3.6 and IBM SPSS version 28.0. Pairwise comparison of means was done using Least Significance Difference (LSD) at $P \leq 0.05$ confidence level. Serological bioassays for CABYV were done with TAS-ELISA and RT-PCR for PBMYV. Total RNA was extracted from the leaf samples using RNeasy Mini Kit **(**Qiagen**)** and CTAB method, then used for double stranded cDNA synthesis using the SuperScript II kit. The samples were pooled then processed with the transposon-based chemistry library preparation kit (Nextera XT, Illumina). The DNA libraries were sequenced (200-bp paired-end sequencing) on the Illumina MiSeq benchtop sequencer platform (Illumina, USA). The reads were used for de novo assembly and contigs aligned to the viral genomes database using CLC Genomics Workbench 10.1.2. The assembled contigs were subjected to a BLASTn search against the GenBank database. Phylogenetic analyses and comparisons were performed using the MEGA X software. The short rains season recorded higher incidence (73.61%) than the long rains season (42.65%). PBMYV alongside CABYV were detected. The novel PBMYV full genome sequences from Kenya (PBMYV_6, PBMYV_7) clustered together implying common ancestry and a possible identity by descent (IBD) with other PBMYV sequences, and had closest sequence identity (91-95%) with PBMYV (KT963000.2, MT966033.1 and MT966038.1) in distinct clades. CABYV6-2 showed 94.5% nucleotide identity with CpPV2 isolate KX599164.1 from Burkina Faso and 83.4% identity with CABYV isolate MG257902.1 from Korea. This study documents the first reports of PBMYV and CABYV poleroviruses in Kenya with a new host record, *A. hypogaea*. This research findings are strategic in the development of sustainable management technologies of the two poleroviruses through multi-pathogenic robust diagnostics, breeding resistant groundnut cultivars and distinct understanding of vector-virus-host pathosystems interactions.

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LIST OF ABBREVIATIONS AND ACRONYMS

AAFP	-Acquisition Access Feeding Period.
AAP	-Acquisition Access Period.
AFLPs	-Amplified Fragment Length Polymorphisms.
AEZ	- Agro-Ecological Zones.
bp	- Base Pair.
BLAST	-Basic Local Alignment Search Tool.
CABYD	-Cucurbit Aphid-Borne Yellows Disease.
CABYV	-Cucurbit Aphid-Borne Yellows Virus.
CCD	-Charge-Coupled Device.
cDNA	-Complementary Dioxyribonucleic Acid.
CP	-Major Coat Protein.
CPm	-Minor Coat Protein.
CTAB	-Cetyltrimethylammonium Bromide.
DAC-ELISA	-Direct Antigen Coated–Enzyme Linked Immunosorbent Assay.
DAS-ELISA	-Double Antibody Sandwich–Enzyme Linked Immunosorbent Assay.
DDBJ	-DNA Data Bank of Japan.
DNA	-Deoxyribonucleic Acid.
dNTPs	-Deoxyribonucleoside Triphosphates.
dsRNA	- Double Stranded Ribonucleic Acid.

ELISA - Enzyme Linked Immunosorbent Assay.

GPRS -Geographical Positioning Remote System.

IAFP -Inoculated Access Feeding Period.

IAP -Inoculated Access Period.

IDM -Integrated Disease Management.

IPM -Integrated Pest Management.

IR -Non-Coding Interaction Region.

IRES -Internal Ribosomal Entry Site.

IRM -Integrated Resistance Management.

LM - Lower Midland.

LSD -Least Significance Difference.

MAbs -Monoclonal Anti-bodies.

MEGA -Molecular Evolutionary Genetics Analysis.

MMUST -Masinde Muliro University of Science and Technology.

MP -Putative Movement Protein.

NACOSTI - National Commission for Science, Technology and Innovation.

NGS - Next Generation Sequencing.

ORF - Open Reading Frame.

P0-P5 -Protein 0 to Protein 5

PBMYD -Phasey Bean Mild Yellows Disease.

PBMYV -Phasey Bean Mild Yellows Virus.

PBS -Phosphate Buffer Saline.

PCR -Polymerase Chain Reaction.

PCR-RFLPs -Polymerase Chain Reaction-Restriction Fragment Length Polymorphisms.

PLWD -People Living With Diability.

Pro -Putative Protease.

PTGS -Post-Transcriptional Gene Silencing.

Rap1 -Replication-Associated Protein.

RdRp -RNA-dependent-RNA-polymerase.

RNA -Ribonucleic Acid.

RT - Reverse Transcribing.

RT-P - Read-Through Protein.

RT-PCR - Reverse Transcription-Polymerase Chain Reaction.

SDS - Sodium Dodecyl Sulphate.

SNPs - Single Nucleotide Polymorphisms.

ssRNA - Single Stranded Ribonucleic Acid.

TAS-ELISA -Triple Antibody Sandwich–Enzyme Linked Immunosorbent Assay.

UM - Upper Midland.

VPgs - Serine Roteases and Genome Linked Viral Proteins.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

1.1.1 Groundnut crop origin and botany

The groundnut (*Arachis hypogaea* L.) is a native crop of southern America that goes by many different names around the world: groundnut in Africa, peanut in the United States of America,'mani' in Spanish, 'amondoim' in Portuguese, 'pistache' in French, 'mungphali' in Hindi, and 'ying zui dou' in Chinese. As an annual oilseed crop, groundnut (*Arachis hypogaea* L.) yields 37.2 million tonnes worldwide from 23.4 million ha (Kayondo *et al*., 2014). Grown for its oilseed, edible nuts, and animal feed, groundnut is a major monoecious and herbaceous annual leguminous crop in the family *Fabaceae* (Upadhyaya *et al*., 2006). The scientific name for this plant, *Arachis hypogaea* Linn., comes from the Greek for "underground chamber" (*hypogea*) and the term for "weed," "*Arachis*" (Holbrook and Stalker, 2003). Depending on the variety, groundnut plants can reach a maximum height of 0.6 m and have a tiny, upright, thin-stemmed appearance with feathery-looking leaves. The leaves are attached near the stem in a manner similar to a leaf, and they are organized in alternate pairs. Blossoms on the groundnut plant can be yellow, orange, cream, or white. These blossoms evolve into pegs, which grow downward into the soil, demonstrating positive geotropism, and eventually stop growing in length to form a pod (Shezi, 2011). The pods are cylindrical in shape and include constriction between the seeds and reticulation on the outer surface. Pods often form underground, although they can also grow in the air. The pods can reach a maximum length of 10 cm and typically contain anywhere from one to five seeds. Variables such as cultivar, planting time, pests, diseases, and weeds all affect pod productivity (Shezi, 2011; Chintu, 2013). Seeds can be found in a wide variety of shapes and colors, from off-

white to deep purple. Each seed has an epicotyl, a major root, and two enormous cotyledons. The cotyledons are the primary storage tissue for the developing seedling and account for about 96% of the total seed weight (Holbrook and Stalker, 2003). The life span of a groundnut plant is strictly limited to the growing season.

1.1.2 Groundnut production and importance

Commercial cultivation of groundnut (*Arachis hypogaea* L.) takes place across the globe, from latitudes 40° N to 40° S, in both tropical and subtropical environments. Over 90% of the world's groundnuts come from the semiarid tropical region, which is characterized by variable rainfall. The semiarid regions are ideal for growing this crop because they rely solely on rain for irrigation. Low inputs, rain-fed cultivation of the crop on marginal soils, a lack of acceptable high-yielding cultivar seed, and the presence of insect pests and illnesses at various phases of the crop's development, all contribute to low yields in groundnut. Groundnuts thrive in temperatures between 250 and 300 degrees Celsius. Since the reproductive phase of groundnut is more vulnerable to heat stress than the vegetative phase, temperatures exceeding 35 ^{0}C are damaging to groundnut output (Kamara, 2010). The crop can withstand dry conditions, but it produces at its highest potential only with evenly spaced annual rainfall of 500–1000 millimeters (Kamara, 2010). The best soils for growing groundnut are deep, well-drained sandy, sandy loam, or loamy sand soils. Nevertheless, the plant can be grown in almost any soil type except particularly heavy ones (made up mostly of clay).

Farmers around the world can benefit financially and nutritionally from growing groundnuts. China, India, and the United States are the top three groundnut producers, followed by countries in sub-Saharan Africa, Central America, and South America (Chintu, 2013). The average yield per hectare for groundnuts in 2009 was 1520 kilograms, and the total production was 36.45 million tons (Muitia, 2011). Only 26% of the highest average were

seen in the Southern Africa region, and the lowest in the Eastern Africa region of sub-Saharan Africa (Kidula *et al*., 2010). Africa is home to 40% of the world's groundnut plantings. Virginia, Espanola/Spanish, Malgache, Kersting, and Roja Tennessee are the most common groundnut cultivars grown on all five continents. The majority of groundnuts planted in Kenya are either bunchy groundnuts, which take 90–100 days to mature, or runner groundnuts, which take 120–150 days. The common varieties grown include Alika, Asyria Mwitunde, Bukene, CG-3, CG-7, Cianda, Homabay Red, Homabay White (CG-2), ICGV-07, ICGV-12991, ICGV-12988, ICGV-90708, ICGV-9991, Local Red, Madiaba Red, Makulu Red, Minipinta, Red Valencia, Serere-116 White, SM-99508, SM-99568, Texas Peanut, Uganda Red and Virginia Purple. Most of the ICGV-90708 crop is produced in the Rift Valley areas of Kenya.

The high quality edible oil (48-50%), protein (26-28%), and carbs (34-36%) found in groundnuts are the primary reasons for its global cultivation (Ayoola *et al*., 2012). It is also high in dietary fiber, vitamins (especially the B group), minerals (including copper, phosphorus, calcium, magnesium, and iron), and protein. The harvested haulms and stalks of groundnuts make good fodder for cattle, and the cake obtained from the oil extraction process is also put to use in the animal feed business. Cardboard, fuel, and filler in the feed and fertilizer industries are just some of the many uses for groundnut shells. As a leguminous crop, it is useful in maintaining cropping systems because of the N_2 it adds to the soil through biological nitrogen fixation (BNF). Two-thirds of the world's harvest is crushed for oil, with the remaining third going to indirect uses like candy. However, usage patterns differ both domestically and internationally. The majority of groundnuts grown in Kenya come from subsistence farmers. Local and international trading in the crop occurs (Kidula *et al*., 2010). Raw, roasted with salt, boiled, and as a key element in cereal flours for pregnant women and babies are just some of the ways the crop is used in the region. It is also partially processed

and sold as cooking oil. Children with malnutrition can benefit from eating groundnuts, which are also used as a snack and in processed form (Onyango, 2017).

1.2 Constraints to groundnut production

The insufficiency of better groundnut cultivars, as well as biotic and abiotic stressors, all act as brakes on groundnut productivity. Although smallholder farmers in underdeveloped nations produce 75% to 80% of the world's groundnut, they often only harvest 500-800 kg/ha despite the crop's potential yield of >2.5 t/ha (Kayondo *et al*., 2014). Farmers in western Kenya only harvest an average of 600-700 kg/ha of groundnuts, which is less than 30-50% of the possible yield (Kidula *et al*., 2010). Major yield losses in vegetable and arable crops are frequently caused by viruses of the genus Polerovirus (family *Luteoviridae*). Plant viruses such as Phasey bean mild yellows virus (PBMYV) and Cucurbit aphid-borne yellows virus (CABYV), are the primary cause of low groundnut yields in western Kenya. The devastating infections that plant viruses cause to crop plants reduce both the quality and yield of the harvest. In addition, viruses are responsible for a higher percentage of new plant infectious illnesses than any combination of fungi, bacteria, phytoplasma, or nematodes (Jones *et al*., 2004). Nearly half (47%) of such diseases in the studied ecosystems are caused by the calculated plant viruses, according to Anderson *et al* (2004). As many as 31 different viruses have been documented as naturally infecting groundnut in various parts of the world (Kumar & Waliyar, 2007). Potyvirus accounts for nine, Tospovirus for six, Cucumovirus, Pecluvirus, Soymovirus, and Umbravirus for two, and Begomovirus, Bromovirus, Carlavirus, Ilarvirus, Luteovirus, Potexvirus, Rhabdovirus, and Tymovirus for one each. As reported by Salem *et al* (2010), 19 of these viruses were initially isolated from groundnut, while the remaining 12 were found in other hosts. With the development of Next Generation Sequencing (NGS), Sanger Sequencing, and other rapidly enhanced molecular diagnostic tools, not only are these preexisting groundnut viruses being found in groundnuts, but also novel viruses not

previously known to infect groundnuts. Other examples are the Phasey bean mild yellows virus (PBMYV; Sharman *et al*., 2021) and the Cucurbit aphid-borne yellows virus (CABYV; Costa *et al*., 2019), both of which have recently been discovered in new farmed and wild hosts. This is leading to a rise in the number of reported viruses that infect groundnuts, including ones that have not yet been found in Kenya. The identification of these new viruses demonstrates the underappreciated variety within the genus Polerovirus and may be associated with the introduction of new diseases in cultivated plant species or virus-like diseases for which the etiological agents have not yet been identified. CABYV is transmitted by at least three aphid species, *Aphis gossypii*, *Myzus persicae*, and *Macrosiphum euphorbiae*, in a circulative, non-propagative way (Kassem *et al*., 2013), whereas PBMYV is transmitted by (*Aphis craccivora*) and by grafting with infected scions (Sharman *et al*., 2021). There are many aphids in Kenya, and western Kenya pathosystems is where the virus's primary hosts (Leguminous beans and Cucurbit crops) are grown. PBMYV has only been documented in Australia (Sharman *et al*., 2021) while CABYV has been detected in adjacent Uganda (Masika *et al*., 2017) and Tanzania (Desbiez *et al*., 2016), from which there is enormous exchange of planting material.

Several different viruses, including the Cucumber mosaic virus (CMV), Groundnut bud necrosis virus (GBNV), Groundnut rosette assistor virus (GRAV), Groundnut rosette virus (GRV), Indian peanut clump virus (IPCV), Peanut clump virus (PCV), Peanut mottle virus (PeMoV), and Tobacco streak virus (TSV), are responsible for the most devastating groundnut viral diseases. In terms of economic impact, viral infections are at the top of the list because they cause significant yield losses on a local, national, and international scale (Gyoutoku *et al*., 2009). Several other crops are also susceptible to natural infection from these viruses, which causes severe yield reduction. Some of the symptoms observed during earlier monitoring efforts in western Kenya's groundnut-growing sub-counties are not

characteristic of any of the primary viruses described in groundnuts (Mukoye *et al*., 2020). This suggests that groundnuts may be harboring several viruses that have not been previously documented in western Kenya.

Insect pests, such as the termite (*Microtermes* spp.), white grub (*Lachnosterna consanguinea* Blanchard), thrip (*Megalurothrips uitatus* Bagnall), aphid (*Aphis craccivora* Koch), and whitefly (*Bemisia tabaci* Gennadius), can cause significant damage to groundnut crops. Drought, low soil fertility, weeds, faulty seed production and certification technologies, and inefficient socio-economic post-harvest processing procedures, all qualify as abiotic pressures. Better disease resistance, tolerance, higher yields, preferred taste and size, and general market acceptability are the goals of plant breeding efforts.

1.3 Statement of the problem

Plant virus diseases including unreported Phasey bean mild yellows disease (PBMYD) and Cucurbit aphid-borne yellows disease (CABYD) devastate groundnut crop in sub-Saharan African countries. Crop science researchers need to redouble their efforts to ensure food security. Farmers in sub-Saharan Africa face failing harvests in groundnut farming as a result of various viral diseases which are difficult to control, making them often unable to feed their families. New and different varied symptom types observed on groundnuts in western Kenya are not typical to any of the major known groundnut viruses, but are associated with PBMYV and CABYV, posing a great challenge in effective diagnosis and management of groundnut virus diseases by the farmers. With the advent of Next Generation Sequencing (NGS) technologies, groundnuts have been shown to host many viruses and some of them like PBMYV and CABYV have not yet been reported on groundnuts in Kenya (Mukoye, 2020). Viruses have been identified as one of the major yield reducing biotic factors in groundnuts leading to increased poverty, malnutrition and food insecurity, hindering the farmer's

economic and social development (Ndisio *et al*., 2017). PBMYV and CABYV Poleroviruses were detected through NGS in groundnut samples from western Kenya in this study. The discovery of these new viruses highlights the underestimated diversity that is under researched within the genus *Polerovirus*, and this could be related to virus-like diseases with yet unidentified causal agents, symptoms or the emergence of new disease scenarios in cultivated plant species. PBMYV and CABYV are new viruses in western Kenya and on groundnut crop as an alternative host. The serological and molecular characteristics of PBMYV and CABYV detected on groundnut isolates were unknown in western Kenya and this would hinder their robust specific and efficient diagnosis and management on the new host plants. Therefore, for precise and adequate understanding of PBMYV and CABYV viruses, there was need for their characterization through molecular, serological and biological methods to document the biology of these new plant virus diseases. PBMYV is transmitted by *Aphis craccivora* and by grafting with infected scions while CABYV is transmitted by at least three aphid species, *Aphis gossypii*, *Myzus persicae* and *Macrosiphum euphorbiae* in a circulative non-propagative manner (Kassem *et al.,* 2013). The generalist viruliferous aphid vectors are abundant in western Kenya, and the main hosts (Leguminous beans and Cucurbits) of the viruses are widely cultivated within western Kenya. This distinct information implies that PBMYV and CABYV Poleroviruses could quickly spread to new unidentified ecological niches, making western Kenya a hotspot of new plant virus diseases, unless specific and robust diagnostic methods for their detection and appropriate management mechanisms are established through this study.

The Polerovirus epiphytology is complex and lack of precise and sensitive molecular diagnostic tools for the virus, limits the understanding of the epidemiology and etiology of PBMYV and CABYV, and further development of appropriate management strategies for these diseases. The inadequacy in documented information available on the serological and

molecular characteristics and misdiagnosis of new viruses in groundnut, has resulted in continued yield losses amongst groundnut farmers. Information on PBMYV and CABYV in western Kenya is currently inadequate and under-researched thereby hindering accurate and specific robust disease diagnostics, pathotyping, phenotyping and genotyping diversity, to create new genetic resources for exploitation of rapid advances in crop breeding for plant virus resistance. Unavailability of antisera bioassays and specific primers to amplify the virus strains occurring in western Kenya for robust detection, should be urgently designed for diagnostics to avoid misdiagnosis and new disease patterns through development of appropriate disease management strategies for the groundnut farmers. Poleroviruses have remained poorly characterized due to unexplored genomic sequences from PBMYV and CABYV that present similar symptoms in infected groundnuts, both natural and experimental fields with respect to system-level genome functions in co-evolution, co-expression, co-infections and mixed infections in biotechnology manipulation studies.

1.4 Justification of the study

The status of PBMYV and CABYV in western Kenya agro-ecological zones was unknown and undocumented, hence there was need to survey and confirm the occurrence, distribution and epidemiological status of these new viruses. The viruses pose a major problem to groundnuts (*Arachis hypogaea* L.) by reducing yields, and unravel other alternative hosts contributing to the etiology of PBMYV and CABYV, that have continued to increase yield losses of groundnut productivity while the knowledge on the viruses is inadequate. There was need to study the prevalence and socio-economic status of PBMYV and CABYV having been diagnosed with various symptoms detected for the first time in western Kenya groundnut fields. This information will be used to provide targeted and rapid diagnostics to farmers and inform best management practices. Several methods have been used to

investigate and manage virus vectors through application of pesticides to reduce vector populations, various cropping patterns to delay the onset and spread of both vector and disease, and cultural practices, but very little success has been achieved with each of these approaches (Abrahamian and Abou-Jawdah, 2014). Therefore, it was also necessary to understand the genomic characterization of RNA diversity for PBMYV and CABYV Poleroviruses among the different host plants in the dynamic environments of western Kenya. The purpose of this study was therefore to generate and contribute distinct knowledge about the serological and molecular characterization of PBMYV and CABYV in groundnut isolates of western Kenya, and study their natural host range, alternative host range, transmission efficiency by aphid vectors and biological characteristics that will help in unravelling the epidemiology and epiphytology of PBMYV and CABYV, necessary for developing sustainable crop protection technologies. In Kenya, genomic RNA analysis of PBMYV (complete genome) and CABYV (partial or complete) has not been done both for the coat protein gene and nucleotide base sequences obtained by RT-PCR using primers of already characterized viruses or newly designed primers. Furthermore, farmers grow groundnut varieties whose resistance to PBMYV and CABYV has not been documented/ascertained, hence this new information is vital and timely in developing sustainable control/management measures.

1.5 General objective

The general objective of this study was to evaluate the prevalence, distribution, serological and molecular characterization of Phasey bean mild yellows virus (PBMYV) and Cucurbit aphid-borne yellows virus (CABYV) infecting groundnut (*Arachis hypogaea* L.) in selected Counties of western Kenya for effective management.

1.5.1 Specific objectives

The specific objectives of this study were;

i. To determine the incidence and distribution of Phasey bean mild yellows virus and Cucurbit aphid-borne yellows virus infecting groundnut (*Arachis hypogaea* L.) in western Kenya.
ii. To assess the serological bioassay of Cucurbit aphid-borne yellows virus in groundnut (*Arachis hypogaea* L.) isolates of western Kenya.
iii. To examine the molecular diagnostic assay of Phasey bean mild yellows virus in groundnut (*Arachis hypogaea* L.) isolates of western Kenya.
iv. To determine the biological characteristics of Cucurbit aphid-borne yellows virus on known and new indicator plants in western Kenya.
v. To evaluate vector transmission of Cucurbit aphid-borne yellows virus on groundnut (*Arachis hypogaea* L.) and Bambara groundnut (*Vigna subterranea*) in western Kenya.
vi. To determine the molecular characterization of Phasey bean mild yellows virus and Cucurbit aphid-borne yellows virus infecting groundnut (*Arachis hypogaea* L.) in western Kenya.
vii. To establish the socio-economic value chain challenges of groundnut (*Arachis hypogaea* L.) production in western Kenya.

1.6 Hypotheses

The research objectives were tested by the following hypotheses;

HO_1: Phasey bean mild yellows virus and Cucurbit aphid-borne yellows virus are not widely distributed in groundnut (*Arachis hypogaea* L.) crop in western Kenya.

HO_2: The serological bioassay of Cucurbit aphid-borne yellows virus in groundnut (*Arachis hypogaea* L.) isolates of western Kenya are not similar to those from elsewhere.

HO_3: The molecular diagnostic assay of Phasey bean mild yellows virus in groundnut (*Arachis hypogaea* L.) isolates from western Kenya are not similar to those from elsewhere.

HO_4: Isolates of Cucurbit aphid-borne yellows virus from western Kenya express no similar symptoms to indicator plants with those from elsewhere.

HO_5: Cucurbit aphid-borne yellows virus is not vector transmitted on groundnut (*Arachis hypogaea* L.) and Bambara groundnut (*Vigna subterranea*) in western Kenya.

HO_6: The molecular characteristics of Phasey bean mild yellows virus and Cucurbit aphid-borne yellows virus from western Kenya are not similar to those from elsewhere.

HO_7: The socio-economic challenges of groundnut (*Arachis hypogaea* L.) production in western Kenya are not similar to those from elsewhere.

CHAPTER TWO

LITERATURE REVIEW

2.1 Occurrence, distribution and genomics of Phasey bean mild yellows virus (PBMYV)

Complete genome studies in Australia reported a novel Phasey bean mild yellows virus (Polerovirus) (PBMYV) in mixed infections with Bean leaf roll virus (BLRV), Faba bean Polerovirus 1 (FBPV-1), Soy-bean dwarf virus (SbDV) and Turnip yellows virus (TuYV) that naturally infects Phasey bean (*Macroptilium lathyroides* L.) (Sharman *et al*., 2021). Transmission studies by the cowpea-groundnut aphid (*Aphis craccivora*) and by grafting with infected scions reported *Fabaceae* hosts of Phasey bean, Faba bean (*Vicia faba*), Chickpea (*Cicer arietum*) and Pea (*Pisum sativum*) as alternative hosts for PBMYV (Wilson *et al.*, 2012), but was not reported in groundnut (*Arachis hypogaea* L.).

This virus is a single stranded positive sense RNA (+ssRNA) in the genus *Polerovirus*. The virus has been reported in Australia (Sharman *et al*., 2021) and now in Kenya (Mabele *et al*., 2022) through this study. Limited and scanty information was available on its occurrence, host range and economic importance. Genetically, Sharman *et al*., (2021) reported two genetically distinct variants of PBMYV (PBMYV-1 and PBMYV-2). The PBMYV-1 as the originally described variant, and PBMYV-2 had a putative recombinant located in ORF5 in that PBMYV-1 and PBMYV-2 shared only 65-66% amino acid sequence identity in the P5 protein.

2.1.1 Aetiology of Phasey bean mild yellows virus (PBMYV)

A recently discovered Polerovirus, called Phasey bean mild yellows virus (PBMYV), infects legumes in Kenya (Mabele *et al*., 2022). Initially, it was found infecting peas in Tasmania (Wilson *et al*., 2012) and Phasey beans in Queensland (QLD), the virus was later discovered infecting Subterranean clover (*Trifolium subterraneum*) in Western Australia (WA) and

Chickpea (*Cicer arietinum*) in New South Wales (NSW) (Sharman *et al*., 2016). Infected Phasey beans turn to pale yellow (Sharman *et al*., 2016), but infected peas show no visible symptoms (Wilson *et al*., 2012). The genetic diversity of poleroviruses is great, and there is abundant evidence of genomic recombination among them. Major production losses in vegetable and arable crops are largely attributable to them, and they have been linked to the spread of novel viral infections around the world (Lotos *et al*., 2016). This new virus has been found in legumes grown in Kenya, and this is cause for serious alarm. In addition to these new Poleroviruses in legumes, several more have been discovered in other regions in recent years (Abraham *et al*., 2006; Abraham *et al*., 2008; Zhou *et al*., 2017).

2.2 Distribution of Cucurbit aphid-borne yellows virus (CABYV)

Since its initial description in 1992 in French melon and cucumber plants, Cucurbit aphid-borne yellows virus (CABYV) has been found in many other countries' cucurbit crops (Al Saleh *et al*., 2015; Omar and Bagdady, 2012; Orfanidou *et al*., 2014; Svoboda *et al*., 2011; Xiang *et al*., 2008a). Resaerch by Lecoq and Desbiez, 2012, Relevante *et al*., 2012 and Cheewachaiwit *et al*., 2017, all report that CABYV is widely distributed and has likely been prevalent for some time across the Mediterranean Basin and Asia. It has been documented from a small number of locations in the Americas and Oceania (Ali *et al*., 2012; Maina *et al*., 2018), but is otherwise widespread around the world. Since CABYV infection can be difficult to recognize based on outward phenotypic symptoms alone, the virus may have gone unnoticed for quite some time before suitable diagnostic methods were established. Ten years before its description (Lecoq *et al*., 1992), CABYV was discovered in France in samples that had been stored in a freezer. A similar preliminary report of CABYV in various countries of the Mediterranean Basin was made on the basis of DAS-ELISA testing several years prior to the publishing of comprehensive 'first report notes' (Lecoq *et al*., 1994). The global spread of CABYV is currently inconsistent. Over 30% of infected plants were found in surveys in

France, Iran, the Ivory Coast, Morocco, Serbia, Sudan, Spain, and Tunisia where CABYV is prevalent (Kassem *et al*., 2007; Lecoq and Desbiez, 2012). In contrast, CABYV is either exceedingly uncommon (less than 2% infected samples) in the Czech Republic, Southern USA, Panama, Venezuela, and French West Indies (Romay *et al*., 2014) or nonexistent in Panama, Venezuela, and French West Indies (Romay *et al*., 2014). Leaf yellowing in melons is verified to be a viral disease caused by CABYV that was initially discovered in 2014 in Korea by next-generation sequencing (NGS) of melons exhibiting yellowing symptoms (Lee *et al*., 2015).

2.3 Symptomatology of Phasey bean mild yellows virus (PBMYV)

Phasey bean mild yellows virus (PBMYV) symptoms range from mild chlorosis/yellowing, chlorotic mottling, chlorotic spots, reddening/browning of leaves, thickening of leaves, reduced leaf size, dwarfing and stunting. However, studies in Australia by Sharman *et al* (2021), indicated that some natural host symptoms of dwarfing, yellowing and chlorosis in grain legumes were not obviously specific for PBMYV due to mixed infections and co-infections. This visual misdiagnosis can only be practically unraveled through laboratory analysis with serological and molecular techniques.

2.4 Symptomatology of Cucurbit aphid-borne yellows virus (CABYV)

Lower and older leaves tend to yellow and thicken as CABYV infection progresses. The initial signs of CABYV infection in melons, cucumbers, squash, and watermelons are spots and mottling on the leaves that gradually consolidate into larger yellow, thickened, and brittle areas (Relevante *et al*., 2012; Lecoq and Desbiez, 2017). Although symptoms are typically restricted to the older leaves, significant variations are seen between cultivar types. Some cultivars develop only very mild symptoms on a select number of leaves, while others turn the entire plant to a bright yellow color (Lecoq *et al*., 1992). Dropping flowers and less fruits

are also common results (Menzel *et al*., 2020). Some crops and regions experience more severe symptoms in the summer than in the winter. Since 1996, CABYV has been known to produce a serious disease in bitter gourd (*Momordica charantia*) in the Philippines known locally as "Namamarako" (Relevante *et al*., 2012). Young leaves may show interveinal chlorosis, while older leaves may have dark green veins. The thickness of the leaves increases, and the tips of the leaves begin to curl upward. Significant crop losses were reported in Thailand in 2005 (Cheewachaiwit *et al*., 2017), India in 2015 (Suveditha *et al*., 2017; Sangeetha *et al*., 2019), and Sri Lanka in 2016 (Abeykoon *et al*., 2018) after the appearance of a similar disease.

More severe CABYV symptoms are seen in co-infections with Potyviruses (Bourdin and Lecoq, 1994), manifesting as severe mosaic distortions, deformations, and filiformism in leaf tissue.

2.5 Pathogenesis of Polerovirus symptoms on Biological Nitrogen Fixation (BNF) efficacy

Groundnut (*Arachis hypogaea* L) is a leguminous crop that fixes free atmospheric nitrogen biologically into the soil, through symbiotic relationship with the root nodule bacteria (*Rhizobia*) that plays an important role in soil remediation. Biological nitrogen fixation (BNF) is a major source of nitrogen for plant protein synthesis, hence the need for best farming practices that enhances BNF. Improved pest management practices that reduces aphid vector populations of cowpea aphid or groundnut aphid (*Aphis craccivora*), cotton aphid or melon aphid (*Aphis gossypii)*, green peach aphid (*Myzus persicae)* and potato aphid (*Macrosiphum euphorbiae)* transmitting Phasey bean mild yellows virus (PBMYV) and Cucurbit aphid-borne yellows virus (CABYV), increases N_2 fixation by the host plant. Increase in N_2 fixation in grain legumes in the cropping systems (intercropping, polyculture,

multiple cropping, crop rotation, agroforestry and use of cover crops) has proven to be microbiologically and economically attractive in improving BNF among leguminous crops. The amount of nitrogen fixed by legumes in tropical soils frequently deficient of N_2 varies widely with host genotype, Rhizobium efficiency, soil P^H (soil acidity), climatic conditions (high temperatures, CO_2 concentration and drought/water deficit) (Mariangela and Milton, 2000), and the methodology used in assessing BNF constrains legume root-nodule formation and function. Limited studies have explored how plant virus diseases influence BNF and in particular, the effect/impact of PBMYV and CABYV yellowing symptoms on BNF has not been documented. On uprooting groundnuts infected with PBMYV and CABYV, they have reduced number and size of root nodules and pods. PBMYV and CABYV symptoms induces photosynthetic physiological stress on the plant, this reduces nodulation process that further reduces BNF and groundnut yield. Genes that control nodulation (nod, ndv), nitrogen fixation (nif, fix), host range (nod, hsp), surface polysaccharide (exo) and energy utilisation (dct, hup) have been identified (Khosro *et al.,* 2012).

Viruses in plants can disrupt the photosynthetic process and the active carriers in the soil, as well as the transmission of molecular signals between the legume and the microsymbiont. There are six different types of bacteria (*Rhizobia*) that can develop nodules on the roots of legumes and fix nitrogen. Although most legume-rhizobial symbioses are located in tropical regions, there has been tremendous progress in understanding the factors that limit nodulation and bacterial nitrate fixation (BNF) in tropical soils in recent years (Mariangela and Milton, 2000). Legumes and bacteria of the *Rhizobium*, *Bradyrhizobium,* and *Azorhizobium* genera work together to fix nitrogen in the soil through a symbiotic relationship.

Increased temperatures and the effects of climate change on ecosystem dynamics, likely contribute to a decline in *Rhizobia* population levels during the dry season, resulting in poor

nodulation of legumes in desert soils. As a result, BNF tends to decline with legume aging, primarily as a result of the concurrent rise in soil N_2 and calcium deficiency, with or without the confounding influence of low pH that affects rhizobial attachment to root hairs, as well as the decline in photosynthetic activity in leaves and stems, which lowers plant vigor (Al-Falih, 2002). While much has been learned over the past few decades, the physiological and molecular foundation for environmental stress tolerance in legume microbe symbiotic systems is still mainly unknown and empirical. This might be because biotic and abiotic stresses affect the host legume, the *Rhizobia*, and the symbiotic couple in different ways. Because of this, more research is needed in genomics, pangenomics, and proteomics to establish how PBMYV and CABYV affect BNF in both legumes and microbes.

2.6 Host range of Phasey bean mild yellows virus (PBMYV)

Phasey bean mild yellows virus (PBMYV) naturally infects Phasey bean (*Macroptilium lathyroides* L.) (Sharman *et al*., 2021). Transmission studies by the cowpea aphid (*Aphis craccivora*) and by grafting with infected scions also reported *Fabaceae* hosts of Faba bean (*Vicia faba*), Chickpea (*Cicer arietum*) and Pea (*Pisum sativum*) as alternative hosts for PBMYV (Wilson *et al.*, 2012), but not reported in groundnut (*Arachis hypogaea* L.) which this study documents.

2.7 Host range of Cucurbit aphid-borne yellows virus (CABYV)

Although groundnut legume has not been reported as being infected by CABYV, the virus has been found in other annual crops such as lettuce, faba bean, and chickpea (Mnari-Hattab *et al*., 2009; Buzkan *et al*., 2017; Kumari *et al*., 2018). There is evidence that CABYV is widespread in Brazil's perennial passion fruit as well (Vidal *et al*., 2018). The CABYV virus has been detected in fodder beet, miner's lettuce, and dill in controlled laboratory settings

(Lecoq *et al*., 1992; Schoeny *et al*., 2019). Multiple weed species have tested positive for CABYV in a range of ecosystems. Numerous wild cucurbits (*Bryonia dioica*, *Citrullus colocynthis*, *Coccinia grandis*, *Cucurbita foetidissima*, *Ecballium elaterium*) and non-cucurbits (*Lamium amplexicaule*, *Capsella bursa-pastoris*, *Chenopodium murale*) are included.

Other alternative hosts include *Datura wrightii* (Shates *et al.*, 2019), *Sysimbrium irio* and *Citrullus colocynthis* (Vafaei and Mahmoodi, 2017), *Ecballium elaterium* (Kassem *et al.*, 2013).

2.8 Transmission of Phasey bean mild yellows virus (PBMYV)

The groundnut aphid, also known as the cowpea aphid (*Aphis craccivora*), is responsible for the transmission of Phasey bean mild yellows virus (PBMYV), as well as grafting with infected scions (Sharman *et al*., 2021). The presence of additional vector biotypes of cotton aphid or melon aphid (*Aphis gossypii*), green peach aphid (*Myzus persicae*), and potato aphid (*Macrosiphum euphorbiae*) on diseased leaves that tested positive for PBMYV with RT-PCR, may indicate a greater diversity of vectors transmitting Phasey bean mild yellows virus (Mabele *et al*., 2022)

2.9 Transmission of Cucurbit aphid-borne yellows virus (CABYV)

The generalist cotton-melon aphid (*Aphis gossypii*), a global crop pest and carrier of several viral plant infections, is the most efficient vector of Cucurbit aphid-borne yellows virus (CABYV). At least three aphid species, including the cotton aphid (*Aphis gossypii*), the green peach aphid (*Myzus persicae*), and the potato aphid (*Macrosiphum euphorbiae*), are responsible for transmitting CABYV (Kassem *et al*., 2013). Following ingestion of infected phloem, CABYV particles enter the aphid's hemocoel via the anterior and posterior midgut,

travel through the hemolymph, and eventually reach the aphid's accessory salivary glands (Reinbold *et al*., 2003). Saliva secretion from the salivary glands carries the virus into the phloem tissue, where it replicates. It takes aphids anywhere from a few hours to a day to acquire the CABYV they need to transfer it. They are virulent over the majority of their life cycle, emerging from a dormant state after a lag time of one to three days (Radcliffe and Ragsdale, 2002). The bug cannot reproduce the virus, therefore it cannot pass it on to its progeny.

2.10 Virion morphology and genome organization of Cucurbit aphid-borne yellows virus

The genome of Cucurbit aphid-borne yellows virus (CABYV) is a single-stranded, positive-sense RNA that is around 5.7 kilobases (kb) in length and is divided into two parts by a 200-nucleotide-long, non-coding internal region (IR) (Figure 1; D'Arcy & Domier, 2005). When genomic RNA is translated, the 5′-proximal ORFs (ORF 0, 1, and 2) produce the proteins P0, P1, and the ribosomal frameshift protein P1-P2. The proteins P3, P4, and the read-through protein P3 - P5 are all products of translation from the 3′-proximal ORFs (ORF 3, 4, and 5), which are transcribed from sub-genomic RNA. It has been found that the P0 protein inhibits post-transcriptional gene silencing (PTGS). Amino acid patterns typical of RNA-dependent RNA-polymerases (RdRP) are present in both P1 and P2, and P1 shares sequence similarity with serine proteases and genome-linked viral proteins (VPgs) of other Poleroviruses (Guilley *et al*., 1994; Mayo and Miller, 1999). Aphids use the P3-P5 combination, which consists of a coat protein (CP) and a mobility protein (MP) (Mayo and Miller, 1999).

In the past several years, researchers have revealed the whole genome sequences of 11 CABYV isolates from France, Spain, China, Japan, and Taiwan (Kassem *et al*., 2013; Knierim *et al*., 2013) and the partial genome sequences of another 100 CABYV isolates from

a variety of other countries. CABYV isolates have been split into the Asian and Mediterranean groups based on phylogenetic analyses (Shang *et al*., 2009).

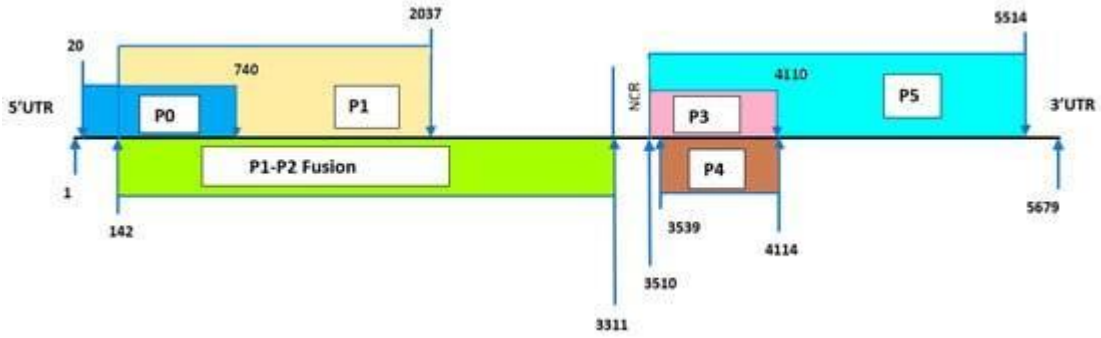

Figure 1: The CABYV BL-4 genomic organization depicted schematically. The arrows show where the nucleotides begin and finish in the protein in question. P1 protein also includes a segment of the P0 protein, and P5 protein incorporates the entirety of the P3 protein. The scale of this diagram is not to scale. (Khanal *et al*., 2021).

2.11 Poleroviruses and their genomic characteristics

A wide variety of hosts can be infected by the *Polerovirus* genus. There are currently 26 officially recognized species (Walker *et al*., 2021). Additionally, there are additional unrecognized species with complete genome accessions reported in GenBank. Some species of Poleroviruses cause devastating illnesses in a wide range of plants, including potato, sugarcane, maize, and beets, and they are found all over the world (Garcia-Ruiz *et al*., 2021). Potato leafroll virus (PLRV) is the most common type of Polerovirus (Taliansky *et al*., 2003; Delfosse *et al*., 2021). Aphids are required vectors for Poleroviruses, and infection is restricted to the phloem. Stunting, yellowing, malformed leaves, and darkening of the primary leaf veins are typical signs (Distéfano *et al*., 2010; Fiallo-Olive *et al*., 2018).

Poleroviruses have been found in a wide variety of crops, including chickpeas and faba beans in Ethiopia (Abraham *et al*., 2006), cotton in Australia and Argentina (Ellis *et al*., 2013;

Distefano *et al*., 2010), strawberry in Canada (Xiang *et al*., 2015), cabbage, maize, and brassicas in China (Zhang *et al*., 2014; Wang *et al*., 2016). Peas were found to be infected with a novel Polerovirus in a recent survey of vegetable crops in Tasmania (Wilson *et al*., 2012). After being identified in Phasey bean (*Macroptilium lathyroides*) in Queensland, the virus was also found in Subterranean clover (*Trifolium subterraneum*) in Western Australia and Chickpea (*Cicer arietinum*) in New South Wales (Sharman *et al*., 2016). Food crop production is severely hampered by the introduction of new viruses and the diseases they induce, many of which have not yet been recognized. Therefore, research on their epidemiology and genomic characterization are required after the discovery of novel Poleroviruses in Kenyan legume crops.

During replication, the Polerovirus genome generates two subgenomic RNAs that are translated in a number of ways (Figure 2; Smirnova *et al*., 2015). Leaky scanning is employed to translate P1 due to different start codons present in ORF0. P1 can be produced alone or fused with P2 when a ribosomal frameshift occurs, both of which are crucial for viral replication (Nickel *et al*., 2008; Smirnova *et al*., 2015; Delfosse *et al*., 2021). It has also been shown that proteolysis can uncouple P1 from VPg (Osman *et al*., 2006). P3a, MP, and CP are all translated by leaky scanning from sub-genomic RNA 1 (Smirnova *et al*., 2015). P4 is a protein involved in transport, specifically plasmodesmic and systemic transport (Ju *et al*., 2017; Delfosse *et al*., 2021).

To switch between hosts and vectors, viruses' genomes need to be malleable (Garcia-Ruiz, 2018; Nigam and Garcia-Ruiz, 2020). Genetics information determines the extent to which a virus varies, evolves, and adapts to its host (Nigam *et al*., 2019; LaTourrette *et al*., 2021). This evolutionary process is mediated by the preferential accumulation of mutations in particular sections of the genome (Nigam *et al*., 2019; LaTourrette *et al*., 2021). This is

shown in the creation of new virus strains or species with novel features. The formation of new species or strains of Poleroviruses has been linked to the prevalence of RNA recombination, as shown by subsequent studies (Dombrovsky *et al*., 2013; Ibaba *et al*., 2017). The RdRp, VPg, and CP coding regions, as well as the non-coding intergenic region between ORF2 and ORF3 (the 5' UTR of subgenomic RNA 1, Miller *et al*., 1995) are hotspots for crossover events.

Mutations in several Polerovirus species or proteins have been mapped. Single-nucleotide polymorphisms (SNPs) were found to be most prevalent between ORFs P2–P4 and at the 5′ end (P0 and P1) and 3′ end (CP-RT) of the genomes of nine Polerovirus species (Huang *et al*., 2005). The P2 component of the P1 - P2 fusion that makes up the RdRp is conserved (Delfosse *et al*., 2021). For example, Hebrard *et al* (2010) and more recently (2018) found that the VPg near the 5' end of the Rice yellow mottle virus (RYMV, genus Sobemovirus) is hypervariable and mediates the establishment of resistance breaking variants.

Multiple proteins may be affected by a mutation in the Polerovirus genome due to the presence of overlapping open reading frames (ORFs; Smirnova *et al*., 2015; Figure 2). However, there is currently no known complete characterization of Polerovirus genomic variation.

2.11.1 Biology of Poleroviruses

Infections caused by Poleroviruses are only found in the phloem and are spread by aphids. Phloem necrosis, leaf streaking, yellowing, rolling, and thickening, and crop stunting are all symptoms of infection. Viruses are acquired through vector phloem feeding, with the viruses first entering the haemocoel of the aphid gut via a receptor-mediated transport pathway, before circulating in the hemolymph and eventually making their way into the accessory

salivary glands. Clathrin-mediated endocytosis mediates transport through epithelial and cellular barriers in the digestive tract and accessory salivary glands (Gray and Gildow, 2003). Viruses such as Turnip yellows virus (TuYV), Beet mild yellowing virus (BMYV), and Cucurbit aphid-borne yellows virus (CABYV) have a potential receptor in the ephrin receptor protein of the aphid *Myzus persicae* Sulzer (Mulot *et al*., 2018). *Schizaphis graminum* Rondani, a type of aphid, has been linked to the spread of the Cereal yellow dwarf virus (CYDVs) RPV (Tamborindeguy *et al*., 2013) through the presence of cyclophilin B. Ghosh *et al* (2019) reported that the whitefly *Bemisia tabaci* Gennadius is responsible for spreading the unnamed Polerovirus known as Pepper whitefly-borne vein yellows virus (PeWBVYV).

Poleroviruses are often found in tandem with other viruses, as described in a number of investigations (Moreno and López-Moya, 2020). As in the case of co-infection with the Umbravirus Pea enation mosaic virus 2 (PEMV2) and the assistor Enamovirus Pea enation mosaic virus 1 (PEMV1), a number of Umbraviruses rely on assistance from Poleroviruses and helper viruses or helper entities. However, this relationship is not obligatory for the assistor Poleroviruses. Whenever Groundnut rosette disease (GRD) infection is observed in Kenya, it is also claimed that the unassigned Groundnut rosette assistor virus (GRAV) is present (Mukoye and Mabele, 2019). GRAV aids Umbravirus Groundnut rosette virus (GRV) in replication and transmission. Although most studies classify GRAV as a Luteovirus, this evidence reveals that it is more closely aligned with the Polerovirus family.

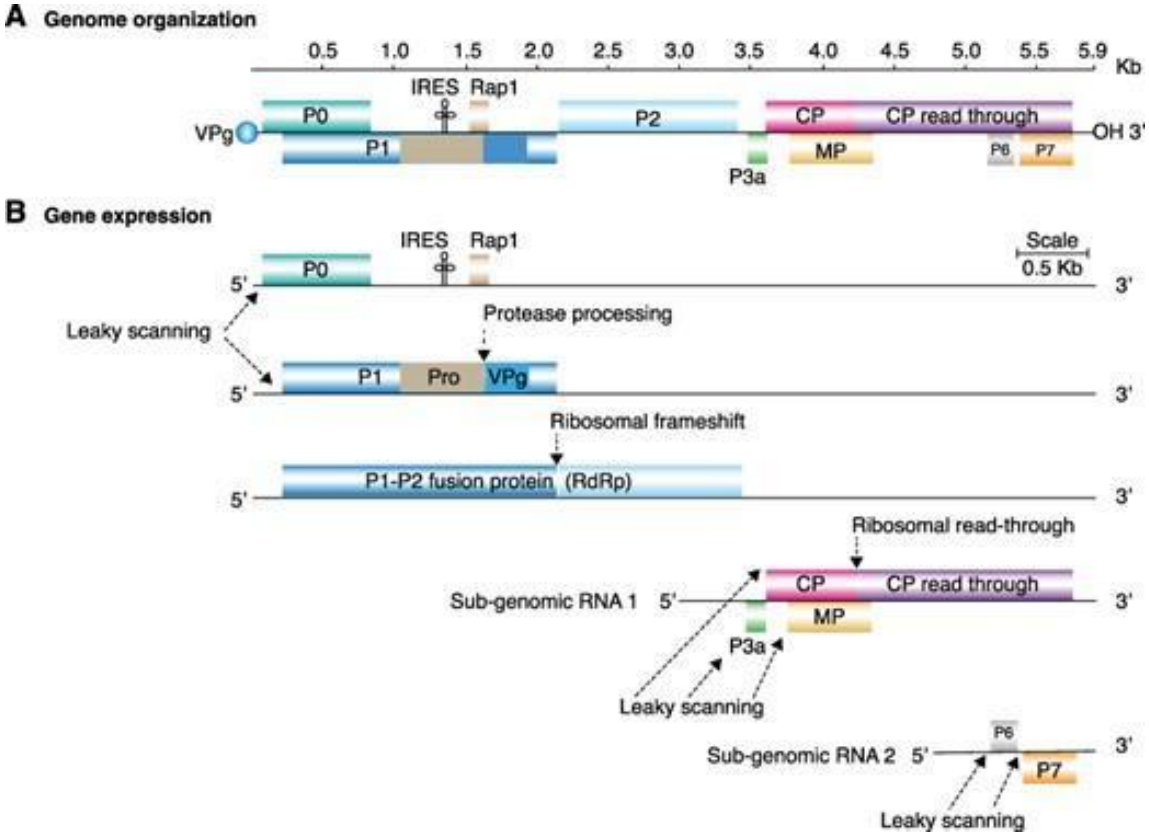

Figure 2: Illustration showing the structure and expression of the Polerovirus genome. Coding regions are shown as boxes with labels, while inactive regions are shown as lines. (A) An overarching structure of the genomes of poleroviruses. PLRV accession number KY856831 was used to get these coordinates. (B) Two sub-genomic RNAs can be formed, and there can be leaky scanning, ribosomal frameshift, and ribosomal read-through in addition to translation via IRES-mediated internal initiation. Proteolysis converts protein 1 into the functional form of VPg. Capsid protein (CP) and read-through domain (RTD), replication-associated protein (Rap1), p3a (protein required for viral spread throughout the body), and internal ribosome entry site (IRES) are all components of a virus. According to (LaTourrette *et al*., 2021).

2.12 Poleroviruses in Kenya

Multiple poleroviruses have been documented in Kenya. Pumpkin plants in Kenya exhibiting mosaic symptoms were sequenced using next-generation sequencing (NGS), revealing a Polerovirus sequence similar to Pepo aphid-borne yellows virus (PABYV) (Kidanemariam *et al.*, 2019). Polerovirus genomes are typically organized into seven putative open reading frames (ORFs), and the nearly full sequence was 5,810 nucleotides long (Kidanemariam *et al.*, 2019). Using the same sequencing technology, Cowpea Polerovirus 1 (CPPV1) full sequence genome was reported in Kenya and possibly the first one in Eastern Africa on cowpea (*Vigna unguigulata*) (Orakha *et al*., 2019). Barley virus G (BVG) and Maize yellow mosaic virus (MaYMV) were reported alongside other viruses infecting Maize in Kenya (Wamaitha *et al*., 2018; Mwatuni *et al*., 2020). Potato leaf roll virus (PLRV) was among the early reported Poleroviruses in Kenya (Were *et al*., 2013; Onditi *et al.*, 2021). There are however very few reports and studies of Poleroviruses in Kenya on grain legumes (Mabele *et al*., 2022a).

2.13 Plant virus evolution, recombination and mutation

Viruses are highly adaptable biological creatures that can cause widespread destruction. To improve their chances of survival, viruses can quickly adjust the rates at which they replicate, spread inside a host, and infect new hosts (Lefeuvre *et al*., 2019). The health of hosts and the environment are both negatively affected as viruses evolve and spread through populations. Multiple influences shape viral evolution (Roossinck, 2008). Mutations and recombinations are the driving forces behind the evolution of viruses, which results in genetic differences and symptom diversity in virus ecology.

Mutation, recombination, and reassortment are the primary mechanisms by which viral genomes evolve and diversify. Compared to double- or single-stranded DNA viruses which

have mutation rates of 10-8 to 10-6, single-stranded RNA and reverse transcribing (RT) viruses tend to have higher mutation rates (10-6 to 10-4 substitutions per nucleotide per cell infection) (Duffy, 2018). High-error-prone RNA-dependent RNA-polymerase and RNA-dependent DNA-polymerase (retrotranscriptases, RT) cause a greater mutation rate in RNA and RT viruses because they lack proof-reading capabilities or base excision repair (Steinhauer *et al*., 1992). Estimated mutation rates in viruses can be calculated either per strand copied or per cell infected.

Due to their prolonged or acute lifestyle, some viruses can have lower mutation rates and generate less variety (Roossinck, 2010). The rate at which mutations occur in plant viruses can also be affected by exposure to mutagens, the activity of host antiviral enzymes, and the results of chance chemical interactions (Duffy *et al*., 2008). Since the likelihood of accumulating harmful mutations is lower with RNA viruses, their genome sizes tend to be smaller (Belshaw *et al*., 2007).

Since plant viruses are subject to lesser immune selection, strong stabilizing selection, strong bottlenecks, or alternative replication modalities, they have lower mutation rates and are genetically more stable than animal viruses (Marco & Aranda, 2005; Sardanyés *et al*., 2009). Natural selection acts on the diverse and novel populations created by mutations, giving rise to new viral variations. High mutation rates of viruses (particularly RNA) are consistent with the theory in which faster replication is encouraged at the cost of fidelity (Furió *et al*., 2005), not because they give more adaptability. A high number of harmful mutations are produced by this method of replication, and viruses exist in a state of error-threshold or mutation-selection equilibrium. A positive mutation entering the population is still possible with all the new varieties being produced. This is crucial for surviving in novel surroundings and avoiding the immune system of the host (Elena *et al*., 2014).

Epistasis, the interplay between mutations that lower or raise the fitness of the virus, is one way in which viruses can reduce the negative impacts of high mutation rates and perhaps better adapt to new settings. Given that epistasis affects the effect of mutations (Whitlock *et al*., 1995), it appears to be the primary driving force underlying across host fitness trade-offs and adaptive processes. Researchers have found that antagonistic epistasis, in which two mutations together have higher fitness than either mutations alone, is common among plant viruses. Most of these investigations have focused on RNA viruses. Host genetics have a major role in determining the strength of this antagonistic epistasis (Bernet & Elena, 2015). Due to their tiny genome size, presence of secondary structures, overlapping genes, and multifunctional proteins, where loss of one function affects many others (Elena *et al*., 2014), RNA viruses are particularly susceptible to the impact of epistasis.

Antagonistic pleiotropy, in which changes that are helpful in one host can be deleterious in another, is another fundamental phenomena that influences the fitness of viruses in their hosts (Montarry *et al*., 2011). Because of this antagonistic pleiotropy, viruses can evolve to cause novel diseases by jumping host or species barriers.

When two different viruses, either RNA or DNA, infect the same host cell, they can exchange pieces of their genome, a process known as recombination (Pérez-Losada *et al*., 2014). According to research by Varsani *et al* (2008) and Holmes (2009), recombination occurs frequently in +RNA, ssDNA, and reverse transcribing viruses but seldom in -RNA viruses. Variations in recombination rates between -RNA and +RNA, ssDNA, and reverse-transcripting viruses may result from biological differences between these virus types. Limiting recombination events is the rapid binding of the genomes of -RNA viruses to the nucleocapsid subunit (Simon-Loriere & Holmes, 2011). Recombination has been found to have significant effects on virulence, pathogenicity, host range, vector transmission, and evasion of host immunity, all of which play a role in the evolution and epidemiology of RNA

and ssDNA viruses (Wang *et a*l., 2022). Therefore, novel viral illnesses might evolve and significant changes in symptomatology can occur as a result of recombination or reassortment of virus genomes (Zanardo *et al*., 2021).

2.14 Plant virus diagnostics and characterization

2.14.1 Next generation sequencing

The use of NGS technology is on the rise as a rapid and efficient way to get whole plant viral genomes (Boonham *et al*., 2014). With the capacity to use total RNA extractions, NGS is quickly becoming the method of choice for sequencing plant virus genomes in their entirety (Adams *et al*., 2009). Problems arise not just with gaining entry to and employing NGS technology, but also with analyzing and making sense of the enormous datasets that are produced (Boonham *et al*., 2014).

For plant viral diagnosis, and notably for the discovery of novel viruses, next-generation sequencing (NGS) represents a promising new tool. Total RNA and DNA can be extracted and sequenced for NGS detection of viral genomes in infected plant material (Kreuze *et al*., 2009; Eichemeier *et al*., 2016). NGS provides a highly sensitive diagnostic tool for the rapid and routine detection of viruses, as it can sequence the whole genomes of both known and undiscovered viruses and can detect numerous viruses from a mixed infection. Due to the fact that NGS is not biased toward any particular virus's pathogenicity, it can be used to detect any and all viruses in a host. Groundnut rosette virus (GRV), Groundnut rosette assistor virus (GRAV), and satellite-ribonucleic acid (sat-RNA) were all found in the same leaf samples (Mukoye *et al*., 2020). The peanut (*Arachis hypogaea* L.) crop was also found to be infected with a number of hitherto unreported viruses. This demonstrates that both well-characterized and as-yet-unidentified plant viruses can be detected by means of Next-generation sequencing.

2.14.1.1 Next generation sequencing chemistry

Fluorescently tagged deoxyribonucleotide triphosphates (dNTPS) are incorporated into a DNA template strand by the Next Generation Sequencing (NGS) DNA polymerase during successive DNA synthesis cycles. At the site of inclusion in each cycle, nucleotides are detected by illumination of a fluorophore. NGS uses massively parallel processing to sequence millions of DNA fragments at once instead of just one.

2.14.1.2 Next generation sequencing technology platforms

Fluorescently tagged deoxyribonucleotide triphosphates (dNTPS) are incorporated into a DNA template strand by the Next generation sequencing (NGS) DNA polymerase during successive DNA synthesis cycles.

The Next generation sequencing (NGS) platforms have emerged due to advances made in the fields of microfluidics, nanotechnology, informatics, machine learning (ML) with internet of things (IoTs) in artificial intelligence (AI) applications and alternative technologies to increase the rapidity and/or high throughput sequencing (HTS) of DNA/RNA deep sequencing (Varshney *et al.,* 2009). NGS collectively describes platforms available or in development other than Sanger sequencing (Kling, 2005; Service, 2006). The platforms have potential to circumvent the limiting factors of Sanger sequencing. For example, sequencing can be multiplexed to a much greater extent by many parallel reactions at a greatly reduced cost (Hudson, 2008). Currently, Roche/454, Solexa and AB SOLiD are the platforms that are predominantly used in crop genetics and breeding applications.

These cutting-edge methods offer rapid throughput DNA sequencing and are themselves quite new. The platforms aid in determining sequence information from single DNA fragments that have been amplified, eliminating the necessity for DNA fragment cloning. These technologies are very dependable due to their high throughput ability. However, they

are also expensive and in some applications, generate short read lengths, non-uniform confidence in base calling in sequence reads, and in particular, deteriorating 3'sequence quality in technologies with short read lengths. Moreover, software developers are constantly improving efficiency of computer algorithms applicable in the sequencing platforms.

(a) The 454 Genome Sequencer FLX instrument (Roche Applied Science)

The basis of this device uses the principle of pyrophosphate detection as described by Nyren and Lundin (1985) and applied in a new method for DNA sequencing reported in 1988 (Hyman, 1988). The technique was further modified into a routinely functioning method for the analysis of 96 samples in parallel in a microtiter plate (Ronaghi *et al.*, 1996). The Genome sequencer instrument introduced by 454 Life Sciences in 2005 is the first next-generation system (Wilhelm, 2009). Specific adapters are ligated to the DNA fragments, which then binds each fragment to a separate bead. In emulsion PCR, water droplets containing one bead and PCR reagents in oil are used to do fragment amplification. Amplification is required because weak light signals cannot be reliably detected during the sequencing-by-synthesis reaction steps without it.

After denaturation, then follows a series of PCR amplification cycles, the beads, each carrying a single amplified fragment, are inserted atop an etched fiber in an optical fiber chip made from bundles of glass fibers. When one end of a bundle of glass fibers is placed in front of a sensitive charge coupled device (CCD) camera, the emitted light can be detected precisely at a certain location. The light guide chip has hundreds of thousands of fibers, each of which is linked to a bead. To begin synthesis of the complementary strand, polymerase enzyme and primers are introduced to the beads, and the reaction mixture is provided to all beads on the chip. A pyrophosphate group is released whenever the polymerase enzyme incorporates a new base into the expanding chain. Since we know which nucleotide was

supplied at each stage, we can use a light signal to track which base was added to the expanding DNA strand. The technique allows for paired-end reads as long as 400-500 bases. Currently mentioned limitations include a somewhat high operating cost and generally reduced reading accuracy in homopolar stretches of identical bases (Schuster, 2008).

(b) The Illumina (Solexa) Genome Analyzer

Solexa uses a DNA polymerase enzyme that can integrate unique reversible terminator nucleotides for the four bases that are fluorescently labeled. This method is based on the sequencing-by-synthesis chemical approach. After denaturation, DNA fragments are ligated on both ends to adapters and immobilized on a solid substrate. Adapters and their equivalent adapters cover the surface of the support in a thick layer. Each single-stranded fragment, which is fixed at one end to the surface, forms a 'bridge' structure by hybridizing with its free end to the complementary adaptor on the support. Surface adapters function as primers for subsequent PCR amplification when added to a mixture containing PCR amplification reagents. DNA 'polonies,' which resemble cell colonies following polymerase amplification, are formed after multiple PCR cycles, and each polonnie contains around a thousand copies of a single-stranded DNA fragment. Primers, four fluorescently labeled reversible terminator nucleotides, and the DNA polymerase are all part of the surface-delivered reaction mixture used for sequencing reactions and DNA synthesis. The charge coupled device (CCD) camera is used to detect the fluorescent dye of the terminator nucleotide after it has been incorporated into the DNA strand and to identify the nucleotide and its position on the support surface.

After the fluorescent dye and the terminator group at the 30-end of the base have been removed, the synthesis cycle can begin again. In the iterative reactions, the maximum length of a sequence read is around 35 nucleotides. Very high sequence throughput on the order of

Gigabases (Gb) per support is possible, thanks to the ability to determine the sequence of at least 40 million polonies in parallel.

The output of the Genome Analyzer II from Illumina is three times that of the original Genome Analyzer. The instrument increases its output per paired-end run from 1 to 3 Gb by using a paired-end module for the sequencer, as well as improved optics and camera components, which enable the device to photograph DNA clusters more effectively across broader areas. More than 50 million reads are recorded per flow cell, yielding at least 1.5 Gb of single-read data every run and at least 3 Gb of data per paired-end run. Single-read runs of 36 cycles take two days to complete, while paired-end runs take four days (Schuster, 2008).

(c) The Applied Biosystems ABI SOLiD system

The ligation chemistry foundation of the ABI SOLiD sequencing system. This method involves binding DNA fragments to beads after adapter ligation. Emulsion polymerase chain reaction (PCR) amplifies DNA fragments on beads using a water droplet in oil emulsion that comprises the amplification chemicals and just one fragment attached per bead. The beads are then placed onto a glass substrate following DNA denaturation.

Initially, an adaptor is hybridized with a primer. After the DNA fragments have been ligated together, a combination of oligonucleotide octamers is hybridized to them. The doublet of the fourth and fifth bases in these octamers is designated by one of four fluorescent labels at the very end. The label's fluorescence is detected, and bases 4 and 5 of the sequence are calculated as a result. The fluorescent label is removed from the ligated octamer oligonucleotides after the fifth base, and the hybridization and ligation cycles are repeated to determine bases 9 and 10 in the sequence, bases 14 and 15 in the next cycle, and so on. Another primer, shorter by one base than the preceding one, can be used to continue the

sequencing process, allowing one to determine bases 3, 4, 8, 9, 13, and 14 in subsequent cycles. The resulting sequence can be read in as few as 35 bases. Errors are less likely to occur when determining each base with a unique fluorescent label. More than 50 million bead clusters' worth of sequences can be calculated in parallel, resulting in a throughput on the order of Gb per run. Applied Biosystems' SOLiD 2.0 platform update boosts data production from 3 Gb to 10 Gb each run. This modification shortens the new system's run time for a fragment library from 8.5 days on the old machine to 4.5 days (Schuster, 2008).

2.14.2 The Enzyme-Linked Immunosorbent Assay (ELISA)

Serological characterization of plant viruses typically employs one of two ELISA formats. Both the direct ELISA, in which the virus (antigen) is bound to an antiviral antibody and detected with an enzyme-labelled virus-specific antibody, and the indirect ELISA, in which an antiglobulin antibody is conjugated to the enzyme used for final detection and test, are examples. Direct ELISA utilizes methods like double-antibody sandwich ELISA (DAS-ELISA), while indirect ELISA bioassays make use of methods like direct antigen-coated ELISA (DAC-ELISA) and triple-antibody sandwich ELISA (TAS-ELISA).

Coating the solid surface with IgG taken from antiserum against a specific virus, and then using the same IgG tagged with an enzyme for detection, is how DAS-ELISA works. The double-antibody sandwich (DAS) method is a type of enzyme-linked immunosorbent assay (ELISA) in which the antigen is sandwiched between immunoglobulins (IgGs). When the substrate of the labeling enzyme is added, a color develops, allowing the reaction to be observed. The antigen concentration in a sample is directly correlated to the hue. Immunoglobulins (IgGs) must be isolated from crude antiserum and an immunoglobulin-enzyme conjugate must be prepared as per the manufacturer's instructions for this process to work.

Another type of indirect ELISA, known as triple antibody-sandwich ELISA (TAS-ELISA), employs an antibody against a virus that was created in two separate animal systems. The procedure is standard practice in the screening of hybridoma during the manufacturing of monoclonal antibodies. In the first stage, the antigen is captured using rabbit polyclonal antiserum. After antigen is added, the hybridoma secreting antibodies are employed to detect it. These antibodies are created by fusing the spleen cells of a mouse inoculated with the virus and malignant cells. Anti-mouse IgG-alkaline phosphatase is used to generate a vivid color and hence visualize the antigen-antibody reaction.

The effectiveness of enzyme linked immunosorbent test (ELISA) as a technique for the identification of plant viruses has been extensively documented. In addition, increased specificity of the test allows for the detection of even closely related strains of the same virus (Abd El-Aziz, 2019).

2.14.3 Polymerase Chain Reaction (RT-PCR) for Reverse Transcription

The RT-PCR is an in vitro nucleic acid amplification technique that uses RNA as the template. RT-PCR was developed after the discovery of retroviral reverse transcriptase in the 1970s. To put it simply, reverse transcriptase is a DNA polymerase that uses RNA as a template to synthesize DNA. Complementary DNA (cDNA) is the final result. cDNA is more stable than RNA because it is resistant to destruction by RNase. The initial RNA is destroyed, double-stranded DNA (dsDNA) is synthesized, and the PCR amplification continues as usual in RT-PCR. With the advent of RT-PCR and the development of RNA extraction tools for both manual and automated RNA purification, RNA analysis in the clinical laboratory has become nearly as quick and similarly sensitive as PCR-based DNA amplification. In order to detect and quantify RNA plant virus infections, RT-PCR is routinely utilized. Therefore, RNA virus molecular characterization and diagnosis can benefit from this type of PCR.

Amplifying RNA targets using reverse transcription (RT)-polymerase chain reaction. The enzyme reverse transcriptase uses RNA as a template to synthesize complementary DNA. Later, the cDNA is used as a template for PCR-based exponential amplification. One or two stages are required to complete RT-PCR. For convenience, RT and PCR can be performed in the same tube using one-step RT-PCR. Primers targeting a specific sequence are required. Two-step RT-PCR involves moving the cDNA from its synthesis tube to a PCR tube. Primers might be gene-specific, random hexamer, or Oligo (dT). In contrast to random primers, which prime anything present, including ribosomal RNA, oligo (dT) primers are favored because they hybridize specifically to the 3' poly (A) tails of mRNAs (transcribed gene sequences). Both approaches have their benefits and drawbacks. One-step reactions facilitate high throughput screening since they require fewer personnel and materials. Multiple messages can be detected from a single RNA sample using two-step processes.

2.15 Polerovirus disease management

Plants with the ability to resist Poleroviruses are uncommon. There are no Polerovirus-resistant cultivars of economically significant crops. Existing varieties only provide limited immunity and protection against certain subsets of the virus. Viruses break genetic resistance within a few years because resistant cultivars impose selection pressure and viruses evolve swiftly. Most Poleroviruses, with the exception of PeWBVYV, are transmitted by aphids, hence measures have been taken to physically restrict their spread. Some crops can benefit from a layer of plastic reflective mulch where aphids can be scared away and repelled by the reflected UV light. Physically preventing the aphids from reaching the plant is also possible with the help of floating row covers made of fine mesh. Mineral oil and aphicides are two examples of topical treatments. Aphids can be eliminated from plants by smothering them

with mineral oil. Similar to the usage of chemical pesticides, aphidicides can be applied to plants (Holste, 2020).

Aphid populations can be kept in check and virus transmission minimized by strategically timing the planting season. Aphid populations in a field could be reduced by planting when aphid populations are low, such as after a brief rainy season. Timing the application of pesticides in conjunction with this tactic is a viable option. Restricting the cultivation of particular crops during certain times of the year by the government will also help minimize the spread of Poleroviruses. These practice is already in place in a number of countries and has global expansion potential. Farmers should also use IRM methods including crop rotation, agroforestry, polyculture, diversification, intensification, and extensification as a belt around IDM and IPM technologies for disease and pest control. Since the aphid vectors are anticipated to be different between maize and soybean, the risk of sustained crop infection is reduced, and the presence of parasitoids and natural enemies is increased. Weeds management in a field is an approach that is less common. Infected weeds may persist from one planting season to the next, especially if they are of the same species as the crop and are not eradicated. It is also recommended that any plant material that has been contaminated by the virus be burned. The aphids that could have fed on diseased plant material and propagated the disease are also killed off in this process. Since aphids are drawn to disturbed ground, farmers should attempt to grow their crops closer together, incorporate cover crops and non-host barrier crops, and till the soil. Poleroviruses can be avoided through preventative measures, but resistant plants are currently the only practical method of directly combating an infection (Holste, 2020). Premenition cropping strategies that limit the activation of PBMYV and CABYV symptoms may be influenced by rogueing of diseased plants and potential alternate hosts.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Determination of incidence and distribution of Phasey bean mild yellows virus and Cucurbit aphid-borne yellows virus in western Kenya

Two disease field sampling surveys to detect and determine virus incidence and severity, was conducted in major groundnut growing areas of western Kenya in Bungoma, Busia, Kakamega and Siaya Counties. Symptomatic and asymptomatic leaf samples were collected from the farmers' fields and taken to the laboratory for serological and molecular analysis. Groundnut fields were sampled during the short rains season (October to December) of 2020 and long rains season (March to May) of 2021.

3.1.1 Description of the study area in western Kenya

Sampling was done in the Kenyan Counties of Bungoma, Busia, Kakamega and Siaya in western Kenya representing the following agro-ecological zones (AEZs): Lower Midland; LM1 (Butula and Teso South), LM2 (Bumula, Bungoma East, Bungoma South, Bungoma West and Busia), LM3 (Siaya, Sirisia and Teso North) (Figure 3). The region experiences biannual long rains season during the months of March to June and short rains season in the months of October to December. The environmental characteristics of the surveyed Agro-ecological zones (AEZs) were as shown in Table 1.

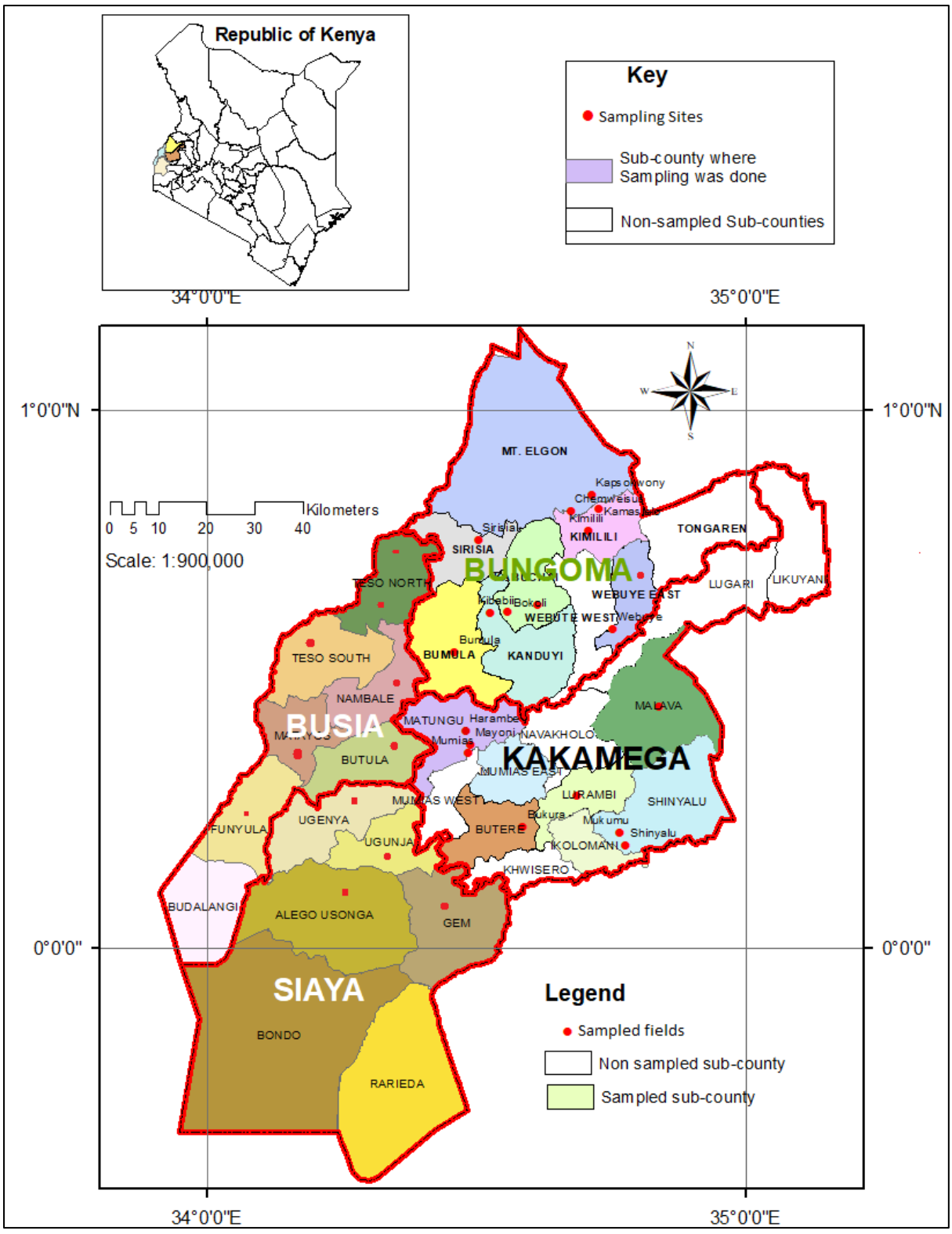

Figure 3: Study site map showing the surveyed Counties of Bungoma, Busia, Kakamega and Siaya in western Kenya. (Source: Map drawn to scale from the Global Positioning System (GPS) data of longitudes and latitudes collected during the field survey).

Table 1: Environmental characteristics of the AEZs covered in the survey in western Kenya

AEZ	Altitude (m)	Rainfall (mm)	Average temp. (°C)
LM1	1350-1500	1600-1800	21.1-22.0
LM2	1350-1550	1350-1650	20.9-22.0
LM3	1200-1400	1200-1450	21.6-22.4

3.1.2 Sampling, sample size and socio-economic survey

Sampling of groundnut farms was done by stopping at regular predetermined intervals of 3-7 km or thereafter when a suitable groundnut farm was found along major motorable roads and feeder roads traversing each sampling area. A total sample size of 673 leaf samples were collected from the total population of 345 groundnut farmers in the four counties. The mathematical estimation of the number of groundnut farmers (345) was chosen purposively from the population size (1000) as respondents for this study, was calculated using the online sample size calculator which is in output agreement with the sample size equation by Kothari and Garg (2019) as shown in this equation:

$$\text{Necessary sample size} = \frac{(\text{Z-score})^2 \times \text{Std Dev} \times (1-\text{Std Dev})}{(\text{Margin error})^2}$$

This equation accounts for the unobserved proportion of groundnut farmers in the four counties from which interviewees were drawn, at a 95% confidence level, within a +/- 5% confidence interval, with a standard deviation of 0.5 and a Z-score of 1.96. In order to conduct a valid significance test at a 95% confidence level and conduct an adequate analysis, a sample size of 1000 groundnut farmers across the 4 counties (250 farmers per county) was estimated to be optimal.

Purposive sampling was used to randomly select fields to go through and visually assess for symptomatic leaves of PBMYV and CABYV on groundnuts. Disease incidence and severity was evaluated for each quadrat using random sampling, with quadrat sizes ranging from 10 to 100 square meters depending on the size of the farm. Each farm's virus activity was documented using a disease diagnostic score sheet. According to Reddy (1991), disease incidence was determined by comparing the number of plants that exhibited signs of a virus to the total number of plants that were seen in the field, as stated in the following equation;

$$\text{Disease incidence} = \frac{\text{Number of virus symptomatic plants}}{\text{Total number of groundnut plants sampled}} \times 100\ \%$$

Virus disease incidence was scored using a rating scale according to Reddy (1991) where: low incidence = 1 – 20 %; moderate incidence = 21 – 49 % and high incidence = 50 – 100 %. The virus disease severity was scored using a severity scale of 0 – 3, where: 0 = No disease, 1 = Mild, 2 = Moderate and 3 = Severe. Visual observation of symptomatic groundnut leaves and those with evidence of aphid vector colonies were collected, placed in a cool box then taken to the laboratory for serological and molecular analysis. Socio-economic data was collected by administering a pre-tested questionnaire (Appendix II) to the groundnut farmers to establish the agribusiness value chain challenges of production, marketing, processing and storage associated with groundnut production. The extensive fields surveyed were geo-referenced using the Geographical Positioning Remote System (GPRS, entrex venture HC GARMIN^{TM}) that recorded latitude, longitude and altitude Global Positioning Systems (GPS).

3.1.3 Survey data analysis

The collected survey data on Phasey bean mild yellows virus (PBMYV) and Cucurbit aphid-borne yellows virus (CABYV) incidence and severity was managed and transformed then subjected to analysis of variance and correlation analysis using R Statistical Software Package Version 3.6 (R Core Team, 2020; Fox and Weisberg, 2019). The incidence and severity data was then transformed and analysis carried out using Pearson's Correlation Coefficient to test the significance of the linear association between disease incidence and virus severity. The socio-economic data was analysed (descriptive statistics for frequencies, means and graphs) using the IBM Statistical Package for the Social Sciences (SPSS) Version 28.0 (IBM SPSS, 2021). Pairwise comparison of means was done using the Least Significance Difference (LSD) at $P \leq 0.05$ confidence level.

3.2 Serological bioassay of Cucurbit aphid-borne yellows virus

Virus detection was carried out using an enzyme-linked immunosorbent assay of antisera raised against Cucurbit aphid-borne yellows virus (CABYV, genus *Polerovirus*, family *Luteoviridae*) (DSMZ, Braunschweig, Germany). The serological methods used in virus detection are based on the principle of cross-reactivity of antisera against viral proteins which includes the micro-precipitin test, the first serological method used to identify viruses (Bhat *et al*., 2010). All serological tests use antiserum prepared against a particular pathogen frequently used for classification and establishment of taxonomic relationships among different groups of viruses (Craig *et al.,* 2004). Enzyme linked immunosorbent (ELISA) assays have been developed to enhance the sensitivity of antigen-antibody reactions during detection of plant viruses. The ELISA protocols are carried out in wells of polystyrene micro-titre plates which have an advantage over other serological techniques since very low concentration (1-10 ng) of the virus can be detected, small amount of antibodies are required,

suitable for large number of samples, viral titre can be determined/quantified and it is cheap in terms of cost (Selvarajan and Balasubramanian, 2008).

The ELISA technique used for detecting CABYV was triple antibody sandwich (TAS)-ELISA (Booonham *et al.*, 2014). Greiner Microlon medium binding microtitre plates were utilized for ELISA reactions with 100 µl/well volume used generally for each reactant. Three intensive washing steps (3 min each) with a washing buffer for 4 min were carried out between incubations. Antibodies and reagents used were acquired from DSMZ, Germany and they included the Coating buffer (pH 9.6) with 1.59 g Sodium carbonate (Na_2CO_3), 2.93 g Sodium bicarbonate ($NaHCO_3$), 0.20 g Sodium azide (NaN_3) and dissolved in 900 ml H_2O, adjusted pH to 9.6 with HCl and made up to 1litre; the PBS (pH 7.4) Phosphate buffer saline with 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) and dissolved in 900 ml H_2O, adjusted pH to 7.4 with NaOH or HCI and made up to 1 litre; PBS-Tween (PBST) of PBS + 0.5 ml Tween 20 per liter; Sample extraction buffer (pH 7.4) of PBST + 2% PVP (Serva PVP-1S Polyvinyl pyrrolidone); Conjugate buffer of PBST + 2% PVP + 0.2% egg albumin (Sigma A-S253); Substrate buffer of 97 ml Diethanolamine, 600 ml H_2O, 0.2 g Sodium azide (NaN_3), and adjusted to pH 9.8 with HCI and made up to 1 liter with H_2O.

3.2.1 Serological detection of CABYV by TAS-ELISA

Sample extraction buffer was used to grind leaf tissues from plants infected with the virus at a ratio of 1:10 (w/v). Following the protocol outlined by Were *et al* (2004) and the manufacturer's instructions, a Triple Antibody Sandwich-Enzyme Linked Immuno Sorbent Assay (TAS-ELISA) was used to detect CABYV. CABYV IgG diluted 1:1000 (v/v) in a coating buffer was used to coat micro titre plates (wells), which were then incubated for 2

hours at 37°C. For blocking, 2% skimmed milk in PBST (200 l/well) was added and incubated the plates for 30 minutes at 37 degrees Celsius. The crushed leaf tissues of virus-infected plants were added to a 1:10 (w/v) solution of sample extraction buffer (PBST + 2% PVP) and incubated at 4°C overnight. MAbs were produced against CABYV, diluted 1:100 (v/v) in conjugate buffer, and applied to the assay plates before being incubated for 2 hours at 37°C to detect a color change. Plant extracts from both healthy and CABYV-infected plants served as the controls. The conjugate of IgG with alkaline phosphatase was added and incubated for 2 hours at 37 degrees Celsius after being diluted 1:1000 (v/v) in conjugate buffer. The procedures for adding substrate, incubating, and measuring absorbance were carried out as stated previously. In order to quantify the p-nitrophenol substrate conversion that led to the yellow color, the absorbance at 405nm (A405) was measured using a Biotek® model (800 TS) spectrophotometer (Labsystems Co., Finland). The positive criteria were set at values twice the average absorbance levels of healthy controls.

3.3 RT-PCR of Phasey bean mild yellows virus (PBMYV)

Reverse transcription polymerase chain reaction (RT-PCR) was used to detect PBMYV in groundnut leaf samples. A total of 24 pooled leaf samples were selected based on typical PBMYV viral symptoms expression observed in the groundnut farms. Total RNA was extracted from leaf samples using RNeasy Mini Kit **(**Qiagen**)** according to the manufacturers' protocol and cetyltrimethylammonium bromide (CTAB) method following these procedure. The groundnut leaf samples (approximately 0.5-1 g) were ground using 2ml of the extraction buffer (CTAB) in mortar and pestle that are sterilized. Transferred the resulting solution (700 µl) to a 2ml sterile centrifuge tube and then mixed the sample by briefly vortexing until the sample was thoroughly re-suspended. Incubated the samples at 65℃ for 15mins for lysing cells completely. Added 700 µl of chloroform: Isoamyl alcohol (24:1) to each tube, homogenized them by vortexing. Centrifuged at 14000 rpm at 4℃ for 10min. Transferred the

upper aqueous phase to a new 1.5ml eppendorf tube. Added 700µl Lithium chloride to precipitate the RNA and then inverted tubes 3 - 4 times to mix the solution. The tubes were incubated overnight at 4℃. Centrifuged the tubes at 14000rpm for 30mins at 4℃ and poured off the salts. The pellet was suspended in 200µl TE buffer containing 1% SDS. Added 100µl NaCl and 300µl ice cold isopropanol and mixed well. Incubated the samples at -20℃ for 30mins. Centrifuged the samples at 14000rpm for 10mins and poured off the salts. The pellet was washed in 500µl of 70% ethanol by centrifuging them at 14000rpm for 5mins at 4℃ and decanted off. Air dried the samples for 20 - 30mins. Re-suspended them in 50µl nuclease free water. Quantification was done using nanodrop technology.

Reverse transcription polymerase chain reaction (RT-PCR) was done using primers as described by Sharman *et a*l (2021). The primer sets used were PhB7F 5'GATCCTTGTGCA AGTTTGTT3' and PhB455R 5'GAATGAGACCTTTGT AAGTA3' with target product size of 450bp. The PCR cycling parameters included an initial denaturation step of 1min at 94°C, then 30 cycles of 30s at 94°C, 60s at 58°C, and 3min at 72°C, and a final extension at 72°C for 10min. The PCR products were analysed using 1.5% agarose gel electrophoresis in 1X TAE buffer and run at 100V, 500mAh for 30min.

3.4 Determination of the biological characteristics of Cucurbit aphid-borne yellows virus

Extensive host range experiments were carried out in a greenhouse with positive and negative controls for each test plant and the pots were arranged in a randomized block design (RBD) in a 10x2 factorial design replicated 3 times, and also screened in insect proof wooden cages with 3 replications, through mechanical inoculation and aphid inoculation of the indicator plants respectively. The indicator plants comprised of leguminous, solanaceous and related weed hosts with CABYV inoculum to ascertain earlier findings, and entomologically identified the cotton-melon aphid (*Aphis gossypii* Glover.), green peach aphid (*Myzus*

persicae Sulzer.) and potato aphid (*Macrosiphum euphorbiae* Thom.). The 10x2 factors included the ten screened plants and the two factors comprised the inoculated and non-inoculated control test plants. Low concentrations of CABYV in host plants makes it essential to develop a reliable and sensitive diagnostic method for the detection of the virus (Irwin and Ruesink, 1986; Kreuze *et al*., 2009; Keshavarz *et al*., 2013).

3.4.1 Germination of test plants for Cucurbit aphid-borne yellows virus screening

Groundnuts (*Arachis hypogaea),* common beans (*Phaseolus vulgaris),* cowpea (*Vigna unquiculata),* soybean (*Glycine max),* thorn apple *(Datura stramonium),* black nightshade *(Solanum nigrum),* Bambara groundnuts (*Vigna subterranea*) and green grams (*Vigna radiata),* were planted in round-shaped plastic pots of 18 cm top diameter, 11 cm base diameter and 18 cm height with small sized holes at the base, filled with small sized gravel rocks to improve drainage, then filled with steam sterilized soil and maintained in the greenhouse. Three seeds per indicator plant were planted in each pot. After germination, the seedlings were thinned to remain with two plants per pot.

3.4.2 Artificial mechanical inoculation of Cucurbit aphid-borne yellows virus

Biological characterization on groundnuts (*Arachis hypogaea),* common beans (*Phaseolus vulgaris),* cowpea (*Vigna unquiculata),* soybean (*Glycine max),* thorn apple *(Datura stramonium),* black nightshade *(Solanum nigrum),* Bambara groundnuts (*Vigna subterranea*) and green gram (*Vigna radiata)* were done with freshly prepared inoculum of CABYV from some of the enzyme-linked immunosorbent assay (ELISA) positive leaf samples from the survey. The ELISA positive leaf samples were ground using a sterilized pestle and mortar, with the aid of dust powdered Carborundum (Silicon carbide, SiC) 320 grit. Prepared ice-cold from scratch The ground tissue was combined with 0.01M Potassium phosphate buffer (K_2HP0_4 + KH_2P0_4), pH 7.0, containing 0.2% Sodium sulfite and 0.01M Mercaptoethanol (1:

6 [w/v] tissue: buffer), then transferred to a falcon tube and left to settle for 5 minutes on ice while standing upright. The sap was frozen until the inoculation process was finished. CABYV was artificially mechanically inoculated by rubbing a suspension of pure virions at a concentration of 100mg/mL in sterile water into the Carborundum-dusted leaves of test plants at the four to six true-leaf stage. Successful infection was confirmed by observing typical viral symptoms of CABYV and further validation through TAS-ELISA diagnosis for CABYV coat protein accumulation.

3.4.3 Vector transmission of Cucurbit aphid-borne yellows virus

Vector transmission was carried out using aphids from a known viruliferous colony, the cotton aphid or melon aphid (*Aphis gossypii* Glover.), green peach aphid (*Myzus persicae* Sulzer.), potato aphid (*Macrosiphum euphorbiae* Thom.) and groundnut aphid or cowpea aphid *(Aphis craccivora* Koch.), sourced from symptomatic groundnut leaves and those plants with evidence of aphid vector colonies that collected during the extensive field survey in Kakamega County, that was near the crop science laboratory facility and taken to the laboratory for entomological identification before subsequent experimentation. The aphids were raised in aphid proof wooden cages that housed round-shaped plastic pots of 22 cm top diameter, 14 cm base diameter and 21 cm in height with small sized holes at the base, filled with small sized gravel rocks to improve drainage, then covered with steam sterilized soil in which groundnut (*Arachis hypogaea*), pumpkin (*Cucurbita pepo*) and black nightshade (*Solanum nigrum*) were planted and raised. The insect vectors were allowed acquisition access feeding period (AAFP) of 48hr and 20 days caging. In groups of 10 - 20 aphids, the viruliferous *Aphis gossypii* and *Myzus persicae* were transferred on healthy disease free groundnuts (*Arachis hypogaea*) and Bambara groundnuts (*Vigna subterranea*) at three leaf stage in insect proof wooden cages for inoculation access feeding period (IAFP) of 72hr and allowed 30 days for symptoms development, and kept under open field environmental

conditions of shielding heavy rains from washing the vector aphids off the plants. Negative controls were set up without the aphid vectors while positive controls had the viruliferous aphids on symptomatic indicator test plants. TAS-ELISA bioassays were done after 8 weeks post inoculation, after which the aphids were killed by spraying them with an aphidicide.

3.5 Determination of the molecular characteristics of Phasey bean mild yellows virus and Cucurbit aphid-borne yellows virus

3.5.1 Next Generation Sequencing of positive samples for PBMYV and CABYV

Whole plant viral genomes can now be obtained rapidly using Next Generation Sequencing (NGS) methods, which are on the rise (Boonham *et al*., 2014). Since NGS can employ total RNA and DNA extractions, it is increasingly being used to sequence entire plant viral genomes, with outstanding results (Adams *et al*., 2009; Pecman *et al*., 2022). In order to determine if PBMYV, CABYV, or another virus was responsible for the various symptoms found in groundnut crops, this study used NGS. Using the RT-PCR Qiagen RNeasy Plant Mini Kit (Qiagen, Germany), total RNA was isolated from the ELISA-positive leaf samples. Nanodrop and 1.5% agarose gel electrophoresis were used to determine the RNA concentration.

The RNA was used in a SuperScript II (Thermo Fisher Scientific, Waltham, USA) based double stranded cDNA synthesis reaction. The cDNA was measured using a Qubit 2.0 Fluorometer (Thermo Fisher Scientific) and purified using a DNA Clean & ConcentratorTM-5 - DNA kit (Zymo Research, Irvine, USA). Following the manufacturer's instructions (Eichmeier *et al*., 2016), the samples were pooled and processed using a transposon-based chemical library preparation kit (Nextera XT, Illumina). In order to evaluate the DNA library's fragment size and structure, an Agilent 2100 Bioanalyzer (Agilent Technologies,

Santa Clara, USA) was used. On the Illumina MiSeq technology (Illumina, USA), we sequenced the indexed, denaturated DNA libraries (200-bp paired-end sequencing).

3.5.2 Library preparation and sequencing of PBMYV and CABYV

The cDNA was prepared using a library preparation kit (Nextera XT, Illumina, USA) that relies on transposon-based chemistry. The DNA library's fragment size distribution was analyzed with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA). Using the Illumina MiSeq benchtop sequencer platform (Illumina, USA), and sequenced the indexed denaturated DNA library (200 bp paired-end sequencing) to obtain 50 nt single-end (SE) reads. After standardization, pooling, and diluting, the libraries were at a final concentration of 6.5pM. The combined libraries were analyzed by the Illumina MiSeq System with a control of 12pM of 1% PhiX. The BecA-ILRI Hub in Nairobi, Kenya was the site of two rounds of paired-end sequencing (2,300 bp).

3.5.3 Sequence data analysis of PBMYV complete genome and CABYV partial genome

FastQC (version 0.11.5) was used to check the quality of the reads. Then the low-quality sequences in the Reads were removed (Haas *et al*., 2013). De novo assembly was performed using the trimmed reads, and the resulting contigs were matched against the viral genomes database in CLC Genomics Workbench 10.1.2. BLASTn was used to search the GenBank database for sequences that were similar to the assembled contigs (Altschul *et al*., 1990). The GenBank was used to extract full and partial PBMYV and CABYV sequences for use in the phylogenetic analysis. Groundnut (*Arachis hypogaea* L.) PBMYV sequences obtained in their entirety were deposited in the DNA Data Bank of Japan (DDBJ).

CHAPTER FOUR

RESULTS

4.1 Virus incidence and distribution of PBMYV and CABYV in western Kenya

A total of 345 farms were surveyed in 4 counties (245 in long rains season and 100 in short rains season). Virus disease symptoms were observed in all the 4 Counties surveyed with a total of 673 samples collected (501 during the long rains and 172 during the short rains season). The disease expressed diversity in symptoms variation across the Counties. The incidence and severity of this visual virus disease symptoms varied across the surveyed Counties.

4.1.1 Major virus symptoms of PBMYV and CABYV observed on groundnuts (*Arachis hypogaea* L.) in western Kenya

In most farms, groundnuts showed severe yellowing and bunching of the young leaves. Leaf mottling and downward curling as well as severe chlorotic blotches were observed. In some cases, stunting of the crop was observed (Figure 4).

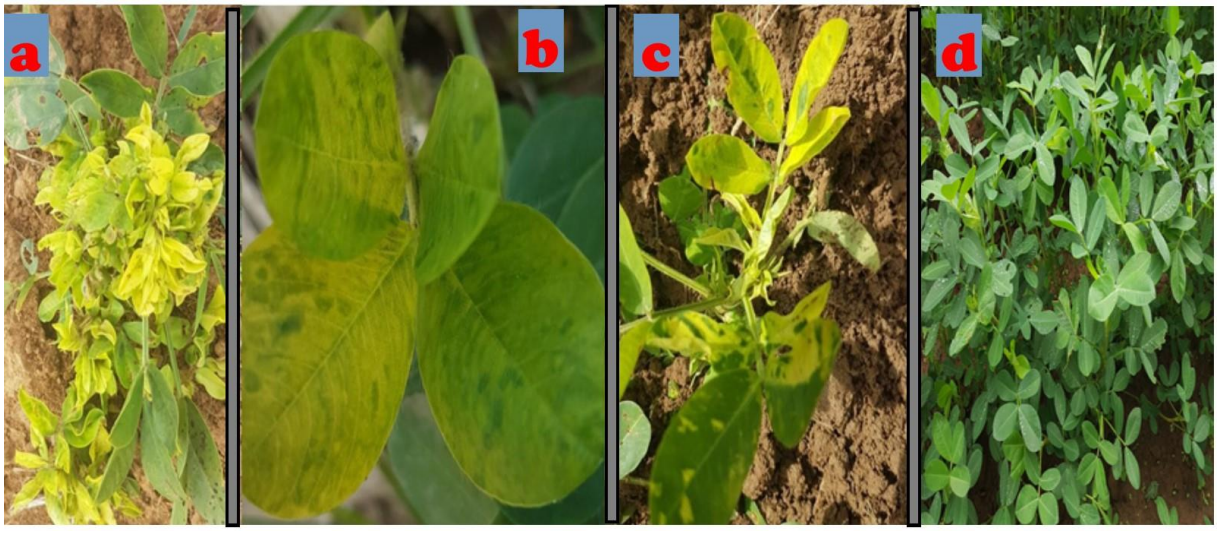

Figure 4: Examples of some major PBMYV and CABYV viral symptoms observed on groundnuts during the survey in western Kenya: (a) Severe chlorosis, puckering, leaf deformation and bunching, (b) leaf mottling, bunching, puckering and curling downwards, (c) severe chlorotic blotches, mosaic, necrosis, leaf crinkling and mottling; and (d) healthy groundnut crop.

4.1.2 Visual virus disease incidence and severity of PBMYV and CABYV

Generally, the PBMYV and CABYV incidence was high during the short rains season than during the long rains season in all the surveyed Counties. The highest mean (73.61%) virus incidence was recorded in Busia in the short rains season while the lowest was 42.65% during the long rains season in the same County (Table 2). There was a statistically significant difference in virus incidence among the Counties ($p<0.0001$). Overall, Busia had the highest PBMYV and CABYV incidence that was significantly different from that of Bungoma, Kakamega and Siaya. The PBMYV and CABYV incidence in Bungoma, Kakamega and Siaya did not vary significantly. Additionally, virus incidence during the short rains season was significantly ($P = 0.0167$) different from that recorded in the long rains season at $p<0.0001$.

The mean virus severity ranged from mild (1) to moderate (2) across all the surveyed Counties and seasons. The short rains season recorded higher severity compared to the long rains season in all the Counties surveyed. The highest mean severity was recorded in Bungoma in the short rains season (2.53) while the lowest was in Busia during the long rains season (1.56) (Table 2). However, the severity was not significantly different between the Counties.

Table 2: Mean virus disease incidence and severity on groundnut across the Counties in western Kenya

County	Season	N	Incidence	S.E	Severity	S.E
			Mean (%)	(+/-)	Mean	(+/-)
Bungoma	Long	76	48.37 [a]	2.015	1.70	.059
	Short	15	73.47[b]	2.828	2.53	.133
Busia	Long	73	59.52 [a]	2.231	1.70	.097
	Short	46	73.61 [b]	2.147	2.28	.111
Kakamega	Long	43	42.65 [a]	2.593	1.56	.084
	Short	23	69.87 [b]	2.101	2.17	.120
Siaya	Long	53	50.77 [a]	2.071	1.57	.103
	Short	16	62.31 [b]	2.949	2.44	.157
Total	Long	245	51.21[c]	1.172	1.64	.043
	Short	100	70.92[d]	1.318	2.32	.066

The severity of PBMYV and CABYV increased with increase in incidence. Where incidence was high, severity was also high and predicted to increase significantly. There was a statistically significant positive correlation P-value of 0.0167 between disease incidence and severity ($r=0.409$, $p<0.0001$) with moderate association (Table 3)

Table 3: Correlation relationship between disease incidence and severity of PBMYV and CABYV in western Kenya

			Incidence	Severity
Pearson Correlation			1	.409**
Sig. (2-tailed)				.000
N			345	345
Bootstrap[b]	Bias		0	-.001
	Std. Error		0	.046
	95% Confidence Interval	Lower	1	.317
		Upper	1	.496

**. Correlation is significant at the 0.01 level (2-tailed).

b. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples

4.1.3 Socio-economic information of the groundnut farmers in western Kenya

(a) Diseases and pests reported by farmers as a challenge in groundnut farming

Eighty percent of the farmers had no knowledge about any disease or pests affecting groundnuts. However, those who had knowledge about groundnut pests and diseases reported stunting, rosetting and yellowing as the main diseases observed. Some pests such as moles, aphids and unnamed caterpillars were also reported (Figure 5).

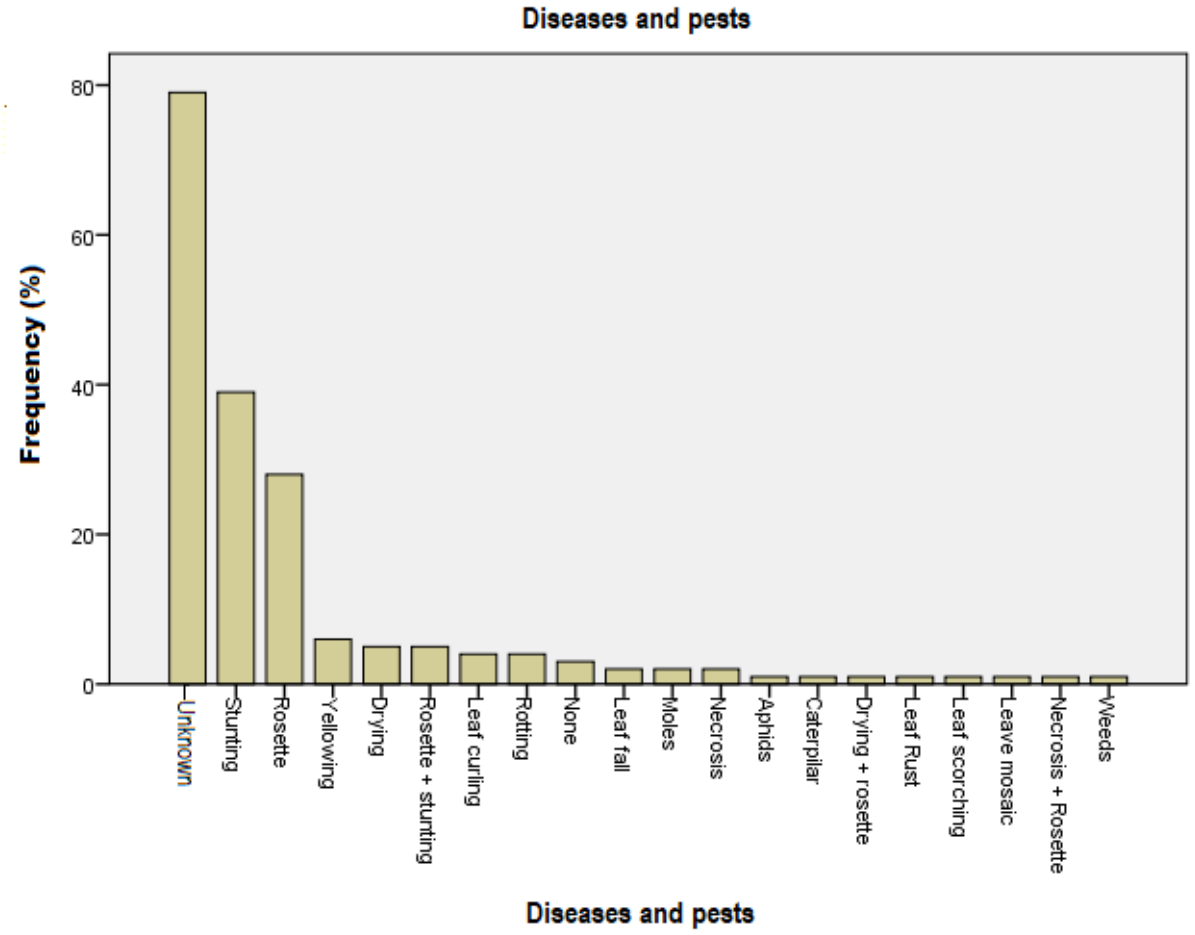

Figure 5: Diseases and pests reported by farmers as a challenge in groundnut farms. The first column of 'unknown' represents farmers who are unaware that groundnuts are infected by any disease, while the 'none' column represents farmers who dispute that groundnuts are infected by diseases. This challenge brings a problem in management since PBMYV and CABYV are among the groundnut virus diseases.

(b) Groundnut varieties grown

The main variety grown was Red Valencia (79.1%), followed by Homabay white – CG2 (7.5%), Homabay red (7.0%), Serere 116 (3.2%), Virginia purple and Uganda red (0.5%) (Figure 6).

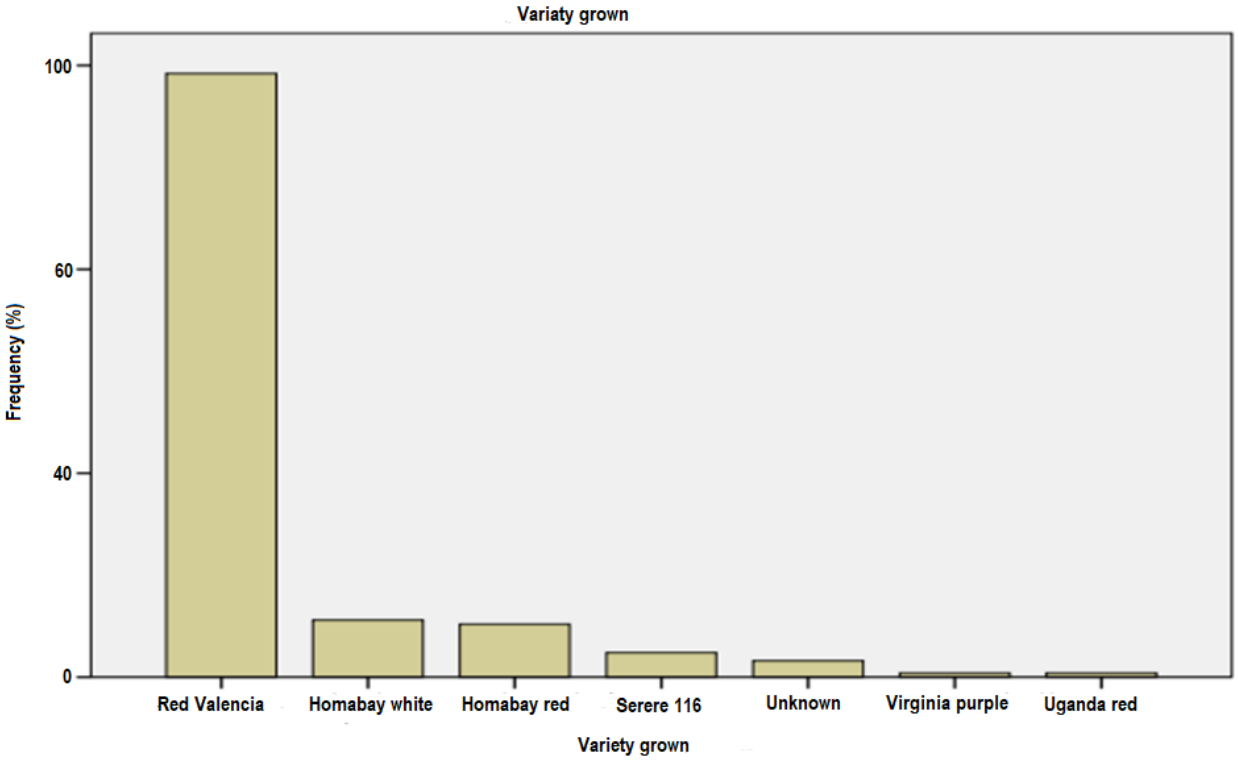

Figure 6: Frequency of groundnut varieties grown in western Kenya. The 'unknown' column indicates varieties grown but unable to be identified by the farmers.

(c) Season of cultivating groundnuts

Majority of the farmers cultivate groundnuts during both long and short rains seasons (81.8%), while few do plant only during the short rains season (13.4%), or during the long rains season (4.3%), while 0.5% allow the groundnut crop to grow as a volunteer crop in parts of their farms especially hedges continuously throughout the year (Figure 7).

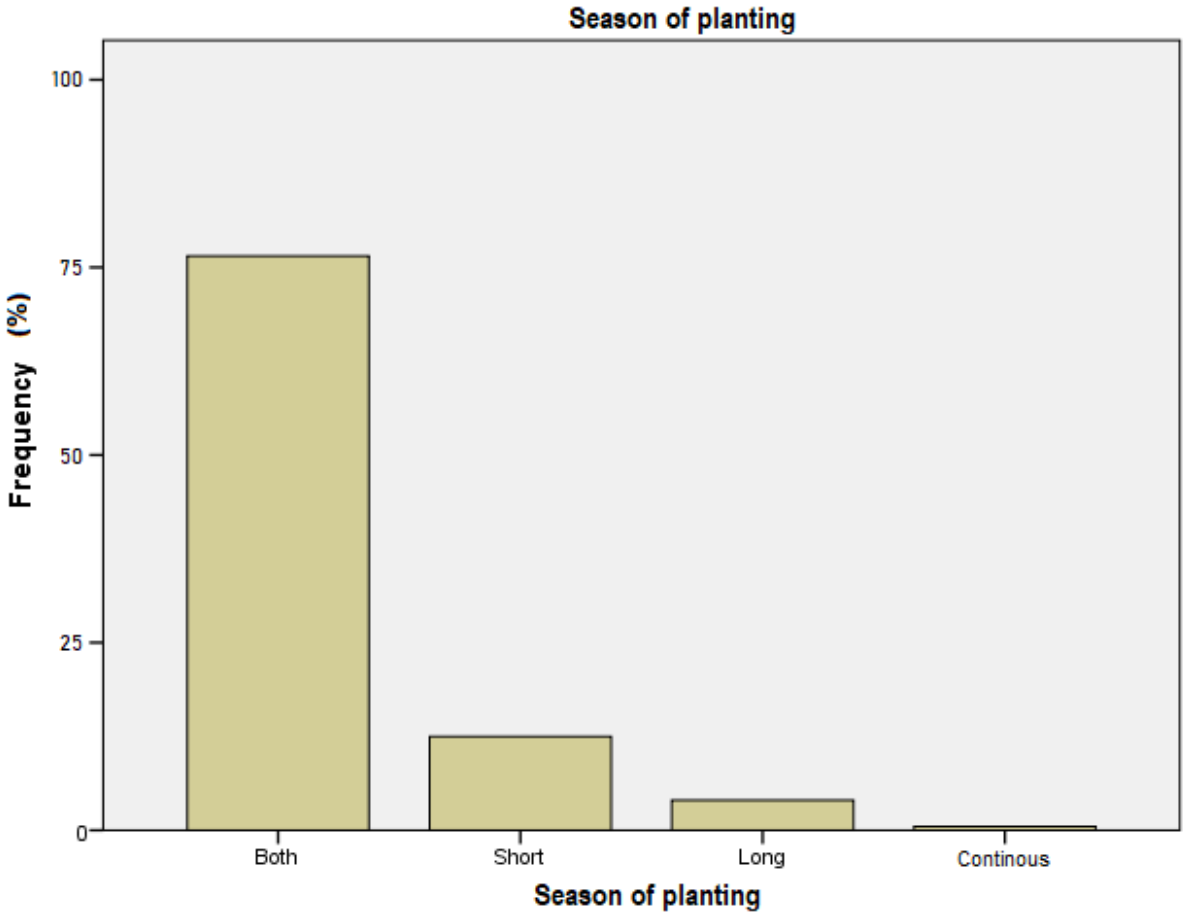

Figure 7: Frequency of planting seasons when groundnuts are cultivated in western Kenya. The continuous column indicates groundnuts that thrive between and during the growing seasons.

(d) Land size and groundnut yield per acre

On average, the farm sizes are 2.3 acres across the surveyed Counties with Bungoma having the largest (3.63 acres) while Siaya the least (1.56 acres). Groundnut production is done on an average small scale land size of 0.23 acres with Bungoma County allocating the largest at 0.36 acres, while Siaya County the least at 0.09 acres. The yield of groundnuts is about 2.48 bags (shelled) which translates to about 223.2 Kgs. On average, Bungoma recorded the highest yield (3.27 bags) while Siaya had the least (1.66 bags) statistically commensurate to

the land size, varieties grown and disease incidence. The selling price averaged at Kshs. 244.48 per 2kgs (Table 4).

Table 4: Average land size and groundnut yield in bags per acre in the surveyed Counties.

County	Farm size (Acres)	Land size under groundnuts (Acres)	Groundnut yield (bags @90kg)	Selling Price@2kg
Bungoma	3.63	0.36	3.27	280.00
Busia	2.22	0.28	2.62	237.86
Kakamega	1.69	0.16	2.44	186.30
Siaya	1.56	0.09	1.66	309.52
Mean Total	2.3	0.22	2.5	253.42

(e) Education level, gender, land ownership and household head of groundnut farmers

Most farmers were female (61%) while 38.5% were males. Household heads were mainly fathers (males) at 69% while few were headed by mothers (females) at 23.5%. Majority of the farmers (59.4%) had primary level of education, followed by secondary level (27.3%), and others (10.2%) while those who had attended informal education were 1.1%. Land ownership was mainly family (66.8%), then individually owned land was at 24.1% while hired land was at 7.5% (Table 5).

Table 5: Education level, gender, land ownership and household head of groundnut farmers

Aspect	Gender		Household head		Education level				Land Ownership		
	M	F	Father	Mother	Informal	Primary	Secondary	Others	Family	Hired	Owned
%tage	38.5	61	69	23.5	1.1	59.4	27.3	10.2	66.8	7.5	24.1

4.2 Serological bioassay of Cucurbit aphid-borne yellows virus and molecular diagnostics of Phasey bean mild yellows virus

4.2.1 Enzyme-Linked Immunosorbent Assay (ELISA) test results for CABYV

During the long rains season, a total of 40 grouped/pooled samples tested positive for CABYV representing 8% of the total 501 samples while 73 grouped/bulked samples representing 42.4% of the total 172 were positive during the short rains season (Table 6; Figure 8)

Table 6: Analyzed ELISA positive pooled/bulked samples for CABYV in the long rains and short rains seasons

County	Number of ELISA +ve samples (pooled)	
	Long rains	Short rains
Bungoma	12	22
Busia	17	26
Kakamega	7	14
Siaya	4	11
Total	**40 (8%)**	**73 (42.4%)**

	1	2	3	4	5	6	7	8	9	10	11	12	
A	1.193	1.175	0.176	0.158	0.167	0.198	0.188	0.169	0.193	0.176	0.163	0.16	405
B	0.987	0.976	1.172	1.176	0.883	0.881	0.175	0.178	0.883	0.863	0.164	0.165	405
C	0.183	0.182	0.878	0.881	0.183	0.186	0.566	0.578	0.171	0.162	0.885	0.884	405
D	0.801	0.878	0.186	0.176	0.771	0.775	1.17	1.175	0.673	0.684	0.185	0.183	405
E	0.16	0.157	1.196	1.202	0.167	0.165	0.673	0.668	0.164	0.107	0.772	0.774	405
F	0.786	0.771	0.195	0.183	0.873	0.865	0.164	0.168	0.669	0.668	0.591	0.588	405
G	0.987	0.976	0.905	0.884	0.173	0.169	0.181	0.167	0.171	0.169	0.199	0.197	405
H	0.672	0.676	0.177	0.165	0.739	0.78	0.181	0.171	0.151	0.161	3.97	3.565	405

Figure 8: The CABYV ELISA plate reader results of a sample plate. Spectrophotometric measurement of optical density (OD) positive values visually coloured red had threshold of >0.312 at 405 nm absorbance. An ELISA microplate reader was used to detect the OD values at 405 nm after 2 hours. The isolates that were considered positive were those with OD values of more than twice the value of the negative control.

4.2.2 Molecular diagnostics of Phasey bean mild yellows virus (PBMYV) with RT-PCR

Out of the 24 pooled samples that were selected for molecular detection of PBMYV, 21 tested positive for the virus (Figure 9).

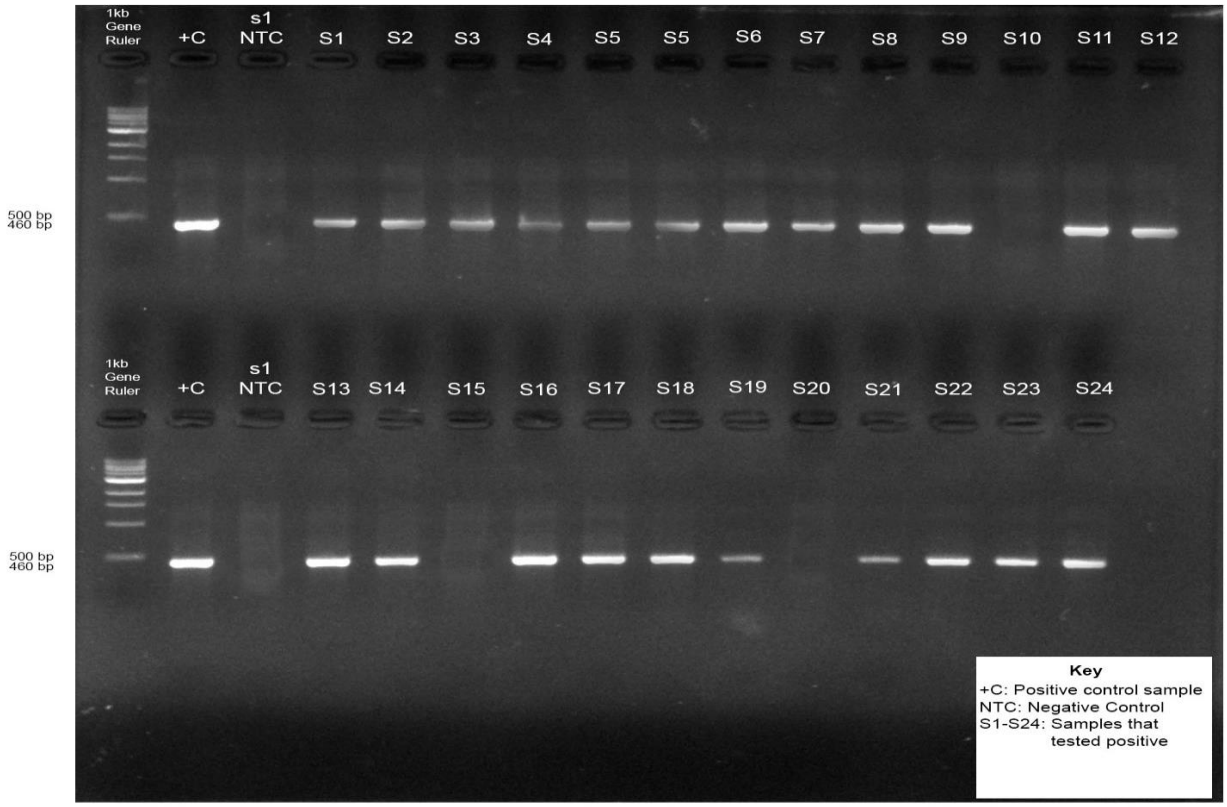

Figure 9: Gel electrophoresis of RT-PCR for PBMYV. A 1kb gene ladder was used and target product size was 450kb. S1-S8 samples from Busia County, S9-S16 samples from Bungoma County, S17-S21 samples from Kakamega County, and S22-S24 samples from Siaya County.

4.3 Biological characteristics of Cucurbit aphid-borne yellows virus on indicator plants

The screened plants replicated in three plots expressed distinct symptoms of bright and mild yellowing, chlorotic blotches, mild and severe mottling, interveinal yellowing, vein clearing, mosaic, leaf curling, leaf bunching, leaf clumping, dwarfing and stunting. For mechanically inoculated plants, only groundnuts tested positive for CABYV for all the three screened groundnut varieties (Red Valencia, Homabay white – CG2 and Virginia purple – CG7) (Table 7; Figure 10).

Table 7: Symptoms expressed by various test plants upon artificial mechanical inoculation with CABYV

Test plant	**Number inoculated mechanically**	**Control**	**Local Symptoms***	**Systemic symptoms***	**ELISA**
Cowpea	18	6	N	VC, M, SM, Y	-
Groundnuts	54	18	N	Y, CB, S, LC	+
Soybean	18	6	N	SM, VY, M	-
Common beans	18	6	N	Y, CB, M	-
Green grams	18	6	N	BY, MM, SM	-
Thorn apple	18	6	N	Y, M	-
Bambara nut	18	6	N	CB, MM, LC	-
Black nightshade	18	6	N	Y, VC, M	-

Key: *BY-Bright yellow, MM-Mild mottle, N-Necrosis, Y-Yellowing, CB-Chlorotic blotches, S-Stunting, SM-Severe mottling, VY-Vein yellowing, VC-Vein clearing, M-Mosaic, LC-Leaf curl.

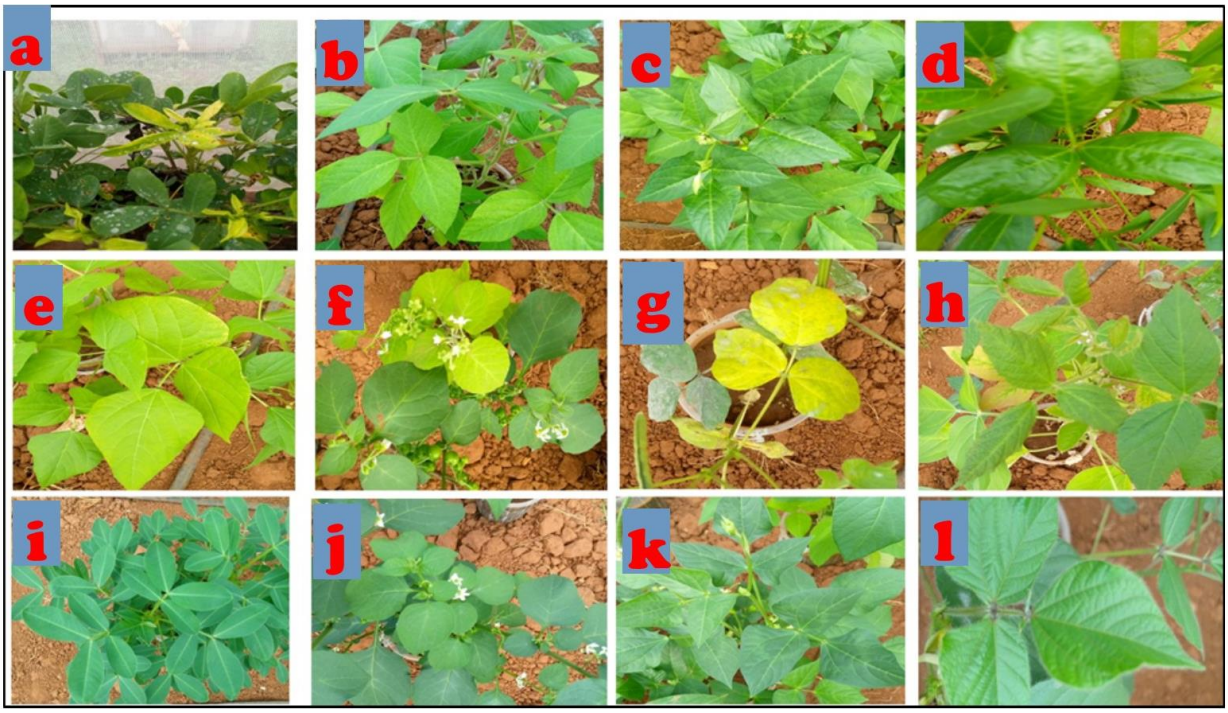

Figure 10: Some of the typical virus symptoms expressed by test plants inoculated with CABYV: **a**-Severe yellowing of young leaves on groundnuts, **b**-mild yellowing and mosaic on soybean, **c**-vein clearing and mosaic on cowpea, **d**-leaf mottling and leaf curling on Bambara groundnuts, **e**-yellowing on common beans, **f**-yellowing on black nightshade, **g**-bright yellowing on green gram, **h**-severe mosaic and vein yellowing on soybean, **i**-healthy groundnut (control), **j**-healthy black nightshade (control), **k**-healthy cowpea (control), **l**-healthy soybean (control).

4.4 Insect vector inoculation characteristics of Cucurbit aphid-borne yellows virus on groundnut test plants

The groundnut (*Arachis hypogaea*) varieties (Red Valencia, Homabay white – CG2 and Virginia purple – CG7) and Bambara groundnuts (*Vigna subterranea*) were aphid inoculated with viruliferous aphids in insect proof wooden cages. Typical viral symptoms of interveinal mild yellowing, mild mottling, chlorotic blotches, mosaic, curling, leaf bunching and stunting

were expressed. All aphid vector inoculated groundnut plants tested positive for CABYV with serological TAS-ELISA bioassays (Table 8; Figure 11).

Table 8: Viral symptoms expressed by groundnuts and Bambara groundnuts upon aphid inoculation with CABYV.

Test plant	**Number inoculated**	**Control**	**Symptoms**	**ELISA**
Groundnut varieties (Red valencia, CG2 and CG7)	9	6	SM, PLD,CB, S, LC	+
Bambara groundnuts	3	2	CB, MM, LC, NL	+

Key*: SM-Severe mottling, **P**-Puckering leaf deformation, **CB**-Chlorotic blotches, **S**-Stunting, **LC**-Leaf curling, **MM**-Mild mottling, **N**-Necrotic lesions. Some of the major virus symptoms expressed in groundnut (*Arachis hypogaea*) and Bambara groundnut (*Vigna subterranea*) upon aphid vector inoculation included interveinal yellowing of leaves and vein necrosis, leaf mottling, downward leaf curling, leaf rolling, leaf mosaic distortions, deformations, filiformism, chlorotic streaking, leaf bunching and stunting of the crop.

Figure 11: Viral symptoms expressed by **a-** groundnut (*Arachis hypogaea*) and **c-** Bambara groundnut (*Vigna subterranea*) upon aphid vector inoculation with CABYV in insect proof wooden cages: **a**- Stunting, bunching, leaf yellowing, puckering and curling on groundnut; **b**-healthy groundnut (control); **c**-chlorosis, streaking, and leaf curling on Bambara groundnut; and **d**-healthy Bambara groundnut (control).

4.5 Molecular characteristics of Cucurbit aphid-borne yellows virus (CABYV) and Phasey bean mild yellows virus (PBMYV) infecting groundnut (*Arachis hypogaea* L.) in western Kenya

4.5.1 Phylogenetic analysis of CABYV and PBMYV isolates from western Kenya

The CABYV isolates from Kenya clustered with other Poleroviruses diversely in varying clades. The CABYV7-3 clustered with several other CABYV isolates available in the GenBank including KR231961.1, KR231959.1 and MG257901.1. CABYV6-2 grouped closest with Cowpea Polerovirus 2 (CpPV2 – KX599164.1) followed by CABYV7-2 which was in a cluster of CpPV2 and PBMYV. CABYV6-1 and CABYV7-1 formed a distinct cluster. CABYV6-2 showed 94.5% nucleotide identity with CpPV2 isolate KX599164.1 from Burkina Faso and 83.4% identity with CABYV isolate MG257902.1 from Korea (Figure 12).

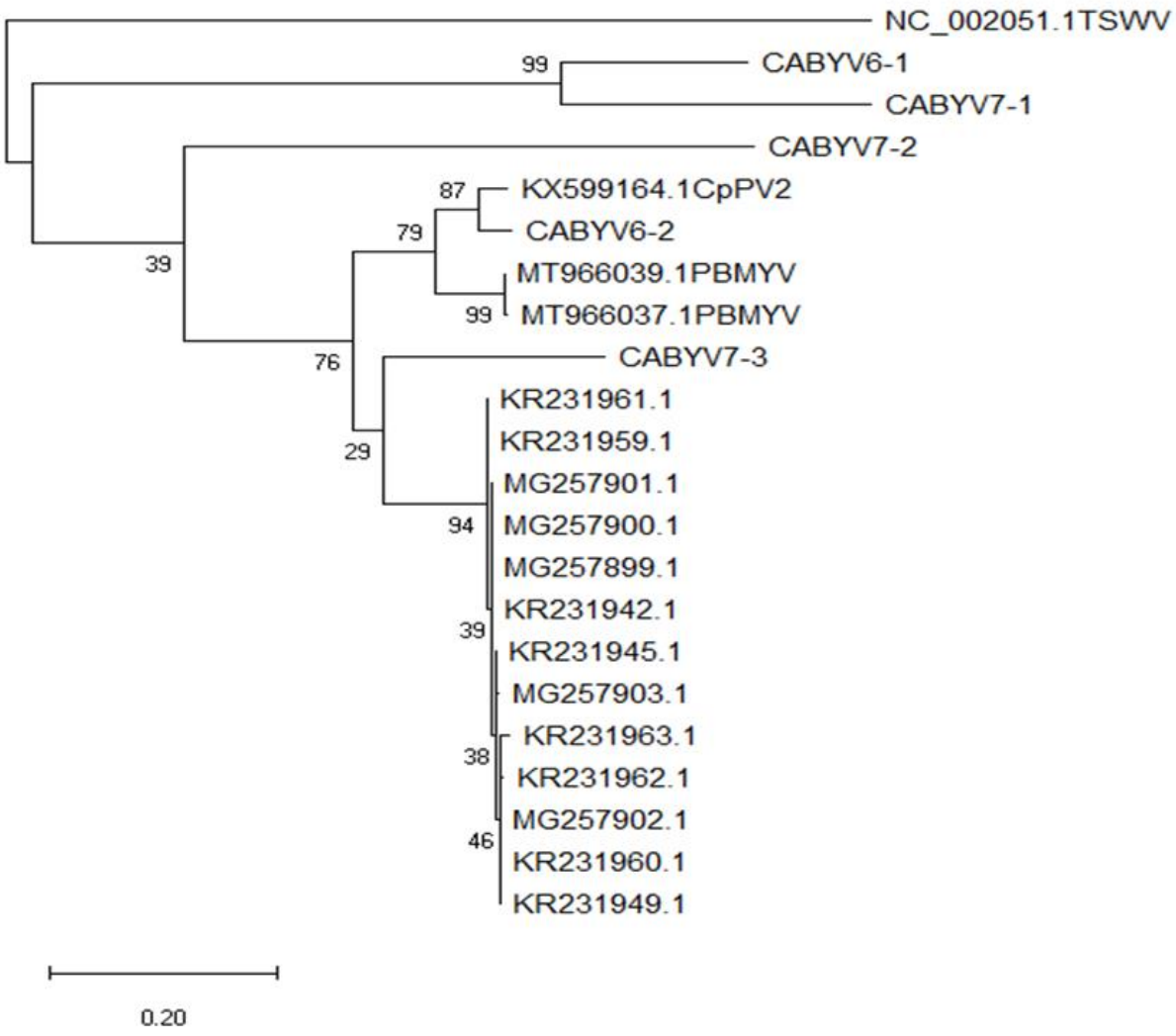

Figure 12: Molecular Phylogenetic analysis of CABYV isolates from western Kenya.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018). Tomato spotted wilt virus *Tospovirus 1* (TSWV – NC-002051.1) was used as the outgroup. The bootstrap values are shown next to the various branches. The phylogenetic tree is drawn to scale, with branch lengths per clade measured in the number of substitutions per site.

The Polerovirus Cucurbit aphid-borne yellows virus (CABYV) was recovered alongside the related Phasey bean mild yellows virus (PBMYV) infecting groundnuts (*Arachis hypogaea* L.). The PBMYV full genome sequences from Kenya (PBMYV_6, PBMYV_7) clustered together with other PBMYV and had closest sequence identity (91-95%) with PBMYV (KT963000.2, MT966033.1 and MT966038.1) (Figure 13). The PBMYV Kenyan strain is similarly diverse to the genetically distinct PBMYV Australian variants described by Sharman *et al* (2021). The PBMYV_6 and PBMYV_7 sequence data were submitted to the DNA Data Bank of Japan (DDBJ) sequence database and were assigned unique accession numbers LC709260 and LC709261 respectively.

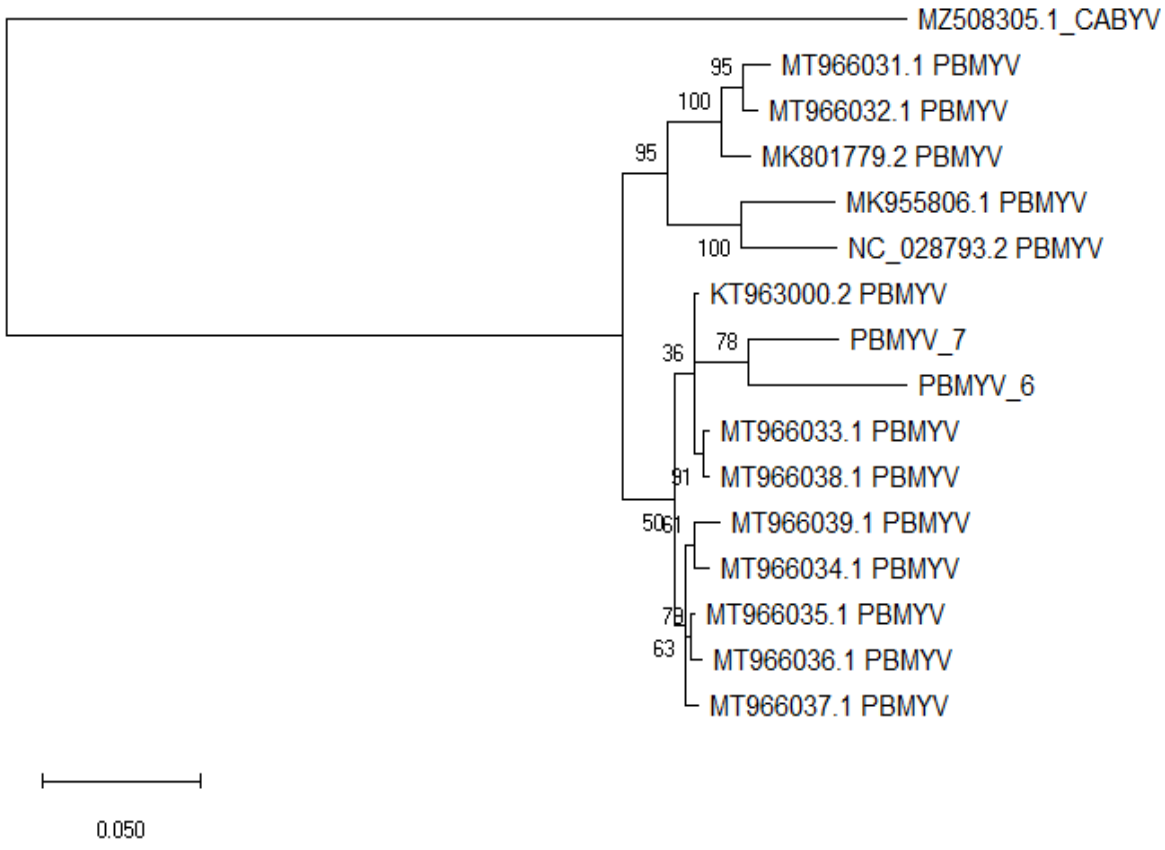

Figure 13: Molecular Phylogenetic analysis of PBMYV isolates from western Kenya.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018). Cucurbit aphid-borne yellows virus *Polerovirus* (MZ508305.1) was used as an outgroup. The bootstrap values are shown next to the various branches. The phylogenetic tree is drawn to scale, with branch lengths per clade measured in the number of substitutions per site.

4.5.2 Phylogenetic relationship of the poleroviruses isolated from groundnuts in Kenya

The Polerovirus isolates (CABYV and PBMYV) from western Kenya clustered separately with other related Polerovirus isolates in the GenBank. CABYV7-3 clustered together with other CABYV isolates, CABYV6-2 clustered with CpPV2 isolates, while CABYV7-1, CABYV7-2 and CABYV6-1 clustered with PBMYV. PBMYV-6 and PBMYV-7 grouped together with PBMYV isolate KT963000.2 and CABYV7-2 (Figure 14).

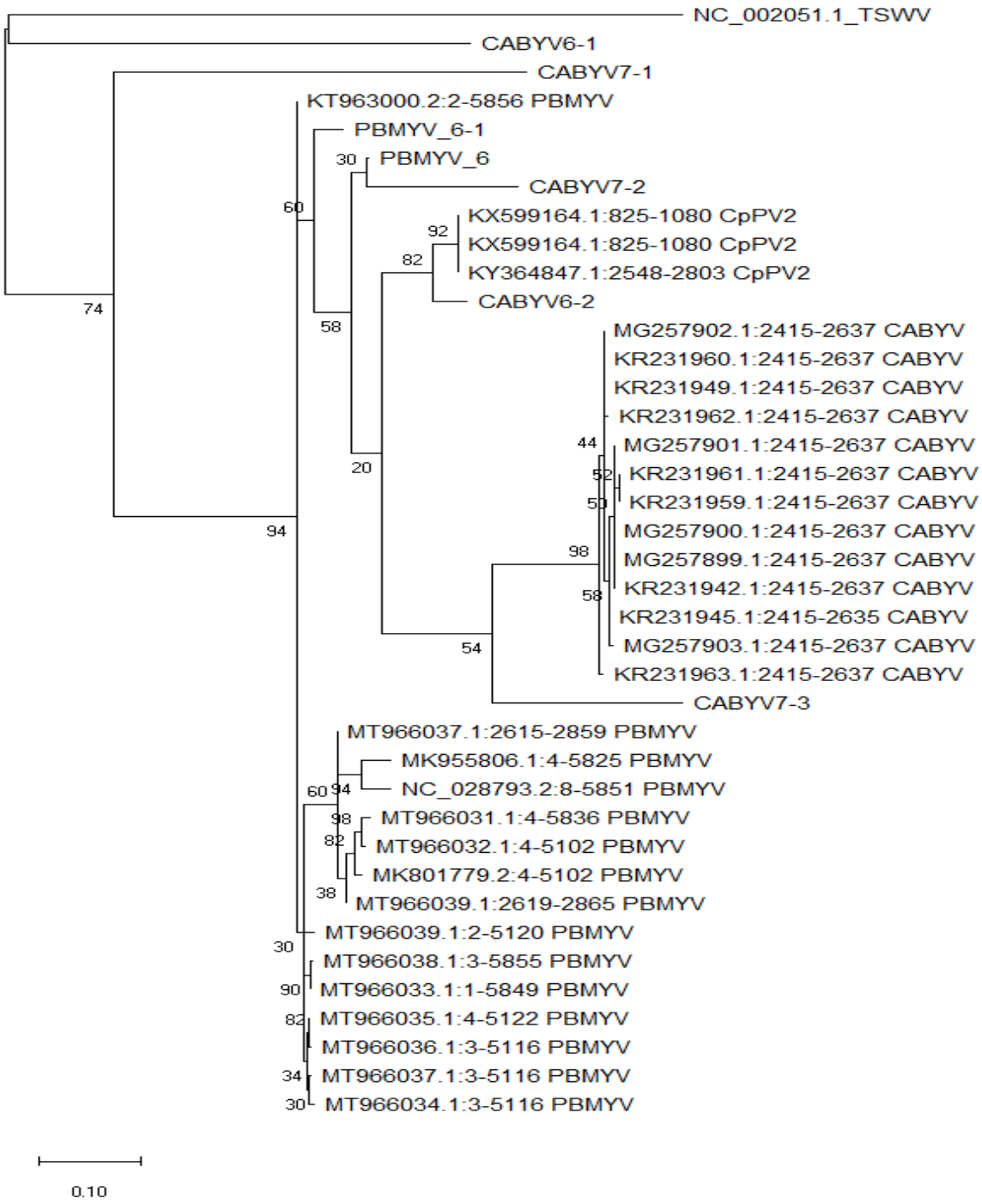

Figure 14. Molecular Phylogenetic analysis and evolutionary relationship of CABYV and PBMYV Polerovirus isolates from western Kenya showing their diversity and ancestry. The bootstrap values are shown next to the various branches. The phylogenetic tree was drawn to

scale with branch lengths per clade measured in the number of substitutions per site as in figures 12 and 13.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura and Nei, 1993). Evolutionary analyses were conducted in MEGA X (Kumar *et al*., 2018). Tomato spotted wilt virus (TSWV – NC-002051.1) was used as the outgroup.

CHAPTER FIVE

DISCUSSION

5.1 Incidence and distribution of PBMYV and CABYV on groundnuts in western Kenya

This study reveals the existence of new viral diseases of PBMYV and CABYV infecting groundnuts in Kenya. These new viruses belong to the genus Polerovirus which majorly displays yellowing colour as its typical symptom type in vegetable hosts, mainly in the C*ucurbitaceae* family (Lotos *et al*., 2014). In most farms that were surveyed, groundnuts showed severe yellowing and bunching symptoms in young leaves. These symptoms were widespread across all the surveyed counties in Kenya. Previous studies reported the major virus disease on groundnuts to be Groundnut rosette disease (GRD) which has a characteristic symptom of rosetting in addition to chlorosis and mosaic (Mukoye *et al*., 2020; Mabele and Were, 2020; Mabele *et al*., 2021). However, this current study documents yellowing symptoms observed on groundnut plants and neighbouring crops which were not rosetted. This indicated that there were other causal agents that were involved in the yellowing symptoms observed on groundnuts. The additional symptoms which included leaf mottling, leaf bunching, puckering and stunting could be associated with mixed infections and co-infections with other viruses including Poleroviruses, Potyviruses, Luteoviruses and Umbraviruses. A study by Mukoye (2020) reported that the groundnut crop is a potential host of many viruses and pests which occur in synergistic and mixed infections.

The virus disease incidence was observed to vary in the two growing seasons. The short rains season recorded higher incidence (73.61%) than the long rains season (42.65%). This could be attributed to the high vector pressure during the short rains as compared to the long rains season when the aphid pressure is low as a result of heavy rains that wash the insects off the

crop, and interfere with their mobility. A study by Mugisa *et al* (2016) established that periods of long rains negatively affected groundnut rosette disease (GRD) progression as aphid vector pressure was low. Were *et al* (2013) reported a positive correlation between potato disease incidence and aphid numbers. This further supports the implication that virus disease incidence variations between the cropping seasons is attributed to the differences in vector pressure. PBMYV and CABYV incidence increased with increase in severity due to early infection leading to intensification, extensification and diversification of the viruses as the plant grows, and build-up of inoculum for vectors to spread to the nearby plants. The polycyclic nature of most plant virus diseases where diseased plants from the previous cropping seasons serve as inoculum sources for initiating subsequent disease spread (Naidu *et al*., 1998a), enhances early infections in subsequent cropping seasons. In Kenya, groundnuts are grown in two cropping seasons (long rains and short rains) although some little percentage grow as volunteer groundnuts throughout the year, and due to fragmentation and limitation of land size to practice shift cultivation, the same piece of land is continuously and routinely used to grow the same or related host crops in the subsequent cropping season. These scenario implies that infected groundnuts and alternate hosts of any of the viruses remaining from the long rains season, serves as the immediate sources of the virus inoculum initiating the disease cycle at early stages of crop development in the short rains cropping season. Such initial infections that occur at early stages of plant growth enhances repeated cycles of infections, thus increasing the infection severity of the disease in the groundnut fields (Waliyar *et al*., 2007). Relevante *et al* (2012) observed that CABYV symptom severity may vary depending on the season, being more pronounced in summer than in winter. This is a similar observation made when the severity of PBMYV and CABYV symptoms on groundnuts were more pronounced during the short rains season when the temperatures are usually higher than in the long rains season with relatively lower temperatures.

5.2 Serodiagnosis assays of Cucurbit aphid-borne yellows virus (CABYV) with TAS-ELISA

Serologically, CABYV was detected in some samples across all the surveyed counties. This confirmed that CABYV was infecting groundnuts in western Kenya. The use of enzyme linked immune sorbent assay (ELISA) for the detection of plant viruses is well documented and proved to be a very valuable detection tool to confirm the presence or absence of the plant viruses that have been visually observed. In addition, the detection of closely related strains of the same virus can be achieved with enhanced specificity of the test (Abd El-Aziz, 2019). The universal commercial antibodies for CABYV used in this serological bioassay, were able to single out CABYV antigens from even those of other related viruses. This confirms that the Kenyan CABYV serological characteristics are similar to those of other isolates from other regions in the world.

5.3 RT-PCR detection of Phasey bean mild yellows virus (PBMYV)

Phasey bean mild yellows virus (PBMYV) was also confirmed in symptomatic leaf samples by RT-PCR. This confirms that the virus occurs in Kenya and on a new host, the groundnut (*Arachis hypogaea* L.) crop. This, together with molecular characterization using NGS proves that PBYMV is infecting groundnuts in Kenya. The central dogma genetics and vector competence of PBMYV transmission by the cowpea-groundnut aphid (*Aphis craccivora* Koch.) and by grafting with infected scions, suggests that all individuals within this vector species are potential vectors, although their populations will differ in their ability to efficiently vector the virus. This findings explain the interaction between PBMYV strains and groundnut host genotypes that determines PBMYV virulence during the long rains season when the temperatures are low, and the short rains when the temperatures are high leading to increased vector colonization of PBMYV-infected plants by aphids, higher concentration of

eggs on symptom-free leaves of diseased plants, positive molecular diagnosis of asymptomatic leaves in plants neighbouring diseased plants, and increased rates of fecundity on PBMYV-diseased plants, although variations may occur within the different phylogeography locations. The two viruses, CABYV and PBMYV, infect groundnuts in a mixed fashion. This concurs with the findings by Mukoye *et al* (2020) that reported groundnuts as a potential host of new viruses in western Kenya. The biological desert of information associated with the discovery of new plant viruses like PBMYV will contribute to the available knowledge on the distinct ability of the PBMYV strains to evolve, and mutate more rapidly through recombination, spillover and spillback, crossover and host jumping events that enables them adapt to new plant species like groundnuts across the different ecosystems.

5.4 Biological characteristics of Cucurbit aphid-borne yellows virus (CABYV)

The screened plants expressed distinct symptoms of bright yellowing, mild mottling, yellowing, chlorotic blotches, stunting, severe mottling, interveinal yellowing, vein clearing, mosaic, leaf curling, leaf bunching, leaf clumping, dwarfing and stunting. The typical symptoms of CABYV on cucurbits is yellowing which appears mostly on older basal leaves of the plant, but gradually expand to the new foliage leaves, and they are indistinguishable to those caused by nutrient deficiencies, a similar phenomenon with cassava brown streak virus (CBSV, *Ipomovirus*) viral symptoms (Osogo *et al*., 2014; Winter *et al*., 2010), and cucurbit chlorotic yellows virus (CCYV, *Crinivirus*) (Abrahamian *et al*., 2013). All inoculated plants have not been reported before as hosts of CABYV and this therefore was an initial trial on identifying new hosts including groundnuts where the virus was detected by NGS. TAS-ELISA tests detected CABYV only in groundnuts for mechanically inoculated plants. The symptoms expressed in other test plants that tested negative for the virus could be as a result of other molecularly related poleroviruses or their co-infections. Several studies describe

Polerovirus co-infections with other viruses (Moreno and López-Moya, 2020). During mechanical inoculations, such co-infecting viruses could be acquired by the test plants leading to the symptoms observed.

Artificial mechanical inoculation of CABYV has not been reported before and the virus had been reported not to be mechanically transmissible. Transmission of CABYV has been reported to only occur by aphid vectors (Kassem *et al*., 2013). The fact that mechanically inoculated groundnuts expressed yellowing, stunting, leaf curling among other typical viral symptoms and tested positive for CABYV is regarded as a new finding. Besides all major cucurbit crops, CABYV has been reported to infect other annual crops including lettuce, faba bean, chickpea, passion fruits and wild cucurbits (Kumari *et al*., 2018; Vidal *et al*., 2018). This study therefore expands the host range diversity of CABYV to include groundnuts crop as its alternative host.

5.5 Insect vector transmission of Cucurbit aphid-borne yellows virus (CABYV)

Aphid inoculated groundnuts (*Arachis hypogaea*) and Bambara groundnuts (*Vigna subterranea*) with the cotton-melon aphid (*Aphis gossypii* Glover.) and green peach aphid (*Myzus persicae* Sulzer.) expressed typical viral symptoms of stunting, leaf yellowing, puckering, chlorosis and leaf curling. Serological analysis of the symptomatic leaves tested positive for CABYV. Although CABYV is aphid transmitted, its successful infection on groundnuts and Bambara groundnuts is a novel finding. This research findings makes a distinct contribution to knowledge by reporting new alternative hosts of CABYV to include groundnuts (*Arachis hypogaea*) and Bambara groundnuts (*Vigna subterranea*). Managed plant pathosystems serve as the basis for advancement in plant virology research especially for the vertically transmitted plant infecting viruses like CABYV by the generalist cotton-melon aphid, *Aphis gossypii* and the generalist green-peach aphid vector, *Myzus persicae*.

This study findings imply that the Kenyan CABYV genotypes are closely associated and similar to the second genotype originating from Europe which does not infect the solanaceous Sacred datura (*Datura wrightii)* (Shates *et al*., 2019; Malmstrom *et al*., 2014) as well as the Kenyan thorn apple *(Datura stramonium* L.), originally from North America as an invasive annual herb and ruderal forb that hosts CABYV vectors, but infects Cucurbits and groundnut legumes. This non-infectivity by some vector biotypes could be as a result of genomic masking which significantly affects virus transmission by aphids and the symptom expression of the resulting plant disease.

5.6 Molecular characteristics of Cucurbit aphid-borne yellows virus and Phasey bean mild yellows virus infecting groundnut (*Arachis hypogaea* L.) in western Kenya

The CABYV isolates from Kenya clustered with other Poleroviruses variedly in different clades. The CABYV7-3 clustered with several other CABYV isolates available in the GenBank including KR231961.1, KR231959.1 and MG257901.1. CABYV6-2 showed 94.5% nucleotide identity with CpPV2 isolate KX599164.1 from Burkina Faso and 83.4% identity with CABYV isolate MG257902.1 from Korea. This implies that the CABYV Polerovirus sequences obtained from NGS belonged to various related viruses in this group. Visual symptomatic identification of CABYV alone is not reliable due to its related symptoms with other Poleroviruses and by extension nutrient deficiency. In some countries, CABYV is one of the most common viruses occurring in cucurbit crops (over 30% infected plants detected during surveys in France, Iran, Ivory Coast, Morocco, Serbia, Sudan, Spain and Tunisia) (Kassem *et al*., 2007; Lecoq and Desbiez, 2012). In other areas, CABYV is either rare (less than 2% infected samples in Czech Republic, southern USA) or absent (Panama, Venezuela, French West Indies) (Ali *et al*., 2012; Romay *et al*., 2014). In Korea, CABYV was first detected by next-generation sequencing (NGS) in melons showing yellowing symptoms in 2014, and it was confirmed that the leaf yellowing symptoms of melon plants was not merely

a physiological disorder or nutrient deficiency, but a virus disease caused by CABYV (Lee *et al.,* 2015). This is the same scenario in Kenya where the occurrence of CABYV could only be isolated by NGS alongside PBMYV Polerovirus.

Phasey bean mild yellows virus (PBMYV) full genome sequences from Kenya (PBMYV_6, PBMYV_7), clustered together with other PBMYV sequences from the GenBank and had closest sequence identity (91-95%) with PBMYV (KT963000.2, MT966033.1 and MT966038.1) isolates. The PBMYV Kenyan strain is similarly diverse to the genetically distinct PBMYV Australian variants described by Sharman *et al* (2021). The PBMYV transmission studies by the cowpea aphid (*Aphis craccivora*) and by grafting with infected scions reported *Fabaceae* hosts of Phasey bean (*Macroptilium lathyroides* L.), Faba bean (*Vicia faba*), Chickpea (*Cicer arietum*) and Pea (*Pisum sativum*) as alternative hosts (Wilson *et al.*, 2012), and in one non-legume host, Scarlet pimpernel (*Anagallis arvensis*) (Sharman *et al*., 2021), suggesting that further hosts outside of the family *Fabaceae* may be found but have not been reported in groundnut (*Arachis hypogaea* L.) which this study has unraveled. This study therefore documents the first report of PBMYV in Kenya with a new host record affecting groundnuts.

The Poleroviruses identified on groundnuts when compared, CABYV7-3 clustered together with other CABYV isolates and CABYV6-2 clustered with CpPV2 isolates, while CABYV7-1, CABYV7-2 and CABYV6-1 clustered with PBMYV. PBMYV-6 and PBMYV-7 clustered together with PBMYV isolate KT963000.2 and CABYV7-2 in a unique clade. This implies that the Polerovirus isolates from Kenya belonged to two distinct evolutionary species, namely CABYV (CABYV7-3), CpPV2 (CABYV6-2) and PBMYV (CABYV7-1, CABYV7-2, CABYV6-1) generating two distinct clades. This findings gives an indication that the two Poleroviruses (PBMYV and CABYV) could be occurring in mixed infections in groundnuts, contributing to the diverse symptoms observed on groundnuts. Poleroviruses are obligatorily

transmitted by aphids, and infections are limited to the phloem. Therefore, Polerovirus symptoms generally include stunting, yellowing, leaf malformation, and discoloration of the main leaf vein (Distéfano *et al*., 2010; Fiallo-Olive *et al.,* 2018). There is a possibility that the two Poleroviruses (PBMYV and CABYV) reported in this study could be acquired and transmitted by aphids simultaneously.

5.7 Socio-economic status of groundnut farming in western Kenya

The average land size owned by smallholder farmers in the surveyed counties was 2.3 acres of land, from which an average of 0.23 acres was allocated to groundnut production. Onyunka *et al* (2017) reported that land allocated to groundnut production around Lake Victoria region was 0.87 acres. This implies that land as an agricultural resource is becoming scarce, hence the smallholder farmers have to balance crop diversification since little land remains available for groundnut farming. One of the contributing factors to smallholder land ownership is the increasing population and the Kenyan culture of subdividing land among the family members. A related socio-economic survey reported average land ownership of 2.1 acres in parts of Nandi County which borders western Kenya (Onyango, 2017). Although groundnut is the main grain legume cultivated in western Kenya (Onyango, 2017), the average yield recorded was 223 Kgs. In sub-Saharan Africa, smallholder farmers routinely obtain yields of 500-800kg/ha as opposed to the potential yield of >2.5t/ha (Kayondo *et al*., 2014). In Kenya, farmers achieve less than 30-50% of the potential yield with an average output of 600-700 kg/ha (Kidula *et al*., 2010). Pests and diseases are the main yield reducing factors and the leading diseases are caused by plant pathogenic viruses like PBMYV and CABYV. According to Anderson *et al* (2004), the calculated plant viruses cause nearly half (47%) of the total plant diseases. About thirty-one (31) viruses were reported to naturally infect groundnuts around the world (Kumar and Waliyar, 2007). This study has reported two new Poleroviruses (PBMYV and CABYV) infecting groundnut farms in Kenya. This

research demonstrates that groundnuts is a potential host of several viruses in Kenya. This implies that the effect of plant viruses on the prospective yield potential of groundnuts is of greater economic importance.

Majority of the groundnut farmers were female at 61% whereas the household heads were male at 69%. The findings on head of household is in line with the African culture where males head households. The head of households are the ones who make major decisions on what crops to plant in a particular season and this affects groundnut production. However, the females were the main actors in groundnut farming, which could imply male dominated households who may not make informed decisions concerning groundnut production. The people who are directly involved with the crop would best determine the best practices to enhance productivity like choice of variety planted and season of planting. Majority of the farmers (59.4%) had primary education. Farmers with higher levels of formal education are more likely to be knowledgeable and able to adopt new emerging technologies and make sound production decisions, although the less educated embrace indigenous cultural technologies in crop management. Any intervention that relies on education levels is therefore more likely to succeed (Onyunka *et al*., 2017). However, less educated farmers cannot effectively understand the impact of pests and diseases on the crops since most of the cultural practices are not practically feasible in virus disease management. This could be one of the reasons why farmers were not practicing any interventions to control pests and diseases on groundnuts which majority (80%) claim are not affected by pests and diseases.

In terms of farmer preferred groundnut varieties, Red Valencia was the most cultivated (79.1%). A similar observation was reported by Mukoye (2020) where Red Valencia was the most cultivated variety in western Kenya. This informs the groundnut sector players that any effort to enhance groundnut productivity should be directed towards the farmer preferred varieties for ease of adoption. There were two groundnut growing seasons, long and short

rains, and most farmers (76.1 - 81.8%) were found to cultivate groundnuts during both seasons. This indicates that the crop is continuously grown as a routine crop in the region alluding to its economic and food security value.

CHAPTER SIX

CONCLUSION, RECOMMENDATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

6.1 Conclusion

This study makes the following conclusions:

1. The two main Poleroviruses reported infecting groundnuts (*Arachis hypogaea* L.) in western Kenya include Phasey bean mild yellows virus (PBMYV) and Cucurbit aphid-borne yellows virus (CABYV). This is the first report of these two viruses in western Kenya with a new host record. The Phasey bean mild yellows virus (PBMYV) and Cucurbit aphid-borne yellows virus (CABYV) disease incidence and severity is high during the short rains season than during the long rains growing season. This findings suggest that severely infected groundnut plants interact synergistically and symbiotically with the viruliferous aphids characterized by increased colonization of PBMYV-infected and CABYV-infected plants by aphids, higher concentration of eggs mutualism on symptom-free leaves of diseased plants, and increased rates of fecundity on PBMYV-diseased and CABYV-diseased groundnuts. Climate change is also likely to alter the incidence and distribution of damaging plant virus disease epidemics through alterations in temperature on development and flight of vectors, CO_2 concentration on compensatory phloem and sap feeding due to increased plant biomass yield, and rainfall impact on vector colonization and dispersal. Climate change also alters the parasitoid-predator associations on the vector, virus titre concentrations and the whole pathosystems interactions of the vector-pathogen-host plant changing the cropping patterns and vector biotype mobility.

2. The serological bioassays of TAS-ELISA detected Cucurbit aphid-borne yellows virus (CABYV) after troubleshooting modifications on the pH of the coating buffer that maximized the adsorption on the multiwell plates, and generated consistent colour development for the positive samples. Cucurbit aphid-borne yellows virus (CABYV) infects groundnuts in mixed fashion with PBMYV. Mixed infections among plant viruses are probably a rule rather than an exception in natural pathosystems.
3. Molecular diagnostics of PBMYV with available primers used in transcription and amplification of the targeted coat protein and nucleotide bases, were not specific for the symptomatic groundnut isolates from western Kenya, therefore subjected to modifications of the hybridization temperatures for development of consistent band sizes in the gel. Testing of samples by molecular techniques that detect multiple viruses simultaneously helps in detecting virus infections being missed out and also reveals new infections occurring in the field.
4. Cucurbit aphid-borne yellows virus (CABYV) can be artificially mechanically transmitted in groundnuts (*Arachis hypogaea* L.), suggesting that there is a possibility for some new variants/strains of Poleroviruses that could be mechanically inoculated in new alternative/natural hosts. This diversification and evolution of Polerovirus strains is a new disease encounter phenomenon/situation/scenario in the groundnut crop within western Kenya agro-ecological zones. However, this study findings imply that the Kenyan CABYV genotypes are closely associated and similar to the second genotype originating from Europe which does not infect the solanaceous Sacred datura (*Datura wrightii)* (Shates *et al*., 2019) as well as the Kenyan Thorn apple *(Datura stramonium)*, but infects Cucurbits and the leguminous groundnut (*Arachis hypogaea* L.).

5. Cucurbit aphid-borne yellows virus (CABYV) can be insect vector transmitted by the cotton-melon aphid (*Aphis gossypii* Glover.) and green peach aphid (*Myzus persicae* Sulzer.) on groundnuts (*Arachis hypogaea* L.) and Bambara groundnuts (*Vigna subterranea*) as new alternative hosts.

6. The genetic diversity of Phasey bean mild yellows virus (PBMYV) full genome sequences from Kenya (PBMYV_6, PBMYV_7), indicates a distinct clustering together with other PBMYV sequences from the GenBank (KT963000.2, MT966033.1 and MT966038.1) with closest sequence identity of 91-95%. The PBMYV Kenyan strains are similarly diverse to the genetically distinct PBMYV Australian variants. The CABYV isolates from Kenya clustered with other Poleroviruses from the GenBank (KR231961.1, KR231959.1 and MG257901.1) variedly in different clades. CABYV6-2 showed 94.5% nucleotide identity with CpPV2 isolate KX599164.1 from Burkina Faso and 83.4% identity with CABYV isolate MG257902.1 from Korea. PBMYV and CABYV genomic divergence is roughly proportional to the evolutionary distance from a common ancestor, and a high degree of sequence diversity over a limited geographic range of co-evolution over a long period of time within their centres of origin.

7. The socio-economic analysis of plant disease epidemics is key in achieving sustainable disease management by understanding the plant disease infestation mechanisms for PBMYD and CABYD. This study findings establish that there are several socio-economic value chain challenges associated with agribusiness productivity of groundnuts (*Arachis hypogaea* L.) in western Kenya. They include production challenges (pests, diseases, land size, certified seed, farm input, labour and extension services); marketing challenges (market price, market availability/location,

produce quantity, produce quality, and marketing information); processing challenges (equipment, quantity, technology, and training) ; storage challenges (facility, cost, location, viability, drying duration, health aspects – aflatoxin, and labour). This study established that the varieties chosen for planting by farmers is influenced by the land size, season, household head, market demand and end use.

6.2 Recommendations

This research findings recommend that:

1. There is need for extensive studies to understand the underlying interactions of mixed infections of PBMYV and CABYV Poleroviruses to explicitly explain their distribution specificity in known and alternative hosts, to understand their persistence in the diverse non-investigated pathosystems, severity in plant stage, economic impact and vector diversity.
2. Regular diagnostics surveillance of the newly reported PBMYV and CABYV Poleroviruses with new primers and antisera to establish a sustainable and optimized analysis framework that evaluates their livelihood impact on the farmers through commercial, regulatory, phytosanitary, biosecurity, health, scientific and agribusiness spheres.
3. Awareness creation among groundnut farmers on PBMYV and CABYV Poleroviruses which have been under-researched, but should not be underestimated to help in intensification and extensification of their symptom diversity identification as emerging plant viruses, and implement early stage management technologies.
4. Further characterization of PBMYV and CABYV Poleroviruses to generate more robust knowledge to supplement the scarce information that has so far been provided by the aid of NGS and metagenomics analysis approaches to unravel their sequence diversity and phylogeography.
5. Breeding for virus immune resistant genes for groundnut varieties/cultivars popularly grown by farmers in western Kenya will be the only practical solution for PBMYV and CABYV management to overcome mixed infections, co-evolution, recombinant strains, spillover, host jumping and superinfections.

6.3 Suggestions for further research

This study suggests further reading and research on the gaps identified that were not exhaustively emphasized including:

1. Sequence the complete genome of Cucurbit aphid-borne yellows virus (CABYV) from groundnuts, and together with the Phasey bean mild yellows virus (PBMYV) complete genome obtained, develop efficient novel methods for sufficiency in rapid diagnostic assays of the pathogen and vector based on species specific markers for use in biosecurity and phytosanitary regulation of each Polerovirus. This will help in identification of factors underlying the appearance/emerging of new virus in mixed infection, co-infection, co-evolution, rapid evolution and diversification of new variants/strains of plant viruses.
2. Investigations on the transmission studies of PBMYV and CABYV to establish their potential vector biotypes, take-off, alighting, settling, flight, infectivity, probing behavior and phenology among the aphids, and further unravel whether these new poleroviruses are also transmitted by the complex whitefly (*Bemisia tabaci*) in the non-investigated pathosystems. This will further help assess comprehensively the economic impact and risks of PBMYV and CABYV in the different agro-ecological zones (AEZs) with respect to environmental land management policy and ownership impacting on farmers' livelihood.
3. Understand the population genetics and explosion of the vectors transmitting PBMYV and CABYV in groundnuts, thereafter establish potential alternative host plants in the environment surrounding the crop for on-season and off-season monitoring of the virus inoculum and fecundity in the major groundnut growing regions. This will generate knowledge on PBMYV and CABYV virus ecology and epidemiology in the different

eco-niches between managed and natural vegetation, and the disturbed non-investigated pathosystems.

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APPENDICES

Appendix I: Disease diagnostic score sheet

SURVEY DISEASE SCORE SHEET

CROP…….VARIETY……..…

Farmer's name……County……..…

Sub-County……Division……

Location……Sub-Location…….….

Village…….Date……..

GPS readings;

Altitude (Metres)……..

Longitude (East or West)……..

Latitude (North or South)…….AEZ…….……

	Disease name…….			
Groundnut variety	No. of plants infected per 10m^2 quadrat	Part infected (root, stem, leaves, pods)	Distribution (whole field, spots)	Severity 0-3
1				
2				
3				
4				
5				

*Severity: 0= No disease; 1=Mild; = Moderate; 3=Severe.

Number of plants infected per $10m^2$: select the area most affected, 10 steps square quadrat, count infected and total plants, (e.g. $^{20}/_{50}$ indicates 20 plants infected out of 50 plants in the 10x10 steps square quadrat).

Appendix II: Questionnaire

QUESTIONNAIRE FOR GROUNDNUT FARMERS' SOCIO-ECONOMIC DATA

A. Household and socio–economic characteristics

Name of the respondent	……	
Age of household head	.....	
Sex of household head (Tick in one box)	Male	Female
County		

1. Formal education (highest level attained)
    i. No formal education (Illiterate)
    ii. Primary school
    iii. Secondary school
    iv. Technical, Vocational and Educational Training (TVET)
    v. College/University
2. Marital status
    i. Single
    ii. Married
    iii. Others (specify)
3. Tenure of land (give acreage)
    i. Communal..... acres
    ii. Private..... acres
    iii. Government/Institutional..... acres
    iv. Family..... acres
4. How long have you been farming.....(Years)

5. Do you farm part – time or full time
    i. Part – time
    ii. Full – time
6. If you are not full time farmer, how much of your time do you devote to farming operation (tick the appropriate)
    i. Less than half
    ii. Half
    iii. More than half
7. Is any member of your household involved in any off–farm activities? (i) YES (ii) NO. If yes, please specify the activity (ies)
    i. Formally employed
    ii. Trading/Business
    iii. Hiring out oxen/farm implements/labour
    iv. Others (specify)
8. Give the groundnut varieties you plant, who plants them and the purpose of production

	Crop	Acreage	Gender	Purpose of production
1.				
2.				
3.				
4.				
5.				

9. How much of the groundnut crop did you sell last year?

Groundnut variety	Quantity produced (bags/tins/basins/kg)		Amount sold (bags/tins/basins/kg)		Price (KShs/kg)	
	Long Rain season	Short Rain season	Long Rain season	Short Rain season	Long Rain season	Short Rain season

B. Labour

1. What family labour is available for production activities (Adults, Youth and PLWD)

Age group	Full time participating in farming activities		Part time participating in farming activities	
	Male	Female	Male	Female

2. Do you use hired labour? (i) Yes (ii) No

3. What kind of hired labour do you use per season (on average)

Long Rains season

Type of hired labour	Number of males	Number of females
Casual		
Permanent		
Village labour exchange		
Adults		
Youth		
PLWD		

Short Rains season

Type of hired labour	Number of males	Number of females
Casual		
Permanent		
Village labour exchange		
Adults		
Youth		
PLWD		

4. For which activities do you hire labour?

	Activity	Casual	Permanent	Village
1.	Land preparation			
2.	Planting			
3.	Weeding			
4.	Harvesting			
5.	On – farm transport			
6.	Others (specify)			

C. Perception of groundnut diseases (Allow description of disease symptom types)

1. Do you know any groundnut diseases (i) Yes (ii) No
    i. What name do you call the disease?
    ii. What do you think causes the disease?
    iii. In your own view how is this disease transmitted?
    iv. How do you try to control the disease?
2. What is the yield loss due to groundnut diseases?
    i. Low (less than 20%)
    ii. Moderate (21 – 40%)
    iii. High (over 50%)
    iv. Total loss (100%)

3. Do you know any variety(s) which is not infected by groundnut diseases? (i) Yes (ii) No. If Yes, name the variety
    i. .....
    ii. .....
    iii. .....
    iv. .....
4. In your view what is the trend of occurrence of the disease you have named over the years?
    i. Increasing
    ii. Same
    iii. Decreasing
5. Do you plant groundnuts in lines/rows? (i)Yes (ii) No (specify spacing)

6. Do you grow groundnuts in pure stand?
    i. Pure stand/sole
    ii. Mixed/intercropped
7. How many times do you weed groundnuts?
    i. Once
    ii. Twice
    iii. Thrice

8. At what stage do you weed the groundnut?

9. What purchased input do you use in production of groundnut?

Input	**Purchased, borrowed or hired**	**Approximate cost**

10. How easy is it for you to obtain the relevant inputs for production?

Type of input		**Input availability**			
Seed	Certified				
	Own				
	Other sources(mention)				
Hoes (‘Jembe’)					
Fertilizers					
Herbicide					
Insecticide					
Fungicides					
Others (specify)					

D. Use, marketing and decision making

1.	What are your uses of groundnut?	
2.	What proportion do you sell?	
3.	What proportion do you retain for seed?	
4.	If sold, where do you sell?	
5.	When do you sell?	
6.	Shelled or unshelled	
7.	Do you sell at once?	

E. Who makes the following decisions?

	Decision	**Who makes?**
1.	How much to plant	
2.	How much seed to retain	
3.	How much to eat	
4.	How much to sell	
5.	When to sell	
6.	Where to sell	

F. Farmer(s) Institutions/Associations/Group(s)

1. Is any member of your household a member of any group/association? Yes/No. If yes, specify the kind of group/association (Name the group/association)

Extension contact group	**Farmer association**	**Others (specify)**

2. What are the major functions of the group/association?

i. When did you become a member of the group/association? (give year)

ii. Why did you become a member of the group (any benefit)?

iii. Does the group/association address groundnut disease(s) issues? Yes/No

iv. If yes, enumerate the groundnut disease(s) issues addressed

3. Have you ever attended field day or demonstration trials? Yes/No

4. Have you ever attended a farmers training course? Yes/No

5. Please give any comment/suggestions relating to groundnut diseases in relation to groundnut production

……..……

……

……

THANK YOU FOR PARTICIPATING.

Appendix III: Location areas of the surveyed groundnut fields in western Kenya

COUNTY	ALTITUDE	LONGITUDE	LATITUDE	SEASON
Busia	1296	E034.31286	N00.31286	Long rain
Busia	1296	E034.31286	N00.31286	Long rain
Busia	1296	E034.31286	N00.31286	Long rain
Busia	1257	E034.23760	N00.29552	Long rain
Busia	1257	E034.23760	N00.29552	Long rain
Busia	1257	E034.23760	N00.29552	Long rain
Busia	1257	E034.23760	N00.29552	Long rain
Busia	1264	E034.23464	N00.29288	Long rain
Busia	1264	E034.23464	N00.29288	Long rain
Busia	1264	E034.23464	N00.29288	Long rain
Busia	1264	E034.23464	N00.29288	Long rain
Busia	1310	E034.28268	N00.31942	Long rain
Busia	1310	E034.28268	N00.31942	Long rain
Busia	1310	E034.28268	N00.31942	Long rain
Busia	1284	E034.32163	N00.32496	Long rain
Busia	1284	E034.32163	N00.32496	Long rain
Busia	1284	E034.32163	N00.32496	Long rain
Busia	1280	E034.32230	N00.32128	Long rain
Busia	1280	E034.32230	N00.32128	Long rain
Busia	1280	E034.32230	N00.32128	Long rain
Busia	1185	E034.19242	N00.40588	Long rain
Busia	1193	E034.20306	N00.41242	Long rain
Busia	1193	E034.20306	N00.41242	Long rain
Busia	1193	E034.20306	N00.41242	Long rain
Busia	1193	E034.20306	N00.41242	Long rain
Busia	1193	E034.20306	N00.41242	Long rain
Busia	1191	E034.20351	N00.41551	Long rain
Busia	1191	E034.20351	N00.41551	Long rain
Busia	1191	E034.20351	N00.41551	Long rain
Busia	1191	E034.20351	N00.41551	Long rain
Busia	1191	E034.20351	N00.41551	Long rain
Busia	1191	E034.20351	N00.41551	Long rain
Busia	1193	E034.20446	N00.41642	Long rain
Busia	1193	E034.20446	N00.41642	Long rain
Busia	1193	E034.20446	N00.41642	Long rain
Busia	1193	E034.20446	N00.41642	Long rain
Busia	1193	E034.20446	N00.41642	Long rain
Busia	1172	E034.13031	N00.41994	Long rain
Busia	1179	E034.10503	N00.41319	Long rain
Busia	1179	E034.10503	N00.41319	Long rain
Busia	1179	E034.10503	N00.41319	Long rain

Busia	1175	E034.10410	N00.40845	Long rain
Busia	1175	E034.10410	N00.40845	Long rain
Busia	1175	E034.10410	N00.40845	Long rain
Busia	1175	E034.10410	N00.40845	Long rain
Busia	1175	E034.10410	N00.40845	Long rain
Busia	1200	E034.11015	N00.43571	Long rain
Busia	1200	E034.11015	N00.43571	Long rain
Busia	1200	E034.11015	N00.43571	Long rain
Siaya	1340	E034.35617	N00.07417	Long rain
Siaya	1340	E034.35617	N00.07417	Long rain
Siaya	1340	E034.35617	N00.07417	Long rain
Siaya	1340	E034.35617	N00.07417	Long rain
Siaya	1367	E034.34445	N00.05377	Long rain
Siaya	1367	E034.34445	N00.05377	Long rain
Siaya	1367	E034.34445	N00.05377	Long rain
Siaya	1290	E034.43913	S00.04043	Long rain
Siaya	1290	E034.43913	S00.04043	Long rain
Busia	1164	E034.31875	S00.23808	Long rain
Busia	1164	E034.31875	S00.23808	Long rain
Busia	1164	E034.31875	S00.23808	Long rain
Siaya	1176	E034.32233	S00.23762	Long rain
Siaya	1176	E034.32233	S00.23762	Long rain
Siaya	1176	E034.32233	S00.23762	Long rain
Siaya	1191	E034.32590	S00.23710	Long rain
Siaya	1191	E034.32590	S00.23710	Long rain
Siaya	1191	E034.32590	S00.23710	Long rain
Siaya	1182	E034.32560	S00.23590	Long rain
Siaya	1182	E034.32560	S00.23590	Long rain
Siaya	1182	E034.32560	S00.23590	Long rain
Siaya	1291	E034.27107	N00.10636	Long rain
Siaya	1291	E034.27107	N00.10636	Long rain
Siaya	1291	E034.27107	N00.10636	Long rain
Siaya	1291	E034.27107	N00.10636	Long rain
Siaya	1282	E034.28773	N00.10851	Long rain
Siaya	1282	E034.28773	N00.10851	Long rain
Siaya	1282	E034.28773	N00.10851	Long rain
Siaya	1282	E034.28773	N00.10851	Long rain
Siaya	1167	E034.18727	S00.03375	Long rain
Siaya	1167	E034.18727	S00.03375	Long rain
Bungoma	1476	E034.54118	N00.61600	Long rain
Bungoma	1476	E034.54118	N00.61600	Long rain
Bungoma	1476	E034.54118	N00.61600	Long rain
Bungoma	1465	E034.54144	N00.61531	Long rain
Bungoma	1465	E034.54144	N00.61531	Long rain

Bungoma	1465	E034.54144	N00.61531	Long rain
Bungoma	1465	E034.54144	N00.61531	Long rain
Bungoma	1404	E034.46411	N00.69718	Long rain
Bungoma	1404	E034.46411	N00.69718	Long rain
Bungoma	1404	E034.46411	N00.69718	Long rain
Bungoma	1404	E034.46411	N00.69718	Long rain
Bungoma	1350	E034.44774	N00.68771	Long rain
Bungoma	1350	E034.44774	N00.68771	Long rain
Bungoma	1350	E034.44774	N00.68771	Long rain
Bungoma	1350	E034.44774	N00.68771	Long rain
Busia	1261	E034.34343	N00.64906	Long rain
Busia	1261	E034.34343	N00.64906	Long rain
Busia	1261	E034.34343	N00.64906	Long rain
Busia	1299	E034.33460	N00.66420	Long rain
Busia	1299	E034.33460	N00.66420	Long rain
Busia	1446	E034.36942	N00.69250	Long rain
Busia	1446	E034.36942	N00.69250	Long rain
Busia	1446	E034.36942	N00.69250	Long rain
Busia	1446	E034.36942	N00.69250	Long rain
Busia	1441	E034.36904	N00.69389	Long rain
Busia	1441	E034.36904	N00.69389	Long rain
Busia	1441	E034.36904	N00.69389	Long rain
Busia	1441	E034.36904	N00.69389	Long rain
Busia	1447	E034.39956	N00.67659	Long rain
Busia	1447	E034.39956	N00.67659	Long rain
Busia	1447	E034.39956	N00.67659	Long rain
Busia	1447	E034.39956	N00.67659	Long rain
Busia	1446	E034.39912	N00.67713	Long rain
Busia	1446	E034.39912	N00.67713	Long rain
Busia	1446	E034.39912	N00.67713	Long rain
Busia	1446	E034.39912	N00.67713	Long rain
Busia	1416	E034.38803	N00.74552	Long rain
Busia	1416	E034.38803	N00.74552	Long rain
Busia	1416	E034.38803	N00.74552	Long rain
Busia	1416	E034.38803	N00.74552	Long rain
Bungoma	1535	E034.76328	N00.61117	Long rain
Bungoma	1535	E034.76328	N00.61117	Long rain
Bungoma	1535	E034.76328	N00.61117	Long rain
Bungoma	1591	E034.79065	N00.62522	Long rain
Bungoma	1591	E034.79065	N00.62522	Long rain
Bungoma	1591	E034.79065	N00.62522	Long rain
Bungoma	1591	E034.79065	N00.62522	Long rain
Bungoma	1591	E034.79065	N00.62522	Long rain
Bungoma	1560	E034.80379	N00.62807	Long rain

Bungoma	1560	E034.80379	N00.62807	Long rain
Bungoma	1560	E034.80379	N00.62807	Long rain
Bungoma	1560	E034.80379	N00.62807	Long rain
Bungoma	1560	E034.80379	N00.62807	Long rain
Bungoma	1628	E034.78019	N00.63291	Long rain
Bungoma	1628	E034.78019	N00.63291	Long rain
Bungoma	1628	E034.78019	N00.63291	Long rain
Bungoma	1628	E034.78019	N00.63291	Long rain
Bungoma	1275	E034.40377	N00.59442	Long rain
Bungoma	1275	E034.40377	N00.59442	Long rain
Bungoma	1275	E034.40377	N00.59442	Long rain
Bungoma	1275	E034.40377	N00.59442	Long rain
Bungoma	1275	E034.40377	N00.59442	Long rain
Bungoma	1344	E034.44003	N00.68440	Long rain
Bungoma	1344	E034.44003	N00.68440	Long rain
Bungoma	1344	E034.44003	N00.68440	Long rain
Bungoma	1374	E034.44807	N00.70103	Long rain
Bungoma	1374	E034.44807	N00.70103	Long rain
Bungoma	1397	E034.44944	N00.70175	Long rain
Bungoma	1397	E034.44944	N00.70175	Long rain
Bungoma	1397	E034.44944	N00.70175	Long rain
Kakamega	1520	E034.78662	N00.14787	Long rain
Kakamega	1520	E034.78662	N00.14787	Long rain
Kakamega	1530	E034.66257	N00.05551	Long rain
Kakamega	1530	E034.66257	N00.05551	Long rain
Kakamega	1530	E034.66257	N00.05551	Long rain
Kakamega	1519	E034.66122	N00.05523	Long rain
Kakamega	1519	E034.66122	N00.05523	Long rain
Kakamega	1519	E034.66122	N00.05523	Long rain
Kakamega	1513	E034.66036	N00.05441	Long rain
Kakamega	1513	E034.66036	N00.05441	Long rain
Kakamega	1513	E034.66036	N00.05441	Long rain
Kakamega	1513	E034.66036	N00.05441	Long rain
Kakamega	1558	E034.74823	N00.00325	Long rain
Kakamega	1558	E034.74823	N00.00325	Long rain
Kakamega	1558	E034.74823	N00.00325	Long rain
Kakamega	1558	E034.74823	N00.00325	Long rain
Kakamega	1558	E034.74823	N00.00325	Long rain
Kakamega	1600	E034.81551	N00.01565	Long rain
Kakamega	1600	E034.81551	N00.01565	Long rain
Kakamega	1600	E034.81551	N00.01565	Long rain
Kakamega	1600	E034.81551	N00.01565	Long rain
Kakamega	1600	E034.81551	N00.01565	Long rain
Kakamega	1589	E034.81600	N00.01505	Long rain

Kakamega	1589	E034.81600	N00.01505	Long rain
Kakamega	1589	E034.81600	N00.01505	Long rain
Kakamega	1589	E034.81600	N00.01505	Long rain
Kakamega	1589	E034.81600	N00.01505	Long rain
Kakamega	1684	E034.82533	N00.03115	Long rain
Kakamega	1684	E034.82533	N00.03115	Long rain
Kakamega	1684	E034.82533	N00.03115	Long rain
Kakamega	1552	E034.72606	N00.12995	Short rain
Busia	1225	E034.33674	N00.65445	Short rain
Busia	1320	E034.35423	N00.73924	Short rain
Busia	1389	E034.38952	N00.71382	Short rain
Busia	1416	E034.37709	N00.69690	Short rain
Busia	1410	E034.37346	N00.69641	Short rain
Busia	1407	E034.39107	N00.71532	Short rain
Busia	1407	E034.39107	N00.71532	Short rain
Busia	1221	E034.17486	N00.36270	Short rain
Busia	1229	E034.17559	N00.36128	Short rain
Busia	1416	E034.37709	N00.69690	Short rain
Kakamega	1552	E034.72606	N00.12995	Short rain
Kakamega	1518	E034.78664	N00.14788	Short rain
Busia	1438	E034.37261	N00.69267	Short rain
Kakamega	1597	E034.70835	N00.07816	Short rain
Kakamega	1552	E034.72606	N00.12995	Short rain
Kakamega	1518	E034.78664	N00.14788	Short rain
Busia	1181	E034.33303	N00.62172	Short rain
Busia	1462	E034.39536	N00.67814	Short rain
Busia	1462	E034.39536	N00.67814	Short rain
Busia	1336	E034.35728	N00.74348	Short rain
Busia	1189	E034.33092	N00.62213	Short rain
Busia	1228	E034.33865	N00.65436	Short rain
Busia	1343	E034.33363	N00.63637	Short rain
Busia	1218	E034.33158	N00.63656	Short rain
Siaya	1267	E034.32716	S00.06093	Short rain
Siaya	1259	E034.32898	S00.05704	Short rain
Busia	1441	E034.39369	N00.67833	Short rain
Busia	1458	E034.39904	N00.68400	Short rain
Busia	1455	E034.39881	N00.67807	Short rain
Siaya	1267	E034.32716	S00.06093	Short rain
Siaya	1267	E034.32716	S00.06093	Short rain
Busia	1467	E034.39631	N00.67923	Short rain
Busia	1469	E034.39760	N00.67960	Short rain
Busia	1469	E034.39760	N00.67960	Short rain
Busia	1390	E034.39028	N00.71010	Short rain
Kakamega	1592	E034.75635	N00.11998	Short rain

Kakamega	1592	E034.75635	N00.11998	Short rain
Busia	1229	E034.17955	N00.36007	Short rain
Busia	1382	E034.38951	N00.71284	Short rain
Busia	1379	E034.38913	N00.71270	Short rain
Busia	1390	E034.39028	N00.71010	Short rain
Busia	1395	E034.39230	N00.71068	Short rain
Busia	1395	E034.39230	N00.71068	Short rain
Busia	1336	E034.35728	N00.74348	Short rain
Busia	1379	E034.38913	N00.71270	Short rain
Busia	1385	E034.38935	N00.71435	Short rain
Busia	1440	E034.37812	N00.69597	Short rain
Busia	1430	E034.37445	N00.69515	Short rain
Busia	1385	E034.38935	N00.71435	Short rain
Busia	1382	E034.38951	N00.71284	Short rain
Busia	1430	E034.37445	N00.69515	Short rain
Busia	1395	E034.39273	N00.71085	Short rain
Busia	1407	E034.39782	N00.70042	Short rain
Busia	1410	E034.39635	N00.70070	Short rain
Busia	1361	E034.36237	N00.73834	Short rain
Busia	1395	E034.39273	N00.71085	Short rain
Busia	1364	E034.36440	N00.74005	Short rain
Busia	1363	E034.36406	N00.74013	Short rain
Busia	1389	E034.38952	N00.71382	Short rain
Busia	1306	E034.27793	N00.31820	Short rain
Busia	1234	E034.15780	N00.32863	Short rain
Bungoma	1307	E034.37951	N00.61641	Short rain
Bungoma	1307	E034.37951	N00.61641	Short rain
Busia	1234	E034.15808	N00.32931	Short rain
Busia	1234	E034.15808	N00.32931	Short rain
Busia	1234	E034.15808	N00.32931	Short rain
Bungoma	1431	E034.47524	N00.71529	Short rain
Bungoma	1431	E034.47524	N00.71529	Short rain
Busia	1306	E034.27793	N00.31820	Short rain
Bungoma	1324	E034.38408	N00.61557	Short rain
Bungoma	1432	E034.47522	N00.71455	Short rain
Bungoma	1432	E034.47522	N00.71455	Short rain
Bungoma	1427	E034.47611	N00.71328	Short rain
Siaya	1274	E034.34568	S00.08004	Short rain
Siaya	1274	E034.34568	S00.08004	Short rain
Siaya	1274	E034.34568	S00.08004	Short rain
Siaya	1274	E034.34568	S00.08004	Short rain
Bungoma	1431	E034.47524	N00.71529	Short rain
Busia	1205	E034.16887	N00.35417	Short rain
Busia	1211	E034.17033	N00.35276	Short rain

Kakamega	1544	E034.66602	N00.06374	Short rain
Busia	1234	E034.15780	N00.32863	Short rain
Busia	1205	E034.16887	N00.35417	Short rain
Busia	1201	E034.17838	N00.36920	Short rain
Bungoma	1324	E034.38408	N00.61557	Short rain
Bungoma	1324	E034.38408	N00.61557	Short rain
Bungoma	1437	E034.47462	N00.47153	Short rain
Busia	1277	E034.15487	N00.31860	Short rain
Bungoma	1427	E034.47611	N00.71328	Short rain
Siaya	1197	E034.34181	S00.32585	Short rain
Busia	1289	E034.33158	N00.33419	Short rain
Busia	1190	E034.16438	N00.36786	Short rain
Siaya	1303	E034.34433	S00.08766	Short rain
Busia	1356	E034.35058	N00.71592	Short rain
Siaya	1303	E034.34433	S00.08766	Short rain
Busia	1277	E034.15487	N00.31860	Short rain
Busia	1199	E034.16658	N00.36056	Short rain
Busia	1199	E034.16658	N00.36056	Short rain
Busia	1286	E034.27865	N00.31569	Short rain
Bungoma	1437	E034.47462	N00.47153	Short rain
Bungoma	1437	E034.47462	N00.47153	Short rain
Bungoma	1441	E034.47380	N00.71567	Short rain
Bungoma	1441	E034.47380	N00.71567	Short rain
Bungoma	1441	E034.47380	N00.71567	Short rain
Busia	1312	E034.28217	N00.31770	Short rain
Busia	1312	E034.28217	N00.31770	Short rain
Siaya	1303	E034.34433	S00.08766	Short rain
Kakamega	1539	E034.69537	N00.06794	Short rain
Bungoma	1427	E034.47611	N00.71328	Short rain
Bungoma	1436	E034.47055	N00.71428	Short rain
Bungoma	1436	E034.47055	N00.71428	Short rain
Bungoma	1436	E034.47055	N00.71428	Short rain
Busia	1182	E034.16068	N00.36627	Short rain
Siaya	1303	E034.34433	S00.08766	Short rain
Kakamega	1539	E034.69537	N00.06794	Short rain
Bungoma	1284	E034.39766	N00.59157	Short rain
Busia	1202	E034.13985	N00.40976	Short rain
Kakamega	1540	E034.66439	N00.06210	Short rain
Kakamega	1540	E034.66439	N00.06210	Short rain
Bungoma	1284	E034.39766	N00.59157	Short rain
Kakamega	1525	E034.66335	N00.05916	Short rain
Bungoma	1284	E034.39766	N00.59157	Short rain
Kakamega	1525	E034.66335	N00.05916	Short rain
Busia	1271	E034.23274	N00.30274	Short rain

Siaya	1223	E034.33007	S00.33214	Short rain
Siaya	1223	E034.33007	S00.33214	Short rain
Busia	1286	E034.27865	N00.31569	Short rain
Bungoma	1284	E034.39766	N00.59157	Short rain
Busia	1274	E034.23130	N00.30292	Short rain
Siaya	1197	E034.34181	S00.32585	Short rain
Siaya	1197	E034.34181	S00.32585	Short rain
Bungoma	1284	E034.39766	N00.59157	Short rain
Kakamega	1525	E034.66335	N00.05916	Short rain
Busia	1274	E034.23130	N00.30292	Short rain
Kakamega	1498	E034.66174	N00.05348	Short rain
Kakamega	1498	E034.66174	N00.05348	Short rain
Kakamega	1498	E034.66068	N00.05534	Short rain
Kakamega	1498	E034.66068	N00.05534	Short rain
Kakamega	1498	E034.66068	N00.05534	Short rain
Busia	1274	E034.23130	N00.30292	Short rain
Bungoma	1271	E034.40218	N00.59605	Short rain
Siaya	1223	E034.33007	S00.33214	Short rain
Busia	1230	E034.18511	N00.35133	Short rain
Bungoma	1271	E034.40218	N00.59605	Short rain
Bungoma	1271	E034.40218	N00.59605	Short rain
Busia	1195	E034.14187	N00.40381	Short rain
Bungoma	1271	E034.40218	N00.59605	Short rain
Busia	1304	E034.28094	N00.31598	Short rain
Busia	1311	E034.27917	N00.31702	Short rain
Busia	1311	E034.27917	N00.31702	Short rain
Bungoma	1271	E034.40218	N00.59605	Short rain
Busia	1277	E034.23585	N00.30755	Short rain
Busia	1195	E034.14187	N00.40381	Short rain
Siaya	1336	E034.35868	S00.07524	Short rain
Siaya	1336	E034.35868	S00.07524	Short rain
Siaya	1336	E034.35868	S00.07524	Short rain
Siaya	1336	E034.35868	S00.07524	Short rain
Busia	1286	E034.27865	N00.31569	Short rain
Siaya	1301	E034.34427	S00.08644	Short rain
Siaya	1301	E034.34427	S00.08644	Short rain
Siaya	1301	E034.34427	S00.08644	Short rain
Bungoma	1283	E034.39667	N00.59429	Short rain
Bungoma	1283	E034.39667	N00.59429	Short rain
Bungoma	1283	E034.39667	N00.59429	Short rain
Bungoma	1283	E034.39667	N00.59429	Short rain
Bungoma	1283	E034.39667	N00.59429	Short rain
Busia	1285	E034.33087	N00.32232	Short rain
Busia	1183	E034.10686	N00.41479	Short rain

Busia	1183	E034.10686	N00.41479	Short rain
Busia	1285	E034.33087	N00.32232	Short rain
Busia	1186	E034.10749	N00.41389	Short rain
Busia	1289	E034.32445	N00.32220	Short rain
Busia	1183	E034.12789	N00.47977	Short rain
Busia	1186	E034.10749	N00.41389	Short rain
Busia	1289	E034.33158	N00.33419	Short rain
Busia	1203	E034.10750	N00.42081	Short rain
Siaya	1303	E034.34433	S00.08766	Short rain
Busia	1320	E034.35423	N00.73924	Short rain
Busia	1284	E034.33161	N00.32302	Short rain
Busia	1284	E034.33161	N00.32302	Short rain
Busia	1183	E034.12789	N00.47977	Short rain
Busia	1320	E034.35423	N00.73924	Short rain
Busia	1320	E034.35423	N00.73924	Short rain
Busia	1320	E034.35423	N00.73924	Short rain
Kakamega	1553	E034.71852	N00.12469	Short rain
Busia	1277	E034.32221	N00.32168	Short rain
Busia	1206	E034.17614	N00.36898	Short rain
Busia	1182	E034.10469	N00.41272	Short rain
Busia	1182	E034.10469	N00.41272	Short rain
Busia	1257	E034.32043	N00.32471	Short rain
Busia	1277	E034.32221	N00.32168	Short rain
Busia	1185	E034.10552	N00.41298	Short rain
Bungoma	1481	E034.53045	N00.60687	Short rain
Bungoma	1514	E034.533119	N00.61361	Short rain
Bungoma	1479	E034.52184	N00.61094	Short rain
Bungoma	1490	E034.526390	N00.617222	Short rain
Bungoma	1509	E034.58068	N00.62845	Short rain
Bungoma	1557	E034.59395	N00.66004	Short rain
Bungoma	1515	E034.60737	N00.66895	Short rain
Bungoma	1538	E034.61226	N00.67426	Short rain
Bungoma	1747	E034.72624	N00.82073	Short rain
Bungoma	1935	E034.72564	N00.85590	Short rain
Kakamega	1469	E034.62708	N00.21789	Short rain
Kakamega	1469	E034.62708	N00.21789	Short rain
Kakamega	1469	E034.62708	N00.21789	Short rain
Kakamega	1469	E034.52639	N00.21789	Short rain
Kakamega	1490	E034.62357	N00.22022	Short rain
Kakamega	1490	E034.62357	N00.22022	Short rain
Kakamega	1490	E034.62357	N00.22022	Short rain
Kakamega	1490	E034.62357	N00.22022	Short rain
Kakamega	1490	E034.62357	N00.22022	Short rain
Kakamega	1490	E034.62357	N00.22022	Short rain

Kakamega	1490	E034.62357	N00.22022	Short rain
Kakamega	1490	E034.62357	N00.22022	Short rain
Kakamega	1490	E034.62357	N00.22022	Short rain
Kakamega	1490	E034.62357	N00.22022	Short rain
Kakamega	1490	E034.62357	N00.22022	Short rain

Appendix IV: NACOSTI Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **816898**

Date of Issue: **14/April/2023**

RESEARCH LICENSE

This is to Certify that Mr.. ANTHONY SIMIYU MABELE of Masinde Muliro University of Science and Technology, has been licensed to conduct research as per the provision of the Science, Technology and Innovation Act, 2013 (Rev.2014) in Bungoma, Busia, Kakamega, Siaya on the topic: Serological and Molecular characterization of Cucurbit chlorotic yellows virus infecting groundnut (Arachis hypogaea) in western Kenya for the period ending : 14/April/2024.

License No: **NACOSTI/P/23/25143**

816898

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

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See overleaf for conditions

THE SCIENCE, TECHNOLOGY AND INNOVATION ACT, 2013 (Rev. 2014)

Legal Notice No. 108: The Science, Technology and Innovation (Research Licensing) Regulations, 2014

The National Commission for Science, Technology and Innovation, hereafter referred to as the Commission, was the established under the Science, Technology and Innovation Act 2013 (Revised 2014) herein after referred to as the Act. The objective of the Commission shall be to regulate and assure quality in the science, technology and innovation sector and advise the Government in matters related thereto.

CONDITIONS OF THE RESEARCH LICENSE

1. The License is granted subject to provisions of the Constitution of Kenya, the Science, Technology and Innovation Act, and other relevant laws, policies and regulations. Accordingly, the licensee shall adhere to such procedures, standards, code of ethics and guidelines as may be prescribed by regulations made under the Act, or prescribed by provisions of International treaties of which Kenya is a signatory to
2. The research and its related activities as well as outcomes shall be beneficial to the country and shall not in any way;
    i. Endanger national security
    ii. Adversely affect the lives of Kenyans
    iii. Be in contravention of Kenya's international obligations including Biological Weapons Convention (BWC), Comprehensive Nuclear-Test-Ban Treaty Organization (CTBTO), Chemical, Biological, Radiological and Nuclear (CBRN).
    iv. Result in exploitation of intellectual property rights of communities in Kenya
    v. Adversely affect the environment
    vi. Adversely affect the rights of communities
    vii. Endanger public safety and national cohesion
    viii. Plagiarize someone else's work
3. The License is valid for the proposed research, location and specified period.
4. The license any rights thereunder are non-transferable
5. The Commission reserves the right to cancel the research at any time during the research period if in the opinion of the Commission the research is not implemented in conformity with the provisions of the Act or any other written law.
6. The Licensee shall inform the relevant County Director of Education, County Commissioner and County Governor before commencement of the research.
7. Excavation, filming, movement, and collection of specimens are subject to further necessary clearance from relevant Government Agencies.
8. The License does not give authority to transfer research materials.
9. The Commission may monitor and evaluate the licensed research project for the purpose of assessing and evaluating compliance with the conditions of the License.
10. The Licensee shall submit one hard copy, and upload a soft copy of their final report (thesis) onto a platform designated by the Commission within one year of completion of the research.
11. The Commission reserves the right to modify the conditions of the License including cancellation without prior notice.
12. Research, findings and information regarding research systems shall be stored or disseminated, utilized or applied in such a manner as may be prescribed by the Commission from time to time.
13. The Licensee shall disclose to the Commission, the relevant Institutional Scientific and Ethical Review Committee, and the relevant national agencies any inventions and discoveries that are of National strategic importance.
14. The Commission shall have powers to acquire from any person the right in, or to, any scientific innovation, invention or patent of strategic importance to the country.
15. Relevant Institutional Scientific and Ethical Review Committee shall monitor and evaluate the research periodically, and make a report of its findings to the Commission for necessary action.

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