EPIDEMIOLOGY AND CHARACTERISATION OF *Groundnut Ringspot Virus* (GRSV) INFECTING GROUNDNUTS AND OTHER PLANTS 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 Crop Protection 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 "Epidemiology and characterisation of *Groundnut Ringspot Virus* (GRSV) infecting Groundnuts (Arachis *hypogaea L*) and other plants in Western Kenya in western Kenya".

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DEDICATION

I dedicate this thesis to my late parents Mr. Gabriel Murere Masinde and Mrs. Mary Namaemba Murere, my wife Emily Nekesa and my sons John Flavian, Mark Dawson, Bravin Wanyonyi and Victor Junior.

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is an annual oilseed legume crop grown by small holder farmers in Kenya for its economic and nutritive value. However, its yield has declined upto 680 kg ha^{-1} than its genetic potential of 1690 kg ha^{-1} attributed to abiotic and biotic stressors. Viruses are among biotic stressors for yield reduction globally. These include; Groundnut ringspot virus (GRSV), Tomato spotted wilt virus (TSWV), among others. GRSV was reported in South Africa, Ghana, Brazil and USA infecting groundnuts, soybeans and others. GRSV and TSWV have similar biological symptoms but differentiated using serological tests. Typical Symptoms for GRSV appears on groundnuts and other plants in western Kenya but no report had been documented on the occurrence of the virus nor its management strategies Kenya. The general objective of this study was to determine the occurrence, distribution and characterisation of GRSV on groundnuts and other host plants in western Kenya. Survey on prevalence of GRSV, was conducted in short and long rain seasons of the years 2019 and 2020 in western Kenya. Simple random sampling (SRS) used in selecting farms visited in groundnut growing regions and disease incidence/ severity recorded and data collected analyzed using post-hoc analysisANOVA. Serological analysis was done on samples collected using polyclonal and monoclonal antisera against GRSV and TSWV respectively. Field trials on the effect of intercropping other legumes with groundnuts on GRSV incidences were laid on a randomized complete block design (RCBD) and replicated three times. Viral incidence and severity recorded and symptomatic leaf samples collected for GRSV ELISA tests. Health tested seeds to GRSV of groundnut varieties and other plant species were planted in plastic pots of a mixture of sterilized loam, sand and organic manure at a ratio of 2:1:1 respectively in greenhouse to screen for their response and host range to GRSV and inoculated with GRSV inoculum. Plants symptomatic development observed at an interval of 5 days for 8 weeks and plant samples for each variety/species collected for GRSV ELISA Tests. Total RNA of Kenyan plant isolates extracted using CTAB and purified by DCC™-5 purification kit then amplified using target primers GRSVnR (5'-GCGGTCTACAGTGTTGCACTT3')andGRSVnF(5'TCTTGTGCATCATCCATTGT-3') using Rt-PCR at 614-bp fragment of the nucleocapsid gene of GRSV corresponding to the part of the nucleocapsid (N) gene. The RT-PCR product taken for Sanger sequencing. Sequence readings trimmed using Bio-edit software and phylogenetic analysis done in MEGA-X. New primers from GRSV sequences of western Kenya was designed using primer3plus software, synthesized and validated using PCR tests. GRSV occurs in surveyed regions with variant incidence; Chwele having the highest incidence (45.04 %) while Kapkateny having the lowest incidence (17.75 %) with significant difference of ($P < 0.05$). Groundnuts planted in pure stand had lowest disease incidence (4%) while intercropped groundnuts had the highest (28%). Screened groundnuts showed Homabay variety being more susceptible with incidence of 31 %, followed by ICGV-9991 with incidence of 28 %. SM99568 variety was tolerant to the virus. Varieties ICGV-90704, ICGV-99048 and ICGV-99019 were resistant to the virus.Screened plants; Pigeon peas, Bambara nut, peas, *Chenopodium album, Galinsoga parviflora* among others, revealed being as host range for the virus. Kenyan GRSV isolates clustered with USA, Ghanaian and South African isolates in GenBank. One of developed primers formed clear bands in a PCR tests with positive samples of western Kenya. GRSV occurs in surveyed counties of western Kenya, which should be a big concern to all stakeholders. Introgression of resistant genes into local groundnuts to gain resistance to the virus with urgency. Farmers should avoid intercropping groundnuts with alternative hosts to reduce transmission of the virus.

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LIST OF ABBREVIATIONS AND ACRONYMS

AEI Agro-ecological Intensification

AEZs - Agro-Ecological Zones.

ANOVA - Analysis of Variance.

CIAT International Centre for Tropical Agriculture

CMV Cucumber mosaic virus

CTAB Cetly Trimethlyamonium bromide

DAS–ELISA Double Antibody Sandwich Enzyme Linked Immunosorbent Assay

dsRNA - Double Stranded Ribonucleic Acid.

ELISA Enzyme Linked Immunosorbent Assay

FAO - Food and Agricultural Organization.

FAOSTAT - Food and Agricultural Organization Statistics.

GBNV Groundnut bud necrosis virus

GRAV Groundnut rosette assistor virus

GRV Groundnut rosette virus

GRSV - Groundnut Ringspot Virus.

ICRISAT - International Crops Research Institute for the Semi-Arid Tropics.

IFPRI International Food Policy Research Institute

IITA International Institute for Tropical Agriculture

ISEM Immunosorbent electron microscopy

ISTA International Rules on Seed Health Testing

KALRO Kenya Agricultural and Livestock Research Organization

LM1 Lower midland zone 1

LM2 Lower midland zone 2

LH Lower Highland

LM Lower Midland

LSD Least Significance Difference

MAb Monoclonal Antibodies.

MMUST Masinde Muliro University of Science and Technology

NGO Non-governmental organization

NGS - Next Generation Sequencing.

ORF - Open Reading Frame.

PBST Phosphate buffered saline with tween

RNA Ribonucleic Acid

RT-PCR - Reverse Transcription Polymerase Chain Reaction.

Sat-RNA – Satellite Ribonucleic Acid.

SSA – Sub-Saharan Africa.

ssRNA - Single Stranded Ribonucleic Acid

TAS ELISA Triple Antibody Sandwich Enzyme Linked Immunosorbent Assay.

UM1 Upper midland zone 1

TSV Tobacco streak virus

PCV Peanut clump virus

PeMoV Peanut mottle virus

CHAPTER ONE

INTRODUCTION

1.0 Background of the study

The title of this research is the Epidermiology and characterisation of Groundnut Ringsport Virus (GRSV) infecting groundnuts in western Kenya. The title focuses on determining the occurrence and distribution of GRSV and diversity of stains in western Kenya. Biological symptoms of GRSV are similar to TSWV, and therefore can only be differentiated either by serological or molecular tests (Webster *et al*., 2015). The typical symptoms of GRSV/TSWV appears on groundnuts and other crops growing in western Kenya, infecting all grown groundnut varieties even including those that have been bred to be resistant to TSWV. This was a motivating factor to study the occurrence and distribution of GRSV infecting groundnuts in western Kenya. This is the first report on occurrence and distribution GRSV in Kenya (Murere *et al*., 2021). The general objective was to determine the occurrence, distribution and characterisation of Groundnut ringspot virus infecting groundnuts and other host plants in Bungoma, Busia and Kakamega Counties of western Kenya. The findings of this study, is to be used by stakeholders; Seed Breeder Companys, Kenya Plant Health Inspectorate Service (KEPHIS), Pest Control Products Board (PCPB), Kenya Agriculture and Livestock Research Organization (KALRO), Researchers in Universities and Farmers in planning and evaluating the strategies in management of the virus to improve on Groundnut production in Kenya. Groundnut (Arachis hypogaea L) is legume crop of global importance grown by farmers for income (Kipkoech *et al.*, 2007) and nutritive value (Bajpai *et al*., 2017). The World annual production is about 44 million tons (USDA, 2018) with China being the largest

producer, followed by India then USA respectively (FAOSTAT, 2015). Groundnut is among preferred crops in Sub-Saharan African countries (Rockstrom *et al*., 2003). Nigeria produces 30% followed by Senegal and Sudan 8%, for total yield of Africa (Upadhyaya *et al*., 2006; Caliskan *et al*., 2008). In Kenya, groundnuts are mainly grown by smallholder farmers, mainly in western Kenya and around Lake Victoria region (Ndisio, 2015). They are roasted or boiled and sold as snack in the streets and for manufacture of peanut butter in factories. Despite of their economic importance, yields of groundnuts in Kenya remains lower; 680kg ha^{-1} against its genetic potential of 1690 Kg ha^{-1} (FAO, 2015), due to biotic and abiotic stressors (Bucheyeki *et al*., 2008).Among stressors, 32 viruses have been documented infecting groundnuts, globally (Mukoye et al., 2020).

1.1 Origin of Groundnuts (*Arachis hypogaea L*)

Groundnut (*Arachis hypogaea L*) is an oilseed legume crop of global importance grown by both smallholder and large commercial farmers, for income (Kipkoech *et al*., 2007) and nutritive value (containing 48 % edible oil and 25 % crude proteins) (Bajpai *et al*., 2017). Groundnut (*Arachis hypogaea L*) is a hybrid of two wild species of groundnuts (*A. duranesis and A. inaensis*) (Saijo *et al*., 2007). Hybridization occurred by breeding wild groundnuts (*Arachis monticola*) in north western Argentina (Krapovickas *et al*., 2007). Artificial selection, made *Arachis hypogea* different from its wild species of its origin (Krapovickas *et al*., 2007). Groundnut landraces have evolved two Subspecies *A. h. fastigiata* growing upright with a shorter crop cycles, while *A. h. hypogaea* grow by spread more on ground with a longer crop cycle (Kochert *et al.*, 1996). Cultivation of groundnuts started in Mesoamerica then introduced in other parts of the world in 16^{th} and 17^{th} centuries with the Spanish, Portuguese, British and Dutch (Isleib *et al.*, 1994).

1.1.1 Botany of groundnut (Arachis hypogaea L)

Groundnuts (Arachis hypogaea L.) is a member of the legume family (Fabaceae) which is an annual crop with their stem growing to a mean height of 30- 50 cm tall (Sharma *et al.*,2006). They have compound pinnate leaves with four leaflets. Leaves have mosaic arrangement pattern on their stem or alternate on the stem each, with leaflets measuring 1-7 cm long and 1-3 cm broad. This arrangement of leaves is very important to enable plant leaves trap sufficient sunlight for photosynthesis (Sun *et al.*, 2022). Peanut yellow pea -like flowers are borne in axillary cluster above the ground (Hasson *et al*., 2010). After the flowers undergoing self- pollination , produce a 'peg' (a short thick stems), at flower base, termed the gynophore, grows downward away from the plant and penetrates into the soil, so the fruiting body develops entirely underground(Shani *et al*., 2010). The pods, usually, containing from one to three seeds, develop only underground (Wang *et al*., 2008). Each seed is covered with thin a papery seed coat. The peanut has well-developed taproot, with numerous lateral roots that extend several inches into the ground.Most roots have nodules (Zhou *et al*., 2014).

1.1.2 Global Groundnut Production

World annual production is about 44 million tons (USDA, 2018) with China being the leading producer a head of India, USA, Nigeria and Indonesia respectively (FAOSTAT, 2015). The crop is grown between 40° N and 40° S (Kumar *et al.*, 2007). In Sub-Saharan African countries, Groundnut is the fifth most widely grown crop, after maize, sorghum, millet and cassava (Rockstrom *et al*., 2003). Nigeria produces 30 % of Africa's total yield, followed by Senegal and Sudan 8 %, Ghana and Chad produce 5 % of total yield of Africa (Upadhyaya *et al*., 2006). Groundnuts grown in rainfall ranging from 500 to 1200 mm and Temperature daily mean of above 20 ^{o}C (Baughman *et al*., 2015). The crop is used for manufacturing domestic goods; furniture polish,

insecticides, paints and lubricating oil. The protein extracted is used in manufacturing textile fibers and their shells used in manufacturing of plastic, fuel, cellulose used paper wallboard (Heuze *et al*., 2017). Groundnut productivity in Africa accounts for 40% of total global yield with S. Africa having the highest production, while E. Africa having the lowest average yields (FAOSTAT 2015). In Sub- Sahara Africa, average yield is 980 kg/ha, which is less than its genetic potential of 1690 kg ha^{-1} (Bucheyeki *et al*., 2008).

1.1.3 Groundnut Production in Kenya

In Kenya, groundnut is mainly grown in western Kenya and around Lake Victoria regions (Ndisio, 2015). Two types of groundnuts (Runners and Bunch) are grown by smallholder farmers, for their economic and nutritive value (Kipkoech *et al.*, 2007). The main varieties grown are; Red Valencia, SM99568, CG7, CG3, ICGV-12991 and ICGV-9991 and ICGV 90704 among others (Ndisio, 2015). These varieties are either grown in purestand or intercropped with other crops (Langat *et al*., 2006). Intercropping groundnuts with either legumes or cereal results into less yields of 30% to the pure stand (Kipkoech *et al*., 2007). The crop is roasted or boiled and sold as snacks in the streets and for manufacturing of peanut butter in factories. Despite of its economic importance, it's yield in Kenya remains lower; 680kg ha^{-1} than it's genetic potential of 1690 Kg ha^{-1} (FAO, 2015), due to unreliable rainfall, lack of high yielding varieties, pests and diseases (Bucheyeki *et al*., 2008). Among diseases are viral diseases caused by; *Tomato spotted wilt virus* (TSWV), *Groundnut bud necrosis virus* (GBNV), *Tobacco streak virus* (TSV), *Groundnut rosette assistor virus* (GRAV) among others are of economic importance in groundnut production .

1.1.4 Economic importance of groundnuts

Groundnut is an oilseed legume crop grown by both commercial and smallholder farmers for food, income and raw materials for manufacturing industries (Ibrahim, 2011). High percentage of world groundnuts, are grown mainly for manufacturing cooking oil and animal feeds (Pande *et al*., 2003). All parts of the plant are used. The nut (kernel) is source of edible oil and protein (Knauft *et al.*, 2005). Their Seeds are consumed either raw / roasted or ground into peanut butter (Upadhyaya *et al.*, 2006). Seeds are scorched to be used as a beverage (Duke, 1981). Also used for manufacture of some products in industries such as soaps, medicines, cosmetics, emulsions for insect control (Gbèhounou *et al*., 2003).

1.1.5 Constraints for groundnut production

Groundnut (*Arachis hypogaea* L.) production is affected by a number of abiotic stressors, which lower its productivity (Upadhyaya *et al*., 2006). Crop productivity has remained below its genetic potential with most of smaller holder farmers obtaining less than 30 – 50 % of the potential yields in Western Kenya (Caliskan *et al*., 2008). The main abiotic constraints; poor soil conditions, unreliable rainfall, fluctuation of temperature, water stress, poor market prices, lack of extension services and poor agronomic practices contributing to low crop yield. Pests and disease are the biotic stressors that lower the crop productivity (Bucheyeki *et al*., 2008). Groundnuts are easily infected with viruses, bacteria and fungi during post and pre harvest stage (Cummins, 1985). The vectors include aphids, thrips and many others (Isleib, *et al.*, 1994). Among diseases transmitted by these vectors are viruses causing yield lose ranging from 25 to 100 % (Duivenbooden *et al*., 2002). The viruses include; Tomato spotted wilt virus, Groundnut bud necrosis virus among others are of economic importance globally. GRSV is among newly reported virus infecting groundnuts

(Webster *et al*., 2011; Mukoye, *et al*., 2020). In Africa, GRSV reported in S.A and Ghana on groundnuts and on soybean (Pietersen *et al*., 2002). In Ghana, GRSV reported co-infecting groundnuts with groundnut rosette disease (Appiah *et al*., 2016).

1.2 Statement of the problem

Groundnut ringspot virus (GRSV) is any of economic important stressor in groundnuts, tomatoes, peppers, soybeans, watermelons and eggplants production worldwide. In Africa, the occurrence of GRSV was reported in South Africa and Ghana infecting groundnuts and soybeans (De breuil *et al*., 2007). In Florida, was reported on Tomatoes, eggplant, pepper and cucumber (Webster *et al*., 2011). The symptoms induced by this virus on groundnuts and other host plants resembles those of *Tomato spotted wilt virus* (TSWV) but can be differentiated either by serological (ELISA) or molecular tests (Pappu *et al*., 2009). The occurrence of GRSV in groundnut producing regions cause severe damage to plants reducing crop yields and quality (Jones, 2005). The necrosis spot caused by GRSV compromise the ability of the plant leaves to intercept radiation for photosynthesis, causing reduction in groundnut quality and productivity of the crop (Culbreath *et al*., 2003). The biological symptoms of GRSV reported in other countries; South Africa, Ghana, USA and Brazil, infecting groundnuts, Soybeans, tomatoes, watermelon among others (Webster et al., 2015), also appears on groundnuts, watermelon, pepper, some legumes and some broad-leafed weeds of western Kenya. In Kenya, no report had been documented on the occurrence, distribution and charactarization of GRSV despite of biological symptoms appearing on Groundnuts, peppers, Tomatoes and watermelon plants in western Kenya. Smallholder farmers continue to experience significant yield loss in groundnuts farming due to viral diseases (Mukoye *et al*., 2020). The GRSV hinders not only productivity of groundnuts but also other host crop such as tomato, capsicum,

Soyabeans among other of economic importance (Rubio, 2020). The occurrence of GRSV on the host crops without proper Knowledge and management strategies could affect international trade due to phytosanitary regulation in international market (MacLeod *et al*., 2010) Therefore, there was need to carry out a research on distribution and genetic diversity characterization of groundnut ringspot virus on groundnuts and other host plants in western Kenya (Webster *et al*., 2011).

1.3 Justification of the study

Groundnut production in western Kenya remains below genetical potential due to abiotic and biotic stressors (Langat *et al*., 2006). Among biotic stressors, there are 32 viral disease that infect groundnuts, lowering both yield and quality of the crop (Kamur *et al.*, 2008). Groundnut ringspot virus reported in Ghana lowering groundnut yields by 69.5 % (Appiah *et al*., 2016), which should be among major concern on food security globally. The symptoms of GRSV appears on groundnuts, Tomatoes and other legumes in western Kenya which interfere with photosynthetic processes on infected plants (Larbi *et al*., 2006) thus lowering yields of groundnuts (Tandzi *et al*., 2020). However, the status about the occurrence and distribution of GRSV had not been known in Kenya, and no research had been done to determine appropriate management strategies of GRSV and its genetic diversity of strains nucleoprotein isolates with those available in GenBank for the purpose of management. There was need for a research on "Epidemiology and characterization of groundnut ringspot virus infecting groundnuts in western Kenya. The aim of the research was to advice stakeholders to breed groundnut varieties that are more resistant to GRSV and farmers be advised to adopt appropriate farming technologies in management of GRSV in groundnuts.

1.4 General objective

To determine the occurrence, distribution and characterization of *Groundnut ringspot virus* infecting groundnuts and other host plants in Bungoma, Busia and Kakamega Counties of western Kenya.

1.4.1 Specific objectives

i. To determine the prevalence and distribution of GRSV in main groundnuts growing areas of western Kenya.
ii. To determine the response of groundnut varieties and other host plants to GRSV in western Kenya.
iii. To determine the diversity of Kenyan GRSV isolates sequenced nucleoprotein genes to reference strains.
iv. To determine the ability of sequenced GRSV strains obtained from groundnut isolates of western Kenya in developing molecular diagnostic tools for PCR.

1.5 Hypothesis

HO_1: GRSV is widely distributed in all surveyed regions with variant incidence and Severity in western Kenya.

HO_2: Many plants are alternative hosts to GRSV and groundnut varieties in western Kenya are susceptible to the virus.

HO_3: Sequenced nucleoprotein genes of GRSV isolates of western Kenya have same genetic sequences with referred strains.

HO_4: Designed diagnostic PCR primers for GRSV have higher ability to detect virus strains of Kenyan isolates than available PCR primers.

CHAPTER TWO

LITERATURE REVIEW

2.1 Groundnut Ringspot Virus (GRSV)

Groundnut ringspot virus (GRSV) is among the emerging viruses in genus of Tospovirus, which are of economic importance on legumes, solanaceae among other families resulting into reduction of yield and marketability of the crop (Mehmet, 2011). *Groundnut ringspot virus* belongs into genus Tospovirus and the family Bunyaviridae (Webster *et al*., 2015). The virus is transmitted by some thrip species of order Thysanoptera (Silva *et al*., 2019). *Groundnut ringspot virus* is among the prevalent member of this genus in Brazil (Bertran *et al*., 2011). The virus results into big losses in solanaceae, legumes among other crops grown globally (Webster *et al*., 2015). Groundnut ringspot virus induces disease symptoms to host plants, similar to those of TSWV. Therefore diagnosing GRSV basing only on biological symptoms induced on host plant become more complicated. Therefore, these viruses can only be identified by using either serological or molecular tests on plant tissue. Was reported GRSV, TCSV, and TSWV co-infecting peanuts, coriander, lettuce, tomatoes and weeds in a study carried out in Brazil (Qingchun *et al*., 2020). The tripartite genomes of tospoviruses, and members of Bunyaviridae allow for reassortment when co-infect the same host plant (Silva *et al*., 2019). Reassortment may occur between a single virus species or between viruses species as observed in GRSV and TCSV in Florida (Silva *et al*., 2019). The characterization of GRSV isolates from Florida revealed of the existence of an LGMTSG genotype with GRSV S and L RNAs and a TCSV M RNA (Yaowapa, 2018). It was reported that the S, M, and L RNAs of TCSV isolates in Florida and Puerto Rico share high percentage level of nucleotide identity with those with the corresponding RNAs of TCSV isolates from South America, therefore have

standard TCSV genotype (Lima *et al.*, 2016). Research on reassortment between intraspecific isolates of TSWV suggested novel biological properties arising from the process (Lima *et al.*, 2016). Biological properties of interspecies of Tospoviruses reassortants have not been reported and no characterisation done. For the reassortment between virus species or stains to take place, must occur sympatrically on the host plant (Bag *et al*., 2012). The occurrence of different species of viruses infecting the same host plant in a given geographic area at the same time, also results into reassortment, this causes the formation of new strains of tospovirus (Bag *et al*., 2012). The presence of a common hosts or thrips makes it possible for multiple viruses species to infect a single individual of that species, which enables reassortment if co-infection of a plant host or insect vector occurs (Webster *et al*., 2015). GRSV, TSWV, and TCSV occurred sympatrically in south Florida. It was noted that GRSV and TSWV occured in the same tomato fields and co-infected the same tomato plant in south Florida.

2.2 Symptoms of GRSV on host plant

Groundnut ringspot virus (GRSV) normally induces a number of typical viral symptoms on groundnuts, these include; leaf mosaic, chlorotic-ring shaped spots on leaves, necrotic ring spot, leaf chlorosis, necrotic patches on leave and stunted plant growth (Camelo *et al.*, 2014). This virus also infect other host plants apart from groundnuts, which include tomato, pepper, soybeans among others. The induced symptoms to tomatoes are; inward cupping of leaves followed by dark spots (Webster *et al.*, 2011). In adverse stage of infection, the plant will show; necrotic spots/ flecks, chlorotic leave spots, deformation of leaves, necrotic lesions on stems and petioles. These symptoms on tomatoes normally affect the quality of the fruit (Adkins *et al.*, 2015). Other symptoms include dark streaks on the main stem and wilting in top

portion of the plant. Fruits may be deformed, showing uneven ripening, with raised bumps on the surface and necrotic rings on the fruits (Webster *et al.,* 2011).These symptoms are similar with those of TSWV on tomatoes. Infected sweet pepper with the virus displays; yellow leave mosaic, necrotic lessons on leaves/ fruits, fruit deformation and leave chlorosis (Eugene *et al*., 2018). These symptoms also have been reported in other host plants; watermelon (Citrullus lanatus), cucumber and wild legumes (Spadotti *et al.,* 2014). Infected Soybeans are asymptomatic but serologically tests positive for the virus. Once the crop is infected by, the virus, become difficult to control (Rubio *et al*., 2020).

2.3 Etiology of Groundnut ringspot virus

Groundnut ringspot virus belongs to genus Tospovirus, transmitted by thrips to host plants (Culbreath *et al*., 2003). The origin of Groundnut ringspot virus has not been reported, although the recent research shows that tospoviruses has a wide diversity of stains with high percentage of recombination and crossing over of genetic materials among tospoviruses. This may have resulted into genesis of GRSV among others (Simon *et al*., 2011). Also it has been noted that Tospoviruses have a wide range of host plants with different response to the viruses with mixed infection, this may have resulted into new viruses among them may have been GRSV due to different response to different strains (Gibbs *et al*., 1999). This virus has quasipherical-enveloped particle of approximately 80 to 120 nm in diameter. Its genome consists of one negative and single- stranded RNA segement (King *et al*., 2012). In Brazil, other species considered vectors of this virus in groundnuts include; Enneothrips *flavens* (Michelotto *et al*., 2017). Biological symptoms induced by GRSV in a host plant are similar to those of TSWV (Adkins *et al*., 2002). These include, yellow leave mosaic, chlorosis ring shaped spots on leaves and deformation of leaves (Webster *et al*., 2011).

2.4 Transmission and Epidemiology of Groundnut ring spot virus

Groundnut ringspot virus transmitted by western flower thrips and common blossom thrip species (Adkins *et al.,* 2015). Tospoviruses are transmitted to groundnuts and other host plants by thrips in their adult and larval stages of their growth only when feeds on an infected plant, then bite a health plant transmitting the virus (Pappu *et al*., 2009; Lewis, 1997). Groundnut ringspot virus is a circulative propagative virus, which implies that the virus multiplies in the body of a vector. Therefore, nymphs feed on GRSV infected host plant to acquire the virus, and then in turn spread by the adult insects to health plants. Transmission of the virus is characterized by replication and systemic invasion of thrips tissues prior to transmission via salivary glands to a host plant causing spotted wilt disease, which is one of the serious diseases infecting Solanaceae, tomatoes, sweet peppers and groundnuts (Pappu *et al.,* 2009). Groundnut ringspot virus generate tubules for viral movements into plant tissue (Adkins *et al*., 2015) but no tubule structure has been noted during infection process (Storm *et al*., 1999). Virus- vector interactions in thrips is characterized by infection of salivary glands which occurs as a result of accumulating GRSV in the Visceral muscles of midgut and transmitted by several thrip species resulting into yield loses and low quality in many parts of the world (Mandal *et al*., 2012). The rate of transmission of GRSV depends on season parameters and host availability (Riley *et al*., 2011). Tospovirus transmission occurs by thrips although few species, known to acquire and transmit the virus (order Thysanoptera). For transmission to occur effectively hatched larvae on tospovirus-infected host plants should be available for successful acquisition and transmission (Webster *et al*., 2015). Viruliferous winged adult thrips are able to migrate and disseminate the virus. Transmission efficiencies vary by virus source, virus isolate and thrips population, therefore knowledge of virus isolates and thrips

population is necessary to identify which thrips species are most likely to transmit viruses in a given geographical area. The western flower thrips (*Frankliniella occidentalis*) are the major vectors of several tospoviruses transmitted into host plants, including TSWV, GRSV, and TCSV, widely spread in United States and other parts of the world. The common blossom thrips (*F. schultzei*) is also an efficient vector of TSWV, GRSV, and TCSV in South America, however this species is not widespread in the United States. However, *F. schultzei*, recently been reported in areas of vegetable production, where GRSV and/or TCSV had been detected in United States and Puerto Rico. The potential of other thrips species to transmit GRSV or TCSV in Florida has not been determined and recorded. This includes the Florida flower thrips (*F. bispinosa*) and tobacco thrips (*F. fusca*) known to transmit tospoviruses and commonly found in the southeastern United States.These viruses known to infect Vegetables in the south-east of United States when climatic condition was favourable. Research has shown that this vector (thrips) normally migrate from northern Florida to other states during spring and summer and returns back to Southern Florida during the winter to follow suitable growing conditions (khan et al., 2020).

2.5 Geographical distribution of GRSV and other Tospoviruses

Groundnut ringspot virus and other Tospoviruses have been reported, occurring and distributed globally (Khan *et al*., 2020). In a research conducted in South east of United States on GRSV and TCSV, indicated solanaceae family being more susceptable to these viruses and displaying typical symptoms of the viruses (Webster *et al*., 2011). Sample from South Florida, serologically tested indicated co-existance of GRSV with TSWV in tomato and pepper samples (Qingchun *et al*., 2020). *Groundnut ringspot virus* and *Tomato spot wilt virus* for the first time were reported occurring in South Carolina and New York infecting Impatiens and lettuce as non-solanaceous crops

(Qingchun *et al*., 2020). *Groundnut ringspot virus* has few strains or less genetic diversity (King *et al*., 2012). The isolates collected from southern and northern Florida and their genome analysed, it was found the reassortants with the TCSV RNA This may have been due to mixed infections of the host plant, resulting into recombination of genetic materials from both GRSV and TSWV strains (King *et al*., 2012). This implies that there is a possibility of crossing over of the genetic materials from one species to the next resulting into many new Tospoviruses/strains (Lima *et al*., 2016). A research on transmission of the virus, was reported that *Frankliniella schultzei* being more aggressive vector in GRSV transmission than *F. occidentalis* (Khan *et al*., 2020). Also noted that TCSV is more easily acquired than GRSV by *F. occidentalis* but upon acquisition, transmission frequencies were similar (Khan *et al*., 2020). Further spread of GRSV and TCSV in the United States is possible and detection of mixed infections highlights the opportunity for additional reassortment of tospovirus genomic RNAs. *Tomato spotted wilt virus* (TSWV), is one of the most economically important plant viruses worldwide, infecting many vegetable and ornamental plant species (Khan *et al*., 2020). TSWV has been the major tospovirus problem of vegetable, peanut, and tobacco producers in the southeastern United States. Intill recently when it has been documented also GRSV being one of viruses of economical in the southeast of United States.

2.6 Typical Tospoviruses symptomatic distribution in Kenya

The occurrence and distribution of symptoms of tospovirus are; leaf mosaic, leaf chlorotic-ringpots, leave necrosis and stunted plant growth was reported in Kenya in 1999 for the first time infecting tomatoes, which was serologically confirmed being TSWV (Wangai *et al.*, 2001). Infected tomatoes displayed; inward cupping of leaves followed by dark spots symptoms that are similar to GRSV symptoms (Webster *et al.*,

2011). In adverse stage of infection, the plant shows; necrotic spots/ flecks, chlorotic spots, deformation of leaves, necrotic lesions on stems and petioles. These symptoms on tomatoes lowers the quality/quantity of the fruits (Adkins *et al.,* 2015). A research conducted in Kenya to establish the awareness obout the virus occurrence and its transmission vector, among farmers through a questionnaire. Tomato leave and fruit samples collected from symptomatic and asymptomatic plants. To investigate for the occurrence, distribution and genetic diversity of TSWV were evaluated in four tomato production areas in Kenya a decade after this incursion. The samples were assayed for TSWV using ELISA and reverse transcriptase-polymerase chain reaction, and the resulting positive samples were sequenced. The occurrence, vectors and alternate hosts was conducted on collected samples from Nakuru, Bungoma, Kirinyaga and Loitokitok to determine the type of tospovirus that was present (Mwangi et al., 2016). The tomato samples tested positive for TSWV. Most of the positive samples came from Nakuru, with only one sample from Bungoma, Kirinyaga and Bungoma testing positive for the TSWV. A phylogenetic analysis based on partial nucleocapsid (N) protein gene sequences suggested that the Kenyan isolates formed a single subgroup nested within a cluster of isolates that came predominantly from Europe. This indicated a single introduction that had undergone limited diversification. The study revealed that the disease has persisted in the area to which it was first introduced but had very limited dispersal to other areas. Commonly planted legumes, brassicas and cucurbits in Kenya that display Tospoviruses symptoms are; Beans, green gram, cowpea, Bambara nut, kales, cabbage, butter nut, pigeon peas, black gram and peas of which some have been tested against TSWV,TCSV among others (Karavina *et al*., 2017). Garden broad leafed weeds commonly found growing or bordering groundnut farms in western Kenya and exhibiting Tospovirus symptoms similar to those of

GRSV, TSWV (Macharia *et al*., 2016). These weeds with viral symptoms may be an alternative host for the Tospoviruses which include; Goat weeds (*Ageratum conyzoides*), pigweed (*Amaranthus retroflexus*), wondering Jew (*Commelina bengalensis*), Sodom apples (*Calotropis procera*), black jack (*Biden Pilosa*), African black nightshade (*Solanum ptychanthum*), wild spinach (*Chenopodium album*),White nightshade (*Solanum americanum*), American burn weed (*Erechtites hieraciifolius*), double thorn (*Oxygonium sinuatam*), sweet potato (Ipomoea batatas), Nile trumpet (*Markhamia lutea*) (Wangai *et al*., 2001).

2.7 Economic importance of GRSV

A wide variety of vegetables and other crops grown by farmers are infected by several viruses of genus tospovirus are highly contagious and their effect on plants are often drastic.They reduce crop yield, quality and marketability (Clark *et al*., 2007). The symptoms induced by GRSV include; leaf mosaic, chlorotic-ring shaped spots on leaves, necrotic ring spot chlorosis and necrotic patches that interfare with leaves from trapping sunlight for effective photosynthesis, which lowers nutrient supply in plants (Thompson *et al*., 2017). In tomatoes, GRSV cause fruits deformed, showing uneven ripening, with raised bumps on the surface and necrotic rings on the fruits (Webster *et al.,* 2011). This reduces the quality of tomato fruits that lower the demand thus less income for farmers. Research on occurrence of GRSV has shown a mojar effect of this virus on groundnuts, pepper, watermelon, soybeans and vegetable production world wide. It was reported that vegetable production in the south east United States shifts north from Florida to other states during spring and summer when GRSV incidence percentage was high and returns south to Florida during the fall and winter to follow suitable growing conditions. This affected the economy of horticulture in Florida. Not only GRSV affects tomatoes but also affect the quality of peppers, soybean,

watermelon and groundnuts. The virus affected quality and yield of groundnuts in Ghana. The same losses was reported in Brazil, South Africa, United States and Argentina.

2.8 Host range for Groundnut Ringspot Virus

Groundnut ringspot virus infect some legumes, which include groundnuts, Soybeans, and Solanaceae plants; pepper, cucumber, tomatillo and some garden weeds (Webster *et al*., 2011). This virus was first reported on groundnuts and solanaceous vegetables in Peninsular (Adkins *et al*., 2010). Then subsequently detected in tomatoes in southwest and southeast of Florida. Later the virus symptoms were observed on pepper, tomatillo and eggplant (Webster *et al*., 2011). The virus has been reported in South Africa on groundnuts (Arachis hypogeae L.). Samples of Soybeans collected from South Africa tested positive for the virus, although its distribution and symptoms was not reported (Pietersen *et al*., 2002). More research on host range to the virus showed that Double Gleam Mix is among the host plant to *Groundnut ringspot virus* (GRSV). The research carried out in 2009 revealed that GRSV was first detected in tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), tomatillo (*Physalis philadelphica*), and eggplant (*Solanum melongena*) in south Florida. *Tomato chlorotic spot virus* (TCSV) was subsequently detected in tomato in south Florida in 2012 and in tomato, pepper, jimsonweed (*Datura stramonium*), and lettuce (*Lactuca sativa*) in Puerto Rico and tomato in the Dominican Republic.

2.9 Molecular structure of GRSV Nucleoproteins

Groundnut ringspot virus belongs to class V viruses which is a negative-sense single-stranded RNA virus in a Tospovirus genus and family of Bunyaviridae (Bernstein *et al*., 2001). GRSV is a widespread member of this genus in most of host plants grown

globally (Karavina *et al*., 2017). The viruses with a tripartite genome, is assigned according to segment length as S (small), M (medium) and L (large) RNAs (figure 1) (Nichot *et al*., 2005). The genomes of GRSV and TCSV each consist of three RNA segments designated small (S), medium (M), and large (L), analogous to TSWV and other tospoviruses. The S RNA is ambisense and encodes a nucleocapsid (N) protein and a nonstructural silencing suppressor protein (NSs). The M RNA is also ambisense and encodes a precursor for two glycoproteins (GN and GC) and a nonstructural movement protein (NSm). The L RNA is negative sense and encodes a multifunction protein (L) including an RNA-dependent RNA polymerase domain.

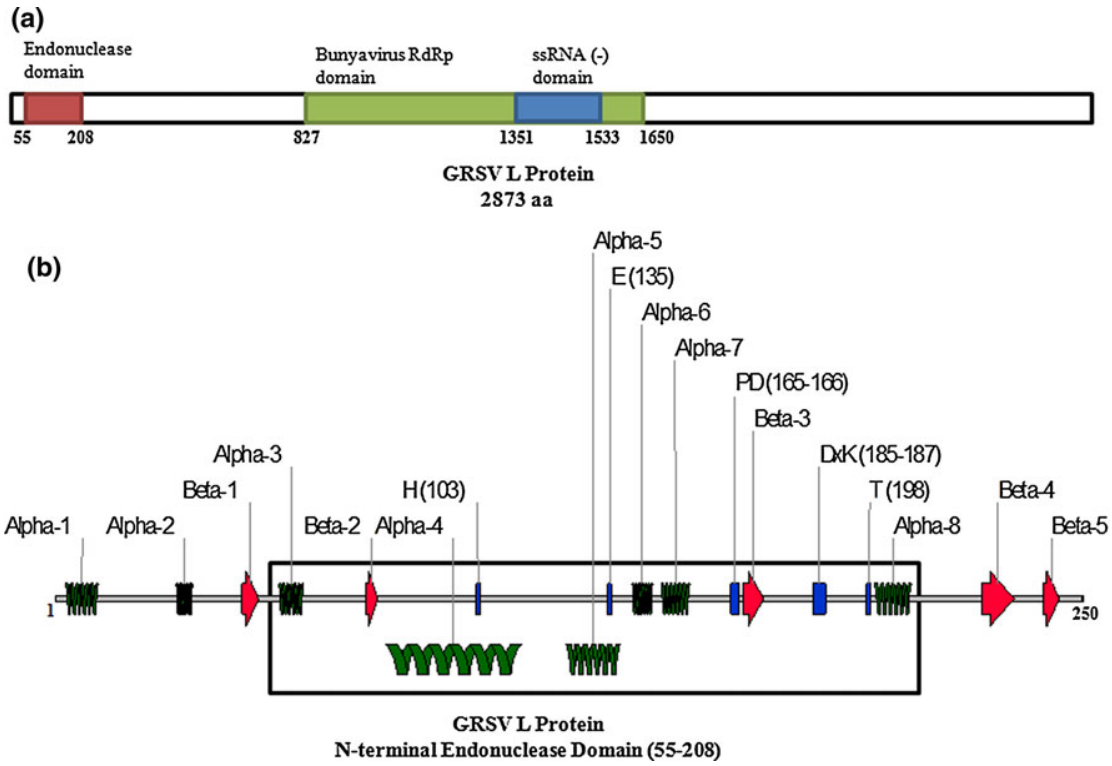


Figure1: Secondary structure of groundnut ringspot virus (GRSV) large protein extracted from infected D. stramonium isolate a) GRSV L. protein (2873 amino acid. b) GRSV Protein N-terminal Endonclease domain (Nichot *et al*., 2005).

2.10 *Groundnut Ringspot Virus* management strategies

Groundnut ringspot virus is transmitted by thrips causing a viral disease (spotted wilt disease) in host plant. The management strategies are;

2.10.1 Chemical control

Chemical control is one of the management strategies commonly used in reducing thrip population to minimizing virus transmission from infected plants to health host plants (Melzer *et al*., 2012). Pesticides mainly used by farmers to control thrips and other vectors include; Organophosphates, imidacloprid, carbamates, abamectin and synthetic pyrethroids (Nyasani *et al*., 2015). When a particular insecticide is over used or wrong dosage used in controlling thrips, normally they develop resistance against them. Therefore, to improve on management strategy, application of new insecticides and those of different modes of action will gives good results (Herron *et al*., 2010). Insecticide application should be done in right dosage to minimize toxic residues into foodstuff (Pappu *et al*., 2009). Most of insecticides recently manufactured are very expensive for farmers than generic ones, attracting farmers in using substandard pesticides due to financial constraints (Popp, 2011).

2.10.2 Physical and cultural control

Physical and cultural management strategies include field hygiene; destruction of remains of viral hosts acting as inoculum and clearing symptomatic/ asymptomatic weeds from the groundnut fields (Coutts *et al*., 2005). Regulating planting dates and crop spacing reduce viral transmission. Screen meshes on greenhouse air vents and sidewalls to reduce thrips entry into greenhouses has been noted to be effective strategy in vectors management into greenhouses (Dietzgen *et al*., 2005). It has been reported that when yellow sticky traps are hanged by farmers in greenhouses may trap thrips from landing on host plant to infect a virus on healthy plants (Dobson *et al*., 2002). The use of organic mulches may result into build- up of thrip predators, which reduce their population in the field. Weeds that attract thrips in farms; especially those with yellow flowers (Compositae and Solanaceae) act as green bridges in groundnut

farms as they are very attractive to thrips, causing groundnut ringspot virus be transmitted to another host plants (Blumthal *et al*., 2005). Such crops that belong in those families should be rooted out to minimize thrip attraction. Cultural practices, like intercropping groundnuts with non-host plants, early planting dates of the crop, adjustment of plant densities, also have been used as alternative method by farmers in management of thrips in farms. It has been reported that *T. tabaci* in bulb onions can be controlled by intercropping onions with carrots (*Daucus carrota*) and spider plants Gachu *et al*. (2012). High densities of plant population in a given farm normally dilutes the number of infected plants and helps healthy plants to shade out neighboring infected plants (Brown *et al*., 1996). Also, compensation of destroyed yield loss due to diseases infection by replacing them with adjacent healthy plants (Culbreath *et al*., 2003). It has been reported that disease incidence and severity is greatly influenced by weather conditions, crops grown during the cool and wet seasons always will be infected less compared to those grown in summer when both vector activities and virus multiplication are high. Therefore, farmers should regulate planting dates of the crop so that susceptible stages of crop development not coincide with peak or increasing thrips populations (Relevante *et al*., 2012). This tends to create a gap between crops with thrips vectors to minimize migratition from infected plants to healthy ones and spread the viruses (Cho *et al*., 1998). This allows greenhouses heated for 4-5 hours at 30°C daily subjecting the vectors to harsh condition thus starving to death (Cloyd, 2009).

2.10. 3. Host plant resistance

Screening and breeding varieties with resistant gene to tospoviruses led to resistant varieties, which were developed from a single genotype (PI 203363) that took place in Brazil to bred cultivars that could carry genes to resist against GRSV infection

(Culbreath *et al*.,2003). It was reported that genotype of the groundnut cultivars has an influence on the phenotypic characteristics of groundnuts. This resulted in breeding of cultivars that are resistant to the virus in United States. The main runner types of groundnut cultivars that have PI203363 alleles have significant proportion of resistant gene for groundnuts (Clevenger *et al*., 2018). Breeding of these groundnuts also resulted into identification of genes that are moderate resistant to GBNV in Asia (Reddy *et al*., 2000; Mandal *et al.,* 2012). Since biological, chemical, cultural and physical methods in management of tospoviruses diseases have limitations in controlling vectors, therefore preferred method to manage the virus in groundnuts is by breeding varieties which are resistant to the virus to improve on crop productivity (Soler *et al*., 2003). Development of resistant host plant is the most effective strategy in managing tospoviruses diseases in plants. The remedies to resolve the incidences of TSWV in South Africa was to breed resistant tomato varieties, which led to breeding of "Stevens" variety that was more resistant to the virus (Thompson *et al*., 1996). Resistant gene Sw-*5b* gene was introgressed into most of fresh market tomato varieties in South Africa. Although their resistance to the virus has been weakened by new strains of the viruses that have evolved in South Africa (Lopez *et al*., 2011), but planting of some tomato varieties that are less attractive to thrips, is recommended to improve productivity in regions with high IYSV occurrence. In Ghana, the high GRSV incidence has resulted into an attempt in breeding programs to improve on the resistance levels of groundnuts against GRSV infection. There being limited natural genes for resistance, and the long duration required to breed resistant plants, has resulted into no genetically-engineered *Tospovirus*-resistant crops grown in Africa. The little effort for coming up with resistant varieties has been due to socio-political

debate on GMOs plants in most countries, and the costs associated with introgression of resistant genes (Bawa *et al*., 2013).

2.11 Methods for detection of plant viruses

A detection method for viruses is very critical in management of viral diseases in plants (Aboul-Ata *et al*., 2011). The method used in detection should be cost effective, convenient and specific for detecting viruses in a plant tissue (McCartney *et al*., 2003). Methods mainly used include; microscopical observation, serological techniques and molecular methods (Lopez *et al*., 2008).

2.11.1 Serological method

Serological method is technique used in diagnosing viruses in plant materials /tissues using specific antibody or antisera developed from animals in responding to antigens (Torrance, 1998). Viruses can be detected if viral antigens used to develop antibody, reacts with antisera present. This kind of technique has been used for routine diagnostic of viruses among many others. Many serological methods have been designed and reported, include; enzyme-linked immunosorbent assay, tissue blot immunoassay and quartz crystal microbalance immunosensors.

2.11.1.1 Enzyme-linked immunosorbent Assay (ELISA)

ELISA is performed in microtitre plate, binding antibodies/ proteins with associated enzyme-substrate reaction (Luminex, 2010). ELISA is a technique used in detecting plant viruses in; inoculum, plant material, insect vectors, and seeds (Naidu *et al*., 2001). Levels of infection is measured based on terms of intensity of viral titre in a sample (Webster *et al.*, 2004). ELISA as a detection method has an advantage over other serological methods since large samples are tested (Vemulapati *et al*., 2014).

Small amount of antibody can be used in detection of viruses in any given sample material. The process can be automated or semi-automated (Naidu *et al*., 2001). Specific antisera have been designed and developed against the target virus (Torrance, 1998). More samples for ELISA are required than for molecular diagnostic methods (Lievens *et al*., 2005). Since ELISA is antibody-antigen based assay, availability of antibody responding against the target agent is an important factor. Therefore, strains of viruses that are related cannot be differentiated correctly by ELISA (Boonham *et al*., 2014). ELISA sensitivity to viruses being tested in a plant tissue can be improved by additional of some additives in extraction buffer (Fegla *et al*., 2013).

2.11.2 Molecular methods

These methods are used to determine most of viruses present in plant tissues or inoculum when their genetic information or codes are available and well known (Kurkela *et al*., 2009). These methods are used as an alternative to serological method, which is commonly used to determine viruses present in a sample in the laboratory due to its high accuracy and sensitivity in the presence of viruses in a sample or plant tissue (Souf, 2016).

2.11.2.1 Polymerase chain reaction (PCR)

PCR is a technique employed to amplify DNA or RNA extracted from a plant tissue or a sample material, or create many identical copies of a particular DNA or RNA sequence in a tiny reaction tube (Cella *et al*., 2013). Before an initiation of a new DNA amplification, the DNA is denatured, two sets of oligonucleotides (primers) annealing to the denatured complementary strand (Pankaj, 2013). Then, primers lead DNA synthesis by the DNA polymerase. Reactions occur in template dependent manner. The target sequences of DNA amplified (Saiki *et al*., 1985). PCR is a technique for

detection of plant viruses in the laboratory in molecular experiments (Webster *et al*., 2004) and a diagnostic tool for detection of strains (Lopez *et al*., 2008). PCR is an effective diagnostic method of viruses. PCR is proceeded in three steps, denaturation above 94^{o}C, annealing of primers at 50-75^{o}C and elongation at 72^{o}C (Makkouk *et al*., 2006; McCartney *et al*., 2003). RT-PCR used for the detection of RNA viruses requires reverse transcriptase which is added at the step of reverse transcription before the regular PCR step (Lopez *et al*., 2008; Webster *et al*., 2004). The RT-PCR technique is sensitive and specific compared to serological methods and is also more reliable than serological methods (Lievens *et al*., 2005; Lopez *et al*., 2008), it has been developed and employed to detect many viruses infecting different plants of economic importance (Drygin *et al*., 2012; Ham, 2003; Peiman *et al*., 2006; Peter *et al*., 2009). RT-PCR also used to detect plant RNA viruses for quarantine purpose (Lee *et al*., 2011). More targets DNA or RNA can be detected at the same time with multiplex PCR in a single reaction although requires several specific primers to detect more than one virus or bacteria (Menzel *et al*., 2002; Singh *et al*., 2000). Although the advantage of this technique has, conventional PCR is more preferred than multiplex PCR, since it involves technical skills in mixing of many compatible primers (Lopez *et al*., 2008). Also, difficult to design specific primer for each target DNA and differentiate the difference of DNA amplification of each size of the gene (Lopez *et al*., 2008). Nested PCR technique is useful when the virus titre is very low, target gene is unstable, and can't be detected by electrophoresis due to low amplification product (Webster *et al*., 2004). The product from primary PCR amplification used for second PCR amplification (Lopez *et al*., 2008).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Survey for occurrence and distribution of GRSV in western Kenya

Extensive diagnostic survey was conducted in the three major groundnut, growing counties (Bungoma, Busia and Kakamega) of western Kenya, covering four agro ecological zones (LM1, LM2, LM3 and UM1), during long and short rain seasons of the year 2019 and 2020 respectively. The survey was done in 11 selected clustered regions of groundnut; Chebich, Chwele, Kapkateny, Kimalewa and Kimilili (in Bungoma County). Alupe, Chakol and Malaba (in Busia County). Then, Matungu, Muhonje and Mumias (in Kakamega County). A total of 536 farms were randomly selected and visited in these clusters during long and short rain seasons in a survey. During short rain seasons (September to December), 276 farms were visited and 260 farms in long rains seasons (March to July) of the year 2019 and 2020 respectively. One to two sampling quadrats measuring 10m x 10m were randomly selected on each farm visited, depending on farm size. Data obtained (GRSV incidence, severity and altitude) was recorded and Symptomatic leaf Samples taken for serological and molecular analysis. A GPS device (Magellan Triton "Windows CE Core 5.0" X11-15302) was used to locate the coordinates and altitude to determine agro- ecological zones of each cluster to avoid biasness or over representation of one agro ecological zone in this study which may have influenced the outcome of incidences and severity of groundnut ringspot virus in western Kenya (Table 1, figure 2).

Table 1: characteristics of the AEZs covered and farms visited in a survey in western Kenya during long and short rain seasons

AEZs	Altitude (m)	Rainfall (mm)	Temp.(ºC)	No. of farms Sampled
UM1	1500-2000	1540-1800	18.0-21.0	92
LM1	1350-1550	1600-1800	21.1-22.0	110
LM2	1350-1500	1350-1650	20.9-22.0	149
LM3	1200-1400	1200-1450	21.6-22.4	185

Key: AEZs- Agro-ecological zones; UM1- upper midlands zone, LM1- lower midland zone 1, LM2- lower midland zone 2 and LM3- lower midland zone 3 (Jaetzold *et al*., 2006).

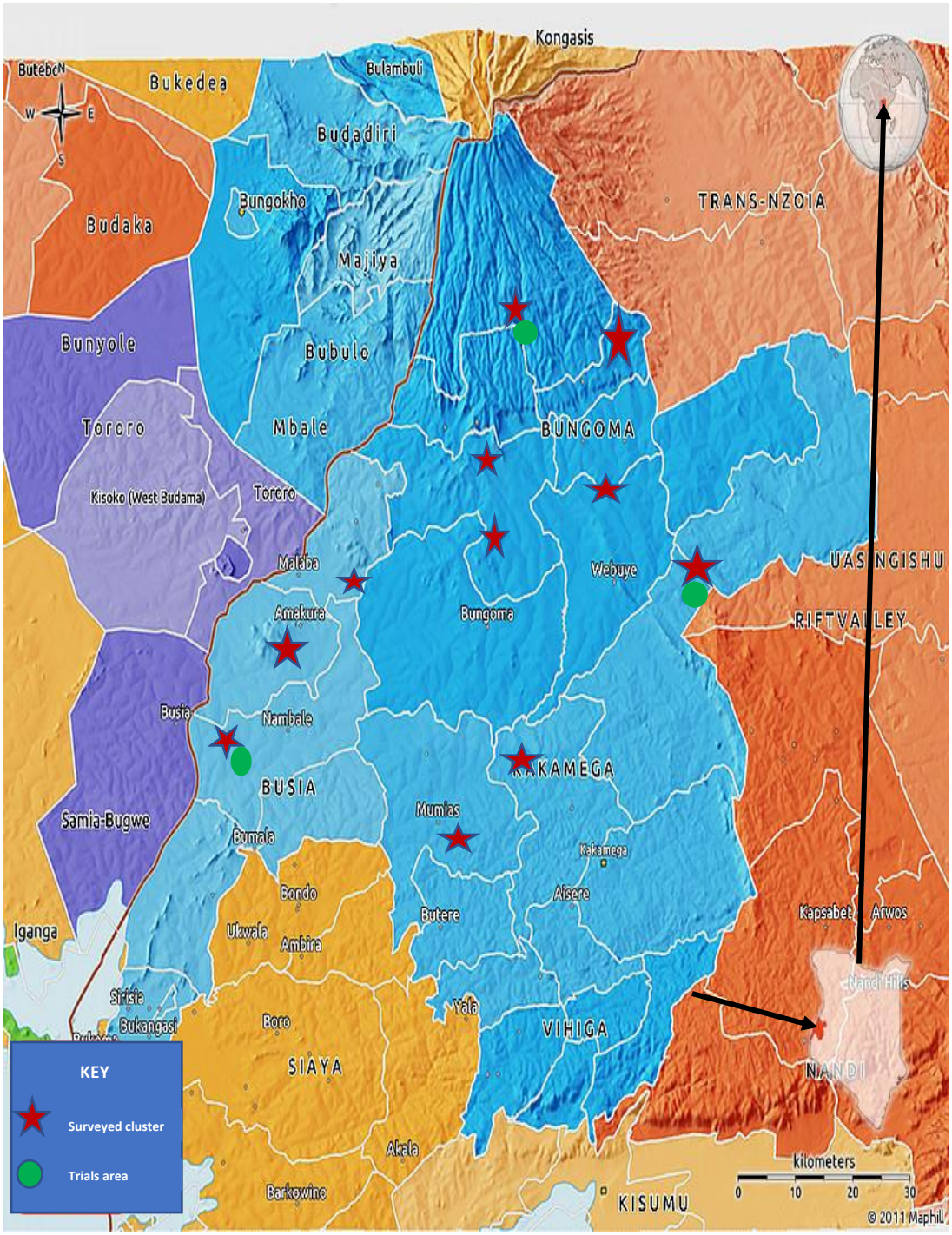


Figure 2: A map of western Kenya showing regions of trials on effect of other legumes intercropped with groundnut varieties on GRSV incidences and severity and surveyed regions for occurrence and distribution of GRSV.

3.1.1 Sample size determination in surveyed clusters of western Kenya

The clusters selected for the study were based on main regions for groundnut growing areas regions in western Kenya covering agro ecological zones in western Kenya (LM1, LM2, LM3 and UM1) reported having good ecological conditions for groungnut growing (Ndiso, 2015). Number of farms growing groundnuts in each selected clusters for study was obtained from County agricultural offices. The number of farms visted (sample size) in each cluster was calculated by using Yamine's formular (Yamine, 1973):

n= N/ (1+N (e)2.

The variables in this formula are:

n = sample size (number of farms visited)

N = Population (number of farms under groundnut farming)

e = the margin error in the calculation

The Slovin's Yamanes formula was used in this study to figure out the minimum number of farms (sample size) in each cluster.The margin error (e) 0.05 was used to calculate the sample size in each region (Table 2).

Table 2. Sample size of farms in surveyed clusters of western Kenya

County	Cluster	Long rains	Short rains	Total farms	AEZs
Bungoma	Chwele	17	19	36	**LM1**
Bungoma	Kapkateny	19	16	35	**UM1**
Bungoma	Kimalewa	15	21	36	**LM1**
Busia	Alupe	38	37	75	**LM2**
Busia	Chakol	27	31	58	**LM3**
Busia	Malaba	31	37	68	**LM3**
Kakamega	Mumias	35	39	74	**LM2**
Kakamega	Matungu	30	29	59	**LM3**
Kakamega	Muhonje	19	19	38	**LM1**
Bungoma	Kimilili	16	14	30	**UM1**
Bungoma	Chebich	13	14	27	**UM1**

3.1.2 Disease incidence and severity determination

Viral symptoms occurrence and variations recorded to determine the disease incidence and severity on each visited farm in the surveyed areas of western Kenya. Type of groundnut varieties grown, neighbouring crops, farm history and sources of seeds grown also were recorded. One to two Sampling quadrats randomly selected on each farm depending on farm size was used to determine viral incidence and severity by calculating the percentage of plants showing GRSV symptoms to total number of plants observed in the field quadrats. The average incidences and severity of the sampled quadrats per farm used as the actual farm disease incidence and severity. The degree of disease (GRSV) incidence was assessed and analyzed according to (Nono-Womdim, 1996) as the proportion of diseased plants in an area.

$$\text{Disease incidence} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

The presence and absence of viral disease on groundnuts scored using a rating scale basing on (Nono-Womdim, 1996) where low incidence = 1-20 %,

moderate incidence = 21-49 % and high incidence = 50-100 %.

Disease severity was scored on a scale of 1-4, as described by (Lyerly *et al*., 2002; Nascimento *et al*., 2006), the scale used to evaluate the severity of symptoms of TSWV and adapted for GRSV since they belong in the same genus and have similar symptoms, where;

1: plants without viral symptoms.

2: plants with symptoms of yellowing leaves, leaf mosaic and/or chlorotic spots,

3: plants with leaf chlorosis, leaf mosaic or chlorotic spots and height reduction,

4; for chlorotic plants and stunting symptoms.

These symptoms were used to score for disease severity in groundnuts and symptomatic leaf samples collected in a cool box and falcon tubes for both serological (DAS-ELISA/ TAS ELISA) and molecular test for GRSV.

3.1.3 Enzymes- Linked Immunosorbent Assay (ELISA)

Detection of GRSV viral titre on leaf samples by serological techniques was based on the ability of the specific antibodies to react in the vitro with their antigens (virus particle), used polyclonal antibodies (IgG) for detection. Microtiter plants (Grainer microloan medium binding) was used and the volume for each reactant, kept at 100µl. between incubations, 3 intensive washing steps each lasting 3 min, carried out by repeated soaking of the plates in washing buffer for 4 min. The following buffers used;

Coating buffer, pH 9.6 (per litre);

1.59 g Sodium carbonate (Na_2CO_3).

2.93 g Sodium bicarbonate ($NaHCO_3$)

0.20 g Sodium oxide (Na_2O) dissolved in 900 ml H_2O

PBS (pH 7.4) phosphate buffer Saline;

8.00 g Sodium chloride (NaCl)

0.20 g monobasic potassium phosphate (KH_2PO_4).

1.15 g Dibasic Sodium phosphate (Na_2HPO_4)

0.20 g potassium chloride (KCl)

0.20 g Sodium oxide (Na_2O)

Was dissolved in 900ml H_2O pH and adjusted from 7.4 to 11 with NaOH

PBS- Tween (PBST);

PBS+0.5 ml Tween 20 per litre

Sample extraction buffer (pH 7.4).

PBST+2% pvp (pvp- is polyvinyl pyrrolidone).

Conjugate buffer

PBST+2% pvp+0.2% egg albumin

Substrate buffer

97 ml diethanolamine

600 ml H_2O

0.20 g Sodium oxide (Na_2O)

Adjusted to pH 9.8 with HCl and make up to 1 litre with H_2O

3.1.4 Double Antibody Sandwich ELISA (DAS ELISA)

Double antibody sandwich ELISA was done as described by Clark and Adams (1977). For detection of GRSV in groundnut leaf samples. Microtiters plates were coated with GRSV IgG diluted 1:1000 (v/v) in coating buffer and incubated for 4 hours at 37 ^{0}C. Sample extracts added and incubate at 4 ^{0}C. Extracts from GRSV commercial positive and negative standards were used as control experiment to check for negative and positive samples to GRSV, respectively. IgG- alkaline phosphate conjugates diluted 1:1000 (v/v) in conjugate buffer added and incubated for 2 h at 37 ^{0}C substrate.

3.1.5 Triple Antibody Sandwich ELISA (TAS ELISA)

TAS ELISA was done as described by (Charoenvilaisiri *et al*., 2021) with minor modifications to detect TSWV. Microlitre plates (96 wells) was coated with TSWV IgG diluted 1:1000 (v/v) in a coating buffer and incubated for 2 h at 37 ºC. Blocking was done by adding 2% skimmed milk in PBST (200 µ1/well) and incubated for 30 min at 37 ºC. Sap extracts sample was added and incubated at 4ºC. Extracts from a healthy plant (groundnut) and those infected with TSWV were used as negative and positive controls, respectively. MAbs raised against TSWV was used in detecting antibodies at dilution of 1:100 (v/v) in conjugate buffer used for detection. 100µ1 of each supernatant dilution was loaded onto microtitre plates and incubate for 2 h at 37ºC. After washing the plates, an alkaline phosphate labeled phosphate as (99Rabbit-anti - mouse) diluted 1:1000 (v/v) in conjugate buffer was added and the plate incubated for 45 min at 37 ºC. The substrate, P-Nitrophonyl phosphate diluted 1mg/ml in substrate buffer was added and incubate for 2 h at 37 ºC.

3.2 Effect of intercropping other legumes with groundnuts on GRSV incidences in western Kenya

Trials on popularly grown groundnut varieties (Red-Valencia, ICGV12991 and ICGV 90704) in western Kenya was conducted in four agro ecological zones (LM1, LM2, LM3, and UM1) in groundnut growing regions of Bungoma, Busia and Kakamega Counties. Two farms in each County were randomly selected based on differences and similarities on topology, soil type, soil fertility, farm history, altitude and latitude. Each selected groundnut variety was intercropped with legumes; cowpea (K80), beans (Rosecoco) and Soybeans respectively. Open field trials were laid on plots (5 x 5) m in a randomized complete block design (RCBD) on each selected farm and replicated three times on each selected farms. Two seeds planted with spacing of 30 cm by 15 cm. Two rows of either soybean, beans, cowpeas were intra cropped with groundnut varieties respectively. Pure stand for each groundnut variety planted separately for a health control (appendix iv).

3.2.1 Sampling design and data analysis

Leaf samples from infected plants at an interval of 1 m on each row of groundnut varieties were, picked for serological and molecular tests for confirmation of GRSV presence/absence for the symptoms displayed. Leaf samples kept in a cool box and falcon tubes taken for serological tests. The GRSV incidences recorded basing on scale of (Nono-Womdim, 1996) and severity calculated according to Lyerly *et al*. (2002) and Nascimento *et al*. (2006) as described in section 3.1.1. The symptoms of Groundnut ringspot virus as described in section 2.2 used to pick leaf samples for DAS ELISA and molecular tests. Leaf samples also tested for TSWV (TAS- ELISA) to determine its occurrence due to similarity in symptoms with GRSV. Data obtained from the research, averaged to obtain mean percentages of each explanatory

parameters and recorded (incidence and severity). The Analysis of variance (ANOVA) differences in incidence and severity between groundnut varieties and between clustered regions of western Kenya analysed. Post hoc ANOVA was used to obtain least significant difference (L.S.D) values to separate the means at P = 0.05. Statistical analysis software, used to obtain correlation between the incidence and severity of GRSV (appendix v)

3.3 Response and Screening of plant species/ varieties to GRSV in western Kenya

Popularly grown groundnut varieties (Red Valencia, SM99568, CG7, ICGV-12991, ICGV-9991, ICGV- 90704, ICGV-99048, ICGV-99019 and Homa bay) in western Kenya, were screened for resistance levels to GRSV. Apart from groundnuts, other legumes, brassicas, cucurbits mainly intercropped or grown adjacent to groundnut farms and broad-leafed weeds in western Kenya, exhibit viral symptoms also screened for their response to GRSV inoculum from groundnuts samples collected in a survey in western Kenya.

3.3.1 Germplasm quality tests

The germplasm of selected groundnut varieties, brassicas, Cucurbits, other common legumes and garden broad-leafed weed, randomly picked for healthy germplasm tests. The selected germplasms were prepared for GRSV test according to international rules for seed health tests (ISTA, 2014). Selected germplasm wiped with cotton wool soaked into 70 % Ethanol, rinsed with distilled water, and then transferred to petri dish water-soaked paper towels and sprout. Sprouting germplasm samples picked for GRSV serological tests, to determine their healthy from the virus

3.3.2 Response of groundnuts and other alternative host to GRSV inoculum

Health tested seeds for GRSV of the selected nine groundnut varieties, tomatoes, soybeans and watermelon were planted in 500 ml plastic pots in sterile soil medium composed of loam soil, manure and sand in the ratio of 2:1:1 in a greenhouse. Each variety/species replicated three times in plastic pots. The plants were arranged in a randomized complete block design (RCBD) in the greenhouse and inoculated with GRSV inoculum at the rate of 2500 µl /plant with a viral load of 1.033 viral titre from groundnut samples collected from a survey in western Kenya. Health controls for each variety/species, were planted separately in a greenhouse from the inoculated varieties/species to avoid contarmination.

3.3.3 Determination of alternative hosts to GRSV in Western Kenya

Commonly planted legumes, brassicas and cucurbits exhibiting symptoms similar to GRSV were screened for alternative host to GRSV. Beans, green gram, cowpea, Bambara nut, kales, cabbage, butternut, pigeon peas, black gram and peas were screened for host range for GRSV in western Kenya.

Garden broad- leafed weeds commonly found growing or bordering groundnut farms in western Kenya and exhibiting viral symptoms similar to those of GRSV were screened to determine alternative hosts for the virus. Goat weeds (*Ageratum conyzoides*), pigweed (*Amaranthus retroflexus*), wondering Jew (*Commelina bengalensis*), Sodom apples (*Calotropis procera*), black jack (*Biden Pilosa*), African black nightshade (*Solanum ptychanthum*), wild spinach (*Chenopodium album*),White nightshade (*Solanum americanum*), American burn weed (*Erechtites hieraciifolius*), double thorn (*Oxygonium sinuatam*), sweet potato (Ipomoea batatas), Nile trumpet (*Markhamia lutea*) planted in 500ml plastic pots, arranged in RCBD in greenhouse and inoculated as described in section 3.3.2.

3.3.4 Inoculum preparation and inoculation

Thirty grams of symptomatic leaf sample isolates from the survey, serologically testing positive for GRSV with viral titre of 1.033 and free from other viral contaminations, was grounded using a sterilized pestle and mortar, and with the aid of dust powdered Carborundum 320 grit. Freshly prepared ice-cold 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), added to the ground tissue, mixed and then transferred to falcon tubes, and allowed to stand for 5 minutes in ice, to settle debris at the bottom of tube. The sap kept on ice, until inoculation completed. The Carborundum dusted on plants under study, acted as an abrasive. The inoculum applied gently on the leaf surfaces at a rate of 2500 µl /plant, using saturated cotton wool swab and excess carborundum and inoculum washed out on the groundnut leaves by spraying gently with sterilized distilled water (Hull, 2009). Hands washed with detergent, before proceeding to the next inoculation, to prevent contamination. The inoculated plants observed on weekly basis for viral symptoms development and recorded. This was repeated for 8 consecutive weeks. Leafy samples of each variety collected and tested by DAS-ELISA for GRSV causal agents.

3.3.5 Inoculation of groundnut varieties/species and alternative hosts

Groundnut positive isolates of 1.033 viral titre to GRSV, macerated and grounded using a pestle and mortar as described in section 3.3. Groundnut varieties, tomatoes, water melon, soy beans, broad leafed weeds and commonly grown crops, inoculated by gently rubbing 2500 µl of the inoculum/plant on leaves dusted with carborundum respectively apart from healthy controls. After inoculation, excess carborundum on plants, gently removed by spraying with water. Tested plants observed for symptom development 3 days after inoculation and thereafter on weekly basis for 8 consecutive

weeks. Data collected included: number of symptomatic plants per variety (disease incidence) and disease severity (using 1-4 scale). Leaf sample collected at 6th week and tested for the virus. DAS - ELISA used as described in section 3.3 and Plants that test positive for GRSV were regarded as susceptible. Viral titre in each variety were, determined by taking the average Spectrophotometric absorbance values (at 405nm) for the positive samples. This was used to grade the resistance levels of different varieties to GRSV, determine alternative host for GRSV and its preference.

3.4 Total RNA Extraction

Total RNA from naturally infected groundnuts leaf samples collected in a survey from farms in western Kenya, was extracted using the CetylTrimethyl Ammonium Bromide (CTAB) method modified from (Giorgio *et al*., 2008). One gram of each Sample isolates placed in a sampling bag and crushed completely. 2 ml of CTAB buffer added to crushed samples, and then transferred the sample solution of 700µl to a 1.5 ml sterile centrifuge tubes and then mixed the sample properly by vortexing until the sample was thoroughly resuspended then allowed to settle for 10 minutes. Samples then incubated at 65º C for lysing cells completely. Added 700µl of chloroform: isoamyl alcohol (24:1) to each tube and homogenized by vortexing then centrifuged at 14,000 rpm at 4º C for 10 minutes. After the centrifugation, the supernatant was transferred to a clean 1.5 micro centrifuge eppendorf tubes. 700µl Lithium chloride added to precipitate the RNA and the samples in tubes inverted 3-4 times for proper mixing. After this the mixture was left at room temperature for approximately 10 minutes to settle. The tubes were incubated overnight at 4º C then centrifuged at 14,000rpm at 4º C for 30 minutes and solution decanted off carefully. The pellet was suspended in 200µl ice- cold TE buffer containing 1% SDS. Added 100µl NaCl and 300µl ice- cold Isopropanol and mixed properly. The sample was incubated at -20º C for 30 minutes and centrifuged at

14,000 rpm and salts decanted off carefully. The liquid was eluted carefully while making sure the pellet is intact. The pellet later washed with 500µl of 70 % ethanol. After air drying the pellet was suspended in 50µl of nuclease free water (NFW). Quantification using the Nano drop was done to ascertain the quantity and quality of the nucleic acid. Later on, the sample was stored at -20º C awaiting to be used in a PCR test.

3.4.1 Reverse Transcription Polymerase chain reaction (RT-PCR)

All set of reactions were carried out in a final volume of 50µl, which consisted of 25µl of master mix, 1µl GRSVnF (5'TCTTGTGCATCATCCATTGT-3') and, 1µl of GRSVnR (5'GCGGTCTACAGTGTTGCACTT-3') which amplify a 614-bp fragment of the nucleocapsid gene of GRSV (DeBreuil *et al*., 2007). Superscript™ III RT/Platinum™ 2 µl, 20µl of Nuclease free water and 1µl of the RNA template was prepared for the required number of reactions. The extracted RNA was denatured at 55 ºC for 30 minutes. The cycling conditions for RT-PCR were: one cycle of reverse transcription at 55 ºC for 30 minutes, one cycle of enzyme inactivation at 94 ºc for 2 minutes, 40 cycles of denaturation at 94 ºC for 15 seconds, 40 cycles denaturation at 94 ºc for 15 seconds, 40 cycles of annealing at 55 ºc for 20 seconds, 40 cycles of extension at 68 ºC for 1 minutes and one cycle of final extension at 68 ºC for 5 minutes. Nested PCR was done. The product was amplified with the Qubit 2.0 Fluorometer Kit amplification module (Thermo fisher Scientific Inc.). Agarose gel (1.5%) was used to confirm the PCR amplification success. The components were mixed gently to ensure all the components are at the bottom of the amplification tube. Then centrifuged briefly in a microcentrifuge.

3.4.2 Visualization of the PCR products

One and half grams of agarose was weighed and then dissolved in 100ml of TBE buffer. The mixture was heated in a microwave for 2 minutes to facilitate dissolving of the agarose. Allowed to cool and then 3µl of gel stain Invitrogen brand added into the mixture and swirled. The mixture poured into a casting tray with combs in place and left to solidify for 20 minutes to form a hard matrix. The combs were then removed and 5µl of each of the sample from the PCR machine was mixed with 3µl loading dye and loaded onto the wells formed by the combs.1kb DNA ladder was also loaded and the casting tray was then placed in gel tank containing TE buffer and connected to an electric power supply. The samples were run at100V for 1hour then observed in Azure™ Gel dock.

3.4.3 Sanger sequencing and phylogenetic analysis

The RT-PCR amplicons were directly sequenced and Bio- edit software was used in sequence editing and generating the consensus sequences. BLAST analysis of the sequence was done to determine library sequences that resembled the query sequence. The resulting nucleotide sequences were and then aligned using the programs Electropherogram quality analysis and CLUSTAL W (Thompson *et al*., 1994). The nucleotide and deduced amino acid sequences of the open reading frame were compared with the corresponding sequences of other tospoviruses deposited in GenBank. Phylogenetic analysis was conducted using MEGA-X software version 10.0 (Tamura *et al*., 2011) and a phylogenetic tree was constructed using the neighbor-joining method with the Kimura 2-parameter model (Kimura, 1980).

3.4.4 Designing New GRSV primers for PCR

Groundnut ringspot virus (GRSV) sequences of sampled groundnuts from western Kenya was used to design new RT-PCR primers. Primer3Plus software (http://primer3plus.com/cgi-bin/dev/primer3plus.cgi (Untergasser *et al*., 2007) was used to design the GRSV primers used in this study. Upon opening Primer3 Plus webpage (http:// fokker.wi.mit.edu/ primer3/ input.htm), the sequences of GRSV were uploaded in the organization of Primer3Plus software web interface (http://primer3plus.com/cgi bin/dev/primer3plus.cgi and http:// sourceforge.net/projects/primer3/files/primer3-web/). The product size of 160 bP was entered with no optimum for the Left (forward) and Right (reverse) primers into the sequence box. Primer Tm of between 55 – 65 °C was chosen with the optimum being 60 °C and a maximum Tm difference between the primers of 5 °C. The product Tm was left blank. A primer % GC content between 40 - 60% with the optimum 50% was chosen and the rest of setting left at default, except GC clamp set at 1, 2 and 3 for the program to pick primers with 1, 2, or 3 G's or C's at the 3' end. The primer Primer3Plus chosen were checked by use of Integrated DNA technologies website Oligo Analyzer (http://www.idtdna.com/SciTools /SciTools .aspx? cat=DesignAnalyze) in the primer3_core main program that uses libprimer3 library. Oligonucleotide Primers sequence (5'- > 3') were Specific in Reference to GRSVnF (5'TCTTGTGCATCATCCATTGT-3') for forward reaction and, GRSVnR (5'GCGGTCTACAGTGTTGCACTT-3') for reverse reaction (Table 3).

Table 3: New Designed RT-PCR primers

	Primer name	Sequence 5" > 3"	Product size	Anneal.Temp
1	GRSV4_F	ACCAGAACCAGGTTGCATTC	160bp	60
	GRSV4_R	ATCGTGACCTTGCCAAAAGT		59.6
2	GRSV4_1_F	GACCAGAACCAGGTTGCATT	161bp	60
	GRSV4_1_R	ATCGTGACCTTGCCAAAAGT		59.6
3	GRSVKE_F	GGCAGATGCAAAATCTGTGA	194bp	59.8
	GRSVKE_R	TTAAGCACTGTGCAGCAACC		60.1
4	GRSVKE6_F	CGTGCACTTTCTCACCTTGA	155bp	60
	GRSVKE6_R	AATGCAACCTGGTTCTGGTC		60

Table 2: Oligonucleotide primers designed and used for the amplification of GRSV

3.4.5 Validation of designed RT-PCR primers

Total RNA from positive samples extracted as described in section 3.4. Four pairs of designed primers; (GRSV4 F/GRSV4R), (GRSV I 4F/GRSV I 4R), (GRSVKE F/ GRSVKE R) and (GRSVKE6 F/ GRSVKE6 R) used in preparation of master mix respectively for RT-PCR to validated their use. A pair of Commercial standard primers (GRSVnF 5'TCTTGTGCATCATCCATTGT-3' for forward reaction and, GRSVnR 5'GCGGTCTACAGTGTTGCACTT-3' for reverse reaction), was used to check the validity of new developed primers, all sets of reactions were carried out in a final volume of 50µl, which consisted of 25µl of master mix, 1µl for forward and 1µl for reverse reaction for each designed pair of primers respectively which amplified at 160 bp fragment of the nucleoproteins genes of GRSV (DeBreuil *et al*., 2007). Superscript™ III RT/Platinum™ 2 µl, 20µl of Nuclease free water and 1µl of the RNA template, was prepared for the required number of reactions. The extracted RNA denatured at 55 ºc for 30 mins. The cycling conditions for RT-PCR were: one cycle of reverse transcription at 55 ºc for 30 minutes, one cycle of enzyme inactivation at 94 ºc for 2 mins, 40 cycles of denaturation at 94 ºc for 15 seconds, 40 cycles of annealing at 55 ºc for 20 seconds, 40 cycles of extension at 68 ºc for 1 minutes and one cycle of final extension at 68 ºc for 5 mins. Nested PCR was done. The product was amplified

with the Qubit 2.0 Fluorometer Kit amplification module (Thermo fisher Scientific Inc.). Agarose gel (1.5%) used to confirm the PCR amplification success. The components were mixed gently to ensure all the components are at the bottom of the amplification tube and centrifuged briefly in a microcentrifuge

3.5 GRSV data analysis

The collected data on GRSV incidence and severity, subjected to analysis of variance (ANOVA), using Statistical Analysis System (SAS) program version 9.3.1 software (SAS Institute, 2013). Pairwise comparisons of means done using Least Significance Differences (LSD) for multiple-means comparison method at $P \leq 0.05$ confidence level.

3.5.1 Sequence analysis

The sequences were trimmed using Bio-Edit version 5.09 software. Trimmed and size-selected reads then mapped to the NCBI viral RefSeq GenBank containing other representatives of all viral genomes with completely sequenced genomes. Results of the mappings were inspected. The remaining reads of each sample were assembled using metaSPAdesV.3.10.1 (Nurk et al., 2017) with default settings. The resulting contigs were submitted to BLAST for comparison against a local download of NCBI genBank nucleotide database of plant viruses using BLASTn (Camacho *et al*., 2009). Final sequences were submitted to the DNA Data Bank of Japan (**DDBJ**).

Phylogenetic analysis was conducted using MEGA-X software version 10.0 with the maximum likelihood model at 1,000 bootstrap replicates (Tamura *et al*., 2016) The sequence obtained were aligned with 18 GRSV complete genome sequences and used phylogenetic tree construction using the neighbor-joining method with the Kimura 2-parameter model (Kimura, 1980).

3.5.2 Designed primer analysis

Designed primers using primer3plus using consensus sequences from this study were validated by PCR on four ELISA positive samples and checked with standard commercial primer. Primer that formed clear band was recommended to be valid while those that failed were recommended for improvement. Validated primer sequence was BLAST to the NBCI for comparison with sequences in the GenBank.

CHAPTER FOUR

RESULTS

4.1 Occurrence and distribution of GRSV in western Kenya

Typical symptoms of groundnut ringspot virus in groundnuts and tomato spotted wilt virus were observed on groundnuts and other plants bordering infected groundnuts in some farms surveyed in some agro ecological zones of western Kenya (plate 1). These include; chlorotic-ring spots, necrosis ring spot, leaf deformation and stunted growth.

Plate 1: a) Groundnut plant serologically tested positive for GRSV and TSWV co-infecting the plant showing typical GRSV symptoms; chlorotic and necrotic ringspots with leaf deformation from Kimilili region in Bungoma County. b) Groundnut plant with no viral symptoms serologically tested negative for GRSV from Mumias in Kakamega County of western Kenya.

Other viral symptoms observed on groundnuts, include, leave mosaic, leaf chlorosis, stunted growth, reduced height of groundnut plant and leaf deformation (plate 2). In tomatoes, plants displayed; inward cupping of leaves, leaves develop bronze cast, dark spots, necrotic spots and flecks, chlorotic areas on leaves, deformation of leaves, necrotic lesions on stems and petioles on tomatoes affecting the quality of the fruit as well.

Plate 2. Infected groundnut plants in western Kenya displaying symptoms of viral symptoms. a) groundnut plant from Alupe in Busia county with stunted growth, b) groundnut plant from Chebich in Bungoma County with leaf mosaic, c) groundnut plant from Muhonje in kakamega county with leaf chlorosis symptoms and d) groundnut plant from Chwele in Bungoma county with deformed leaves, necrosis and stunted growth.

4.1.1 Viral incidences in the Surveyed areas of western Kenya

Viral disease incidences in all agro ecological regions surveyed varied from cluster to cluster; Chwele (LM1) had the highest disease mean incidence of 45.04 %, with maximum mean of 80 %. Chebich (UM1) was second with mean incidence of 41.19 % followed by Kimilili (LM1) with viral incidence of 39.19 %. While Kapkateny (LM1) region had the lowest disease incidence (17.75 %) with maximum incidence of

60 %. Most of surveyed regions of western Kenya showed moderate disease incidence (25.12 to 37.83 %). (Table 4).

Table 4. Viral disease incidences in groundnut growing regions of western Kenya

County	Cluster	Mean Incidence	Max Incidence	Std error	AEZs
Bungoma	**Chwele**	**45.04**	**80**	**2.43**	**LM1**
Bungoma	**Kapkateny**	**17.75**	**60**	**2.19**	**UM1**
Bungoma	**Kimalewa**	**28.26**	**75**	**1.92**	**LM1**
Busia	**Alupe**	**37.83**	**68**	**2.18**	**LM2**
Busia	**Chakol**	**25.12**	**56**	**3.02**	**LM3**
Busia	**Malaba**	**25.61**	**80**	**2.19**	**LM3**
Kakamega	**Mumias**	**27.36**	**80**	**3.23**	**LM2**
Kakamega	**Matungu**	**25.67**	**70**	**2.69**	**LM3**
Kakamega	**Muhonje**	**35.72**	**75**	**3.57**	**LM1**
Bungoma	**Kimilili**	**39.75**	**75**	**2.15**	**UM1**
Bungoma	**Chebich**	**41.19**	**76**	**2.67**	**UM1**

4.1.2 Viral disease severity in surveyed regions of western Kenya

Disease severity observed varied from cluster to cluster; Chwele had the highest disease severity (2.99) with maximum severity of (4) and minimum of (1), followed by Chebich with disease severity of (2.94) then Kimilili with severity of (2.90). While Kapkateny had the lowest with disease mean severity of (2.22).(Table.5)

Table 5. Disease severity in groundnuts growing regions in western Kenya

County	Cluster	Mean Severity	Max Severity	Min Severity	Std Error
Bungoma	Chwele	2.99	4	1	0.10
Bungoma	Kapkateny	2.22	4	1	0.11
Bungoma	Kimalewa	2.65	4	1	0.09
Busia	Alupe	2.88	4	1	0.13
Busia	Chakol	2.38	4	1	0.12
Busia	Malaba	2.40	4	1	0.12
Kakamega	Mumias	2.65	4	1	0.13
Kakamega	Matungu	2.42	4	1	0.11
Kakamega	Muhonje	2.82	4	1	0.15
Bungoma	Kimilili	2.90	4	1	0.14
Bungoma	Chebich	2.94	4	1	0.19

Kapkateny cluster (UM1) in Bungoma County, Chakol Cluster (LM3) in Busia County, Matungu cluster (LM3) in kakamega County and then Malaba Cluster (LM3) in Busia County, all had mean severity of below 2.5. These shows that most symptoms displayed were leaf mosaic, chlorotic leaf spots, chlorotic spots, and necrotic leaf spot. On the other hand, Chwele cluster (LM1) in Bungoma County, Chebich cluster (UM1) in Bungoma County and Muhonje cluster (LM1) of Kakamega County among others had mean severity above 2.5 (Table 5).

4.1.3 Correlation between incidence and severity

There was a positive correlation ($r = 0.745$; $P < 0.001$) between mean incidence and mean severity on the data collected from selected clusters in the survey in western kenya during the long and short rain seasons. Chwele region had both highest mean incidence and severity, followed by Chebich region, and the Kimilili cluster

respectively. Kapkateny had both the lowest mean incidence/ severity. This showed that an increase in incidence correlated with an increase in severity (Table 6).

Table 6. Correlation between incidences and severity in surveyed clusters.

County	Cluster	Mean incidence	Max Incidence	Mean Severity	Max Severity
Bungoma	Chwele	**45.04**	**80**	2.99	4
Bungoma	Kapkateny	**17.75**	**60**	2.22	4
Bungoma	Kimalewa	**28.26**	**75**	2.65	4
Busia	Alupe	**37.83**	**68**	2.88	4
Busia	Chakol	**25.12**	**56**	2.38	4
Busia	Malaba	**25.61**	**80**	2.40	4
Kakamega	Mumias	**27.36**	**80**	2.65	4
Kakamega	Matungu	**25.67**	**70**	2.42	4
Kakamega	Muhonje	**35.72**	**75**	2.82	4
Bungoma	Kimilili	**39.75**	**75**	2.90	4
Bungoma	Chebich	**41.19**	**76**	2.94	4

4.1.4 Disease incidence and severity variations in rain seasons

Disease incidence and severity during short rain seasons varied from those of long rain seasons in all surveyed regions of western Kenya. In overall, short rain season recorded higher viral incidence and severity than the long rain seasons. Chwele Region had the highest disease incidence (55.08 %) during short rain seasons compared to 35.00 % of long rains seasons followed by Chebich region with disease incidence of 50.38 % during short rain season to its long rain incidence of 32.00 %. Kimilili was third with disease incidence of 48.50 % during short rain seasons and (31.00 %) for long rain seasons: Kapkateny had incidence of 25.50 % during short rains season compared to long rains season (13 .00 %). Disease severity also varied from long rain seasons to

short rain seasons in all clusters, like in disease incidence, Severity in short rain seasons, Chwele had the highest mean severity with 3.40 for short rain seasons and 2.46 for long rain seasons. Kapkateny had the lowest mean severity of 2.44 for short rain season and 2.00 for long rain seasons respectively (Figure.5).

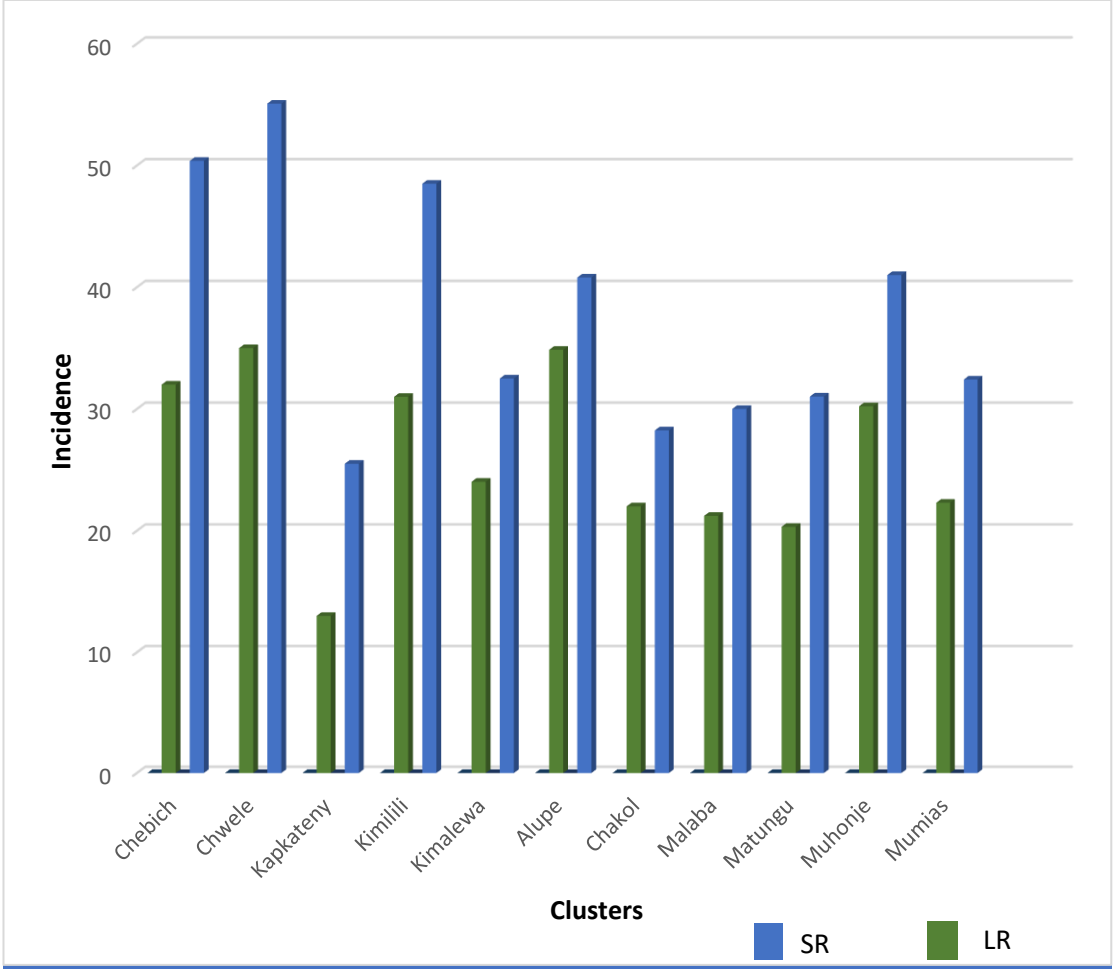


Figure 3: Graph for visual variation in viral incidence in surveyed regions during long and short rains seasons of western Kenya

4.1.5 Correlation of incidences to severity between rain seasons

The incidences and severity of GRSV between rain seasons showed positive correlation in incidences and severity. During short rains the incidences and severity was high with variation from region to region. Chwele had the highest GRSV incidence/Severity (55 %), (3.4), followed by Chebich with disease incidence and

severity 50.38 % and 3.00, respectively. During long rains, Chwele had disease incidences of 35 % and severity of 2.56 followed by Chebich with incidence of 32 % and severity of 2.52, (Table 7)

Table 7: GRSV incidence and severity for rain seasons in surveyed regions

County	Clusters	Seasons	N	Incidence %	Severity
Bungoma	**Chebich**	Long Rains	18	**32.00**	2.52
		Short Rains	13	**50.38**	3.00
	Chwele	Long rains	31	**35.00**	2.56
		Short rains	37	**55.08**	3.40
	Kapkateny	Long rains	26	**13.00**	2.00
		Short rains	20	**25.50**	2.44
	Kimilili	Long rains	18	**31.00**	2.14
		Short rains	22	**48.50**	3.00
	Kimalewa	Long rains	30	**24.02**	2.30
		Short rains	41	**32.50**	3.00
Busia	**Alupe**	Long rains	17	**34.86**	2.60
		Short rains	20	**40.80**	2.70
	Chakol	Long rains	22	**22.00**	2.00
		Short rains	20	**28.24**	2.62
	Malaba	Long rains	15	**21.22**	2.44
		Short rains	19	**30.00**	3.00
Kakamega	**Matungu**	Long rains	19	**20.32**	2.52
		Short rains	26	**31.02**	3.00
	Muhonje	Long rains	15	**30.22**	2.50
		Short rains	14	**41.00**	2.74
	Mumias	Long rains	17	**22.30**	2.00
		Short rains	29	**32.42**	2.80

4.1.6 Serological tests

The symptomatic leaf samples collected from the survey were subjected to serological tests by ELISA. Some leaf samples either tested positive for GRSV or TSWV while others tested positive for both viruses. Samples from Chwele had the highest number of samples (6 leaf samples) testing positive for GRSV followed by samples from

Kimalewa and Mutungu with two leave samples testing positives for the virus. However, samples from Chebich tested positive for TSWV. Sample collected from Kimilili region tested positive for both GRSV and TSWV (Table 8).

Table 8. ELISA tests for GRSV /TSWV on samples collected in W. Kenya

County	Cluster	Samples (N)	GRSV	TSWV	AEZs
Bungoma	Chwele	12	6(+)	_	LM1
Bungoma	Kapkateny	13	_	1(+)	UM1
Bungoma	Kimalewa	11	2(+)	_	LM1
Busia	Alupe	19	1(+)	_	LM2
Busia	Chakol	10	_	_	LM3
Busia	Malaba	6	_	_	LM3
Kakamega	Mumias	10	_	_	LM2
Bungoma	Kimilili	9	1(+)	1(+)	UM1
Kakamega	Muhonje	7	_	_	LM1
Bungoma	Matungu	11	2(+)	_	LM3
Bungoma	Chebich	10	_	4 (+)	UM1

4.1.7 Effect of GRSV on groundnut productivity

The groundnuts from western Kenya serologically tested positive for GRSV with symptoms; leave chlorosis, leaf mosaic, stunted growth, reduced stem height necrotic leaf spot, yielded less or no nuts compared to those testing negatives for the virus with non-symptomatic groundnuts in the same regions (Table. 9), (plate. 3).

Table 9. Effect of GRSV on groundnut productivity

County	Cluster	AEZs	N	Symptoms	ELISA	Yield Nuts/plant
Bungoma	**Chwele**	LM1	6	Leaf mosaic, reduced height	+	01
Bungoma	**Chwele**	LM1	6	No viral symptoms	_	11
Bungoma	**Kimalewa**	LM1	2	Leaf necrotic spots, ringspot, leaf mosaic	+	00
Bungoma	**Kimalewa**	LM1	6	No viral symptoms	_	10
Bungoma	**Kimilili**	UM1	1	Leaf mosaic	+	02
Bungoma	**Kimilili**	UM1	6	No viral symptoms	_	14
Busia	**Alupe**	LM2	1	Ringspots, Stunted growth, leaf mosaic, leaf chlorosis	+	00
Busia	**Alupe**	LM2	6	No viral symptoms	-	10
Kakamega	**Matungu**	LM3	2	Leaf mosaic, reduced height,	+	01
Kakamega	**Matungu**	LM3	6	No viral symptoms	_	12

Plate 3. Groundnuts plants obtained from Western Kenya in a survey showing the effect of GRSV on crop yields; a) infected groundnuts with GRSV having no nuts, b) Health groundnuts having nuts

4.2 Effect of intercropped legumes on GRSV incidences and severity on groundnuts in western Kenya

Typical viral symptoms on intercropped groundnut varieties (Red Valencia, ICGV 12991, and ICGV 90704) were; leaf chlorotic ringspots, leaf necrotic spot, stunted growth, leaf chlorosis, leaf mosaic, leaf deformation or a combination of all. In all treatments; (groundnuts + beans), (groundnuts + soybeans) and (Groundnuts + cowpeas), but with variant incidence. Stunted growth, yellowing leaf mosaic and leaf necrosis with reduced height, mainly observed in trials with Red Valencia + Soybeans, Red Valencia+ beans and Red Valencia+ Cowpeas (plate.3).

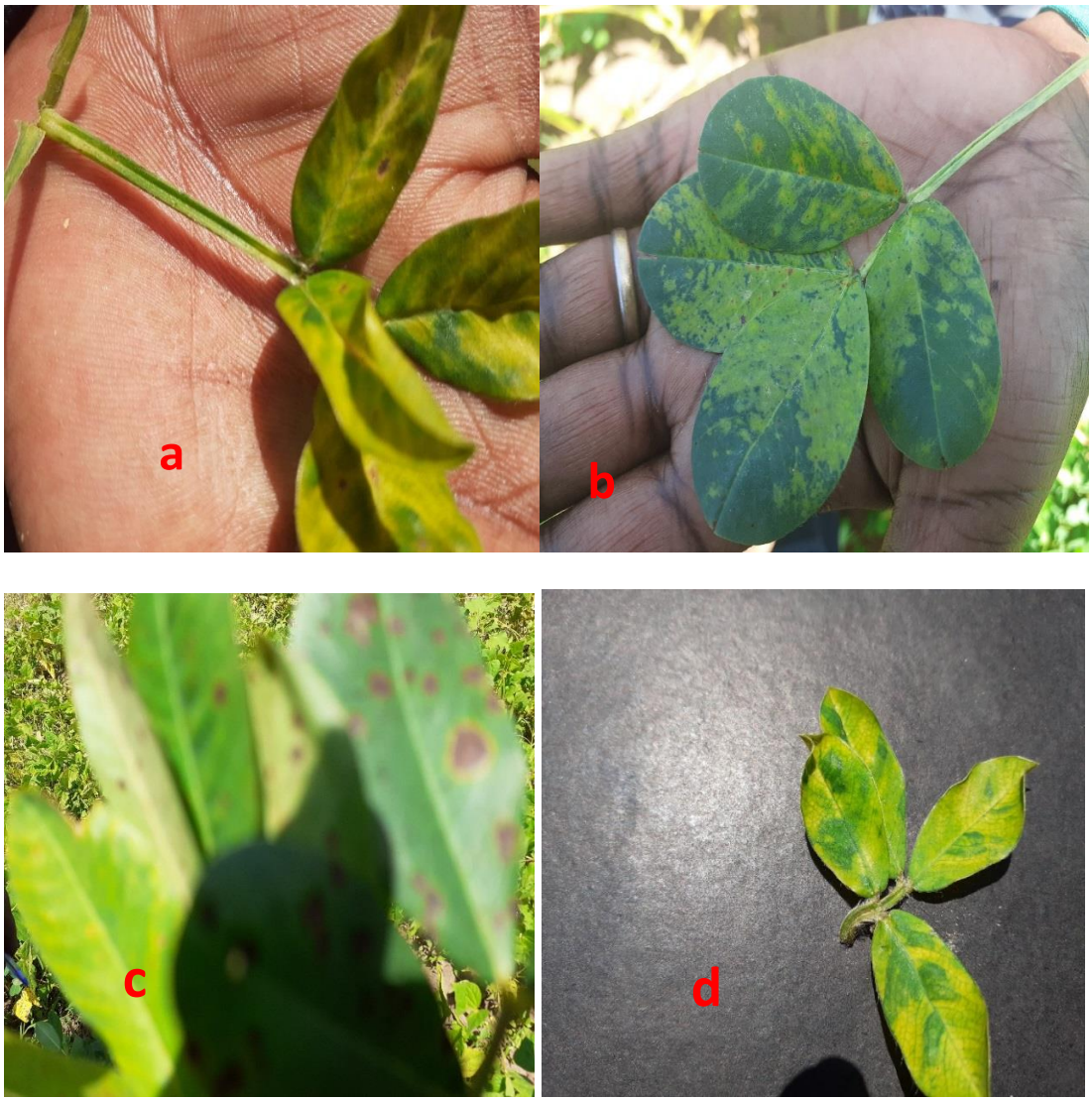


Plate 4. Intercropped groundnut varieties from Bungoma County in Chebich open field Experimental trial of western Kenya a) leaf from a Red Valencia groundnut intercropped with Soybeans showing necrotic spots and chlorosis leaf veins. b) Leaf of Red Valencia groundnut intercropped with soy beans showing leaf mosaic symptoms. c) Leaf of ICGV129991 groundnut intercropped with soy beans showing necrotic ringspot on leaves and leaf mosaic. d) Leaf of ICGV129991 groundnut variety displaying upward leaf curling, leaf chlorosis. These are typical symptoms for GRSV but serologically tested positive for TSWV

4.2.1 Viral incidences on intercropped groundnut varieties in western Kenya

Viral disease incidences varied from treatment to treatment although with no significant difference noted among varieties intercropped apart from those planted in pure stand (less than 0.05). Red Valencia intercropped with beans had the highest mean

incidence of (28 %) with maximum incidence of (80 %), followed by red Valencia intercropped with soy beans (27 %), ICGV-90704 intercropped with beans had viral incidence of (24 %) respectively. All groundnut varieties planted in pure stand had lower disease incidence compared to the same variety intercropped with other legumes. ICGV-12991 pure stand had the lowest mean incidence of (4 %) with a maximum incidence of (8 %). Followed by pure stand of ICGV-90704 groundnut variety with viral incidence of 6 % and lastly with pure stand of Red Valencia variety with viral incidence of 8 % (Table.10).

Table 10: Viral incidences on intercropped groundnuts varieties in western Kenya

Treatments	Mean Incidence (%)	Max incidence (%)	Min Incidence (%)	Std error
ICGV-12991 + Beans	13	40	0.00	3.99
ICGV-90704 + Beans	24	56	8	6.05
Red Valencia + Beans	28	80	0	7.08
ICGV-12991 + Soy beans	11	36	0.0	5.24
ICGV-90704 + Soy beans	17	24	0.0	3.32
Red Valencia + Soy beans	26	50	0	5.04
ICGV-12991 + Cowpeas	17	48	0	5.65
ICGV-90704 + Cowpeas	18	40	0	5.83

Red Valencia + Cowpeas	20	56	0	6.90
ICGV-12991 + (mixture of legumes)	8	32	24	4.00
ICGV-90704 + (mixture of legumes)	9	16	8	4.00
Red Valencia + (mixture of legumes)	11	24	0	7.06
ICGV-12991 Purestand	4	8	0	
ICGV-90704 Purestand	6	12	0	4.00
Red Valencia Purestand	8	16	8	0.00

4.2.2 Disease severity on intercropped groundnut varieties in western Kenya

Disease severity ranged from 1 to 4. The groundnut variety ICGV-90704 intercropped with Beans had the highest severity of (3.24) with a maximum severity of (4) followed by Red Valencia variety intercropped with soybeans having disease severity of (3.10). Red Valencia intercropped with beans had a mean severity of (2.90) while pure stand of all groundnut varieties had the lowest disease mean severity than those intercropped with other legumes. Pure stand of Red Valencia had the lowest mean severity (1), followed by pure stand of ICGV-12991groundnut variety with severity of 1.09 and lastly pure stand of ICGV-90704 variety with severity of 1.24 (Figure.8).

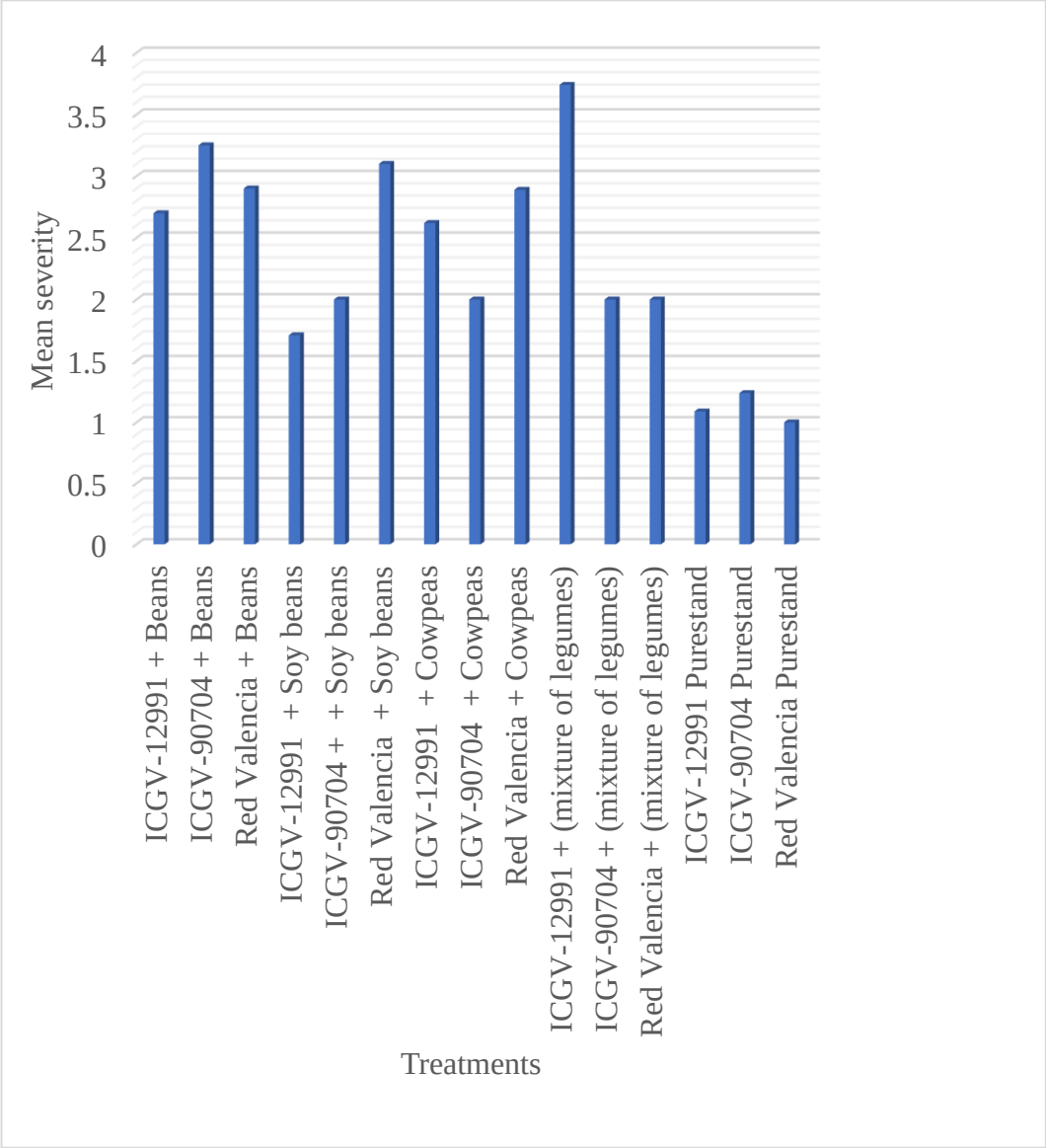


Figure 4: Graph showing disease mean severity on groundnuts in field trials on effect of other legumes on intercropped groundnut varieties from Alupe KALRO (Busia County), Chebich (Bungoma County), and Muhonje (Kakamega County) of western Kenya

4.3 Susceptibility of groundnut varieties to GRSV in Western Kenya

Nine groundnut varieties screened for resistance to GRSV showed variant symptoms; leaf mosaic, chlorotic leaf spots, necrotic leaf spots, chlorotic ringspots, reduced height and stunted growth with different incidences and severity for each variety screened for resistence levels. Homabay groundnut variety had the highest disease incidence of 42% with disease severity of 3.55, followed by ICGV-9991 variety with disease

incidence of 31 % and disease severity of 3. Groundnut varieties; ICGV-90704, SM99568 and ICGV-99019, displayed no disease symptoms. Groundnut SM99568 variety displayed no viral disease symptom but tested positive to GRSV. All control experimental plants (-CP) for each variety serologically tested negative for the virus with no viral symptom (Table 11), (Plate: 5).

Plate 5. Showing visual symptoms of screened groundnut varieties for resistance to GRSV in response to groundnut positive inoculum. a) CG7 groundnut variety with Leaf chlorosis, reduced height, stunted growth, b) ICGV-9991 groundnut variety with Leaf mosaic, reduced height and necrotic leaf spot c) Red Valencia variety with Chlorotic leaf spot, leaf chlorotic, leaf mosaic d) SM99568 groundnut variety with no disease symptom. e) ICGV-12991groundnut variety with Leaf chlorosis, leaf mosaic, necrotic leafspot. f) Homabay groundnut variety showing Chlorotic ringspot, stunted growth, leaf mosaic and leaf necrotic spots. These are GRSV symptoms of positive isolates collected from survey for inoculation.

Table 11. Screened groundnuts for resistance to GRSV in western Kenya

ID	Variety	Group	N	Incidence	Severity	Symptoms	ELISA
WKGV001	ICGV-12991	Bunch	9	16	1.8	Leaf chlorosis, leaf mosaic, necrotic leaf spot.	+
WKGV001CP	ICGV-12991	Bunch	3	0	1	Absence of viral disease symptoms	_
WKGV002	CG7	Runners	9	23	2.8	Leaf chlorosis, reduced height, stunted growth, chlorotic ringspot.	+
WKGV002CP	CG7	Runners	3	0	1	Absence of viral disease symptoms	_
WKGV003	ICGV-99019	Bunch	9	0	1	Absence of viral disease symptom	_
WKGV003CP	ICGV-99019	Bunch	3	0	1	Absence of viral disease symptom	_
WKGV004	ICGV-99048	Bunch	9	0	1	Absence of viral disease symptoms.	_
WKGV004CP	ICGV-99048	Bunch	3	0	1	Absence of viral disease symptoms.	_
WKGV006	SM99568	Bunch	9	0	1	Absence of viral disease symptoms.	+
WKGV006CP	SM99568	Bunch	3	0	1	Absence of viral disease symptoms.	_
WKGV007	ICGV-9991	Bunch	9	31	3	Leaf mosaic, reduced height, chlorotic leaf spot and necrotic leaf spot.	+
WKGV007CP	ICGV-9991	Bunch	3	0	1	Absence of viral disease symptoms	_
WKGV008	Red Valencia	Bunch	9	26	1.66	Chlorotic leaf spot,	+

						leaf chlorotic, leaf mosaic.	
WKGV008CP	Red Valencia	Bunch	3	0	1	Absence of viral disease symptoms	_
WKGV009	Homabay	Runners	9	42	3.55	Chlorotic ringspot, stunted growth, leaf mosaic, reduced height and leaf necrotic spots	+
WKGV009CP	Homabay	Runners	3	0	1	Absence of viral disease symptoms	_
WKGV011	ICGV-90704	Runners	9	0	1	Absence of viral disease symptoms	_
WKGV011CP	ICGV-90704	Runners	3	0	1	Absence of viral disease symptoms	_

Key. ID with CP are health control experiment for the variety.

4.3.1 Alternative hosts to GRSV in crops grown in western Kenya

Common crops grown legumes, brassicas and cucurbit in western Kenya; Bambara nut, beans, Cowpeas, black grams, green grams, cabbage, kales, butternuts. These plants showed symptoms of groundnut inoculum that were inoculated (plate. 6).

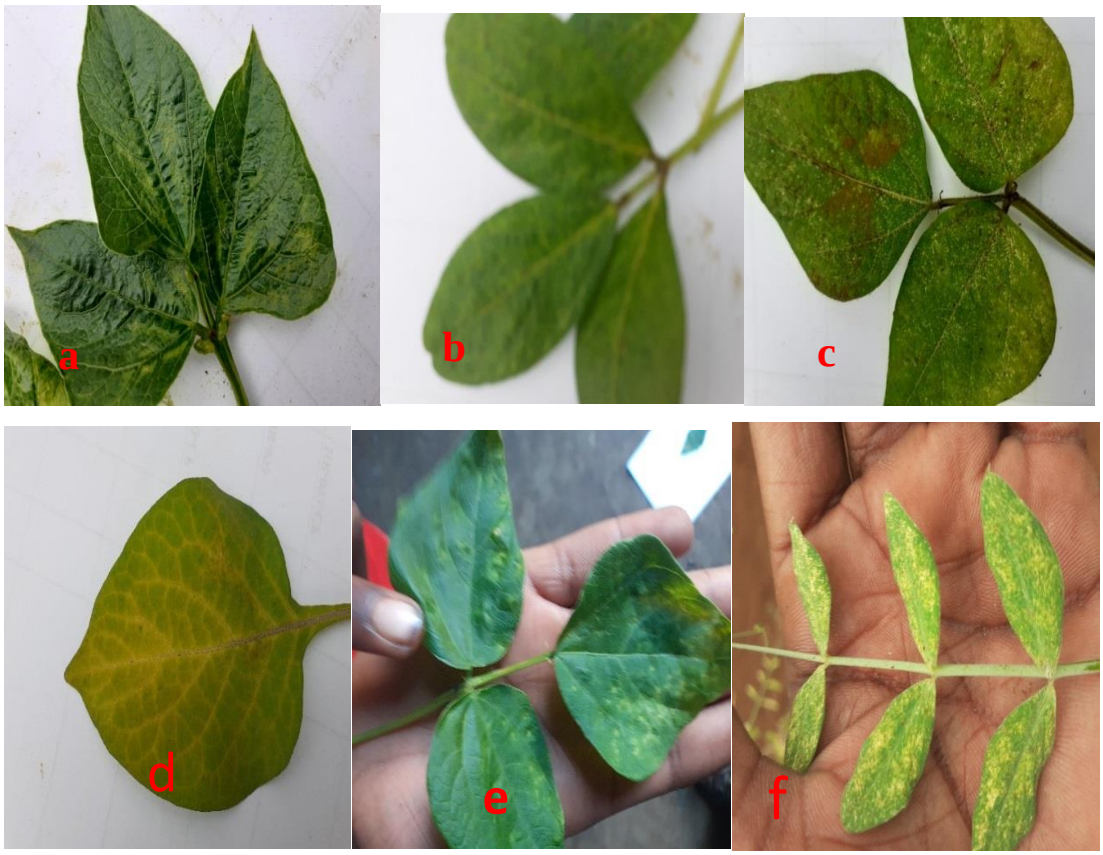


Plate 6. Crops leaves, screened for host range to GRSV. a) cowpea leave with leave mosaic and chlorotic leave spots, b) Bambara nut leaf with leaf mosaic and chlorotic leave veins, c) green gram leaf with chlorotic leaf spots, d) cabbage leaf with chlorotic leaf veins, e) bean leaf with chlorotic leaf spot and leaf mosaic and f) peas leaf with leaf mosaic, these are symptoms of groundnut positive isolates used for inoculation. These plants serologically tested positive for GRSV.

These plants exhibited variant response to GRSV groundnut inoculum after inoculated with positive isolates collected from a survey (Table 12).

Table 12. Response of Legumes and Brassicaceae crops to GRSV positive isolates

ID	Name	Family	N	Incidence %	Severity	Symptoms	ELISA
KHRL021	Pigeon peas	Leguminosae	9	14	2	Leaf mosaic, chlorotic leaf spot	+
KHRL021C	Pigeon peas	Leguminosae	3	14	1	No viral disease symptoms	_
KHRL022	Bambara nut	Leguminosae	9	28	3	Leaf mosaic, chlorotic leaf spot, leaf deformation, upward leaf curling	+
KHRL022C	Bambara nut	Leguminosae	3	28	1	No viral disease symptoms	_
KHRL023	Green gram	Leguminosae	9	0	1	No viral disease symptoms	_
KHRL024	Black gram	Leguminosae	9	0	1	No viral disease symptoms	_
KHRL024C	Black gram	Leguminosae	3	0	1	No viral disease symptoms	_
KHRL025	Peas	Leguminosae	9	43	3.2	Leaf mosaic, leaf chlorosis, necrotic leaf spot. Leaf vein chlorosis.	+
KHRL025C	Peas	Leguminosae	3	0	1	No viral disease symptoms	_
KHRL026	Cowpeas	Leguminosae	9	21	2.4	Leaf mosaic, chlorotic leaf spot, leaf curling and leaf chlorosis.	+
KHRL026C	Cowpeas	Leguminosae	3	0	1	No viral disease symptoms	_

KHRL027	Beans	Leguminosae	9	0	1	No viral disease symptoms.	_
KHRL027C	Beans	Leguminosae	3	0	1	No viral disease symptoms.	_
KHRL028	Cabbage	Brassicaceae	9	29	2.6	Leaf mosaic, leaf chlorosis, leaf vein chlorosis, necrotic leaf spots	+
KHRL028C	Cabbage	Brassicaceae	3	29	1	No viral disease symptoms	_
KHRL029	Butternut	Cucurbitaceae	9	40	3.4	Leaf mosaic, chlorotic ringspot, leaf vein chlorosis, necrotic leaf spot and leaf chlorosis	+
KHRL029C	Butternut	Cucurbitaceae	3	0	1	No viral disease symptoms	_
KHRL030	Kale	Brassicaceae	9	0	1	No viral disease symptoms.	_
KHRL030C	Kale	Brassicaceae	3	0	1	No viral disease symptoms.	_

4.3.2 Alternative hosts of broad-leafed weeds to GRSV in Western Kenya

Broad-leafed garden weeds commonly growing in farm gardens in western Kenya; goat weeds (*Ageratum conyzoides*), pigweed (*Amaranthus retroflexus*), wondering Jew (*Commelina bengalensis*), Sodom apples (*Calotropis procera*), black jack (*Biden Pilosa*), African black nightshade (*Solanum ptychanthum*), wild spinach (*Chenopodium album*), White nightshade (*Solanum americanum*), American burn weed (*Erechtites hieraciifolius*), double thorn (*Oxygonium sinuatam*), sweet potato

(Ipomoea batatas), Nile trumpet (*Markhamia lutea*) showed different response and symptoms on the groundnut positive isolates to GRSV (Table 13), (plate. 7) and (Figure. 5).

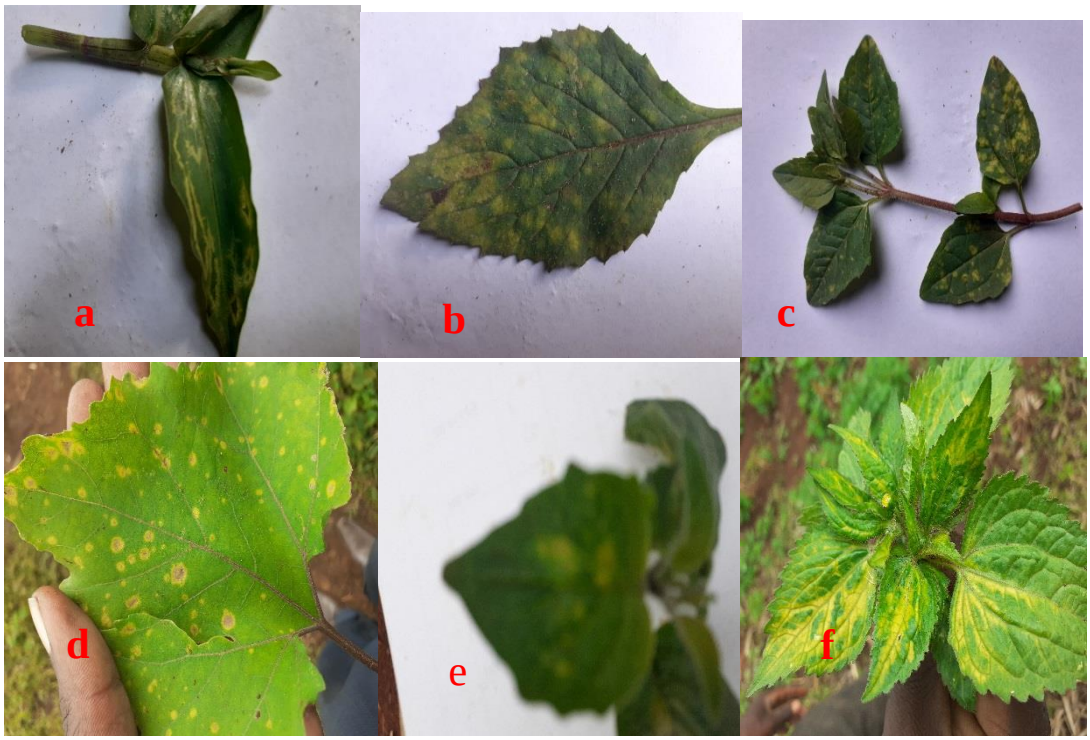


Plate 7. Screened broad-leafed weeds for GRSV host range tested serologically positive for GRSV indicating disease symptom for the virus. a), leaf of wondering Jew with chlorotic ringspots, b) A leaf of American burn weed showing leaf mosaic, c) a leaf of Sphaeranthus indicus with chlorotic spots, d) a leaf of Chenopotum album; with necrotic and chrolotic leaf spots, e) a leaf of solanum americanum with leaf mosaic and f) a leaf of Ageratum conyzoides with leaf chlorosis. The symptoms displayed by these plants was on groundnut innoclum used.

Table 13. Screened broad-leafed weeds for alternative hosts to GRSV

ID	Name	Family	N	Incidence %	Severity	Symptoms	ELISA
KHRW041	Achyanthes bidentate	Amaranthaceae	9	4,8	1	No viral disease symptoms	_
KHRW42	Amaranthus retroflexus	Amaranthaceae	9	26	2	Leaf mosaic,leaf chlorosis and necrotic leaf spot.	+
KHRW43	Bidens Pilosa	Asteraceae	9	5.5	1	No viral disease symptoms	_
KHRW44	American burn weed	Asteraceae	9	55	2.6	Leaf chlorosis, leaf mosaic, leaf deformatin and leaf curling	+
KHRW45	Commelina benghalensis	Commelinaceae	9	28	2	Leaf chlorosis, chlorotic ringspot, leaf mosaic.	+
KHRW46	Datura stramonium	Solanaceae	9	46	3.6	Leaf mosaic, leaf chlorosis, chlorotic leaf spot.	+

KHRW47	Chenopodium album	Amaranthaceae	9	34	3	Leaf necrosis, chlorotic leafspot, leaf deformation and leaf chlorosis.	+
KHRW48	Solanum incanum	Solanaceae	9	43	3.8	Leaf mosaic, leaf chlorosis, necrotic leaf spot.	+
KHRW49	Ageratum conyzoides	Asteraceae	9	0	1	No viral disease symptom	_
KHRW50	Oxygonium sinuatam	Polygonaceae	9	0	1	No viral disease symptoms	_
KHRW51	Solanum Americanum	Solanaceae	9	58	3.4	Leaf vein chlorosis, necrotic leaf spot and leaf deformation.	+
KHRW52	Solanum ptychanthum	Solanaceae	9	59	3.5	Leaf mosaic, leaf chlorosis, necrotic leaf spot.	+

KHRW53	Ipomoea batatas	Convolvulaceae	9	0	1	No viral disease symptoms.	_
KHRW54	Persea Americana	Lauraceae	9	0	1	No viral disease symptoms	_
KHRW55	Markhamia lutea	Bignoniaceae	9	0	1	No viral disease symptoms	_
KHRW56	Amaranthus rudis	Amaranthaceae	9	0	1	No viral disease symptoms	_
KHRW57	Ageratum conyzoides	Asteraceae		26	2.4	Chlorotic leaf spot, necrotic leaf spot	+
KHRW58	Galinsoga parviflora	Asteraceae	9	31	2.6	Chlorosis ringspot, leaf mosaic.	+

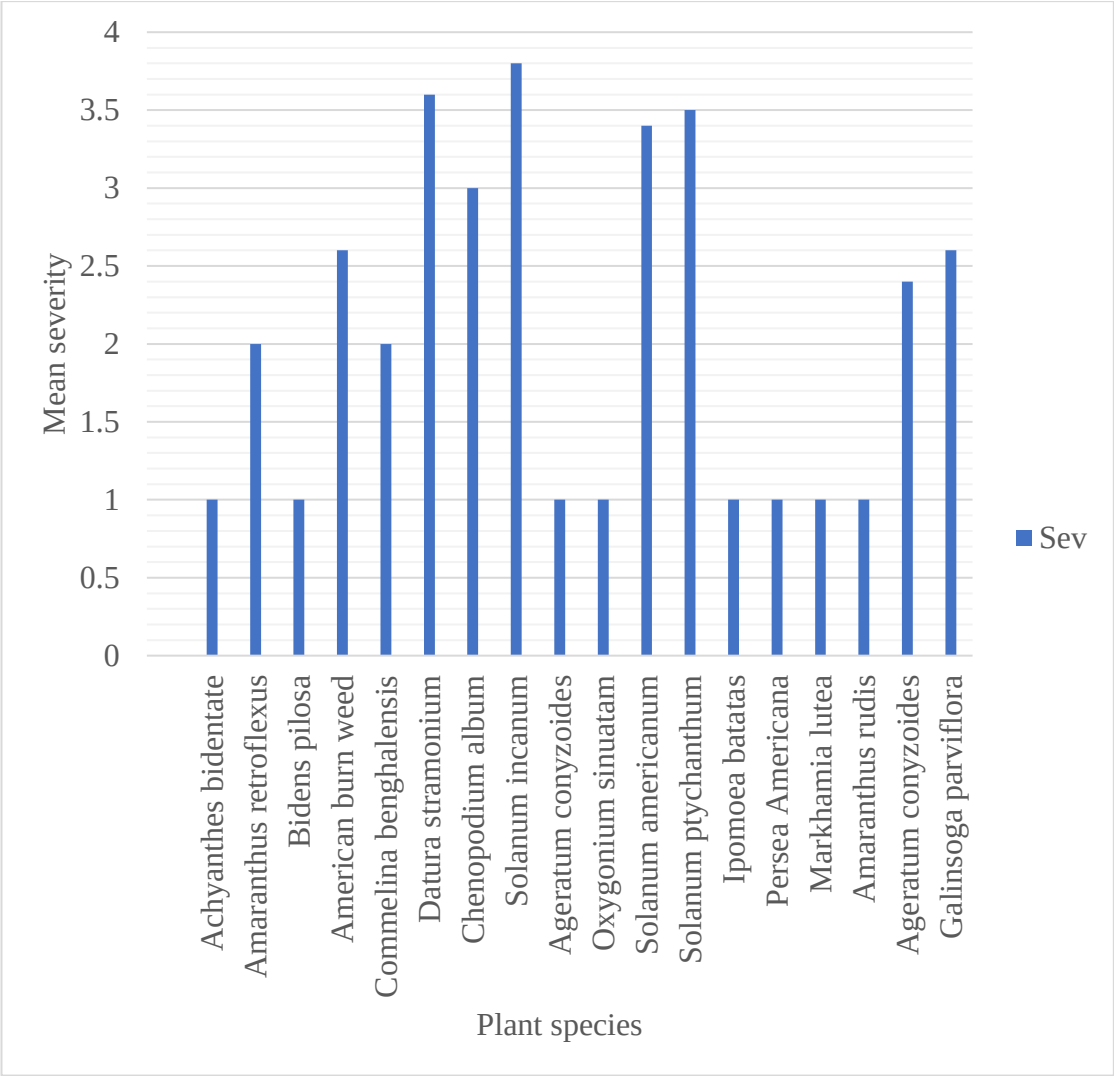


Figure 5: Graph showing variations in mean severity on screened broad-leafed weeds for host range to GRSV in western Kenya

4.3.3 Response of alternative host to groundnut GRSV positive isolates

Tomatoes, Watermelon, Soybeans and groundnuts responded differently to GRSV inoculum from groundnuts samples. The virus was more virulent to tomato plant than any other host plant screened for response to GRSV groundnut inoculum. Visual symptoms exhibited were chlorotic leaf spot, necrotic leaf spots and leaves with leave deformation, necrotic patches on stems and fruits were also noted. Viral symptoms noted on groundnuts and Soybeans was leaf mosaic and chlorotic leaf spots. Groundnuts than in soybeans. After inoculation, tomatoes exhibited disease symptoms

after three weeks; Leaf chlorosis, necrotic parches appeared both on leaves. Groundnuts displayed disease symptoms stem and fruits symptoms were more Disease incidence and severity were observed and recorded also symptom variation and development were recorded progressively (plate.8), (Table.14) and (Figure .6).

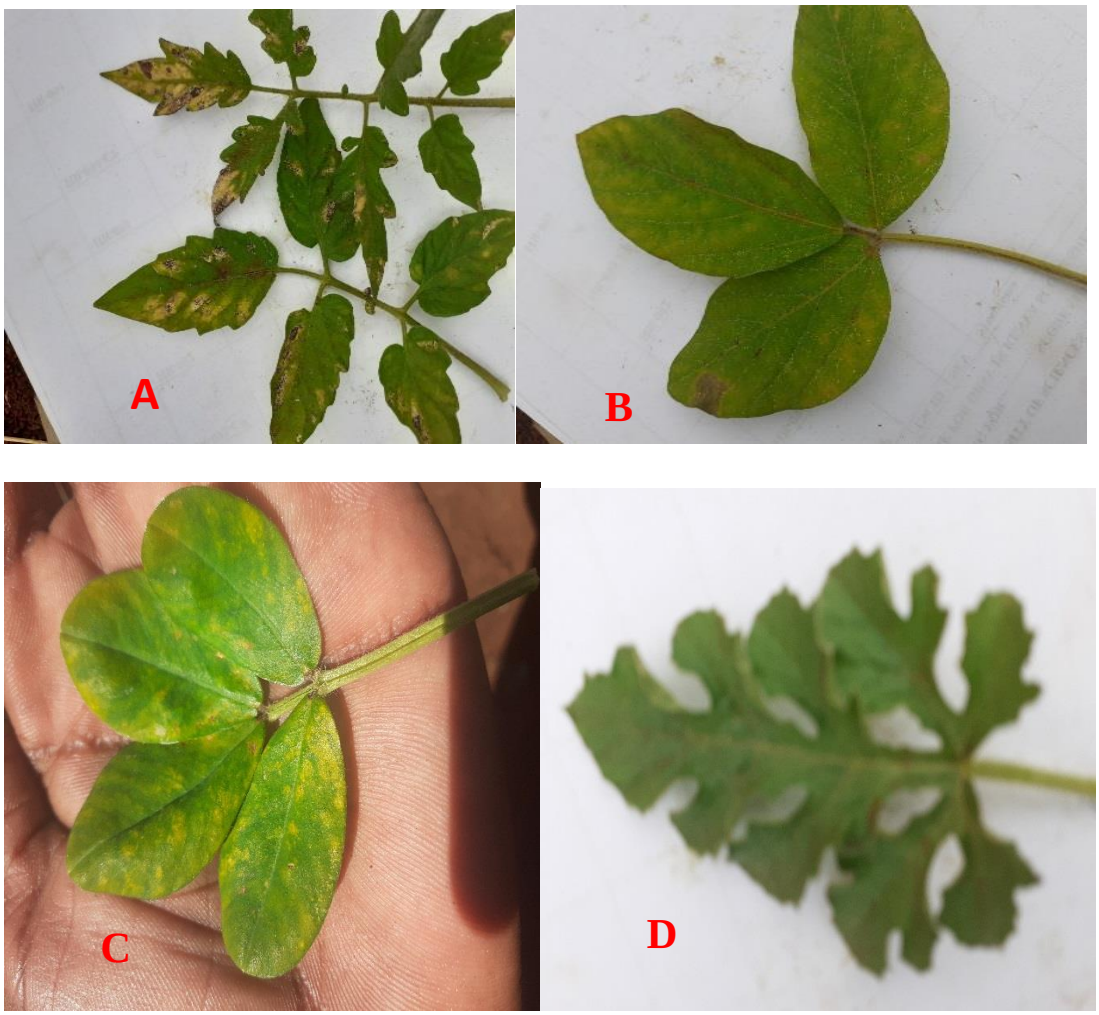


Plate 8. Showing visual symptoms of screened host plants to GRSV in response to groundnut positive inoculum isolates. A) a leaf from tomato plant with necrotic and chlorotic patches, B) a leaf from Soy bean with leaf mosaic and necrotic leaf spot, C) a leaf from groundnut with leaf mosaic and chlorotic leaf spots and D) a leaf from watermelon with no disease symptoms.

Table 14: Symptomatic, ELISA tests and scores for alternative hosts to GRSV

ID	Species	Family	N	Incidence	Severity	Symptoms	ELISA
HR91	Tomato	Solanaceae	6	80	3.8	Leaf chlorosis, upward leaf curling, necrotic sperks on stems	+
HR91C	Tomato	Solanaceae	3	0	1	Absence of viral disease symptoms	_
HR92	Watermelon	Curcurbitaceae	6	0	1	Absence of viral disease symptoms	+
HR92C	Watermelon	Curcurbitaceae	3	0	1	Absence of viral disease symptoms	_
HR93	Soy beans	Leguminosae	6	32	2.4	Necrotic leaf spot, leaf mosaic	+
HR93C	Soy beans	Leguminosae	3	0	1	Absence of viral disease symptoms	_
HR94	Groundnuts	Leguminosae	6	64	3.2	Chlorotic ringspot,necrotic spots,leaf mosaic, reduced height.	+
HR94C	Groundnuts	Leguminosae	3	0	1	Absence of viral disease symptoms	_

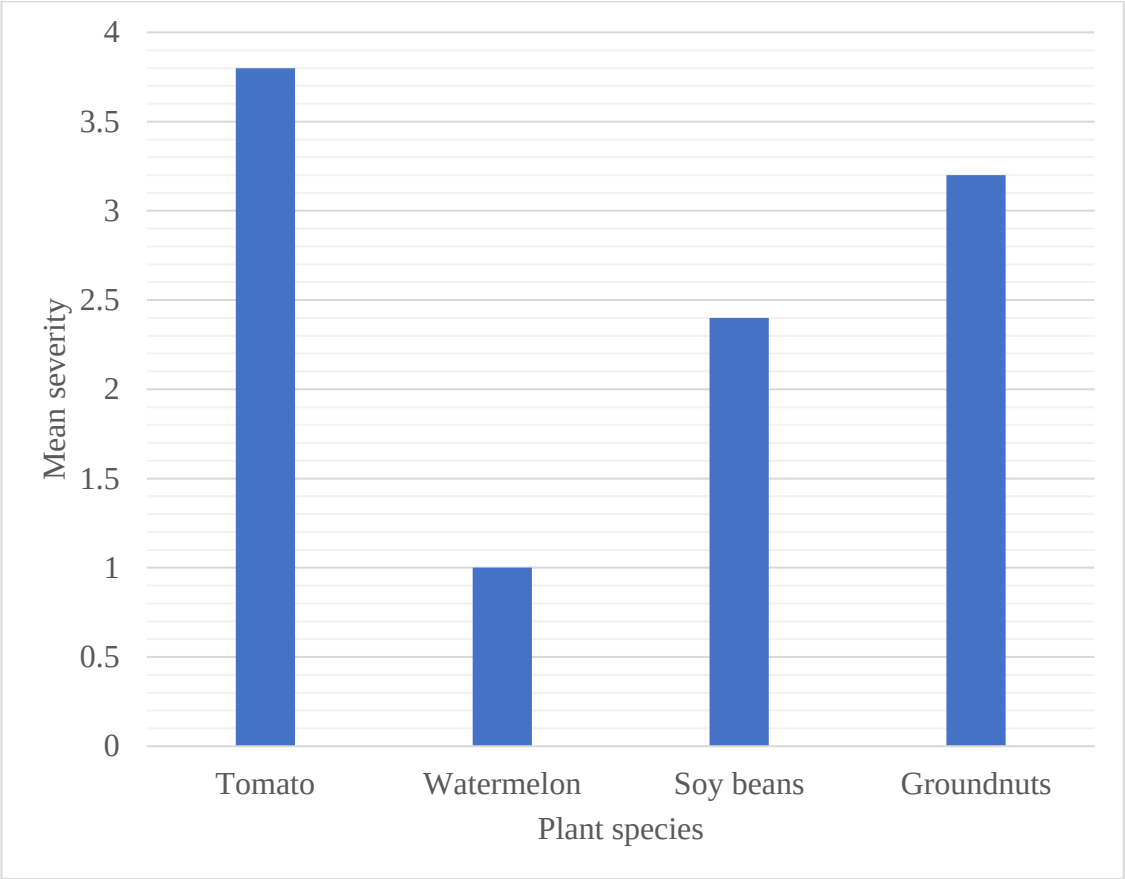


Figure 6: Graph of variations in severity in screened host plants in response to GRSV to positive isolates inoculum from serologically tested positive isolates of groundnuts in western Kenya.

4.4 Detection of GRSV in leaf samples by RT-PCR

Leaf samples of symptomatic ELISA positive collected during the survey in Bungoma, Busia and Kakamega Counties of western Kenya, tested by RT-PCR to detect GRSV using target GRSV primers. Showed, six samples tested positive for GRSV (Plate.9).

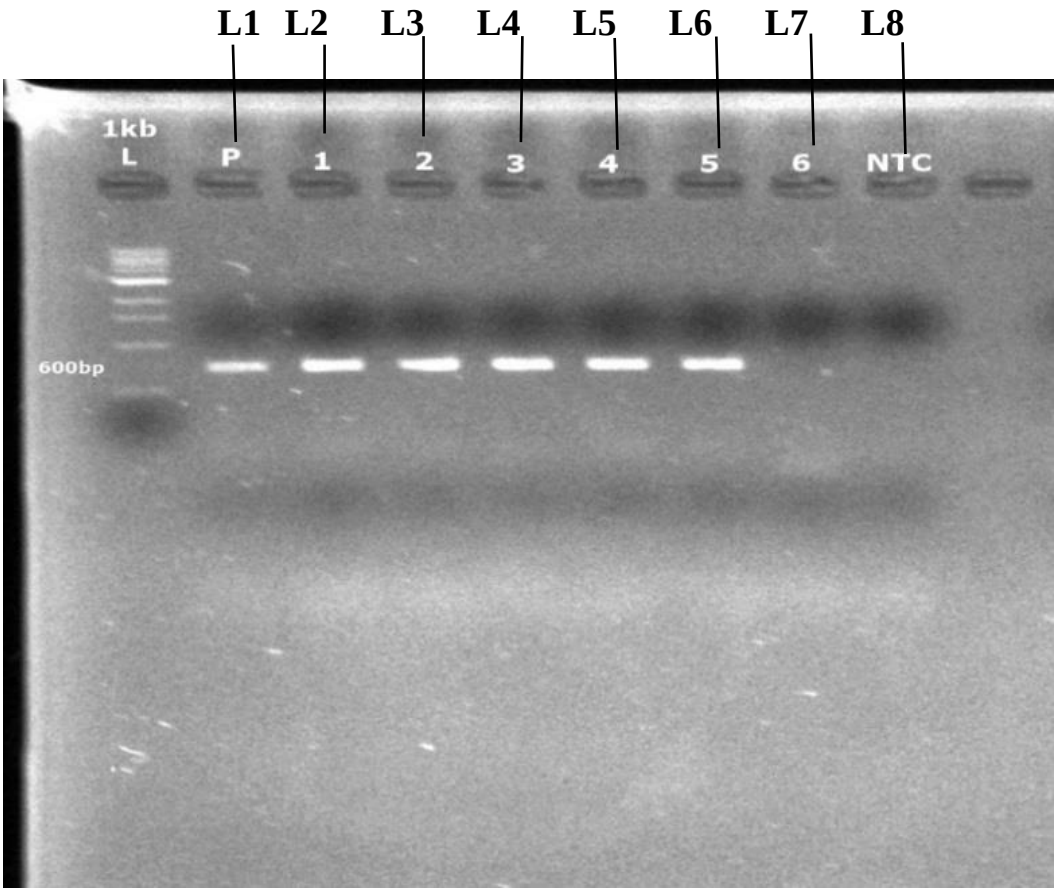


Plate 9: Gel electrophoresis of RT-PCR amplified RNA using primers specific for GRSV for symptomatic samples collected in a survey conducted in western Kenya. Expected band size was 600 bp. Lane L- 1 kb ladder, L1- positive control, L2- GRSV-KE1 groundnut sample from Chwele with stunted growth with necrotic leaf spot, L3- GRSV-KE2 groundnut sample from Alupe with leaf mosaic, L4- GRSV-KE3 groundnut sample from Chwele L5- GRSV-KE4 groundnut sample from Matungu L6- GRSV-KE5 groundnut sample from Kimalewa with leaf mosaic and necrotic leafspots L7- GRSV-KE6 groundnut sample from Chwele with chlorotic leafspots L8- Negative control

4.4.1 Diversity of Kenyan GRSV isolates nucleoproteins

The Six Kenyan GRSV isolates nucleoprotein(N) gene sequences (600 bp) was compared with GRSV isolates nucleoproteins (N)/nucleocapsid proteins gene sequences available in GenBank from other countries. The comparison revealed that Kenyan isolates had 96.13 to 99.98 % identity with GRSV isolates available in GenBank. Kenyan Isolates; GRSV-KE1 with accession number (LC616779), GRSV-KE2 (LC616780), GRSV-KE3 (LC616781), GRSV-KE4 (LC616782), GRSV-KE5 (LC616783) and GRSV-KE6 (LC616784) had very close percentage identity with isolates from different alternatives of various counties. Brazilian soybean Isolate LEM (MH686229.1) had closest identity of 99.93% followed by USA infecting insect isolate +GRSV (HQ634665.1) had identity of 99.82 %. Brazilian Pisum sativum isolate (ER1) (KY778230.1) had identity of 99.30 %. Tomato isolate (SA-05) of accession number (MH742958.1) from South Africa had identity of 99.28 %. Groundnut isolates from South Africa (SA-05) (Accession number S54327.1) and Ghana (GRSV-N-Gh) of accession number KT345728.1 had identity of 97.56 and 98.02 % respectively with Kenyan isolates. Other host plants with close identity with Kenyan isolates; watermelon isolate (GRSV leaves) (MN364668.1) from Brazil had identity of 96.89 %, Solanum americanum isolate (11.102) of accession number KM007024 from USA had identity of 96.13 % and Glycine max isolate (S30) of accession number MG029625 from Brazil had identity of 96.62 %. In general, all western Kenya isolates exhibited close identity and grouped together with some isolates, from Argentina, Brazil, Ghana, USA, South Africa of all tested alternative host (Table 15).

Table 15: Kenyan GRSV isolates nucleoproteins in comparison with isolates of other countries in GenBank

Description	Scientific name	Host plant	Country	Query cover %	E Value	Per Ident %	Acc Les	Accession number
Groundnut ringspot virus isolate	GRSV	Tomato	S. Africa	98	0.0	99.28	3038	**MH742958.1**
Groundnut ringspot virus isolate	GRSV	Peanut	Argentina	90	0.0	96.38	777	**MT423636.1**
Groundnut ringspot virus isolate	GRSV	Peanut	Argentina	90	0.0	96.38	777	**MT423645.1**
Groundnut ringspot virus isolate	GRSV	Peanut	Argentina	90	0.0	97.40	777	**MT423626.1**
Groundnut ringspot virus isolate	GRSV	Watermelon	Brazil	98	0.0	96.89	3074	**MN364668.1**
Groundnut ringspot virus isolate	GRSV	Peanut	Brazil	98	0.0	97.04	3069	**KY400110.1**
Groundnut ringspot virus isolate	GRSV	Solanum Americanum	USA	76	0.0	96.13	542	**KM007024.1**
Groundnut ringspot virus isolate	GRSV	Soybean	Brazil	97	0.0	99.93	3040	**MH686229.1**
Groundnut ringspot virus isolate	GRSV	Peanut	Brazil	76	0.0	96.93	522	**KF511798.1**
Groundnut ringspot virus isolate	GRSV	Pisum sativum	Brazil	78	0.0	99.30	557	**KY778230.1**
Groundnut ringspot orthotospovirus isolate	GRSTV	Glycine max	Brazil	81	0.0	96.62	562	**MG029625.1**
Groundnut ringspot virus isolate	GRSV	Thrips	Brazil	99	0.0	97.04	3074	**MG797643.1**
Groundnut ringspot virus isolate	GRSV	Soybean	S. Africa	98	0.0	98.67	857	**AF487516.1**
Groundnut ringspot virus isolate	GRSV	Soybean	S. Africa	98	0.0	98.37	857	**AF487517.1**
Groundnut ringspot virus isolate	GRSV	Peanut	Ghana	88	0.0	98.02	768	**KT345728.1**

Groundnut ringspot virus isolate	GRSV	Groundnut	S. Africa	98	0.0	97.56	928	**S54327.1**
Groundnut ringspot virus isolate	GRSV	Groundnut	Kenya		0.0	99.99	6041	**LC616779** ★
Groundnut ringspot virus isolate	GRSV	Groundnut	Kenya		0.0	99.98	6041	**LC616781** ★
Groundnut ringspot virus isolate	GRSV	Groundnut	Kenya		0.0	99.98	6041	**LC616782** ★
Groundnut ringspot virus isolate	GRSV	Groundnut	Kenya		0.0	99.9	6041	**LC616780** ★
Groundnut ringspot virus isolate	GRSV	Infecting insect	USA	83	0.0	99.82	569	**HQ634665.1**
Groundnut ringspot virus isolate	GRSV	Groundnut	Kenya		0.0	99.82	6041	**LC616784** ★
Groundnut ringspot virus isolate	GRSV	Groundnut	Kenya		0.0	100	6041	**LC616783** ★

Table14: showing accession number of GRSV isolates nucleoproteins in GenBank from different countries and sources with identity percentage to Kenyan isolates. Kenyan isolates accession numbers are flagged with a star.

4.4.2 Phylogenetic analysis of Kenyan isolates

The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei., 1993). Groundnut rosette assistor virus (LC480463.1) was used as an out-group that gave a better rooting stability than the other possible Tospoviruses which could have give very close similarities since are of the same genus; Tomato chlorotic spot virus (TCSV), Irish yellow spot virus (IYSV), Tomato spotted wilt virus (TSWV), Impatians necrotic spot virus (INSV). Phylogenetic tree constructed in MEGA- X for evolutionary comparison revealed the clustering of the six Kenyan isolates forming two groups with other isolates available

in GenBank. Kenyan isolates have a common ancestral origin with isolates from South Africa, Ghana, Brazil and USA. GRSV-KE5 (LC616783) and GRSV-KE6 (LC616784) had most recent evolutionary origin with USA isolate (HQ634665) having closest clustering with 99.82 % identity. The comparison reveals that Kenyan isolates GRSV-KE1 (LC616779) and, GRSV-KE3 (LC616781) are monophyletic (have same recent evolutionary origin on phylogenetic tree) with 100 % identity. GRSV-KE2 (LC616780) and GRSV- KE4 (LC616782) originate from the same node on the phylogenic tree with 100 % identity. Kenyan isolate GRSV-KE6 (LC616784) , Brazilian Isolate with accession number MG797643.1 and Ghanian isolate of accession number KT345728.1 are monophyletic (have same recent evolutionary origin on phylogenetic tree) with 97.04 % similar identity. Kenyan groundnut isolates GRSV-KE4 (LC616782), GRSV-KE3 (LC616781) and GRSV-KE1 (LC616779) showed monophyletic relationship with soybeans isolates (AF487516.1), (AF487517.1) and groundnut isolates SA (S543227.1) of South Africa and Peanut isolate (KT345728.1) from Ghana with identity of 98.67%, 98.37%, 97.56% and 98.02% respectively. Kenyan isolates had paraphyletic relationship with Brazilian isolates; peanut isolate (KF511778.1), Pisum sative isolate (KY778232.1), Glycine max isolate (MG029625.1) and Thrips isolate (MG797643.1) with identity of 96.93%, 99.31%, 96.62% and 97.04 respectively. Although the Kenyan isolates had common ancestral origin with some isolates available in GenBank, formed divergent cluster with the following isolates; tomato isolate (MH742958.1) of South African with identity 99.28%, Brazilian watermelon isolate (MN364668.1) with identity of 96.89 %, USA Solanum americanum isolate (KM007024) with identity of 96.13 %. and Brazilian soybeans isolate (KY400110.1) with identity of 99.93 %. Some isolates of different species available in GenBank also exhibited divergent from Argentinian

peanut isolates; MT423639.1, MT423642.1, MT423626.1, all having identity of 96.38 and MT423626.1 with identity of 97.40 %. In general, all western Kenya isolates exhibited closest identity and grouped together with some South African, Brazilian, Ghanaian isolates (Figure.7) and (plate.10).

4.4.3 Phylogenetic tree of Kenyan isolates

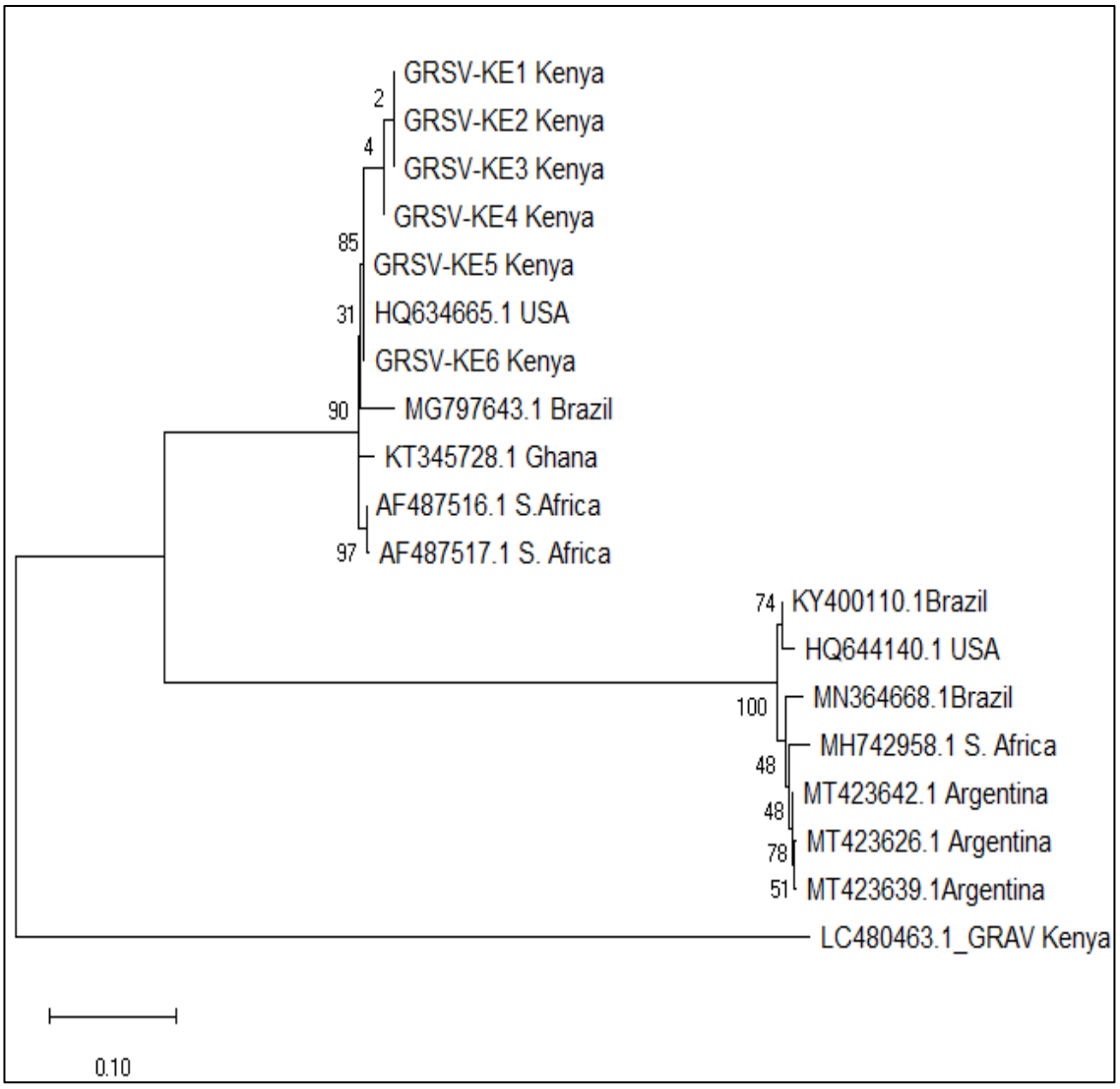


Figure 7. Phylogenetic analysis of six GRSV Kenyan isolates and some GRSV isolates from GeneBank. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [Tamura and Nei, 1993]. The percentage of trees in which the associated taxa clustered together is shown next to the branches (1000 replications). Evolutionary analyses were conducted in MEGA X [Kumar et al., 2018]. LC480463.1 (Groundnut rosette assistor virus - Kenya) was used as an outgroup

4.5 Gel Electrophoresis of New designed primers from Kenyan GRSV sequences

The PCR Master Mix of 25µl of new designed primers GRSV4 F (5" ACCAGAACCAGGTTGCATTC 3") for forward reaction and GRSV4R (5" ATCGTGACCTTGCCAAAAGT 3") for reverse reaction formed clear band with GRSV positive samples collected from western Kenya (plate 3).

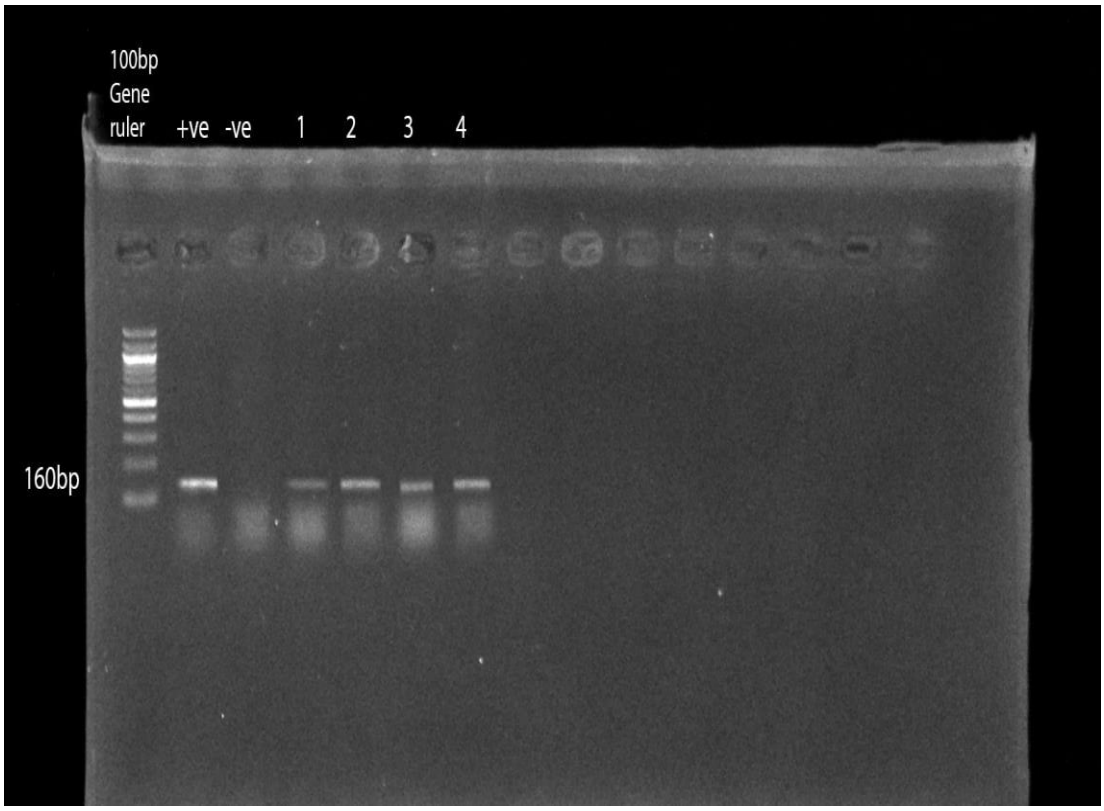


Plate 10: Gel electrophoresis of RT-PCR amplified RNA using designed validated primer for GRSV on positive samples collected in a survey conducted in western Kenya. Expected band size was 160 bp. Lane L- 100bp gene ruler, +Ve - positive control, -Ve -Negative control L1- GRSV-KE1 groundnut sample from Chwele with stunted growth with necrotic leaf spot, L2- GRSV-KE2 groundnut sample from Alupe with leaf mosaic, L3- GRSV-KE3 groundnut sample from Chwele L4- GRSV-KE4 groundnut sample from Matungu

4.5.1 Validation of Developed RT-PCR primers

The PCR Master mix of 25µl of commercial standard primers GRSVnF (5'TCTTGTGCATCATCCATTGT-3') for forward reaction and, GRSVnR (5'GCGGTCTACAGTGTTGCACTT-3') for reverse reaction formed clear bands with all serologically tested positive samples collected in western Kenya to check the validity of designed primers in this study (Plate 11).

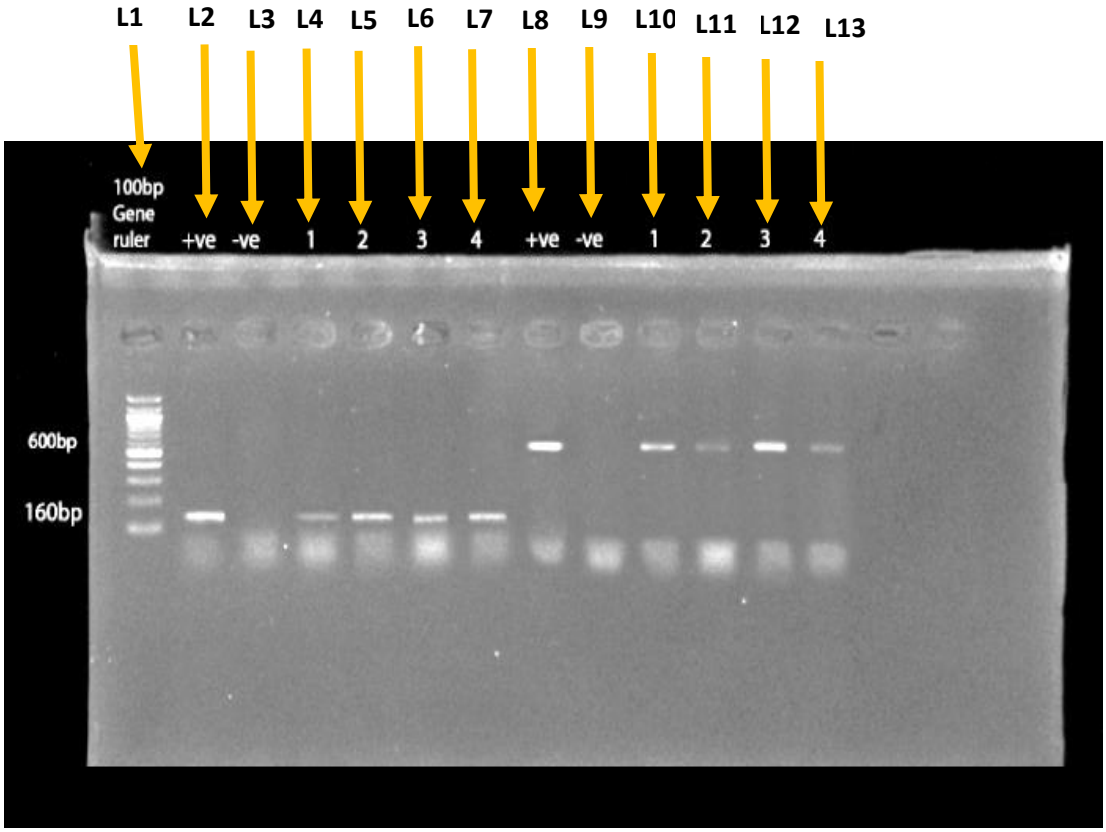


Plate 11: Gel electrophoresis of RT-PCR amplified RNA using Commercial standard primer GRSV4F (5' ACCAGAACCAGGTTGCATTC -3') and GRSV4R (5' ATCGTGACCTTGCCAAAAGT-3') as control experiment on designed primers for GRSV on positive samples collected in a survey conducted in western Kenya. And checked with GRSV target primer GRSVnF (5'TCTTGTGCATCATCCATTGT-3') and, GRSVnR (5'GCGGTCTACAGTGTTGCACTT-3'). Expected band size was 160 bp. Lane L1- 100bp gene ruler,L2- +Ve positive control,L3 -Ve -Negative control L4- GRSV-KE1 groundnut sample from Chwele with stunted growth with necrotic leaf spot, L5- GRSV-KE2 groundnut sample from Alupe with leaf mosaic, L6- GRSV-KE3 groundnut sample from Chwele L7- GRSV-KE4 groundnut sample from Matungu , L8- +Ve positive control, L9- -Ve Negative control, L10- KE1 Isolate using GRSV target primer, L11- KE2 isolate using GRSV target primer, L12- KE3 Isolate using target primer and L13- KE4 Isolate using GRSV target primer.

4.5.2 Diversity of New Developed GRSV primers for RT-PCR

Designed primer GRSV4_R (5" ATCGTGACCTTGCCAAAAGT 3") of product size of 160 bp with Temp. 59.6 ^{0}C. correlated 100 % query cover and 100 % identities with nucleoproteins/ nucleocapsid proteins (N) gene of isolates of accession numbers in GenBank; (MH742958.1) a whole genome of groundnut ringspot orthotospovirus isolate S4 nucleocapsid protein (N) mRNA of query length 567, (MT423646.1) complete nucleic acid genome of groundnut ringspot isolate Villa-Ascasubi nucleoprotein(N) gene of query length 777, (MT423643.1) a complete genome groundnut ringspot virus isolate general-Fotheringham nucleoprotein(N) gene of nucleic acid of query length 777, (MT423636.1) a complete genome of groundnut ringspotvirus isolate Gigena nucleoprotein (N) gene of nucleic acid of query length 777, (MT423634.1) a complete genome of groundnut ringspot virus isolate Manfredi-B nucleoprotein(N) gene of nucleic acid of query length 777, (MT423633.1) a complete genome of groundnut virus isolate General-Deheza-C nucleoprotein (N) gene of nucleic acid of query length 777, (MT423631.1) a complete genome of groundnut ringspot virus isolate Rio-Cuarto-A nucleoprotein (N) gene of nucleic acid of query length 777, (MT423630.1) a complete genome of groundnut virus isolate General-Deheza-C nucleoprotein (N) gene of nucleic acid of query length 777 , (KT345728.1) a complete genome of groundnut ringspot virus isolate GRSV-N-Gh nucleocapsid protein (N) gene of nucleic acid of query length 768, (HQ634665.1) a complete genome of groundnut ringspot virus isolate +GRSV nucleocapsid protein(N) gene of partial compound of nucleic acid of query length 569 , (DQ973171.1) a complete genome of groundnut ringspot virus from Argentina nucleocapsid protein (N) gene of nucleic acid of query length 614 and (AF487517.1) a complete genome of

groundnut ringspot virus isolate 95/0137 nucleoprotein (N) gene of nucleic acid of query length 857.

The primer designed GRSV4F (5" GACCAGAACCAGGTTGCATT 3") with product size of 161 bp and Temp. of 60 ^{0}C. correlated 100 % query cover and 100 % identities with nucleoproteins /nucleocapsid proteins (N) gene of isolates of accession numbers in the GenBank; (MW467981.1) a complete genome of groundnut ringspot orthotospovirus nucleocapsid protein (N) gene of nucleic acid of query length 441, (KY778230.1) a complete genome of groundnut ringspot virus isolate ERI nucleocapsid protein gene of nucleic acid of query length 557, (MT215224.2) a complete genome of groundnut ringspot orthotospovirus isolates 3 nucleocapsid protein (N) gene of nucleic acid of query length 432, (MT215222.2) a complete genome of groundnut ringspot orthospovirus isolate 1 nucleocapsid protein (N) gene of nucleic acid of query length 432, (HQ634665.1) a complete genome of groundnut ringspot virus +GRSV nucleocapsid protein (N) gene of nucleic acid of query length 569, (S54327.1) a complete genome of nucleoprotein (S RNA) groundnut ringspot virus isolate SA-05, genomic RNA nucleic acid of query length 928 and (MH686229.1) a complete genome of groundnut ringspot virus isolate LEM segment S sequences of nucleic acid of query length 3040.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussions

This study revealed occurrence of GRSV in groundnut growing regions of western Kenya lowering productivity of groundnuts. Groundnut ringspot virus is distributed in Bungoma, Busia and Kakamega counties of western Kenya with variant incidence and severity. Typical symptoms of GRSV as described in section 2.10 (Pappu *et al*., 2009) was observed in all surveyed regions and its occurrence confirmed by Serological and molecular tests (Adkins *et al*., 2002). This is the first report in Kenya about the occurrence and distribution of GRSV (Murere *et al*., 2022) and 3rd country in Africa behind Ghana and South Africa where GRSV has been reported (Murere *et al*.,2022). Although these symptoms had been noted on host plants since 19th century (Pittman, 1927), very little has been done in African countries to study on the occurrence of GRSV. This may be due to high cost or lack of modern facilities involved in serological and molecular tests (Rubio, 2020). This implies that the virus may have lasted longer period infecting host plants and lowering their productivity without good information about its occurrence and management strategies (Islam *et al*., 2017). Although in some regions, groundnut plants displayed typical GRSV/TSWV symptoms but serologically their samples tested negative for the viruses, this implies that symptoms displayed may have been from other viruses that have same symptoms infecting groundnuts in these regions (Zongo *et al*., 2018). The difference between incidences and severity in all agro ecological zones surveyed in western Kenya could imply that, GRSV inoculum level differ due to differences in geographical and climatic conditions (Andre *et al*., 2018) or the virus detected serologically could be

having a wide host range imply that the virus could be existing in areas where groundnut is not grown (Fermin *et al*., 2018).

It was observed that in Kimilili region, GRSV and TSWV co- infect groundnuts. This may indicate the existence of GRSV and TSWV transmitting vectors in the region which transmitted RNA stains of GRSV and TSWV into host plants (Okuda *et al*., 2003). Reassortment of GRSV and TSWV genetic material into a host plant may have resulted into multiple infection (Vijaykrishna *et al*., 2015; Dietzgen *et al*., 2016). Disease incidence and severity had positive direction of linear relationship ($r = 0.559$, $p < 0.05$) with moderate magnitude of association, implying that severity increased dependently with viral incidence (Chuang *et al*., 1987), which is a characteristic of non- seed borne viruses where GRSV and TSWV belong (Gallitelli, 2000). This may imply that the source of infection in most farms was from other host plants transmitted by (vectors) thrips (Cardoso *et al*., 2003). Therefore, disease symptom development occurred progressively with time and disease became more severe at later stage of growth thus serving as source of inoculum in the field for transmission (Hanssen *et al.*, 2011), leading to late infection of plants thus moderate viral incidence and reduced built up of viral titre which led to moderate severity of the disease (Takahashi *et al*., 2019).

Disease incidence and severity was high during short rain seasons compared to long rain season. This may have been due to population intensity of thrips (vectors) which had reduced in number during long rain seasons, due to heavy rains which may have washed them out of the host plants (groundnut) thus minimizing their activities in transmission of GRSV to host plants (Agneroh *et al*., 2012). In short rain seasons, abiotic factors were conducive for vectors to multiply and carry on their activities effectively thus transmission of GRSV was high which resulted into high disease

incidence than long rain seasons (Kone *et al*., 2017), which might have resulted into thrips hibernating due to heavy and long rain period thus transmission being lowered and disease pressure being reduced on host plants resulting into low disease incidence and severity (Saroj *et al*., 2019; Karavina *et al*., 2017).

Field trials on effect of other legumes on incidences and severity of GRSV on groundnuts revealed that intercropping other legumes that are alternative host to the virus with groundnuts increases disease pressure on groundnuts than having pure stand which is contrary to the finding of (Ratnadass *et al*., 2012). Groundnuts intercropped with cowpeas had high disease incidence and severity than those of same varieties planted in pure stand, which agree with the findings of (Farrell, 1976). Red Valencia intercropped with beans had the highest disease incidence (28 %), followed by Red Valencia intercropped with soybean (27 %), ICGV-90704 intercropped beans was third with disease incidence of 24%. ICGV-12991 variety intercropped with cowpeas was fifth with an incidence of 17%. The variation in viral incidence and severity in groundnuts varieties may have been due to having different strain-specific resistance gene (Elena *et al*., 2014). The genetic interaction between GRSV strain(s) and groundnut varieties have relationship, which make them to have a variation in incidence (Paul *et al*., 2002). The incidences were higher in intercropped groundnuts to pure stand; Red Valencia, ICGV-90704 and ICGV-12991 with an incidence of 8 %, 6 % and 4 % respectively. There was significant difference ($p < 0.05$) between intercropped groundnut varieties with pure stand. This implies that intercropping of other legumes may be alternative host to the virus (GRSV) with groundnuts tend to increase disease pressure on groundnuts (Jones, 2009). Most of legumes produce colored flowers; thrips are attracted more by pink flowers and yellow flowers. Soybeans, beans and cowpeas produce flowers of these colors, which may attract thrips

in the farm thus increasing population intensity for transmission of the virus (Blumthal *et al*., 2005). Disease incidence and severity on intercropped groundnut varieties had moderate positive correlation. This means that severity was dependent to disease incidence indicating transmission of the virus was by vectors (Singh, 2020).

Screened groundnut varieties (Red Valencia, ICGV-12991, CG7, ICGV-9991, Homabay, ICGV-99048, ICGV-99019, ICGV-90704 and SM99568) for resistance to GRSV had different response to GRSV inoculum (Appiah *et al*., 2016). This implies that groundnut genotypes are of diversity genetic materials that gives them a variation in response to GRSV gene interaction and association (Jone, 2014). Red Valencia, ICGV-12991, CG7, ICGV-9991 and Homabay exhibited symptoms similar with plant samples of inoculum collected in a survey; leaf mosaic, chlorotic leaf spot, necrotic leaf spot, reduced stem height and stunted growth symptoms (Ganesan *et al*., 2007). Homabay variety was more susceptible to GRSV with incidence of 42 % and severity of 3.55, followed by ICGV-9991 with incidence of 31 % and severity of 3.00, then Red Valencia with an incidence of 26 % and severity of 1.66. This is an indication that these varieties are susceptible to the virus but due to genetic diversity of their genotypes resulted into response variations to the virus (Rubio *et al*., 2013). Viral symptomatic development in ICGV-12991 and CG7 became more visible and severity increased with time of plant growth. This implies that some varieties may be having mechanisms of reducing virulence or resisting to viral multiplication, which slow down viral establishment, but with time the system becomes overwhelmed and the disease symptoms are expressed (Lima *et al*., 2000). Groundnut SM99568 variety phenotypically displayed no disease symptoms after inoculation, but serologically tested positive for the virus. This means that the variety has genes that are tolerant to viral. The crop appears healthy but is a host plant for the virus (Hull, 2002). This

variety when planted, thrips may pick the virus from them and transmit to other alternative hosts or crops of economic importance (Cunniffe *et al*., 2021). Groundnuts; ICGV-99048, ICGV-99019 and ICGV-90704 displayed no viral symptom and tested negative for the virus after mechanical inoculation. This indicates that they are resistant to GRSV (Kazuhiro *et al*., 2018), implying that these varieties may defend themselves from virus infection and colonization by RNA silencing and resistant (R) gene-mediated mechanisms (Giuseppe *et al.*, 2021).

Commonly grown crops or intercropped with groundnuts; pigeon peas, Bambara nut, green gram, black gram, peas, cowpeas, beans, cabbage, butter nut and kales in western Kenya (Onyango *et al*., 2019) and display viral symptoms (Haile *et al*., 2017). Pigeon peas, Bambara nut, peas, cowpeas, cabbage and butternut displayed disease symptoms similar to those of plant inoculation used, and serologically testing positive for the virus (Webster *et al*., 2011). This implies that they were infected by the virus therefore are alternative hosts to the virus (Ocimati *et al*., 2018). This study has revealed for the first time, Pigeon peas, Bambara nut, peas and butternut being plant alternative hosts to the virus. Such crops should not be intercropped with groundnuts or crops of economic importance as will act as source of GRSV inoculum for transmission by thrips to other crops (Boari *et al*., 2002).

Response of screened tomatoes to GRSV groundnut inoculum was higher (80 %) than groundnuts (64 %) and Soyabean (32 %). Watermelon displayed no viral symptoms and tested negative for the virus by ELISA (Webster *et al*., 2015). Implying that tomatoes are more susceptible to GRSV groundnut strains than groundnuts and soybeans. This may be, tomatoes lack RNA Antiviral silencing gene mechanism to defend the crop from being attacked by the virus (Sheikh *et al*., 2018). Soybeans displayed viral symptoms, which is contrary to the report (Pietersen *et al*., 2002),

indicating that soybeans show no disease symptoms but serologically test positive for GRSV. Watermelon was asymptomatic but serologically tested positive for the virus. This may indicate that watermelon is tolerant to GRSV strains of groundnut inoculum (Maksimov *et al*., 2019).

Among broad leafed-weeds, screened for alternative hosts to GRSV; *Amaranthus retroflexus, American burn weed, Commelina benghalensis, Datura stramonium, Chenopodium album, Solanum incanum, Solanum americanum, Solanum ptychanthum, Ageratum conyzoides and Galinsoga parviflora* exhibited GRSV symptoms and serologically tested positive for the virus (Webster *et al*., 2015). This implies that these weeds are alternative host for the virus (Boari *et al*., 2002). This study has revealed; American *burn weed, Commelina benghalensis, Chenopodium album, Solanum incanum, Solanum ptychanthum, Ageratum conyzoides and Galinsoga parviflora* for the first time being among the alternative host for virus. Such weeds should completely be eliminated from groundnut farms or other crops of economic importance that are alternative host for the virus (Wisley *et al*., 2005). These weeds when left in farms act as primary inoculum on which thrips pick GRSV strains for transmission to other host plants (Webster *et al*.,2015), thus posing great threat not only to groundnuts but also other crops of economic importance (Webster *et al*., 2011).

The Kenyan GRSV isolates nucleoproteins (N) Blasts aligned 96.13-99.98 % with those available in the GenBank. This implies that there is close origin among GRSV nucleoproteins from western Kenya with those available in the GenBank (Chen, 2015). Which confirms that they are not new viruses (King *et al*., 2012). The six GRSV isolate nucleoproteins (N) of western Kenya (GRSV-KE1, GRSV-KE2, GRSV-KE3, GRSV-KE4, GRSV-KE5 and GRSV-KE6) clustered with each other on phylogenetic tree with identity of 100 %. This implies that they had same recent evolutionary origin of

same geographical region thus shared same transmitting vectors and alternative host for the virus (Erkenbrack *et al*., 2019). This finding concurs with (Wangai *et al*., 2001) and (Appiah *et al.*, 2017) who observed closer identity between sequences from the same geographical region as compared to those from separate geographical regions. The six Kenyan GRSV isolates nucleoproteins had 96.13 to 99.82 % identity with isolates of different species available in the GenBank; soybeans, watermelon, tomato, Solanum americanum, Pisum sativum, thrips and infecting insects of South Africa, Ghana, Brazil and USA. This implying that the genetic sequence of Kenyan GRSV isolates of groundnuts was same with GRSV genetic sequences from other plant species (Kweon *et al*., 2020). This is an indication that GRSV RNA is more stable to mutation from one alternative host to the next, thus very few strains of GRSV occurs in host species (Peris *et al*., 2010). Which implies that although the virus is, picked by thrips from one host plant to other alternative host of different species does not undergo gene alteration along the transmission process (Sharp *et al*., 2011).

Kenyan GRSV isolates; GRSV-KE4 (LC616782), GRSV-KE2 (LC616780), GRSV-KE3 (LC616781) and GRSV-KE1 (LC616779) exhibited closest evolutionary origin with groundnut isolates of United States of America (HQ634665.1) with identity of 98.56 %, Ghanaian's groundnut isolate (KT345728.1) with 98.02 % identity and Brazilian groundnut isolate (MG797643.1) with identity of 96.93 %. It is worth noting that the Kenyan isolates had highest identity to 99. 82 % with USA isolates of infecting insect (HQ634665.1) having same closest evolutionary origin with GRSV-KE6 (LC616784) and GRSV-KE5 (LC616783). This implies that the Kenyan isolates had recent evolutionary origin with those from South Africa, Ghana and Brazilian isolates that may be the reason why they clustered together with closest identity (Harkins *et al*., 2017). Kenyan nucleoproteins of GRSV-KE6 (LC616784) and GRSV-KE5

(LC616783) had same evolutionary node on phylogenetic tree and clustered together with infecting insect isolate (HQ634665.1) from USA with identity of 99.83 %, may imply that the infecting insects of GRSV in USA belong in the same genus with vectors transmitting GRSV into Kenyan groundnuts (Jehle *et al*., 2006).

Kenyan GRSV isolates had close identity 96.13 – 99.93 % with Solanum americanum isolate (KM007024) of USA, peanut isolate (MT423642.1) of Argentina, peanut isolate (MT423626.1) of Argentina, watermelon isolate (MN364668.1) of Brazil, peanut isolate (KY400110.1) of Brazil, tomato isolate (MH42958.1) of South Africa and soybean isolate (MH686229.1) of Brazil but did not cluster together. This implies that these isolates had same ancestral origin but due to wide variation in geographical region resulted into differences in environmental conditions causing variations in evolution of GRSV (Appiah *et al.*, 2017). In general, all GRSV nucleoprotein gene sequences in this study and those in GenBank shared 96.13-100% nucleotide identity. This implies that GRSV nucleoproteins are highly conserved across a wider geographical region globally (Zheng *et al*., 2005). Basing on this characteristic of fitness of GRSV gene to mutation, it's easier to breed pathogen resistant cultivars of groundnuts through genetic engineering that can be used globally to safe-guard for food security (Deom *et al*., 2000; Appiah *et al*., 2017).

New developed primer from western Kenya sequences GRSV4_F (5' ACCAGAACCAGGTTGCATTC- 3') for forward reaction and GRSV4_R (5' ATCGTGACCTTGCCAAAAGT-3') for reverse reaction, formed clear bands with GRSV positive samples collected in western Kenya in a PCR. It implies that a pair of primers developed is valid to be synthesized into a PCR primer as one of standard commercial primers. Their sequences blasts with GRSV isolates from other countries with 100 %, which is an indication that (GRSV4F/GRSV4R) new primers have the

ability to be used on other GRSV isolates from other countries in a PCR tests. Those primers designed that failed to form a clear band may have been due to primer dimer problem which may be solved by increasing the annealing temperature, increase time/temperature of template denaturation, decrease primers concentration or GC content to be between 40 and 60 % with 3' of a primer ending in G or C to promote binding (Rozen *et al*., 2000).

5.2 Conclusion

The research reveals that Groundnut ringspot virus (GRSV) occurs and distributed in all surveyed Counties (Bungoma, Busia and Kakamega) of western Kenya with variant incidences in agro- ecological zones although in some regions (Kimilili) the virus co-exists with Tomato spotted wilt virus (TSWV) infecting groundnuts and exhibiting same symptomatic characteristics on host plants grown in Kenya. It's for the first time the occurrence and distribution of Groundnut ringspot virus is reported in Kenya and 3rd Country in Africa behind Ghana and South Africa in occurrence of the virus.

When groundnut varieties are intercropped with other legumes; Beans, cowpeas and soybeans in western Kenya increases viral disease pressure on groundnuts. This may be due to different flower colours displayed by these legumes, which attract vectors (thrips) in the field, which transmit the virus to the crops grown in western Kenya that are susceptible to GRSV. Some legumes are alternative host to the virus, which may act as primary inoculum for the virus on which vectors, pick to transmit to health crops.

Groundnut varieties grown in western Kenya have different resistance levels to GRSV. Groundnut varieties; ICGV-99048, ICGV-99019 and ICGV-90704, are more resistant to the virus while SM99568 is tolerant to GRSV. Most varieties grown in western

Kenya; Red Valencia, ICGV-12991, CG7, ICGV-9991 and Homabay are more susceptible to the groundnut ringspot virus.

Some of crops of economic importance mainly intercropped with groundnuts or planted adjacent to groundnut farms in western Kenya; pigeon peas, Bambara nut, green gram, black gram, peas, cowpeas, beans, cabbage, butternut and kales are among the alternative hosts to Groundnut ringspot virus. Also some broad-leafed weeds growing in groundnut farms which had not been reported as alternative host for the virus; American *burn weed, Commelina benghalensis, Chenopodium album, Solanum incanum, Solanum ptychanthum, Ageratum conyzoides and Galinsoga parviflora* are among are alternative hosts to GRSV.

The Kenyan GRSV strains infecting groundnuts in western Kenya are similar to GRSV strains infecting groundnuts and other plant species in other countries, as the Kenyan nucleoproteins sequences had very high percentage of identity with other GRSV strains nucleoproteins/nucleocapsid deposited in the GenBank from other countries. GRSV is very stable to mutation during the process of transmission.

New designed and developed primers from GRSV sequences of western Kenya; GRSV4 F (5" ACCAGAACCAGGTTGCATTC 3") and GRSV4R (5" ATCGTGACCTTGCCAAAAGT 3"), have the potential of being used to synthesis a standard commercial PCR primers to amplify RNA of GRSV isolates and be used both locally and globally for PCR tests.

5.3 Recommendations

The findings of this study reveals the occurrence and distribution of Groundnut ringspot virus in western Kenya that lowers the productivity of groundnuts in this region. As a matter of urgency, introgression of resistant gene into local groundnut varieties be done by Seed breeders, KALRO and other research centre to come up with varieties that are resistant to GRSV infection to achieve food security globally.

Farmers should be advised that, Planting of groundnuts in purestand should highly be recommended than intercropping with other legumes which may be alternative host to the virus to minimize transmission of GRSV. In addition, weeds and other crops that are alternative host to the virus should be discouraged from being planted adjacent to to groundnut farms and weeds be rooted out of groundnut farms to minimize transmission of the virus to targeted crop in the field.

Since Kenyan isolates showed closest identity with strains from Ghana, South Africa, and USA isolates, therefore KEPHIS to reinforce importation regulation and rules on contaminated farm inputs to control vectors being imported into the Country.

New designed and developed primers from GRSV sequences of Kenya; GRSV4 F (5" ACCAGAACCAGGTTGCATTC 3") and GRSV4R (5" ATCGTGACCTTGCCAAAAGT 3"), be considered for testing by Oligonucleotide synthesis Companies, for use in synthesizes of standard commercial primers.

Some groundnuts displayed typical biological symptoms for GRSV/TSWV but serologically tested negative for both viruses. This implies that some symptoms induced were from different viruses, therefore there is need to carry ou more research are recommended to determine if new viruses of genus tospovirus occurs in western Kenya.

REFERENCES

Adams AN, Clark MF (1977). Characteristics of the Microplate Method of Enzyme-Linked Immunosorbent Assay for the Detection of plant viruses. Great Britain. J gen. Virol.34; 475-483

Adkins S.T, Webster C.G., Perry K., Lu X., Horsman L., Frantz G., Mellinger C. (2010). First report of *Groundnut ring spot virus* infecting tomato in south Florida. *Plant Health Progress* doi: 10.1094/PHP-2010-0707-01-BR

Agneroh T.A., Kouadia K. T, Soro K. Pohe. J (2012). Identification and distribution of disease virus. Lagenaria siceraria standlet Citrullus sp.in Cote Ivoire. Animal and Plant Science.13 (2); 1758-1770.

Ajayi O. C (2007). User Acceptability of Sustainable Soil Fertility Technologies: Lessons from Farmers 'Knowledge, Attitude & Practice in Southern Africa, *Journal of SustainableAgriculture,* Vol. 30; 21-40.

Anderson. P.K., Cunningham A.A. Patel. N.G., Morales. F.J., Epstem P.R., Daszak P. (2004). Emerging infectious diseases of plants; Pathogen pollution, climate change and technology drivers. Trend. Ecoli. Vol. 119; 35-42

Appiah, A. S., Offei, S. K., Tegg, R. S., & Wilson, C. R. (2016). Varietal response to groundnut rosette disease and the first report of *Groundnut ringspot virus* in Ghana. *Plant Diseases.* 100(5):946-952.

Bajpai, R., Singh, P., P. D, Sobha and Singh. (2017). Study on seed dormancy and Longevity Behaviour of groundnut (Arachis hypogea L.) Genotypes, International Journal of Pure and applied Biosciences. 5(4):399-403,

Baughman, Todd; Grichar, James; Black, Mark; Woodward, Jason; Porter, Pat; New, Leon; Baumann, Paul; McFarland, Mark (2018).Texas Peanut Production Guide" (PDF). Texas A&M University. Vol. 16:4-10

Bawa AS, Anilakumar KR (2013). Genetically modified foods: safety, risks and public concerns. Journal of food science and Technology. 50: 1035-1046

Bernstein E, Caudy A, Hammond S, Hannon G (2001). Role for a bidentate ribonuclease in the intiation step of RNA interference.Nature 409: 363-366

Bertran AGM, Oliveira HS, Nagata T, Resende (2011). Molecular characterization of the RNA-dependent RNA polymerase from groundnut ringspot virus.Arch. Virology.156 (8): 1425-9

Bucheyeki, T. L., Shenkalwa, E. M., Mapunda, T. X. & Matata, L. W. (2008). On-farm evaluation of promising groundnut varieties for adaptation and adoption in Tanzania. *African Journal of Agricultural research*. 3:531-600.

Buerkert, A. (2002). Multi-site time-trend analysis of soil fertility management effects on crop production in sub-Saharan West Africa, *Experimental1T andAgriculture*. Vol. 38: 163- 183.

Burko YS, Shleizer B, Yanai O, Shwartz L, Zelnik ID, Jacob-Hirsch J, Ori O (2013). A role for apetala1/fruitfull transcription factors in tomato leaf development.Plant cell, 25;270-283.https://doi.org/10.1105/tpc.113.113035

Boari A.J., Maciel-Zambolim E., Lau D.D., Lima G.S.A., Kitajima E.W., Brommonschenkel S.H., Zerbini F.M., 2002. Detection and partial characterization of an isolate of *Groundnut ringspot virus* in *Solanum sessiliflorum*. *Fitopatologia Brasileira.*

27: 249-253.

Boonham N., Smith P., Walsh K., Tame J., Morris J., Spence N., Bennison J., Barker I., 2014. The detection of *Tomato spotted wilt virus* (TSWV) in individual thrips using real-time fluorescent RT-PCR (TaqMan). *Journal of Virological Methods* 101: 37-48.

Caliskan S, Arslan M, Arioglu H (2008). Effect of sowing dates and growth duration on growth and yield of groundnut in Mediterranean-Type environment in Turkey. (Elsevier). Field Crop Research (105):131-140

Camelo-Garcia V.M., Lima E.F.B., Mansilla-Cordova P.J. Rezende J.A.M., Kitajima E.W., Barreto M., 2014. Occurrence of *Groundnut ringspot virus* on Brazilian peanut crops. *Journal of General Plant Pathology* 80: 282-286.

Cella LN, Blackstock D, Yates MA, Mulchandani A, Chen W (2013). Detection of RNA viruses: current technologies and future perspectives. Critical Reviews in Eukaryotic Gene Expression.Vol.23:125-137.

Chapman EJ, Hilson P, German TL (2003) Association of L protein and in vitro Tomato spotted wilt virus RNA-Dependent RNA polymerase activity. Intervirology 46:177–181.

Chuang. T.Y., Jeger M. J. (1987). Relationship between incidence and severity of banana leafspot in Taiwan; Phytopathology 77: 1537- 1541

Culbreath, a. k.; Tubbs, r. s.; Tillman, b. l.; Beasley, j. p.; Branch, w. d.; holbrook, c. c.; Smith, a. r.; Smith, n. b. (2003). Effects of seeding rates and cultivar on *tomato spotted wilt* of peanut. *Crop protection*, Vol. 53: 118-124, 2013

Coutts BA, Jones RA (2005). Suppressing spread of tomato spotted wilt virus by drenching infected sources or healthy recipient plants with neonicotinoid insecticides to control thrips vectors. Annimal and Applield Biology. 145:95-103

de Avila AC, de Haan P, Kormelink R, Resende Rde O, Goldbach RW, Peters D.(1993). Classification of tospoviruses based on phylogeny of nucleoprotein gene sequences. Journal of General Virology.74 (2):153–159

de Breuil S., Abad J.A., Nome C.F., Giolitti F.J., Lambertini P.L., Lenardon S., 2007. *Groundnut ringspot virus*: an emerging Tospovirus inducing disease in peanut crops. *Journal of Phytopathology*. 155: 251-254.

Dietzgen RG, Twin J, Talty I, Selladurai S, Carroll ML, Coutts BA, Berryman DI, Jones RAC (2005). Genetic variability of Tomato spotted wilt Virus in Australia and validation of real time RT-PCR for detection in single and bulked leaf samples. Animal and Applied Biology 146:517-530.

Duijsings D, Kormelink R, Goldbach R (2001). In vivo analysis of the TSWV cap-snatching mechanism: single base complementarity and primer length requirements. EMBO J 20(10):2545–2552

Dulvenbooden, N.V., Abdoussalam, S. & Moamed, A.B (2002). Impact of climate change on agricultural production in the Sahel-Part 2. Case study for groundnut and Cowpea in Niger, *Climatic Change*, Vol. 24, 2002, 349-368.

Elena. S.F, Fraile A, Garcia, Arena F (2014). Evolution and emergence of plant viruses. Adv. Virus Res.88, 161-191

FAOSTAT.(2017). Peanut (groundnuts with shell) production in 2016. Food and Agricultural Organization of the United Nations, Statistics Division.

Farrell J.A.K, (1976). Effects of intersowing with beans on the spread of groundnut rosette virus by Aphis craccivora Koch (Hemiptera aphidadea) in Malawi. Bulletin of Entomological Research. 66:331-138.

Fermin G, Velentina I, Casar G, Dennis G (2004). Engineered Resistance against papaya ringspot virus in Venezualan Transgenic papayas. Plant disease.Vol. 88(5):88-95

Gachu SM, Muthomi JW, Narla RD, Nderitu JH, Olubayo FM, Wangacha JM (2012). Management of thrips (Thrips tabaci) in bulb onions by use of vegetable intercrops. International Journal of Agriscience.2 (5):393-402

Gallitelli. D. (2000). The ecology of cucumber mosaic virus and Sustainable agriculture. Virus Res. 71; 9-21.

Giorgio G, Irene P, Ivana G (2008). Arapid and effective method for RNA extraction from different tissues of grapevine and other woody plants.Phytochemical analysis.19 (6):520-525

Hanssen I.M., Van Esse H. P, Ballester A.R, Hogewoning S.W., Parra N.O., Paelema A., Lievens B., Bovy A.G., Thomma B. P. (2011). Differential tomato transcriptomic responses induced by pepino mosaic virus isolates with differential aggressiveness. Plant Physiology. 156: 301- 318.

Heuzé V., Thiollet H., Tran G., Edouard N., Bastianelli D., Lebas F., 2017. Peanut hulls. Feedipedia, a programme by INRA, CIRAD, AFZ and FAO.

Hull R, (2009). Mechanical inoculation of plant viruses.https;//doi.org/10.1002/9780471729259.mc16b06s13.

Ibrahim U (2011). Synopsis of research presented at the Department of Agronomy, Ahmadu Bello University, in partial Fulfillment of the requirement for the award PhD in Agronomy June, 2011. PP35.

Islam WJ, Zhang M, Adnan A, Noman M, Zaynab M, Wu Z (2017). Plant virus ecology: a glimpse of recent accomplishments.Applied Ecology and Environmental Research .15(1):691-705.

Isleib TG. Wynne JC, Nigam SN (1994). Springer.com, 552-623.

Jauron, Richard (Eds). (2011). Growing Peanut in the Home Garden/ Horticulture and Home Pest. Intergrated pest management-lowa state university.5:124-140.

Jehle JA, Nguyen HT (2009). Quantitative analysis of the seasonal and tissue- specific. Expression of CryIAb in transgenic Maize Mon810.Journal of Plant Diseases and Protection New series-114(2):82-87.

Jones R.A.C (2009). Plant virus emergence and evolution origins, new encounter Scenarios, factors. Driving emergence effects of changing world conditions and prospect for control virus Research. 141; 113-130.

Kaaye A, Eigel W, Harris C. (2006). PeanutAflatoxin levels on farms and in markets of Uganda. Peanut science.33:16-23

Kassie M, Shifera WB, Muricho G (2011). Agriculture technology crop income and poverty alleviation in Uganda. Elvsier. World Development. 39 (10):1784-1795

Karavina. C, Gubba.A. (2017). An African perspective on Tospoviruses. Journal of plant pathology. 99:5-16.

Kipkoech AK, Kimenye LN, Ndung'u KW (2009). Effect of combining organic residues with Minjungu phosphate rock.absoption and availability of phosphorus and maize production in soils of Western Kenya.Springer.86 (3): 317-329

Krapovickas, Antonio; Gregory, Walton C. (2007). Translated by David E. Williams and Charles E. Simpson." Taxonomy of the genus Arachis (Leguminosae)'. (PDF). IBONE. 16 (Supl.): 1–205

.Krapovickas A, Vanni RO, Pietrarelli JR, Simpson CE (2013).The peanut Land races from Peru. Bonplandia. 22: 19-90

Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*. 35:1547-1549

Langat M.C, Okirar MA, Oumu JP, Gesimba RM (2006). The effect of intercropping groundnut (Arachis hypogea L.) with Sorghum on yield and Cash income. Agriculture Tropica ET subtropica, 39(2): 89-90

Lee M, Lee S, Zadeh AD, Kolozic PA, (2003). Distinct sites I E-cadherin regulate different steps in Drosophila tracheal tube fusion.Development 130(24): 5989-5999

Lima M, de Avila AC, Resende RO, Nagata T (2000) Lentamente identificac¸a˜o de espe´cies de tospovı´rus em tomateiro e pimenta˜o no sub-me´dio do Vale do Sa˜o Francisco e no Distrito Federal. Summa Phytopathological 26:205–210.

Lopez Y, Burrow MD (2011). Development and validation of CAPS markers for the high oleate trait in peanuts. Procedure of American Peanut Research Education Society 36:25-26

Lyerly, J. H.; stalker, H. J.; moyer, j. w.; hoffman, k. (2002). Evaluation of *arachis* species for resistance to tomato spotted wilt virus. *Peanut Science*. 29 (2):79-84

Marsalis, Mark; Puppala, Naveen; Goldberg, Natalie; Ashigh, Jamshid; Sanogo, Soumaila; Trostle, Calvin (Eds). (2015). New Mexico Peanut Production. Circular. New Mexico State University.645: 116, -2015.

Mehmet AS (2011). Occurrence of pepper mild mottle virus in green house-grown pepper (Capsicum annuum L.) in west Mediterran region of Turkey. African journal of biotechnology. 10(25): 4976-4976

Michelotto MD, De Godoy IJ, Pirotta MZ, Dos Santos JF, Finoto EL, Pereira FA (2017). Resistance to thrips (Enneothrips flavens). In wild and amphidiploid Arachis species. PLOS ONE 12(5): e0176811.doi:10. 1371

Miller J. (1991). Significance of Mycotoxins for health and Nutrition in' Champ B, Highley E, Hocking A, Pitt J. (eds). Fungi and Mycotoxins in stored products, ACIAR proceedings No.36, Australia: Australian Centre for International Agricultural

Moretzsohn, Márcio C.; Gouvea, Ediene G.; Inglis, Peter W.; Leal-Bertioli, Soraya C. M.; Valls, José F. M.; Bertioli, David J. (2013). A study of the relationships of cultivated peanut (Arachis hypogaea) and most closely related wild species using intro sequences and microsatellite markers.Annals of Botany. 111 (1): 113–126.doi:10.1093/aob/mcs237. ISSN0305-7364.PMC3523650. PMID23131301.

Mukoye B, Mabele AS, Ndonga MFO, Mangeni BC, Were HK (2020). Distribution of groundnut rosette disease and sequence diversity of groundnut rosette virus associated satellite RNA (Sat-RNA) in western Kenya. International of Genetics and Molecular Biology, 12(1): 22-29.

Murere LW, Mukoye B, Were HK, Kollenberg M (2021).Epidemiology and Occurrence of Groundnut ringspot virus(GRSV) infecting Groundnuts in western Kenya.

International Journal of Academic and Applied Research (IJAAR). ISSN:2643-9603 (5). 13-25

Mutegi E, Sagnard F, Muraya M, Kanyenji B, Rono B, Mwongera C, Labuschagne M (2010). Eco geographical distribution of wild weedy and cultivated sorghum bicolar (L) Moench in Kenya. Implication for conservation and crop-to- wild gene.flow genetic .resource and crop evolution, 57: 243-253

Nascimento, I. C. D.; Pensuk, V.; Costa, N. P. D.; Assis filho, F. M. D.; Pio-Pibeiro, g.; deom, c. m.; Sherwood, J. (2006). Evaluation of peanut genotypes for resistance to *Tomato sspotted wilt virus* by mechanical and thrips inoculation. *Pesquisa Agropecuária Brasileira*. 41(6): 937-942.

Nichot S,BeatyB,Elliott R,Goldbach R, Plyusnin A, Schmaljohn C, Tesh R (2005) Bunyaviridae. In: Fauquet C, Mayo M, Maniloff J, Desselberguer U, Ball L (eds) Virus taxonomy: VIIIth report of the ICTV. Elsevier/Academic Press, 36: 695–716.

Notomi T, Hiroto O, Harumi M, Tushihiro Y, Keik W, Nobuyuki A, Tetsu H (2000). Loop-Mediated isothermal amplification of DNA. Nucleic Acids Research. 28:63-67.

Okello DK, Birum M, Deom CM, (2010). Overview of groundnuts research in Uganda, past, present and future. African Journal of Biotechnology 9(39), 6448-6459.

Okello, D. K., Monyo, E., Deom C.M., Ininda, J., & Oloka, H. K. 2013. Groundnut production guide for Uganda: Recommended practices for farmers. National Agricultural Research Organisation, Entebbe. ISBN (6): 978-997

Okoko EN, Rees DJ, Kwach JK, Ochieng P (1999). Participatory evaluation of groundnut production Southwest Kenya. In Towards increased use of Demand Driven

Technology. KARI/DFID, NARD. Project, end of project conference 23rd-26th March 1999, Nairobi Kenya, 305-307

Olmos A., Bertolini E., Gil M., Cambra M (2005). Real-time assay for quantitative detection of non-persistently transmitted *Plum pox virus* RNA targets in single aphids. *Journal of Virological Methods,* 128: 151-155.

Pankaj K (2013). Methods for rapid virus identification and quantification. Materials and methods, 3:207

Pappu HR, Jones RA, Jain RK (2009). Global status of tospovirus epidemics in diverse cropping systems: successes achieved and challenges. Virology Research journal 141(2):219–236.

Parida MM, Horioke K, Ishida H (2005). Rapid detection and differentiation of dengue virus Serotypes by a Real-Time reverse transcription-Loop Mediated ISO-Thermal amplification Assay. Journal of clinical microbial. 43:2895-2903.

Paul.X. Kover and Barara.A. Schaal (2002). Genetic variation for disease resistance and tolerance among Arabidopsis thalian accession. Peer reviewed journal of Sciences, 14: 11270-11274.

Peris. J.B., Davis. P. Cueres JM., Nebot. M.M. Senjuan R. (2010). Distribution of fitness effects caused by single-nucleotide substitution in bacteriophase F1. Genetics society of America, 85:603-609.

Pietersen G, Morris J (2002). Natural occurrence of groundnut ringspot Virus on soybean in South Africa. Plant disease. 86(10): 119-127

Poppy J (2011). Cost-benefit analysis of crop protection measures.Springer. Journal of consumer protection and food safety, 6: 105-112.

Putnam, D.H.; Oplinger, E.S.; Teynor, T.M.; Oelke, E.A.; Kelling, K.A.; Doll, J.D.(Eds). (2015). “Peanut”. Alternative Field Crops Manual, New CROP Center, Purdue University: 216-226.

Relevante, C. A.; Cheewachaiwit, S.; Chuapong, J.; Stratongjun, M.; Salutan, V. E.; Peters, D.; Balatero, C. H.; Hoop, S. J.(2012). *Emerging new Poleroviruses and Tospoviruses affecting vegetables in Asia and breeding for resistance*. Food and Fertilizer Technology Center for the Asian and pacific region location of publication, 657:1-12

Resende R.O., de Haan P., de Avila A. C., Kitajima E.W., Kormelink R., Goldbach R., Peters D. (1991). Generation of envelope and defective interfering RNA mutants of tomato spotted wilt virus by mechanical passage. *Journal of General Virology,* 72: 2375-2383.

Rockstrom J, Barron J, Fox P, (2003). Water productivity in rain-fed Agriculture: challenge and opportunities for smallholder farmers in drought-prone tropical agro ecosystems. In Kijne J W, Baker R, Molden D (Eds). Water productivity in agriculture: limits and opportunities for improvement. Centre for Agriculture and Biosciences International, 5:145-162.

Rozen S, Skaletsky H (2000). Primer3 on the WWW for general users and for biologist programmer.Springer.Methods molecular biology, 132: 365- 386.

Saponari M., Manjunath K., Yokomi R.K.(2008). Quantitative detection of *Citrus tristeza virus* in citrus and aphids by realtime reverse transcription-PCR (TaqManR). *Journal of Virological Methods*, 147: 43-53.

Saroj, (2019). Effects of climate change in agricultural insect’s pest. ACTA scientific agriculture (ISSN, 2581-365) 3:1-7

Seijo, Guillermo; Graciela I. Lavia; Aveliano Fernandez; Antonio Krapovickas; Daniel A. Ducasse; David J. Bertioli; Eduardo A. Moscone A, (2007). Genomic relationships between the cultivated peanut (Arachis hypogaea, Leguminosaea) and its close relatives revealed by double GISH". America Journal of Botany. 94 (12): 1963-1973.

Silva A O, Marana M S A, Richard K, Renalo O R, (2019).Tomato chlorotic putatively incorporated a genomic segment of GRSV upon a reassortment event.Journal of National center for biotechnology information, 11(10):339-347

Singh BB, Ajeigbe HA, Tawawali SA, Fernandeza, Rivera S, Abubakar M (2003). Improving the production and utilization of cowpeas as food and fodder. Elsevier. Field Crops Research journal, 84(12):169-177.

Souf S, (2016). Recent advances in diagnostic testing for viral infections. Bioscience Horizons, 9:1-11.

Spadotti D.M.A., Leao E.U., Rocha K.C.G., Pavan M.A., Krause-Sakate R., 2014. First report of *Groundnut ringspot virus* in cucumber fruits in Brazil. *New Diseases Reports* 29:25-25.

Reddy, D. V. R. (1991). Groundnut viruses and virus diseases;Distribution,identification and control. Annual Review of.Plant Pathology.70:665-678.

ICRISAT Groundnut Project Annual Progress Report for 1996. *Chitedze Research Station,* PPO Box1096, Lilongwe, Malawi.

ISTA(2014). International Rules for Seed Testing. International seed testing Association, Bassersdorf.

Sharp. P.M., Simmonde. P (2011). Evaluating the evidence for virus/host co evolution. Curr. Opin.vol 1:436-441

Smartt. J. (1994). The groundnut in farming systems and the rural economy. *Ascientific basis for improvement.*38 (8): 664-699

Takahashi Y, Hiroaki S, Yuki Y, Chiaki M, Toyoaki A, Muthaiyan P, Natesan S, Norihiko T, Naito K (2019). Domesticating Vigna stipulacea: A potential legume crop with Broad Resistance to Biotic stress. Frontier in plant science, 10:1-7

Tamura K. and Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* 10:512-526.

Tang ZB, Zhihong G, Hui T, Yuning H (2006). Inferring direct regulatory targets from expression and genome location analysis: a comparison of transcription factor deletion and over expression. BCM Genomics 7:215-225

Tandzi Ngoune Liliane, Mutengwa Shelton Charles (2020). Factors affecting yield of crops, agronomy-climate change, Amanullah, intechopen, 10:77- 92

Tomita N, Mori Y, Kanda H (2008). Loop-mediated Isothermal amplification (LAMP) of gene sequences and simple visual detection of products. Nat Protoc. (3): 877-882

United States department of agriculture - USDA, Foreign Agricultural Service. *World Agricultural Production*. 2018. Circular Series, January 2018. Available at: https://apps.fas.usda.gov/ psdonline/ circulars/production.pdf. Access at: 25 jan.2018.

Upadhaya D, Gowda CLL, Pundir RPS, Gopel V, Reddy Singh, (2006). Development of core subset of finger millet germplasm using geographical origin and data on 14 quantitative traits.Springer. Genetic Resources and crop evolution 53(4): 679-685

Van Poelwijk F, Boye K, Oosterling R, Peters D, Goldbach R (1993) Detection of the L-protein of Tomato spotted wilt virus.Journal of Virology 197:468–470.

Villa TC, Maxted N, Scholten MA, Ford-Lloyd BV (2005). Defining and identifying crop landrace.Plant Genetic resources and crop evolution 3:373-384

Wangai, A. W., Pappu, S. S., Pappu, H. R., Okoko, N., Deom, C. M. & Naidu, R. A. (2001). Distribution and characteristics of groundnut rosette disease in Kenya.*Plant Disease*,85:470-474.

Webster C.G., Perry K., Lu X., Horsman L., Frantz G., Mellinger C., Adkins S.T.(2018). First report of *Groundnut ring spot virus* infecting tomato in south Florida.American phytopathological society journal, 37:333-339

Woomer PL, Tungani J, Odhiambo G, Mwaura FM (2005). Striga Management options in Western Kenya. African Crop science conference proceedings 7:479-484

Zheng L, Zhou M, Chai, Q, Parrish J, Xue D, Patrick SM, Chen D, Shen B (2005). Novel function of the flap endonuclease 1 complex in processing stalled DNA replication forks. EMBO Rep 6(1): 83-91

APPENDICES

Appendix i: Survey/ Field Trials Disease Score Sheet

Farmer's name……County……...…

Subcounty……...……Village…….…. Date……

CROP……. VARIETY…….…

Cropage……. Plot history……...

Crops in adjacent fields……. Rain seasons……

GPS readings; Altitude (Meters)…….……Longitude……

Latitude (North or South) ……. AEZ…….……

	Disease name…….			
Treatments	**Incidence** (% of plants affected per quadrat)	**Part affected** (stem, leaves, pods flowers, fruits)	**Distribution** (whole field, spots)	**Severity 1-4**
1				
2				
3				
4				

*Severity: 1= No disease; 2=Mild; 3= Moderate; 4=Severe.

Number of plants affected 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:Masinde Muliro University Of Science And Technology

RESEARCH WORK PLAN- AUGUST 2019

Research protocol

Effect of intercropping legumes and groundnut varieties on GRSV incidence and severity in western Kenya

Trial layout and design

The study will be conducted in chebich and Kimalewa in Bungoma County, Alupe KALRO in Busia County and Muhonje in Kakamega County

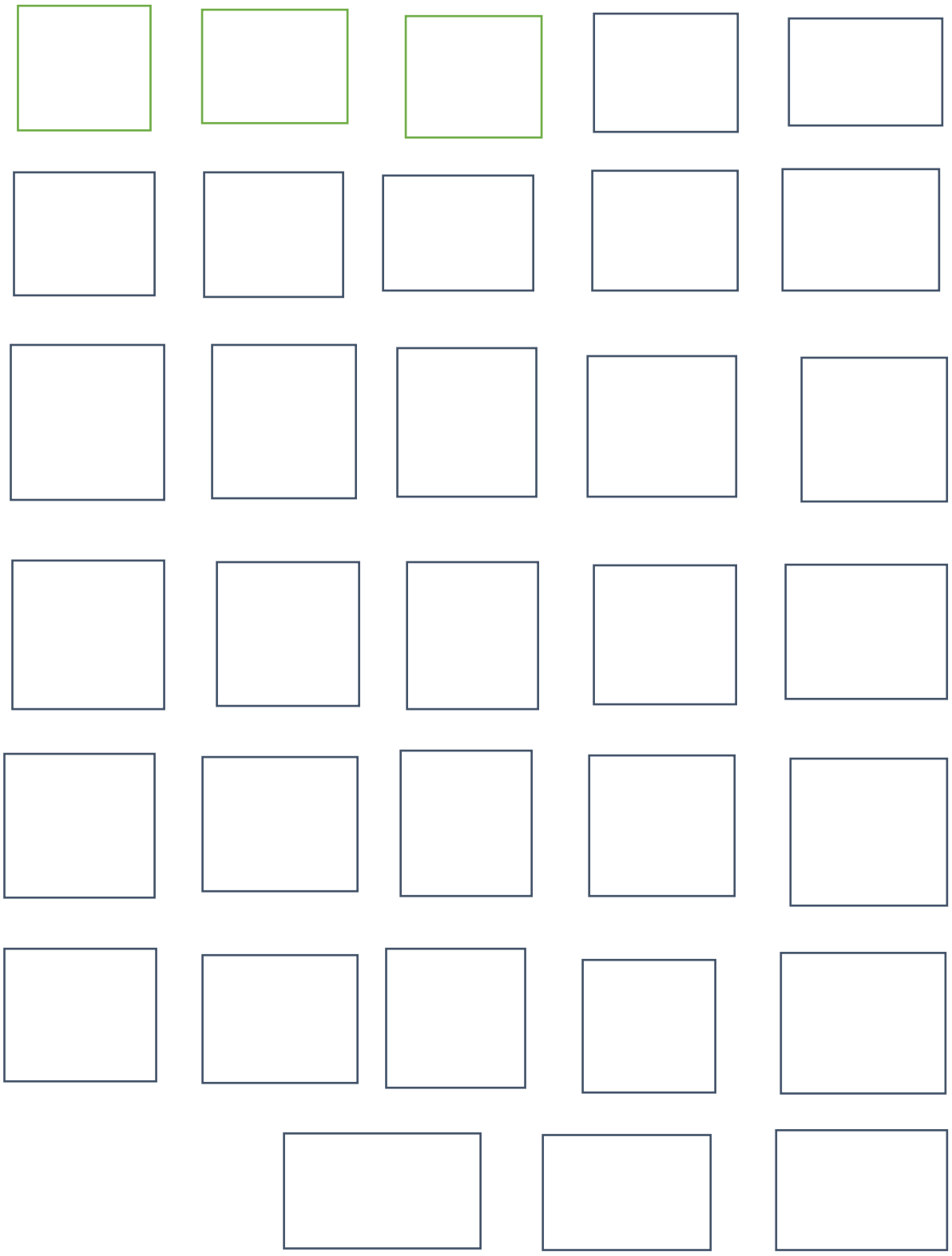
Two farms will be randomly selected based on soil type, altitude, rainfall. Temperature and land topology.

- On each farm, the trials will consist of six experimental treatments as follows:

    1. Red Valencia (groundnuts)+ Cowpea (K80)
    2. ICGV 90704 (groundnuts) + Cowpea (K80)
    3. ICGV12991 (groundnut) + Cowpea (K80)
    4. Red Valencia (groundnut) + Beans (Rosecoco)
    5. ICGV 90704 (groundnut) + Bean (Rosecoco)
    6. ICGV12991 (groundnuts) + Bean (Rosecoco)
    7. Red Valencia (Groundnuts) + Soya peas
    8. ICGV 90704 (groundnuts) + Soya peas
    9. ICGV12991 (groundnuts) + Soya peas

10. Groundnuts + Cow peas + Beans + Soya peas

The experiment will be laid in randomized complete block design within each farm in muhonje(Kakamega), Chebich (Bungoma), Alupe (Busia)



MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

RESEARCH WORK PLAN- JULY 2020

Research protocol

Screening groundnut varieties for resistance levels to GRSV and determine unknown host range for GRSV in western Kenya

(a) Screening resistance levels of groundnut varieties to GRSV.

- Preparation of growth medium of clay soil + sand+ organic manure at ratio of (2:1:1) respectively.
- Growth medium placed in 500 cm^3 pots for planting of seeds of crops for study.
- Seeds used for study be tested for seed health (ISTA 2014)
- Two seeds of each variety be planted in pot and replicated five times (ICGV 12991, Red Valencia, ICGV 90704, TSV 10, Homa bay, ICGV 9991, CG7, ICGV-99019, ICGV-99048, SM 99568) respectively.
- Treatments will be arranged in a complete randomized block design in a green house.
- One pot of each groundnut variety be arranged separately to be a control experiment in each treatment.
- Groundnut varieties be inoculated after two leaves development after germination.
- Symptom development recorded at an interval of five days for 8 consecutive weeks.
- Leaf samples picked for pCR to determine viral titre for each variety.

(b) Determining legumes and common weeds host range for GRSV

- Commonly grown legume (beans, cowpeas, peas, green gram, Bambara,) and common broad leafed weed species (Chick weed, pig weed, night shade, wondering jew, Galinsoga parviflora, Lantana camara, Bidens Pilosa, Datura stramonium, Solanum incanum, Solanum suave and Crotalaria polysperma) be researched on.
- The procedure stated in (a) will be used in this study.

(c) Inoculum preparation and innoculation

- The serologically tested positive isolates for GRSV grounded using a sterilized pestle and mortar.
- Added Freshly prepared ice-cold 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),
- Groundnut tissue, mixed and transferred to a falcon tube, and allowed to stand for 5 minutes in ice, to settle debris at the bottom of tube. The sap will be kept on ice, until inoculation is completed.
- The Carborundum 320 grit be dusted on plants under research to act as an abrasive.
- The inoculum be applied gently on the leaf surfaces, using saturated cotton wool swab.

- Excess carborundum and innoculum be washed out on the groundnuts, Legumes and weeds leaves by spraying gently with sterilized distilled water.

Appendix iii: Photographs of intercropped groundnuts in Alupe Busia County


ND NUTS (R-VA-
PURE STAND


R. VA. GROUNDNUT
+
BEANS

Appendix iv: Post hoc. ANOVA RESULTS

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Virus incidence	Between Groups	35990.738	10	3599.074	8.603	.000
	Within Groups	230083.961	550	418.334		
	Total	266074.699	560			
Virus severity	Between Groups	15.862	10	1.586	1.963	.035
	Within Groups	444.366	550	.808		
	Total	460.228	560			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) claster label	(J) claster label	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Virus incidence	ALUPE	CHAKOL	5.690	4.074	.949	-7.48	18.86
		CHEBICH	-5.475	4.487	.980	-19.98	9.03
		CHWELE	-16.064*	3.564	.000	-27.58	-4.54
		KAPKATENY	13.675*	3.616	.008	1.99	25.36
		KIMALEWA	3.165	3.529	.998	-8.24	14.57
		KIMILILI	2.315	4.018	1.000	-10.67	15.30
		MALABA	4.232	3.756	.989	-7.91	16.37
		MATUNGU	5.762	3.992	.937	-7.14	18.67
		MUHONJE	-4.192	4.590	.998	-19.03	10.64
		MUMIAS	4.073	3.992	.995	-8.83	16.98
	CHAKOL	ALUPE	-5.690	4.074	.949	-18.86	7.48
		CHEBICH	-11.165	4.843	.432	-26.82	4.49
		CHWELE	-21.755*	4.003	.000	-34.69	-8.82
		KAPKATENY	7.984	4.049	.669	-5.10	21.07

		KIMALEWA	-2.526	3.971	1.000	-15.36	10.31
		KIMILILI	-3.376	4.412	1.000	-17.64	10.89
		MALABA	-1.458	4.175	1.000	-14.95	12.04
		MATUNGU	.071	4.388	1.000	-14.11	14.26
		MUHONJE	-9.883	4.938	.648	-25.84	6.08
		MUMIAS	-1.617	4.388	1.000	-15.80	12.57
		ALUPE	5.475	4.487	.980	-9.03	19.98
		CHAKOL	11.165	4.843	.432	-4.49	26.82
		CHWELE	-10.590	4.422	.372	-24.88	3.71
		KAPKATENY	19.149*	4.464	.001	4.72	33.58
	CHEBICH	KIMALEWA	8.639	4.394	.673	-5.56	22.84
		KIMILILI	7.790	4.796	.872	-7.71	23.29
		MALABA	9.707	4.579	.563	-5.09	24.51
		MATUNGU	11.237	4.774	.399	-4.19	26.67
		MUHONJE	1.283	5.284	1.000	-15.80	18.36
		MUMIAS	9.548	4.774	.649	-5.88	24.98
		ALUPE	16.064*	3.564	.000	4.54	27.58
		CHAKOL	21.755*	4.003	.000	8.82	34.69
		CHEBICH	10.590	4.422	.372	-3.71	24.88
		KAPKATENY	29.739*	3.535	.000	18.31	41.17
	CHWELE	KIMALEWA	19.229*	3.446	.000	8.09	30.37
		KIMILILI	18.379*	3.946	.000	5.62	31.13
		MALABA	20.296*	3.679	.000	8.41	32.19
		MATUNGU	21.826*	3.919	.000	9.16	34.49
		MUHONJE	11.872	4.526	.239	-2.76	26.50
		MUMIAS	20.137*	3.919	.000	7.47	32.80
		ALUPE	-13.675*	3.616	.008	-25.36	-1.99
		CHAKOL	-7.984	4.049	.669	-21.07	5.10
		CHEBICH	-19.149*	4.464	.001	-33.58	-4.72
	KAPKATENY	CHWELE	-29.739*	3.535	.000	-41.17	-18.31
		KIMALEWA	-10.510	3.499	.096	-21.82	.80
		KIMILILI	-11.360	3.993	.144	-24.27	1.55
		MALABA	-9.443	3.729	.288	-21.50	2.61

		MATUNGU	-7.913	3.966	.653	-20.73	4.91
		MUHONJE	-17.867*	4.567	.005	-32.63	-3.10
		MUMIAS	-9.602	3.966	.355	-22.42	3.22
		ALUPE	-3.165	3.529	.998	-14.57	8.24
		CHAKOL	2.526	3.971	1.000	-10.31	15.36
		CHEBICH	-8.639	4.394	.673	-22.84	5.56
		CHWELE	-19.229*	3.446	.000	-30.37	-8.09
		KAPKATENY	10.510	3.499	.096	-.80	21.82
	KIMALEWA	KIMILILI	-.850	3.914	1.000	-13.50	11.80
		MALABA	1.067	3.644	1.000	-10.71	12.85
		MATUNGU	2.597	3.887	1.000	-9.97	15.16
		MUHONJE	-7.357	4.498	.867	-21.90	7.18
		MUMIAS	.908	3.887	1.000	-11.65	13.47
		ALUPE	-2.315	4.018	1.000	-15.30	10.67
		CHAKOL	3.376	4.412	1.000	-10.89	17.64
		CHEBICH	-7.790	4.796	.872	-23.29	7.71
		CHWELE	-18.379*	3.946	.000	-31.13	-5.62
		KAPKATENY	11.360	3.993	.144	-1.55	24.27
	KIMILILI	KIMALEWA	.850	3.914	1.000	-11.80	13.50
		MALABA	1.917	4.120	1.000	-11.40	15.24
		MATUNGU	3.447	4.336	.999	-10.57	17.46
		MUHONJE	-6.507	4.892	.963	-22.32	9.31
		MUMIAS	1.758	4.336	1.000	-12.26	15.77
		ALUPE	-4.232	3.756	.989	-16.37	7.91
		CHAKOL	1.458	4.175	1.000	-12.04	14.95
	MALABA	CHEBICH	-9.707	4.579	.563	-24.51	5.09
		CHWELE	-20.296*	3.679	.000	-32.19	-8.41
		KAPKATENY	9.443	3.729	.288	-2.61	21.50

		KIMALEWA	-1.067	3.644	1.000	-12.85	10.71
		KIMILILI	-1.917	4.120	1.000	-15.24	11.40
		MATUNGU	1.530	4.095	1.000	-11.71	14.77
		MUHONJE	-8.424	4.679	.780	-23.55	6.70
		MUMIAS	-.159	4.095	1.000	-13.39	13.08
		ALUPE	-5.762	3.992	.937	-18.67	7.14
		CHAKOL	-.071	4.388	1.000	-14.26	14.11
		CHEBICH	-11.237	4.774	.399	-26.67	4.19
		CHWELE	-21.826*	3.919	.000	-34.49	-9.16
		KAPKATENY	7.913	3.966	.653	-4.91	20.73
	MATUNGU	KIMALEWA	-2.597	3.887	1.000	-15.16	9.97
		KIMILILI	-3.447	4.336	.999	-17.46	10.57
		MALABA	-1.530	4.095	1.000	-14.77	11.71
		MUHONJE	-9.954	4.870	.618	-25.70	5.79
		MUMIAS	-1.689	4.312	1.000	-15.63	12.25
		ALUPE	4.192	4.590	.998	-10.64	19.03
		CHAKOL	9.883	4.938	.648	-6.08	25.84
		CHEBICH	-1.283	5.284	1.000	-18.36	15.80
		CHWELE	-11.872	4.526	.239	-26.50	2.76
	MUHONJE	KAPKATENY	17.867*	4.567	.005	3.10	32.63
		KIMALEWA	7.357	4.498	.867	-7.18	21.90
		KIMILILI	6.507	4.892	.963	-9.31	22.32
		MALABA	8.424	4.679	.780	-6.70	23.55
		MATUNGU	9.954	4.870	.618	-5.79	25.70
		MUMIAS	8.265	4.870	.837	-7.48	24.01
		ALUPE	-4.073	3.992	.995	-16.98	8.83
		CHAKOL	1.617	4.388	1.000	-12.57	15.80
	MUMIAS	CHEBICH	-9.548	4.774	.649	-24.98	5.88
		CHWELE	-20.137*	3.919	.000	-32.80	-7.47
		KAPKATENY	9.602	3.966	.355	-3.22	22.42

		KIMALEWA	-.908	3.887	1.000	-13.47	11.65
		KIMILILI	-1.758	4.336	1.000	-15.77	12.26
		MALABA	.159	4.095	1.000	-13.08	13.39
		MATUNGU	1.689	4.312	1.000	-12.25	15.63
		MUHONJE	-8.265	4.870	.837	-24.01	7.48
		CHAKOL	.278	.179	.901	-.30	.86
		CHEBICH	.136	.197	1.000	-.50	.77
		CHWELE	.109	.157	1.000	-.40	.62
		KAPKATENY	.372	.159	.408	-.14	.89
	ALUPE	KIMALEWA	-.065	.155	1.000	-.57	.44
		KIMILILI	.087	.177	1.000	-.48	.66
		MALABA	-.145	.165	.999	-.68	.39
		MATUNGU	-.168	.175	.997	-.74	.40
		MUHONJE	.208	.202	.995	-.44	.86
		MUMIAS	.187	.175	.993	-.38	.75
Virus severity		ALUPE	-.278	.179	.901	-.86	.30
		CHEBICH	-.142	.213	1.000	-.83	.55
		CHWELE	-.169	.176	.997	-.74	.40
		KAPKATENY	.094	.178	1.000	-.48	.67
	CHAKOL	KIMALEWA	-.343	.175	.672	-.91	.22
		KIMILILI	-.190	.194	.996	-.82	.44
		MALABA	-.423	.183	.433	-1.02	.17
		MATUNGU	-.446	.193	.426	-1.07	.18
		MUHONJE	-.070	.217	1.000	-.77	.63
		MUMIAS	-.090	.193	1.000	-.71	.53
	CHEBICH	ALUPE	-.136	.197	1.000	-.77	.50

		CHAKOL	.142	.213	1.000	-.55	.83
		CHWELE	-.027	.194	1.000	-.65	.60
		KAPKATENY	.236	.196	.982	-.40	.87
		KIMALEWA	-.201	.193	.994	-.83	.42
		KIMILILI	-.048	.211	1.000	-.73	.63
		MALABA	-.281	.201	.950	-.93	.37
		MATUNGU	-.304	.210	.935	-.98	.37
		MUHONJE	.072	.232	1.000	-.68	.82
		MUMIAS	.052	.210	1.000	-.63	.73
	CHWELE	ALUPE	-.109	.157	1.000	-.62	.40
		CHAKOL	.169	.176	.997	-.40	.74
		CHEBICH	.027	.194	1.000	-.60	.65
		KAPKATENY	.263	.155	.839	-.24	.77
		KIMALEWA	-.175	.151	.987	-.66	.31
		KIMILILI	-.022	.173	1.000	-.58	.54
		MALABA	-.254	.162	.894	-.78	.27
		MATUNGU	-.277	.172	.878	-.83	.28
		MUHONJE	.099	.199	1.000	-.54	.74
		MUMIAS	.078	.172	1.000	-.48	.63
	KAPKATENY	ALUPE	-.372	.159	.408	-.89	.14
		CHAKOL	-.094	.178	1.000	-.67	.48
		CHEBICH	-.236	.196	.982	-.87	.40
		CHWELE	-.263	.155	.839	-.77	.24
		KIMALEWA	-.437	.154	.144	-.93	.06
		KIMILILI	-.285	.175	.873	-.85	.28
		MALABA	-.517	.164	.063	-1.05	.01
		MATUNGU	-.540	.174	.074	-1.10	.02
		MUHONJE	-.164	.201	.999	-.81	.48
		MUMIAS	-.185	.174	.993	-.75	.38

		ALUPE	.065	.155	1.000	-.44	.57
		CHAKOL	.343	.175	.672	-.22	.91
		CHEBICH	.201	.193	.994	-.42	.83
		CHWELE	.175	.151	.987	-.31	.66
		KAPKATENY	.437	.154	.144	-.06	.93
	KIMALEWA	KIMILILI	.153	.172	.998	-.40	.71
		MALABA	-.079	.160	1.000	-.60	.44
		MATUNGU	-.103	.171	1.000	-.65	.45
		MUHONJE	.273	.198	.952	-.37	.91
		MUMIAS	.253	.171	.926	-.30	.80
		ALUPE	-.087	.177	1.000	-.66	.48
		CHAKOL	.190	.194	.996	-.44	.82
		CHEBICH	.048	.211	1.000	-.63	.73
		CHWELE	.022	.173	1.000	-.54	.58
	KIMILILI	KAPKATENY	.285	.175	.873	-.28	.85
		KIMALEWA	-.153	.172	.998	-.71	.40
		MALABA	-.232	.181	.972	-.82	.35
		MATUNGU	-.256	.191	.961	-.87	.36
		MUHONJE	.121	.215	1.000	-.57	.82
		MUMIAS	.100	.191	1.000	-.52	.72
		ALUPE	.145	.165	.999	-.39	.68
		CHAKOL	.423	.183	.433	-.17	1.02
		CHEBICH	.281	.201	.950	-.37	.93
		CHWELE	.254	.162	.894	-.27	.78
		KAPKATENY	.517	.164	.063	-.01	1.05
	MALABA	KIMALEWA	.079	.160	1.000	-.44	.60
		KIMILILI	.232	.181	.972	-.35	.82
		MATUNGU	-.023	.180	1.000	-.61	.56
		MUHONJE	.353	.206	.827	-.31	1.02
		MUMIAS	.332	.180	.752	-.25	.91

		ALUPE	.168	.175	.997	-.40	.74
		CHAKOL	.446	.193	.426	-.18	1.07
		CHEBICH	.304	.210	.935	-.37	.98
		CHWELE	.277	.172	.878	-.28	.83
		KAPKATENY	.540	.174	.074	-.02	1.10
	MATUNGU	KIMALEWA	.103	.171	1.000	-.45	.65
		KIMILILI	.256	.191	.961	-.36	.87
		MALABA	.023	.180	1.000	-.56	.61
		MUHONJE	.376	.214	.804	-.32	1.07
		MUMIAS	.356	.189	.733	-.26	.97
		ALUPE	-.208	.202	.995	-.86	.44
		CHAKOL	.070	.217	1.000	-.63	.77
		CHEBICH	-.072	.232	1.000	-.82	.68
		CHWELE	-.099	.199	1.000	-.74	.54
		KAPKATENY	.164	.201	.999	-.48	.81
	MUHONJE	KIMALEWA	-.273	.198	.952	-.91	.37
		KIMILILI	-.121	.215	1.000	-.82	.57
		MALABA	-.353	.206	.827	-1.02	.31
		MATUNGU	-.376	.214	.804	-1.07	.32
		MUMIAS	-.021	.214	1.000	-.71	.67
		ALUPE	-.187	.175	.993	-.75	.38
		CHAKOL	.090	.193	1.000	-.53	.71
		CHEBICH	-.052	.210	1.000	-.73	.63
		CHWELE	-.078	.172	1.000	-.63	.48
	MUMIAS	KAPKATENY	.185	.174	.993	-.38	.75
		KIMALEWA	-.253	.171	.926	-.80	.30
		KIMILILI	-.100	.191	1.000	-.72	.52
		MALABA	-.332	.180	.752	-.91	.25
		MATUNGU	-.356	.189	.733	-.97	.26
		MUHONJE	.021	.214	1.000	-.67	.71

*. The mean difference is significant at the 0.05 level.

CORRELATION

Correlations

Control Variables			Virus incidence	Virus severity
claster label	Virus incidence	Correlation	1.000	.559
		Significance (2-tailed)	.	.000
		Df	0	558
	Virus severity	Correlation	.559	1.000
		Significance (2-tailed)	.000	.
		Df	558	0

Appendix v: Graph of mean severity of viral disease on groundnuts in surveyed clusters in western Kenya.

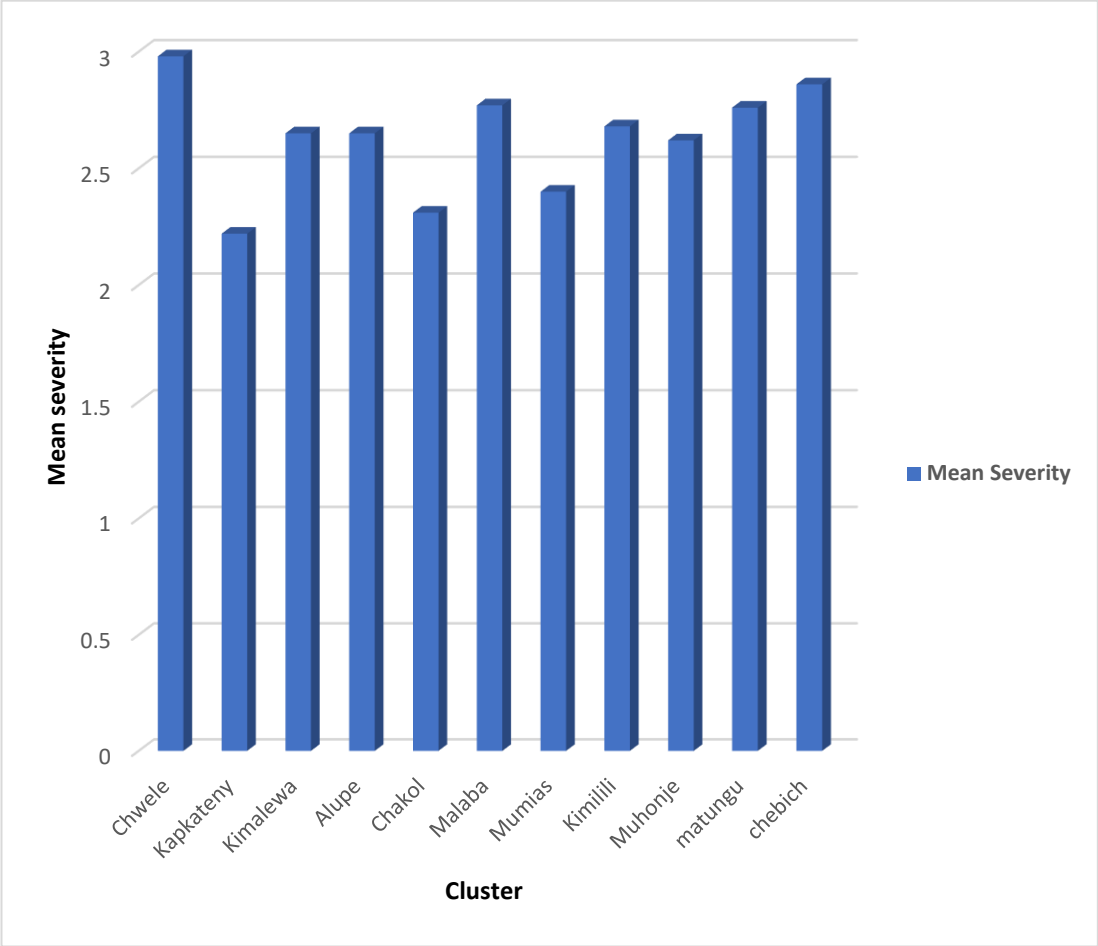
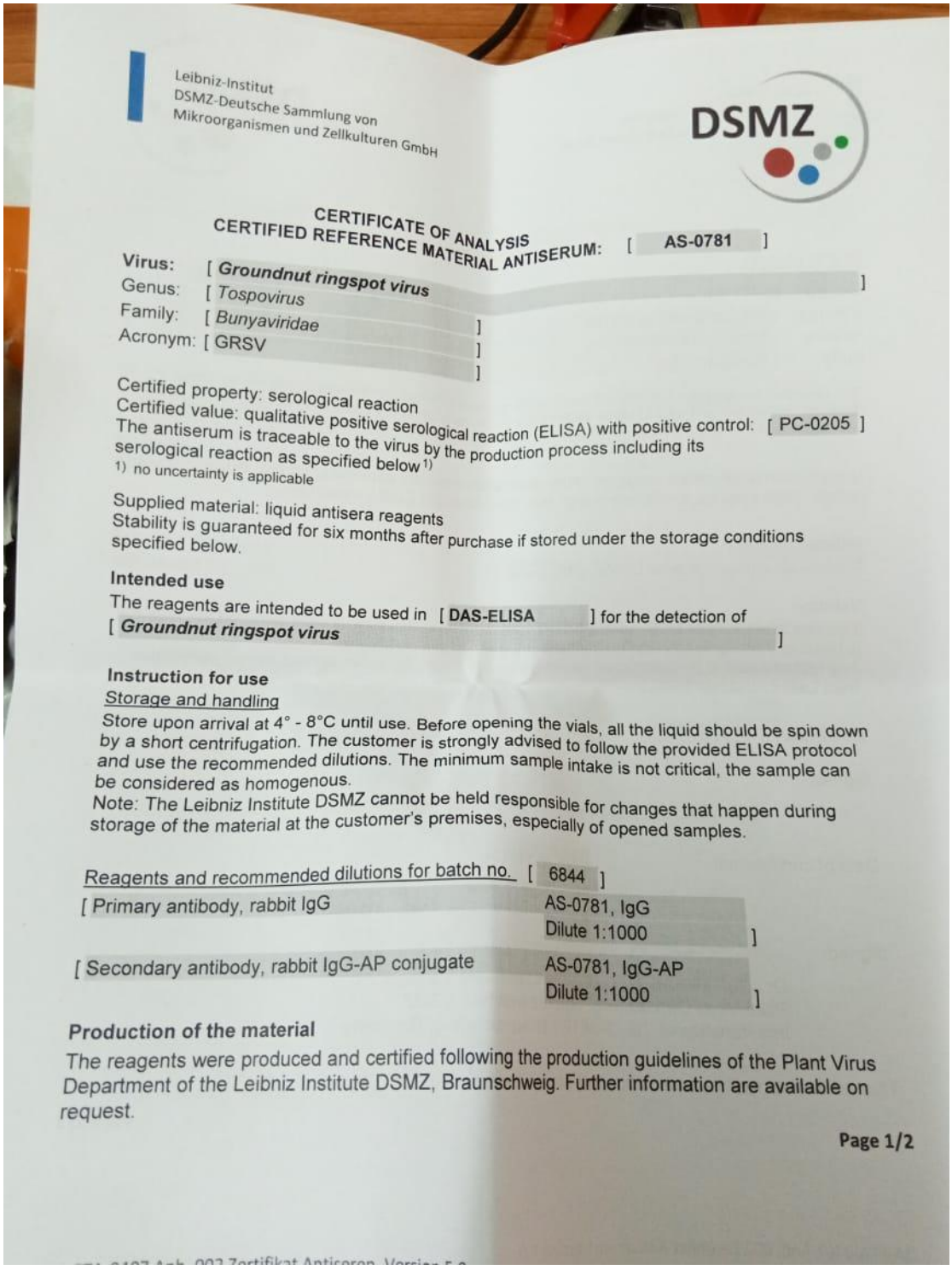


Figure 5: Graph of mean severity of viral disease on groundnuts in surveyed clusters in western Kenya**.**

Appendix vi:GRSV antisera



Leibniz-Institut
DSMZ-Deutsche Sammlung von
Mikroorganismen und Zellkulturen GmbH

DSMZ

CERTIFICATE OF ANALYSIS
CERTIFIED REFERENCE MATERIAL ANTISERUM: [AS-0781]

Virus: [***Groundnut ringspot virus***]
Genus: [*Tospovirus*]
Family: [*Bunyaviridae*]
Acronym: [GRSV]
]

Certified property: serological reaction
Certified value: qualitative positive serological reaction (ELISA) with positive control: [PC-0205]
The antiserum is traceable to the virus by the production process including its serological reaction as specified below [1)]
1) no uncertainty is applicable

Supplied material: liquid antisera reagents
Stability is guaranteed for six months after purchase if stored under the storage conditions specified below.

Intended use
The reagents are intended to be used in [**DAS-ELISA**] for the detection of
[***Groundnut ringspot virus***]

Instruction for use
Storage and handling
Store upon arrival at 4° - 8°C until use. Before opening the vials, all the liquid should be spin down by a short centrifugation. The customer is strongly advised to follow the provided ELISA protocol and use the recommended dilutions. The minimum sample intake is not critical, the sample can be considered as homogenous.
Note: The Leibniz Institute DSMZ cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened samples.

Reagents and recommended dilutions for batch no. [6844]

[Primary antibody, rabbit IgG	AS-0781, IgG Dilute 1:1000]
[Secondary antibody, rabbit IgG-AP conjugate	AS-0781, IgG-AP Dilute 1:1000]

Production of the material
The reagents were produced and certified following the production guidelines of the Plant Virus Department of the Leibniz Institute DSMZ, Braunschweig. Further information are available on request.

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Appendix vii: Viral severity on intercropped groundnut varieties in western Kenya

Treatments	Mean Severity	Max Severity	Min Severity	Std error
ICGV-12991 + Beans	2.7	4	1	0.45
ICGV-90704 + Beans	3.25	4	2	0.25
Red Valencia + Beans	2.90	4	1	0.38
ICGV-12991 + Soy beans	1.71	4	1	0.47
ICGV-90704 + Soy beans	2.00	4	1	0.47
Red Valencia + Soy beans	3.1	4	1	0.31
ICGV-12991 + Cowpeas	2.62	4	1	0.35
ICGV-90704 + Cowpeas	2.00	4	1	0.49
Red Valencia + Cowpeas	2.89	4	1	0.24
ICGV-12991 + (mixture of legumes)	3.74	4	1	0.00
ICGV-90704 + (mixture of legumes)	2.00	3	1	1.00
Red Valencia + (mixture of legumes)	2.00	3	1	0.58
ICGV-12991 Purestand	1.09	2	1	0.00
ICGV-90704 Purestand	1.24	1	1	1.00
Red Valencia Purestand	1	1	1	0.00

Appendix viii:Control experiment for screened broad-leafed weeds for alternative hosts to GRSV

ID	Name	Family	N	Inci %	Sev	Symptoms	ELISA
KHRW041 CP	Achyanthes bidentate	Amaranthaceae	3	0	1	No viral disease symptoms	_
KHRW42 CP	Amaranthus retroflexus	Amaranthaceae	3	0	1	No viral disease symptoms	__
KHRW43 CP	Bidens Pilosa	Asteraceae	3	0	1	No viral disease symptoms	_
KHRW44 CP	American burn weed	Asteraceae	3	0	1	No viral disease symptoms	_
KHRW45 CP	Commelina benghalensis	Commelinaceae	3	0	1	No viral disease symptoms	_
KHRW46 CP	Datura stramonium	Solanaceae	3	0	1	No viral disease symptoms	_
KHRW47 CP	Chenopodium album	Amaranthaceae	3	0	1	No viral disease symptoms	_
KHRW48 CP	Solanum incanum	Solanaceae	3	0	1	No viral disease symptoms	_
KHRW49 CP	Ageratum conyzoides	Asteraceae	3	0	1	No viral disease symptom	_
KHRW50 CP	Oxygonium sinuatam	Polygonaceae	3	0	1	No viral disease symptoms	_
KHRW51 CP	Solanum americanum	Solanaceae	3	0	1	No viral disease symptoms	_
KHRW52 CP	Solanum ptychanthum	Solanaceae	3	0	1	No viral disease symptoms	_
KHRW53 CP	Ipomoea batatas	Convolvulaceae	3	0	1	No viral disease symptoms.	_
KHRW54 CP	Persea Americana	Lauraceae	3	0	1	No viral disease symptoms	_

KHRW55 CP	Markhamia lutea	Bignoniaceae	3	0	1	No viral disease symptoms	_
KHRW56 CP	Amaranthus rudis	Amaranthaceae	3	0	1	No viral disease symptoms	_
KHRW57 CP	Ageratum conyzoides	Asteraceae	3	0	1	No viral disease symptoms	_
KHRW58 CP	Galinsoga parviflora	Asteraceae	3	0	1	No viral disease symptoms	_

Appendix: ix: RT-PCR for groundnut samples

Report On Molecular Testing Of Groundnut leaves For Groundnut Ringspot Virus Using Reverse Transcriptase PCR

A total of 6 samples were tested for the above virus.

RNA Extraction using CTAB

For 6 samples requiring 2mls each

2% PVP for 15 mls = 0.3g

1 % Sodium Sulphite for 15mls =0.15g

Mixed well at room temperature.

Procedure

- Ground samples (leaves, approximately 0.5-1 g) using 2 ml 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 resuspended.
- Incubated the samples at 65℃ for 15 mins for lysing cells completely.
- Added 700 µl of chloroform: isoamyl alcohol (24:1) to each tube, homogenize them by vortexing.
- Centrifuged at 14000 rpm at 4℃ for 10 min.
- Transferred the upper aqueous phase to a new 1.5 ml 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 30 mins
- Centrifuged the samples at 14000rpm for 10mins and poured off the salts.
- The pellet was washed in 500µl of 70% ethanol by centrifuging them 14000rpm for 5mins at 4℃ and decanted off.
- Air dried the samples for 30mins
- Resuspended them in 50µl nuclease free water.
- Quantification done.

The Quantification results were satisfactory to proceed to pcr.

Detection of the virus using RT-PCR

PCR Master Mix

Component	1X
2X Reaction Mix	25µl
Forward primer	1 µl

Reverse primer	1 µl
Superscript™ III RT/Platinum™	2 µl
Nuclease free water	20 µl
Template RNA	1.0

The components were mixed gently to ensure all the components are at the bottom of the amplification tube. Then centrifuged briefly in a microcentrifuge.

Thermal cycle for the Reaction

Temperature regime for the conventional PCR

55^0C	30mins	1
94^0C	2mins	
94^0C	15s	40
55^0C	30s	
68^0C	1min	
68^0C	5mins	1
4^0C	∞	

GEL ELECTROPHORESIS

1.5% gel was prepared as follows:

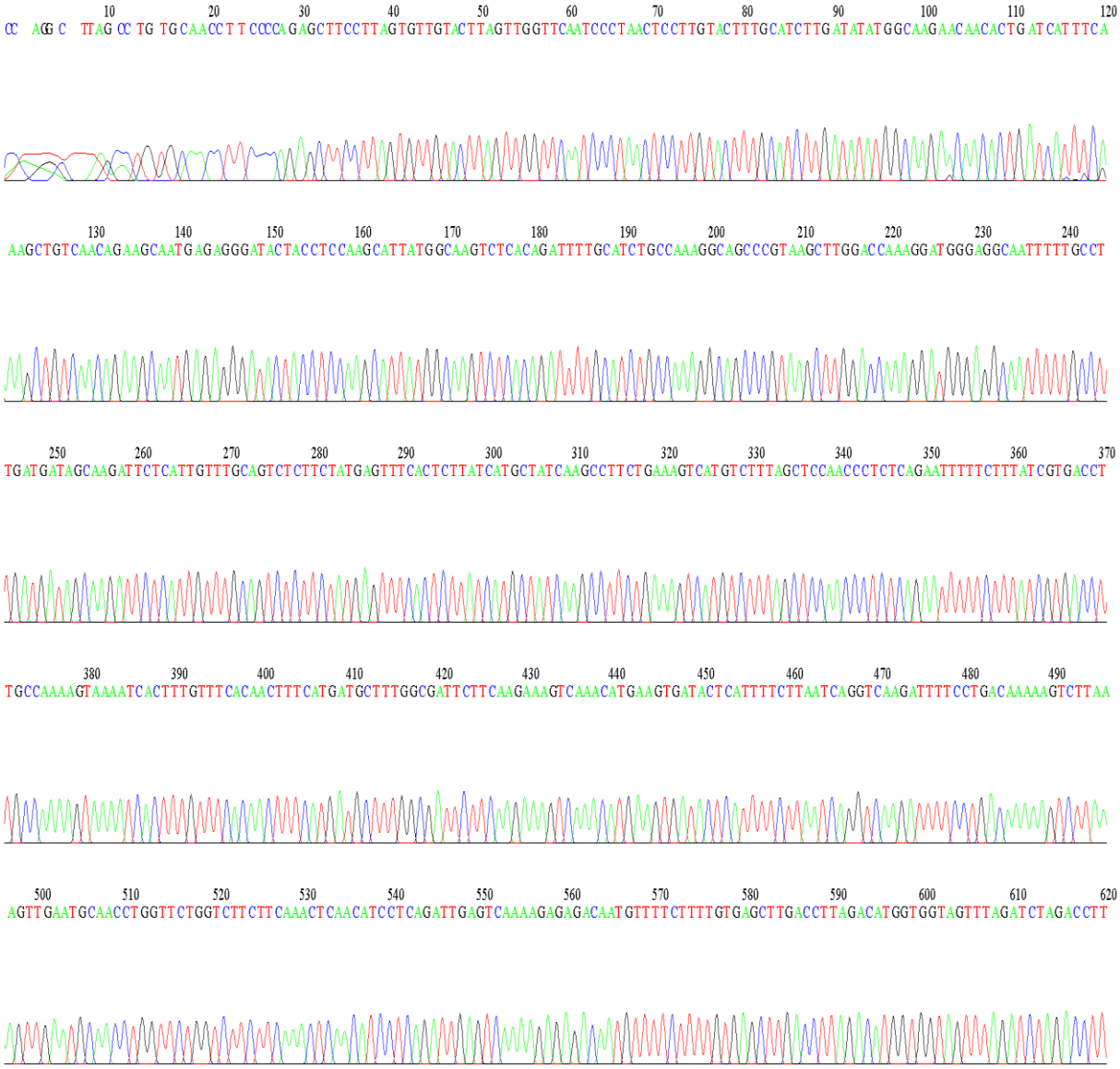
- 1.0g of agarose was weighed and put into a conical flask.
- 100mls of 1% TAE was added and heat to boil
- 3µl of gel stain Invitrogen was added and swirled to mix.
- The gel was cast. After solidifying it was immersed in the gel tank
- 5µl of the sample was mixed with 3µl of loading dye and put in the wells.
- 1kp ladder ladder was run against the samples.
- The samples were run at100V for 1hour then observed in Azure™ Gel dock

L –Ladder

Bands observed at 600 bp

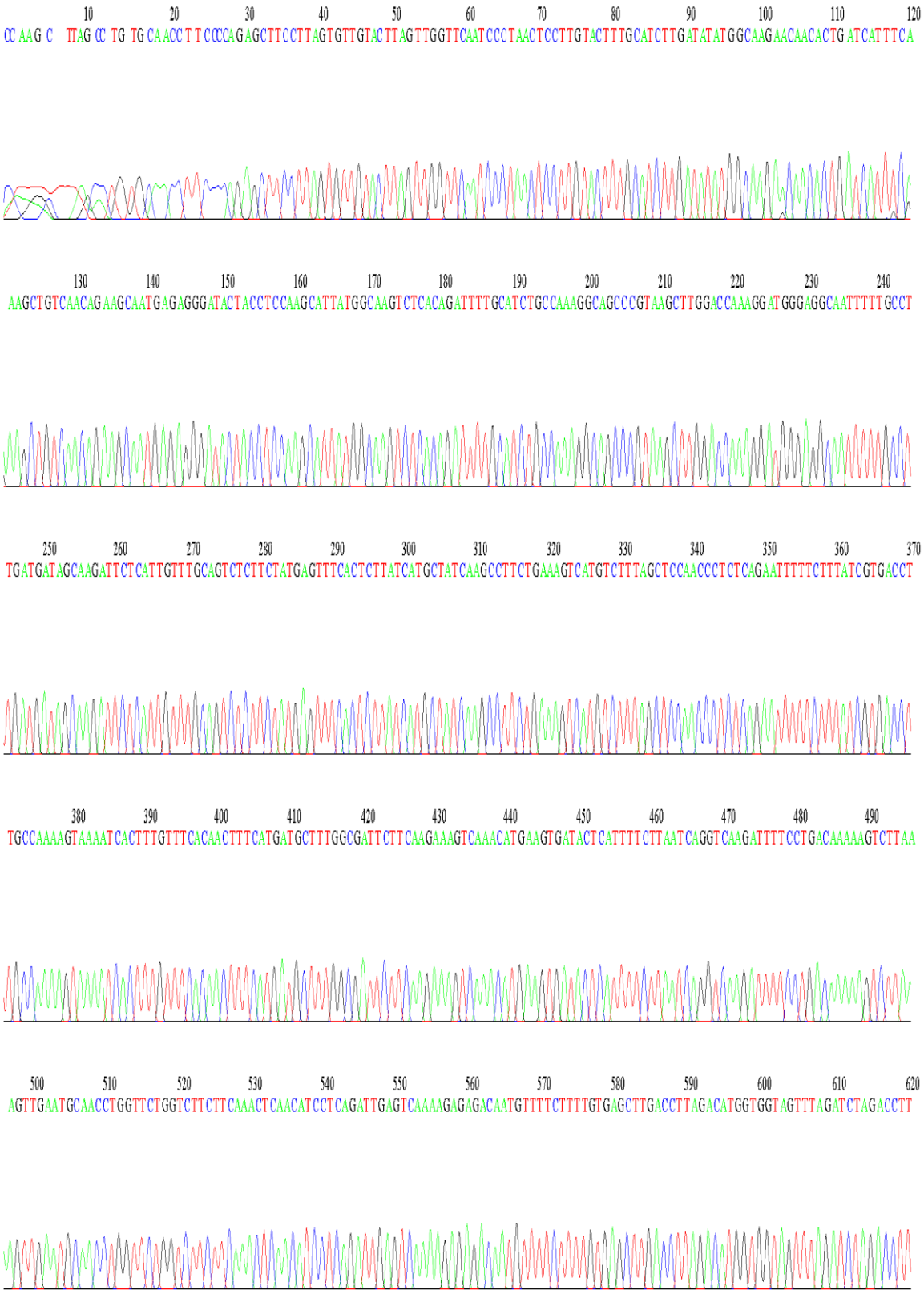
Appendix x: Groundnuts sequences

File: 1_GRSVnF.ab1 Run Ended: 2020/9/28 21:33:12 Signal G:2818 A:4146 C:5910 T:6119
Sample: 1_GRSVnF Lane: 44 Base spacing: 14.22075 1858 bases in 23433 scans
Page 1 of 2



File: 2_GRSVnF.ab1 Run Ended: 2020/9/28 21:33:12 Signal G:2373 A:3182 C:4148 T:4262

Sample: 2_GRSVnF Lane: 42 Base spacing: 14.135621 1919 bases in 23632 scans Page 1 of 2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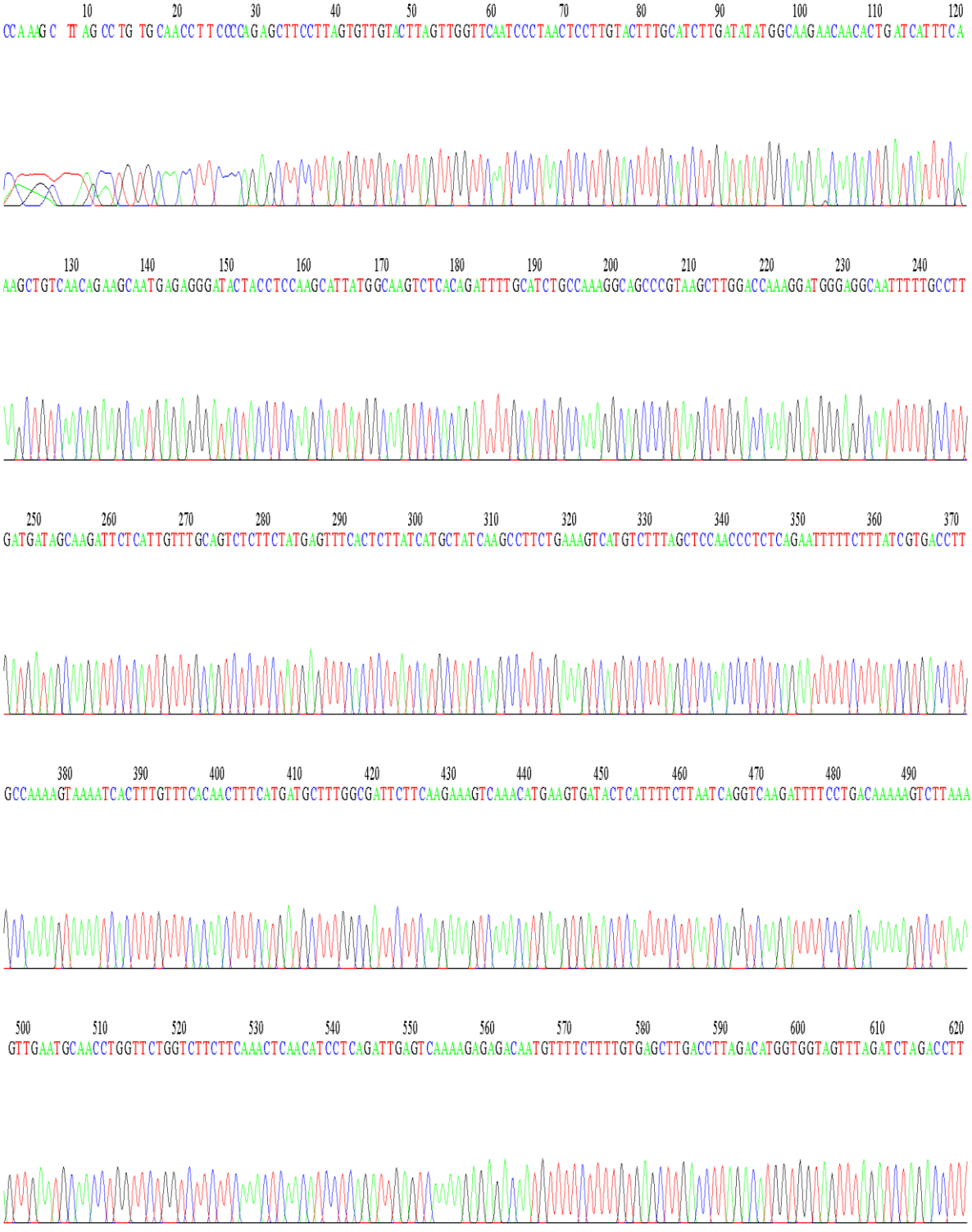
CC AA G C TTAG CC TG TG CAACCTT CCCCAGAGCTTCCTTAGTGTTGTACTTAGTTGGTTCAATCCCTAACTCCTTGTACTTTGCATCTTGATATATGGCAAGAACAACACTG ATCATTTCA
130 140 150 160 170 180 190 200 210 220 230 240
AAGCTGTCAACAG AAGCAATGAGAGGGATACTACCTCCAAGCATTATGGCAAGTCTCACAGATTTTGCATCTGCCAAAGGCAGCCCGTAAGCTTGGACCAAAGGATGGGAGGCAATTTTTGCCT
250 260 270 280 290 300 310 320 330 340 350 360 370
TGATGATAGCAAGATTCTCATTGTTTGCAGTCTCTTCTATGAGTTTCACTCTTATCATGCTATCAAGCCTTCTGAAAGTCATGTCTTTAGCTCCAACCCTCTCAGAATTTTTCTTTATCGTGACCT
380 390 400 410 420 430 440 450 460 470 480 490
TGCCAAAAGTAAAATCACTTTGTTTCACAACTTTCATGATGCTTTGGCGATTCTTCAAGAAAGTCAAACATGAAGTGATACTCATTTTCTTAATCAGGTCAAGATTTTCCTGACAAAAAGTCTTAA
500 510 520 530 540 550 560 570 580 590 600 610 620
AGTTGAATGCAACCTGGTTCTGGTCTTCTTCAAACTCAACATCCTCAGATTGAGTCAAAAGAGAGACAATGTTTTCTTTTGTGAGCTTGACCTTAGACATGGTGGTAGTTTAGATCTAGACCTT

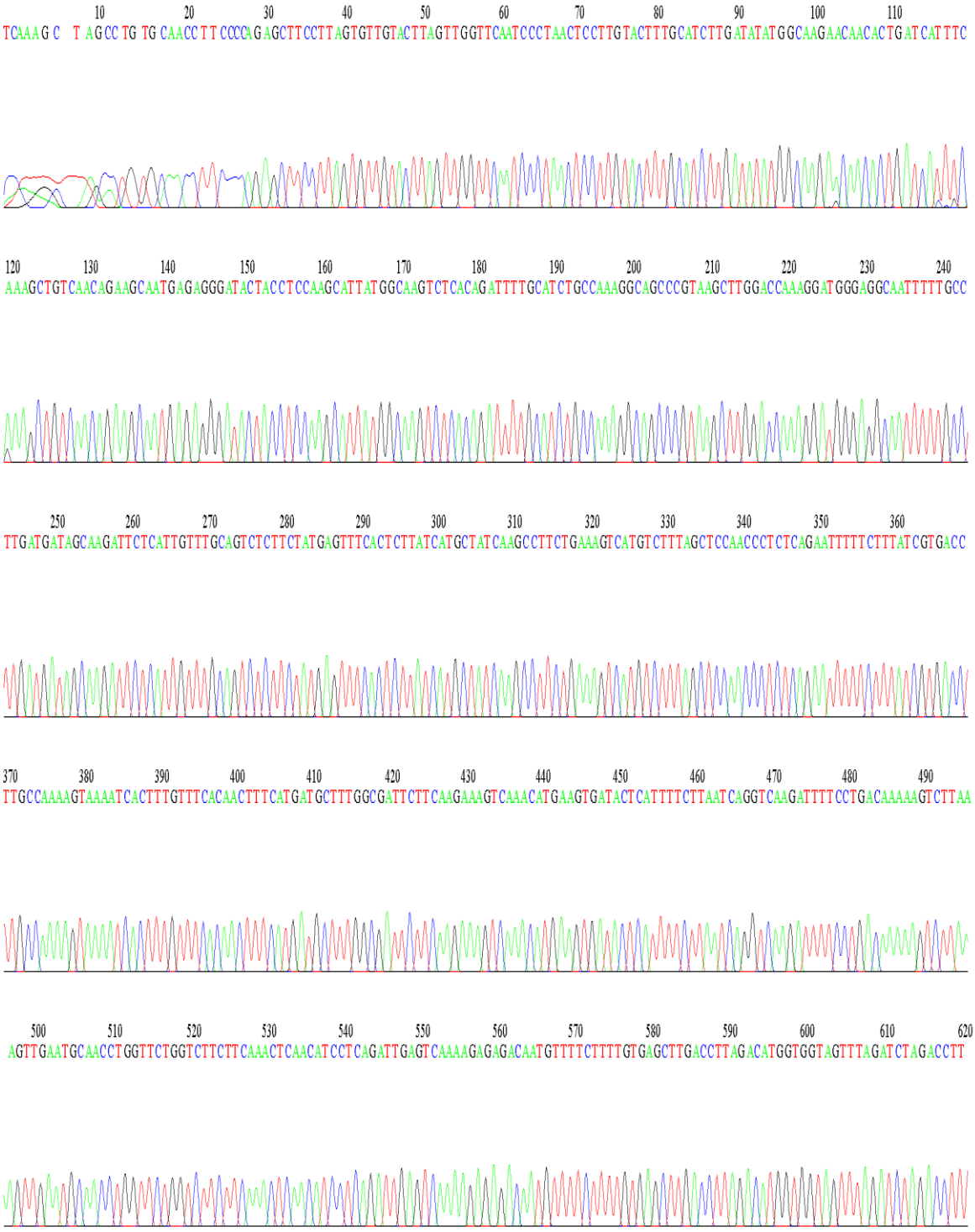
File: 3_GRSVnF.ab1 Run Ended: 2020/9/28 21:33:12 Signal G:3495 A:5078 C:7338 T:7666

Sample: 3_GRSVnF Lane: 40 Base spacing: 14.227506 1881 bases in 23558 scans Page 1 of 2

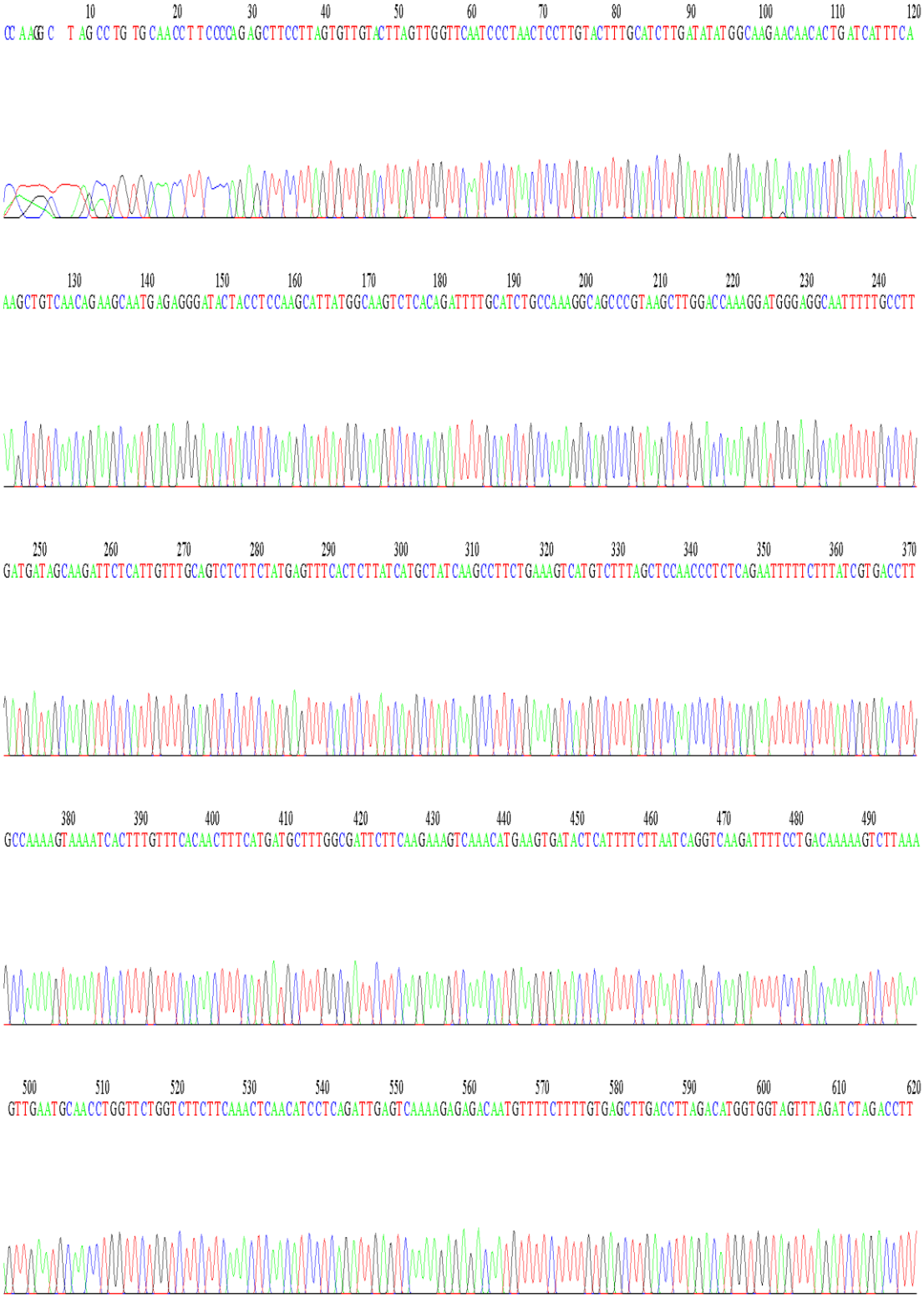


File: 4_GRSVnF.ab1 Run Ended: 2020/9/28 21:33:12 Signal G:1529 A:2259 C:3203 T:3756

Sample: 4_GRSVnF Lane: 38 Base spacing: 14.266343 2067 bases in 23130 scans
Page 1 of 2



File: 5_GRSVnF.ab1 Run Ended: 2020/9/28 21:33:12 Signal G:2795 A:4418 C:6270 T:7210
Sample: 5_GRSVnF Lane: 36 Base spacing: 14.2449465 1935 bases in 23270 scans Page 1 of 2


10 20 30 40 50 60 70 80 90 100 110 120
CC AAGG C T AG CC TG TG CAACCT T CCCCAG AGCTTCCTTAGTGTTGTACTTAGTTGGTTCAATCCCTAACTCCTTGTACTTTGCATCTTGATATATGGCAAGAACAACACTG ATCATTTCA
130 140 150 160 170 180 190 200 210 220 230 240
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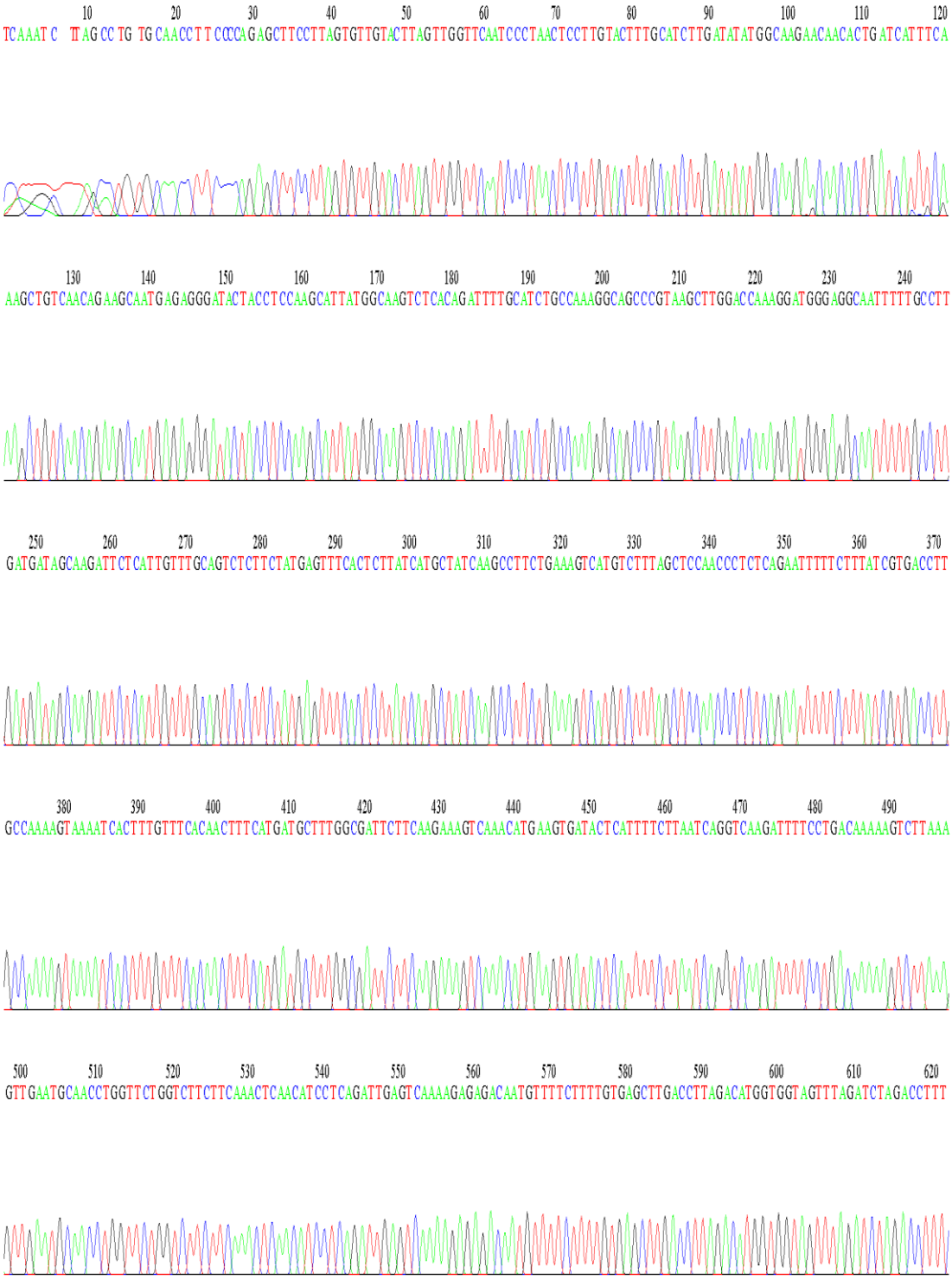
250 260 270 280 290 300 310 320 330 340 350 360 370
GATGATAGCAAGATTCTCATTGTTTGCAGTCTCTTCTATGAGTTTCACTCTTATCATGCTATCAAGCCTTCTGAAAGTCATGTCTTTAGCTCCAACCCTCTCAGAATTTTTCTTTATCGTGACCTT
380 390 400 410 420 430 440 450 460 470 480 490
GCCAAAAGTAAAATCACTTTGTTTCACAACTTTCATGATGCTTTGGCGATTCTTCAAGAAAGTCAAACATGAAGTGATACTCATTTTCTTAATCAGGTCAAGATTTTCCTGACAAAAAGTCTTAAA
500 510 520 530 540 550 560 570 580 590 600 610 620
GTTGAATGCAACCTGGTTCTGGTCTTCTTCAAACTCAACATCCTCAGATTGAGTCAAAAGAGAGACAATGTTTTCTTTTGTGAGCTTGACCTTAGACATGGTGGTAGTTTAGATCTAGACCTT

File: 5_GRSVnF.ab1 Run Ended: 2020/9/28 21:33:12 Signal G:2795 A:4418 C:6270 T:7210

Sample: 5_GRSVnF Lane: 36 Base spacing: 14.2449465 1935 bases in 23270 scans Page 2 of 2

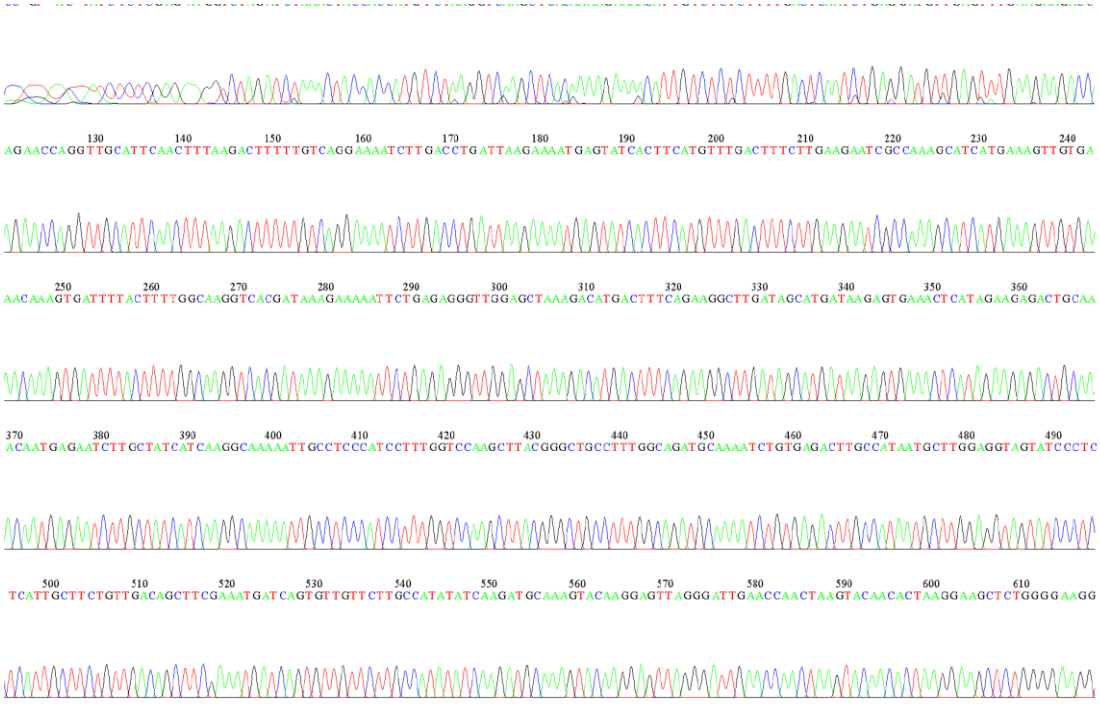
File: 6_GRSVnF.ab1 Run Ended: 2020/9/28 21:33:12 Signal G:2747 A:4188 C:5822 T:6935
Sample: 6_GRSVnF Lane: 34 Base spacing: 14.359551 1877 bases in 22538 scans Page 1 of 2



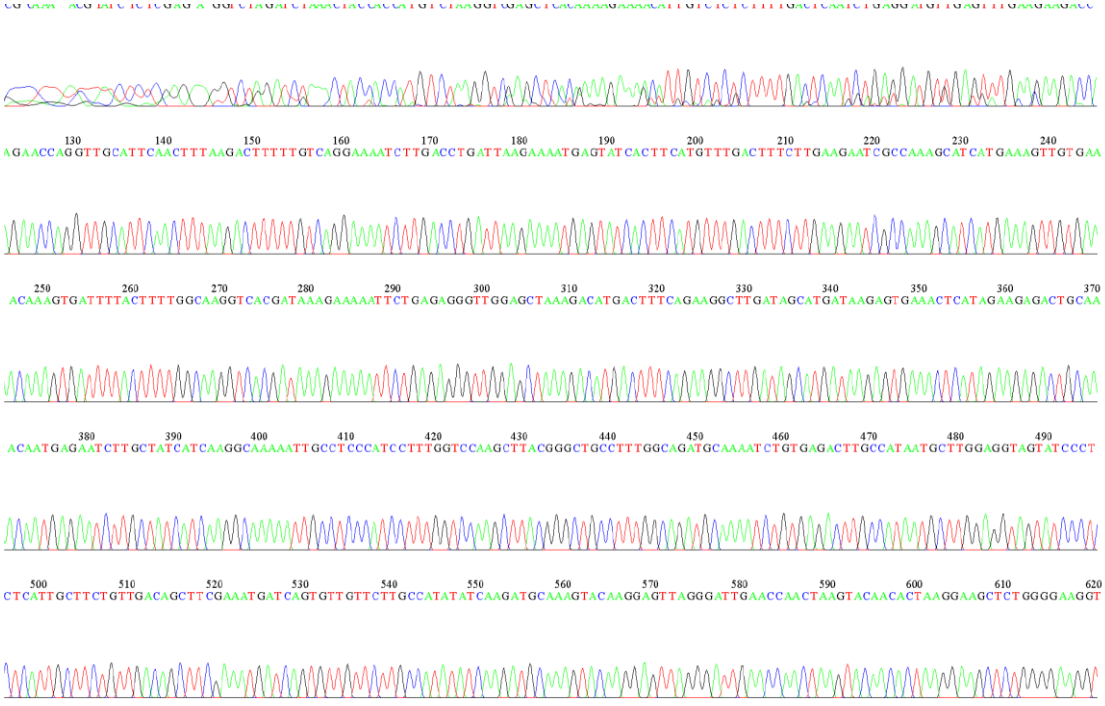
File: 6_GRSVnF.ab1 Run Ended: 2020/9/28 21:33:12 Signal G:2747 A:4188 C:5822 T:6935
Sample: 6_GRSVnF Lane: 34 Base spacing: 14.359551 1877 bases in 22538 scans Page 2 of 2

File: 1_GRSVnR.ab1 Run Ended: 2020/11/28 0:48:0 Signal G:3146 A:4687 C:5069 T:5546

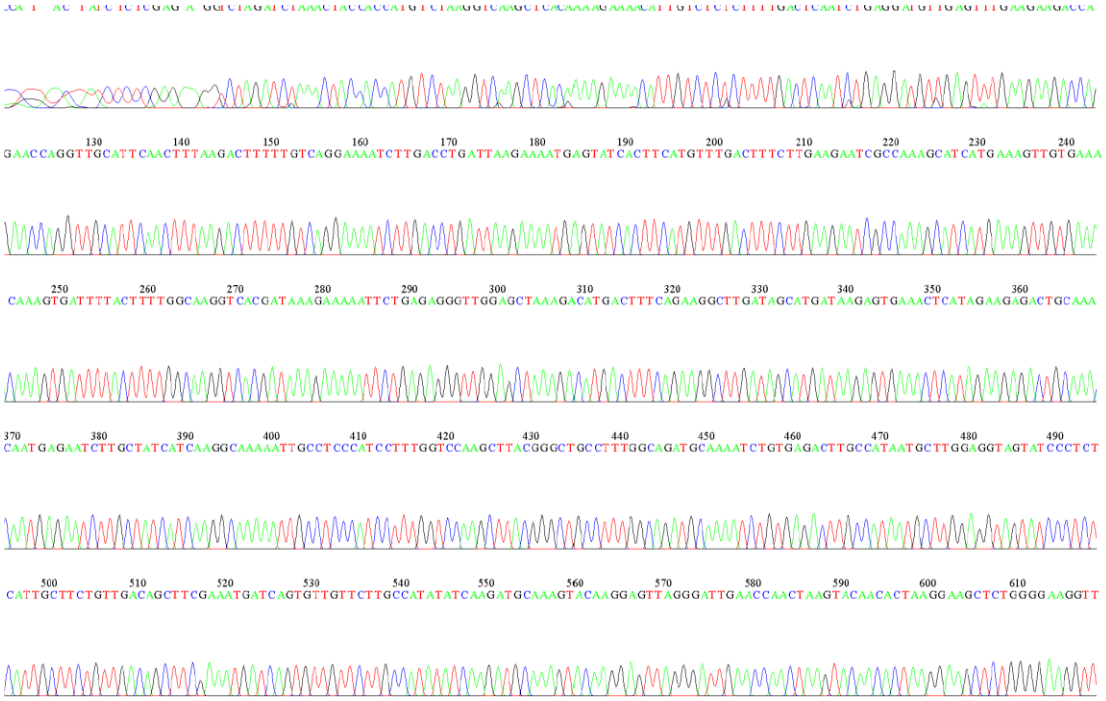
Sample: 1_GRSVnR Lane: 72 Base spacing: 14.512681 679 bases in 22733 scans Page 1 of 2



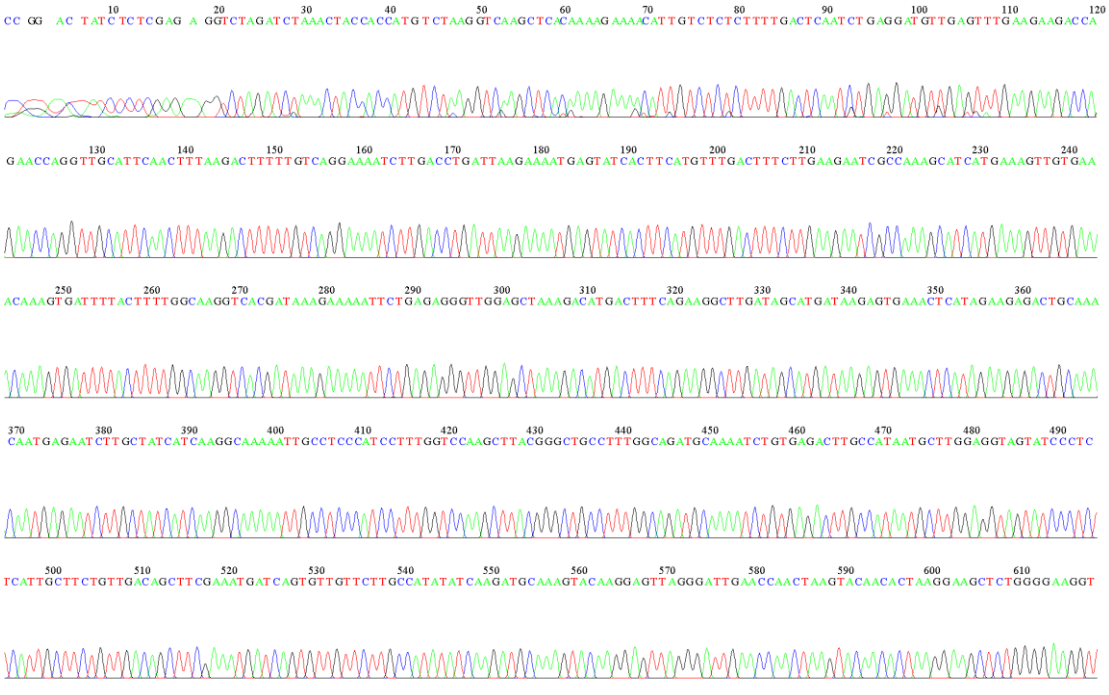
File: 2_GRSVnR.ab1 Run Ended: 2020/11/28 0:48:0 Signal G:1362 A:1485 C:1514 T:1784
Sample: 2_GRSVnR Lane: 70 Base spacing: 14.594162 1623 bases in 20523 scans Page 1 of 2



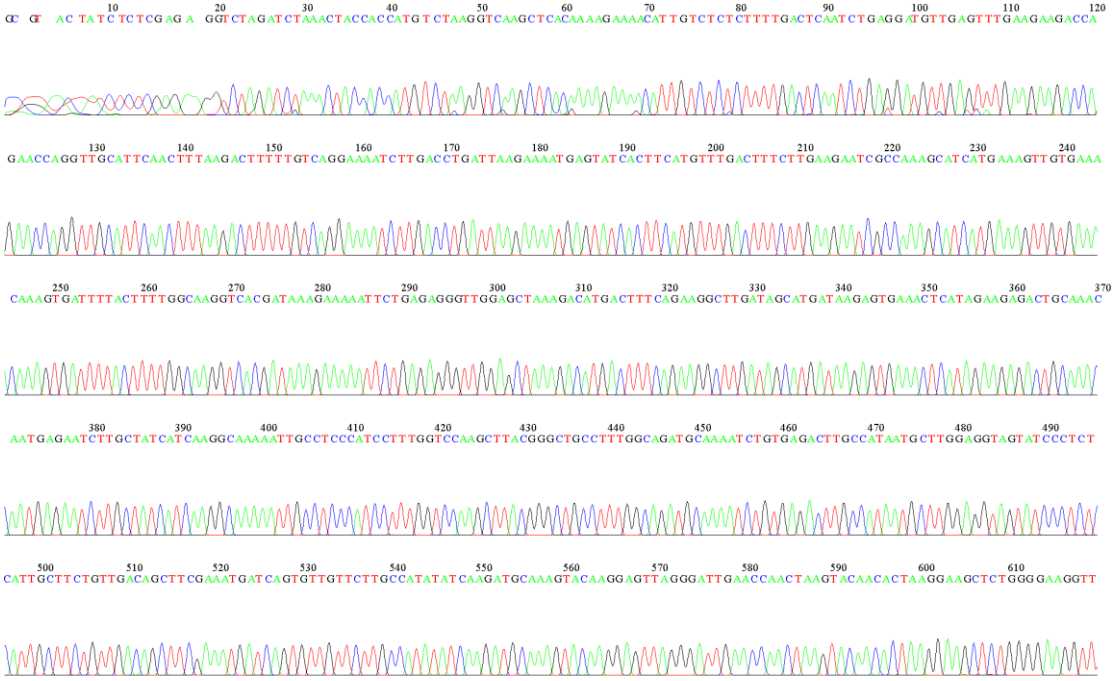
File: 3_GRSVnR.ab1 Run Ended: 2020/11/28 0:48:0 Signal G:2945 A:4279 C:4883 T:5023
Sample: 3_GRSVnR Lane: 68 Base spacing: 14.611421 676 bases in 21395 scans Page 1 of 2



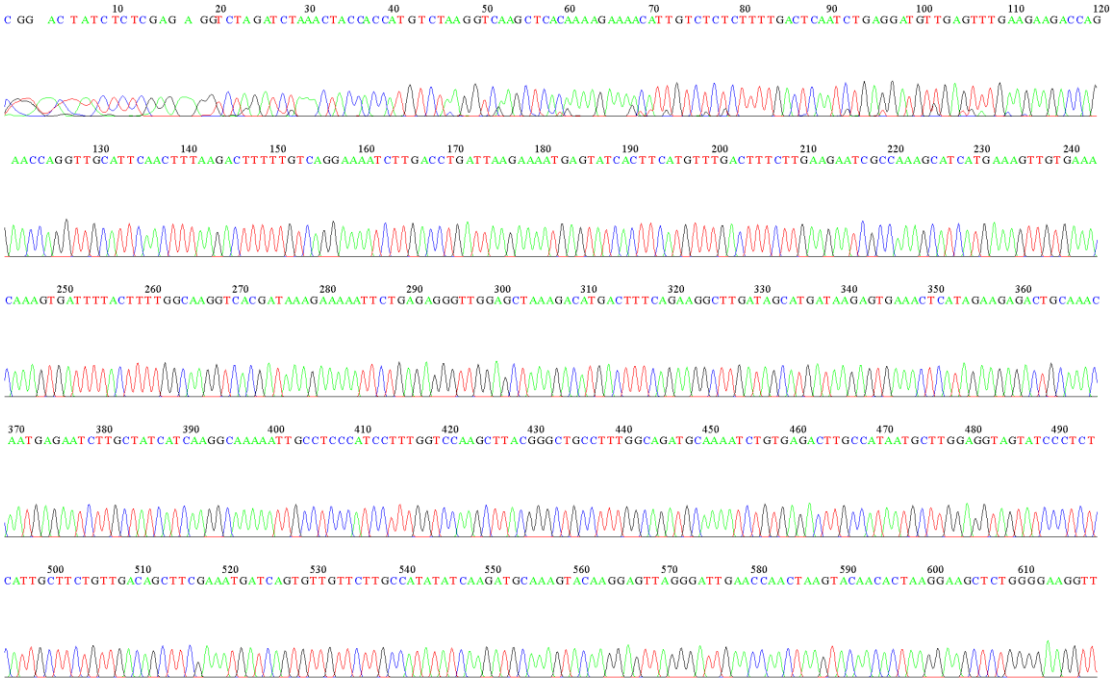
File: 4_GRSVnR.ab1 Run Ended: 2020/11/28 0:48:0 Signal G:3324 A:5297 C:5912 T:6696
Sample: 4_GRSVnR Lane: 66 Base spacing: 14.678369 1783 bases in 21363 scans Page 1 of 2



File: 5_GRSVnR.ab1 Run Ended: 2020/11/28 0:48:0 Signal G:1241 A:2262 C:2161 T:2901
Sample: 5_GRSVnR Lane: 95 Base spacing: 14.89618 677 bases in 20775 scans Page 1 of 2



File: 6_GRSVnR.ab1 Run Ended: 2020/11/28 0:48:0 Signal G:2305 A:4128 C:4315 T:5318
Sample: 6_GRSVnR Lane: 93 Base spacing: 14.753741 1533 bases in 20849 scans Page 1 of 2



Appendix: xi:Six Kenyan Groundnuts Isolates Nucleoproteins Sequences

GRSVn1

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TTTGACTCAATCTGAGGATGTTGAGTTTGAAGAAGACCAGAACCAGGTTG
CATTCAACTTTAAGACTTTTTGTCAGGAAAATCTTGACCTGATTAAGAAA
ATGAGTATCACTTCATGTTTGACTTTCTTGAAGAATCGCCAAAGCATCAT
GAAAGTTGTGAAACAAAGTGATTTTACTTTTGGCAAGGTCACGATAAAG
AAAAATTCTGAGAGGGTTGGAGCTAAAGACATGACTTTCAGAAGGCTTG
ATAGCATGATAAGAGTGAAACTCATAGAAGAGACTGCAAACAATGAGAA
TCTTGCTATCATCAAGGCAAAAATTGCCTCCCATCCTTTGGTCCAAGCTTA
CGGGCTGCCTTTGGCAGATGCAAAATCTGTGAGACTTGCCATAATGCTTG
GAGGTAGTATCCCTCTCATTGCTTCTGTTGACAGCTTCGAAATGATCAGT
GTTGTTCTTGCCATATATCAAGATGCAAAGTACAAGGAGTTAGGGATTGA
ACCAACTAAGTACAACACTAAGGAAGCTCTGGGGAAGGTTGCTGCACAG
TGCTTAAAAGCAAAGGATTTACAATGGAAA

>GRSVn2

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CATTCAACTTTAAGACTTTTTGTCAGGAAAATCTTGACCTGATTAAGAAA
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GAAAGTTGTGAAACAAAGTGATTTTACTTTTGGCAAGGTCACGATAAAG
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ATAGCATGATAAGAGTGAAACTCATAGAAGAGACTGCAAACAATGAGAA
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CGGGCTGCCTTTGGCAGATGCAAAATCTGTGAGACTTGCCATAATGCTTG
GAGGTAGTATCCCTCTCATTGCTTCTGTTGACAGCTTCGAAATGATCAGT
GTTGTTCTTGCCATATATCAAGATGCAAAGTACAAGGAGTTAGGGATTGA
ACCAACTAAGTACAACACTAAGGAAGCTCTGGGGAAGGTTTGCACAGTG
CTTAAAAGCAAAGGATTTACAATGGATGATTGGCACAAAGAAAAAAAAA
AA

>GRSVn3

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AACTACCACCATGTCTAAGGTCAAGCTCACAAAAGAAAACATTGTCTCTC
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GCATTCAACTTTAAGACTTTTTGTCAGGAAAATCTTGACCTGATTAAGAA
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TGAAAGTTGTGAAACAAAGTGATTTTACTTTTGGCAAGGTCACGATAAAG
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ATAGCATGATAAGAGTGAAACTCATAGAAGAGACTGCAAACAATGAGAA
TCTTGCTATCATCAAGGCAAAAATTGCCTCCCATCCTTTGGTCCAAGCTTA
CGGGCTGCCTTTGGCAGATGCAAAATCTGTGAGACTTGCCATAATGCTTG

GAGGTAGTATCCCTCTCATTGCTTCTGTTGACAGCTTCGAAATGATCAGT
GTTGTTCTTGCCATATATCAAGATGCAAAGTACAAGGAGTTAGGGATTGA
ACCAACTAAGTACAACACTAAGGAAGCTCTGGGGAAGGTTTGCACAGTG
CTTAAAAGCAAAGGATTTACAATGGAAAGGGCC

>GRSVn4

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AAAAATTCTGAGAGGGTTGGAGCTAAAGACATGACTTTCAGAAGGCTTG
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CGGGCTGCCTTTGGCAGATGCAAAATCTGTGAGACTTGCCATAATGCTTG
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ACCAACTAAGTACAACACTAAGGAAGCTCTGGGGAAGGTTTGCACAGTG
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>GRSVn5

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CGGGCTGCCTTTGGCAGATGCAAAATCTGTGAGACTTGCCATAATGCTTG
GAGGTAGTATCCCTCTCATTGCTTCTGTTGACAGCTTCGAAATGATCAGT
GTTGTTCTTGCCATATATCAAGATGCAAAGTACAAGGAGTTAGGGATTGA
ACCAACTAAGTACAACACTAAGGAAGCTCTGGGGAAGGTTGTGCACAGT
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>GRSVn6

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GAAAGTTGTGAAACAAAGTGATTTTACTTTTGGCAAGGTCACGATAAAG
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ATAGCATGATAAGAGTGAAACTCATAGAAGAGACTGCAAACAATGAGAA

TCTTGCTATCATCAAGGCAAAAATTGCCTCCCATCCTTTGGTCCAAGCTTA
CGGGCTGCCTTTGGCAGATGCAAAATCTGTGAGACTTGCCATAATGCTTG
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GTTGTTCTTGCCATATATCAAGATGCAAAGTACAAGGAGTTAGGGATTGA
ACCAACTAAGTACAACACTAAGGAAGCTCTGGGGAAGGTTGCTGCACAG
TGCTTAAAAGCAAAGGATTTACAATGG

Appendix xii:Synthesized oligonucleotide for RT-PCR

Your oligonucleotide order 1052008, ordered on 21 Jun 2021, has been dispatched and will be delivered to you soon.

Thank you for your order

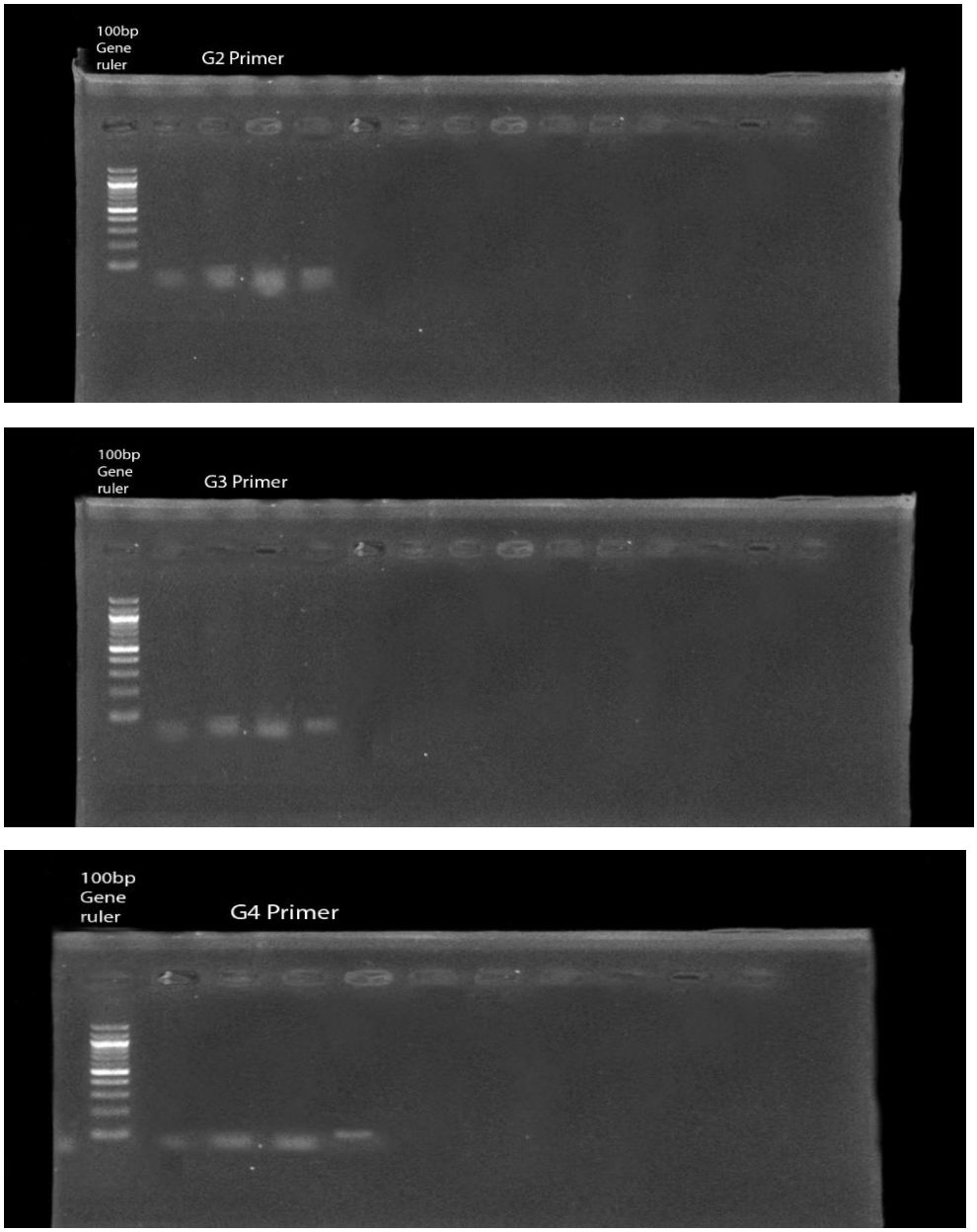
Detail of Order

Name	Sequence	Bases	3'	5'	Scale	Purification
GRSV4F	ACC AGA ACC AGG TTG CAT TC	20	None	None	0.05	Standard
GRSV4R	ATC GTG ACC TTG CCA AAA GT	20	None	None	0.05	Standard
GRSV41F	GAC CAG AAC CAG GTT GCA TT	20	None	None	0.05	Standard
GRSV41R	ATC GTG ACC TTG CCA AAA GT	20	None	None	0.05	Standard
GRSVKEF	GGC AGA TGC AAA ATC TGT GA	20	None	None	0.05	Standard
GRSVKER	TTA AGC ACT GTG CAG CAA CC	20	None	None	0.05	Standard
GRSVKE6F	CGT GCA CTT TCT CAC CTT GA	20	None	None	0.05	Standard
GRSVKE6R	AAT GCA ACC TGG TTC TGG TC	20	None	None	0.05	Standard

Sincerely

Your Inqaba Biotec Team

Appendix xiii:GRSV Primer validation



Appendix xiv: Surveyed farms in western Kenya

Farm ID	County	Cluster	Alt	Longitude	Latitude	Rain seasons
KAP 01	Bungoma	Kapkateny	1660	34^0 36’20.7841E	0^0 48’19.0031N	Short rains
KAP 02	Bungoma	Kapkateny	1660	$34^0$36’21.1440E	0^0 48’17.4722N	Short rains
KAP 03	Bungoma	Kapkateny	1676	34^0 36’19.7816E	0^0 48’56.4124N	Short rains
KAP 04	Bungoma	Kapkateny	1690	34^0 36’31.7146E	0^0 48’44.8734N	Short rains
KAP 05	Bungoma	Kapkateny	1739	34^0 36’29.7816E	0^0 48’17.4174N	Short rains
KAP 06	Bungoma	Kapkateny	1671	34^0 38’22.7842E	0^0 48’21.4798N	Short rains
KAP 07	Bungoma	Kapkateny	1672	34^0 38’23.2846E	0^0 48’35.6734N	Short rains
KAP 08	Bungoma	Kapkateny	1672	34^0 38’25.7826E	0^0 48’54.4719N	Short rains
KAP 09	Bungoma	Kapkateny	1652	34^0 38’26.2846E	0^0 48’15.4321N	Short rains
KAP 10	Bungoma	Kapkateny	1599	34^0 36’22.7842E	0^0 48’13.4324N	Short rains
KAP 11	Bungoma	Kapkateny	1587	34^0 36’21.7246E	0^0 48’69.2334N	Short rains
KAP 12	Bungoma	Kapkateny	1607	34^0 36’23.7826E	0^0 48’19.4700N	Short rains
KAP 13	Bungoma	Kapkateny	1676	34^0 36’22.7843E	0^0 48’29.1325N	Short rains
KAP 14	Bungoma	Kapkateny	1678	34^0 36’27.3846E	0^0 48’18.9870N	Short rains
KAP 15	Bungoma	Kapkateny	1680	34^0 36’23.7844E	0^0 48’19.4951N	Short rains
KAP 16	Bungoma	Kapkateny	1698	34^0 36’18.5846E	0^0 48’39.7234N	Short rains
KIM 01	Bungoma	Kimilili	1624	34^0 36’20.7847E	0^0 48’12.9653N	Short rains
KIM 02	Bungoma	Kimilili	1623	34^0 36’20.8846E	0^0 48’14.0098N	Short rains
KIM 03	Bungoma	Kimilili	1619	34^0 36’18.7849E	0^0 48’19.4008N	Short rains
KIM 04	Bungoma	Kimilili	1591	34^0 36’22.1846E	0^0 48’49.5312N	Short rains
KIM 05	Bungoma	Kimilili	1602	34^0 36’27.7842E	0^0 48’16.1006N	Short rains
KIM 06	Bungoma	Kimilili	1600	34^0 36’26.7346E	0^0 48’19.4730N	Short rains

KIM 07	Bungoma	Kimilili	1597	34^0 36’34.7844E	0^0 48’15.0034N	Short rains
KIM 08	Bungoma	Kimilili	1579	34^0 36’43.7847E	$0^0$48’14.4014N	Short rains
KIM 09	Bungoma	Kimilili	1598	$34^0$36’20.3440E	$0^0$48’89.4700N	Short rains
KIM 10	Bungoma	Kimilili	1589	34^0 36’21.8846E	$0^0$48’91.4003N	Short rains
KIM 11	Bungoma	Kimilili	1593	34^0 36’23.7849E	$0^0$48’19.4004N	Short rains
KIM 12	Bungoma	Kimilili	1600	34^0 37’25.7946E	$0^0$48’19.4768N	Short rains
KIM 13	Bungoma	Kimilili	1614	34^0 36’27.7843E	$0^0$48’19.5834N	Short rains
KIM 14	Bungoma	Kimilili	1612	34^0 36’29.3846E	$0^0$48’19.5009N	Short rains
KL001	Bungoma	Kimalewa	1647	34^0 37’22.7844E	0^0 47’19.6983N	Short rains
KL002	Bungoma	Kimalewa	1632	34^0 37’24.5846E	0^0 47’19.9001N	Short rains
KL003	Bungoma	Kimalewa	1642	34^0 37’26.7847E	0^0 47’55.7053N	Short rains
KL004	Bungoma	Kimalewa	1647	34^0 37’28.2846E	$0^0$47’67.4788N	Short rains
KL005	Bungoma	Kimalewa	1597	34^0 37’30.5840E	$0^0$47’08.0034N	Short rains
KL006	Bungoma	Kimalewa	1600	34^0 37’32.4841E	$0^0$47’16.4994N	Short rains
KL007	Bungoma	Kimalewa	1638	34^0 37’34.7446E	$0^0$47’19.4712N	Short rains
KL008	Bungoma	Kimalewa	1578	34^0 37’36.3846E	$0^0$47’10.3334N	Short rains
KL009	Bungoma	Kimalewa	1634	34^0 37’23.7842E	$0^0$47’13.4321N	Short rains
KL010	Bungoma	Kimalewa	1623	34^0 37’24.7841E	$0^0$47’18.5005N	Short rains
KL012	Bungoma	Kimalewa	1642	34^0 37’26.7840E	0^0 47’19.4734N	Short rains
KL013	Bungoma	Kimalewa	1597	34^0 37’21.6844E	0^0 47’36.4999N	Short rains
KL014	Bungoma	Kimalewa	1567	34^0 37’24.9843E	0^0 47’45.5008N	Short rains
KL015	Bungoma	Kimalewa	1568	34^0 37’23.7836E	0^0 47’32.7770N	Short rains
KL016	Bungoma	Kimalewa	1600	34^0 37’25.7846E	0^0 47’75.4734N	Short rains
KL017	Bungoma	Kimalewa	1558	34^0 37’26.4846E	0^0 47’64.4009N	Short rains

KL018	Bungoma	Kimalewa	1636	34^0 37’27.5846E	0^0 47’12.3334N	Short rains
KL019	Bungoma	Kimalewa	1637	$34^0$37’27.3440E	0^0 47’19.4000N	Short rains
KL020	Bungoma	Kimalewa	1632	34^0 37’27.7846E	0^0 47’15.7734N	Short rains
KL021	Bungoma	Kimalewa	1647	34^0 37’26.9846E	$0^0$45’56.4098N	Short rains
CH001	Bungoma	Chwele	1690	34^0 36’25.7834E	0^0 45’84.4734N	Short rains
CH002	Bungoma	Chwele	1665	34^0 36’20.1046E	0^0 45’19.6008N	Short rains
CH003	Bungoma	Chwele	1671	34^0 36’20.7246E	0^0 45’15.9002N	Short rains
CH004	Bungoma	Chwele	1623	34^0 36’20.6546E	0^0 45’27.5022N	Short rains
CH005	Bungoma	Chwele	1617	34^0 36’20.7855E	0^0 45’19.3942N	Short rains
CH006	Bungoma	Chwele	1614	34^0 36’20.7116E	0^0 45’13.4008N	Short rains
CH007	Bungoma	Chwele	1620	34^0 36’22.7346E	0^0 45’19.5003N	Short rains
CH008	Bungoma	Chwele	1623	34^0 36’23.7843E	0^0 45’10.9004N	Short rains
CH009	Bungoma	Chwele	1602	34^0 36’24.4446E	0^0 45’37.4004N	Short rains
CH010	Bungoma	Chwele	1600	34^0 37’25.7846E	0^0 45’42.4700N	Short rains
CH011	Bungoma	Chwele	1599	34^0 36’25.6646E	0^0 45’19.4333N	Short rains
CH012	Bungoma	Chwele	1665	34^0 35’24.7822E	0^0 45’18.5222N	Short rains
CH013	Bungoma	Chwele	1671	34^0 36’25.7871E	0^0 45’19.0001N	Short rains
CH014	Bungoma	Chwele	1671	34^0 36’26.7846E	0^0 45’67.4006N	Short rains
CH015	Bungoma	Chwele	1679	34^0 36’27.7116E	0^0 45’63.4734N	Short rains
CH016	Bungoma	Chwele	1655	34^0 36’28.7879E	0^0 45’79.9065N	Short rains
CH017	Bungoma	Chwele	1679	34^0 35’29.7846E	0^0 45’20.4734N	Short rains
CH018	Bungoma	Chwele	1673	34^0 37’21.7846E	0^0 45’24.7004N	Short rains
CH019	Bungoma	Chwele	1678	34^0 36’22.7846E	0^0 45’19.8009N	Short rains
CHEB01	Bungoma	Chebich	1712	34^0 36’30.7846E	$0^0$48’87.7888N	Short rains

CHEB02	Bungoma	Chebich	1702	$34^0$36’20.3440E	0^0 48’98.4734N	Short rains
CHEB03	Bungoma	Chebich	1643	34^0 36’21.7846E	0^0 48’14.4734N	Short rains
CHEB04	Bungoma	Chebich	1609	34^0 35’23.7846E	0^0 48’19.4734N	Short rains
CHEB05	Bungoma	Chebich	1668	34^0 36’24.7846E	$0^0$48’17.4734N	Short rains
CHEB06	Bungoma	Chebich	1669	34^0 36’25.7832E	$0^0$48’18.4734N	Short rains
CHEB07	Bungoma	Chebich	1675	34^0 36’26.7846E	$0^0$48’19.4734N	Short rains
CHEB08	Bungoma	Chebich	1666	34^0 36’27.7887E	$0^0$48’34.4700N	Short rains
CHEB09	Bungoma	Chebich	1671	34^0 36’21.1246E	$0^0$48’19.8009N	Short rains
CHEB10	Bungoma	Chebich	1659	34^0 36’23.7996E	$0^0$48’10.4734N	Short rains
CHEB11	Bungoma	Chebich	1634	34^0 36’27.7831E	0^0 48’18.9002N	Short rains
CHEB12	Bungoma	Chebich	1669	34^0 36’24.7823E	0^0 48’39.4009N	Short rains
CHEB13	Bungoma	Chebich	1643	34^0 36’29.5446E	0^0 48’89.4734N	Short rains
CHEB14	Bungoma	Chebich	1639	34^0 36’26.6546E	0^0 48’15.4734N	Short rains
AL001	Busia	Alupe	1141	34^0 26’42.7446E	0^0 29’19.2345N	Short rains
AL002	Busia	Alupe	1157	34^0 26’43.7855E	0^0 29’37.5555N	Short rains
AL003	Busia	Alupe	1178	34^0 26’44.7851E	0^0 29’19.7954N	Short rains
AL004	Busia	Alupe	1200	34^0 26’43.7853E	0^0 29’10.0034N	Short rains
AL005	Busia	Alupe	1232	34^0 26’36.2346E	0^0 29’99.4224N	Short rains
AL006	Busia	Alupe	1216	34^0 26’31.7226E	0^0 29’31.4778N	Short rains
AL007	Busia	Alupe	1256	34^0 26’34.7834E	0^0 29’43.8095N	Short rains
AL008	Busia	Alupe	1248	34^0 26’51.7809E	0^0 29’64.7854N	Short rains
AL009	Busia	Alupe	1235	34^0 26’64.4646E	0^0 29’10.4755N	Short rains
AL010	Busia	Alupe	1265	34^0 26’89.7846E	0^0 29’19.5634N	Short rains
AL011	Busia	Alupe	1200	$34^0$26’21.3440E	0^0 29’74.4775N	Short rains

AL012	Busia	Alupe	1209	34^0 26’22.7846E	0^0 29’86.5560N	Short rains
AL013	Busia	Alupe	1270	34^0 26’23.5646E	0^0 29’45.4709N	Short rains
AL014	Busia	Alupe	1264	34^0 26’24.7887E	0^0 29’44.4664N	Short rains
AL015	Busia	Alupe	1198	34^0 26’25.7556E	0^0 29’19.9999N	Short rains
AL016	Busia	Alupe	1200	34^0 26’23.7812E	0^0 29’17.4734N	Short rains
AL017	Busia	Alupe	1210	34^0 26’24.4346E	0^0 29’54.4005N	Short rains
AL018	Busia	Alupe	1206	34^0 26’23.7842E	0^0 29’19.0049N	Short rains
AL019	Busia	Alupe	1210	34^0 26’24.6746E	0^0 29’92.4099N	Short rains
AL020	Busia	Alupe	1206	34^0 26’25.7996E	0^0 29’18.4730N	Short rains
AL021	Busia	Alupe	1207	34^0 26’23.7813E	0^0 29’10.4786N	Short rains
AL022	Busia	Alupe	1209	34^0 26’32.7846E	0^0 29’17.6664N	Short rains
AL023	Busia	Alupe	1197	34^0 26’67.7811E	0^0 29’15.4734N	Short rains
AL024	Busia	Alupe	1199	34^0 26’71.7822E	0^0 29’16.8007N	Short rains
AL025	Busia	Alupe	1201	34^0 26’43.7834E	0^0 29’12.9005N	Short rains
AL026	Busia	Alupe	1222	34^0 26’64.7746E	0^0 29’56.4734N	Short rains
AL027	Busia	Alupe	1200	34^0 26’87.7855E	0^0 29’76.4734N	Short rains
AL028	Busia	Alupe	1202	34^0 26’91.7877E	0^0 29’19.7850N	Short rains
AL029	Busia	Alupe	1213	34^0 26’19.7126E	0^0 29’19.4224N	Short rains
AL030	Busia	Alupe	1198	34^0 26’28.7226E	0^0 29’13.9009N	Short rains
AL031	Busia	Alupe	1148	34^0 26’27.7846E	0^0 29’19.6008N	Short rains
AL032	Busia	Alupe	1167	34^0 26’27.7855E	0^0 29’16.9999N	Short rains
AL033	Busia	Alupe	1165	34^0 26’20.7899E	0^0 29’86.4716N	Short rains
AL034	Busia	Alupe	1169	$34^0$26’20.3440E	0^0 29’19.9934N	Short rains
AL035	Busia	Alupe	1172	34^0 26’20.7846E	0^0 29’76.5009N	Short rains

AL036	Busia	Alupe	1183	34^0 26’20.7811E	0^0 29’43.4734N	Short rains
AL037	Busia	Alupe	1200	34^0 26’21.7833E	0^0 26’52.4734N	Short rains
CK001	Busia	Chakol	1373	34^0 25’22.4446E	0^0 26’67.4734N	Short rains
CK002	Busia	Chakol	1400	34^0 25’25.5546E	0^0 26’75.4777N	Short rains
CK003	Busia	Chakol	1389	34^0 25’28.7666E	0^0 26’89.4734N	Short rains
CK004	Busia	Chakol	1354	34^0 25’21.7446E	0^0 26’91.4555N	Short rains
CK005	Busia	Chakol	1334	34^0 25’26.7846E	0^0 26’34.4734N	Short rains
CK006	Busia	Chakol	1309	34^0 25’28.7822E	0^0 26’49.4004N	Short rains
CK007	Busia	Chakol	1310	34^0 25’57.1146E	0^0 26’19.0034N	Short rains
CK008	Busia	Chakol	1298	34^0 25’26.7336E	0^0 26’10.6740N	Short rains
CK009	Busia	Chakol	1294	34^0 25’27.7833E	0^0 26’42.0986N	Short rains
CK010	Busia	Chakol	1298	34^0 25’26.7834E	0^0 26’87.8765N	Short rains
CK011	Busia	Chakol	1300	34^0 25’23.7846E	0^0 26’19.0032N	Short rains
CK012	Busia	Chakol	1311	34^0 25’21.3346E	0^0 26’11.0094N	Short rains
CK013	Busia	Chakol	1309	34^0 25’22.7996E	0^0 26’14.4705N	Short rains
CK014	Busia	Chakol	1310	34^0 25’23.7846E	0^0 26’16.9865N	Short rains
CK015	Busia	Chakol	1306	34^0 25’24.7800E	0^0 26’18.4709N	Short rains
CK016	Busia	Chakol	1303	34^0 25’25.7811E	0^0 26’10.4798N	Short rains
CK017	Busia	Chakol	1323	34^0 25’27.7126E	0^0 26’11.9834N	Short rains
CK018	Busia	Chakol	1342	34^0 25’28.7216E	0^0 26’13.4734N	Short rains
CK019	Busia	Chakol	1351	34^0 25’29.7436E	0^0 26’15.7007N	Short rains
CK020	Busia	Chakol	1364	34^0 25’20.3440E	0^0 26’17.5754N	Short rains
CK021	Busia	Chakol	1373	34^0 25’22.7830E	0^0 26’19.0034N	Short rains
CK022	Busia	Chakol	1376	34^0 25’24.7822E	0^0 26’29.4554N	Short rains

CK023	Busia	Chakol	1376	34^0 25’25.0046E	0^0 26’16.1232N	Short rains
CK024	Busia	Chakol	1394	34^0 25’27.1146E	0^0 26’14.9865N	Short rains
CK025	Busia	Chakol	1399	34^0 25’23.7811E	0^0 26’10.4985N	Short rains
CK026	Busia	Chakol	1400	34^0 25’21.7666E	0^0 26’17.7776N	Short rains
CK027	Busia	Chakol	1394	34^0 25’20.7834E	0^0 26’45.4734N	Short rains
CK028	Busia	Chakol	1392	34^0 25’22.0246E	0^0 26’34.4707N	Short rains
CK029	Busia	Chakol	1367	34^0 25’24.0146E	0^0 26’19.4005N	Short rains
CK030	Busia	Chakol	1364	34^0 25’21.7853E	0^0 26’11.0034N	Short rains
CK031	Busia	Chakol	1356	34^0 25’22.7123E	0^0 26’13.4009N	Short rains
MA001	Busia	Malaba	1296	34^0 27’23.7432E	0^0 27’15.5555N	Short rains
MA002	Busia	Malaba	1296	34^0 27’24.7846E	0^0 27’17.8875N	Short rains
MA003	Busia	Malaba	1296	34^0 27’25.3346E	0^0 27’19.4707N	Short rains
MA004	Busia	Malaba	1257	34^0 27’25.7834E	0^0 27’10.4734N	Short rains
MA005	Busia	Malaba	1257	34^0 27’24.7222E	0^0 27’21.4706N	Short rains
MA006	Busia	Malaba	1257	34^0 27’23.7111E	0^0 27’19.4734N	Short rains
MA007	Busia	Malaba	1257	34^0 27’22.2222E	0^0 27’10.4705N	Short rains
MA008	Busia	Malaba	1264	34^0 27’26.7846E	0^0 27’19.4074N	Short rains
MA009	Busia	Malaba	1264	34^0 27’32.7046E	0^0 27’21.5734N	Short rains
MA010	Busia	Malaba	1264	34^0 27’35.7800E	0^0 27’32.4765N	Short rains
MA011	Busia	Malaba	1264	34^0 27’39.7234E	0^0 27’44.9970N	Short rains
MA012	Busia	Malaba	1310	34^0 27’76.3440E	0^0 27’52.4708N	Short rains
MA013	Busia	Malaba	1310	34^0 27’21.7846E	0^0 27’16.7008N	Short rains
MA014	Busia	Malaba	1310	34^0 27’23.7006E	0^0 27’19.4734N	Short rains
MA015	Busia	Malaba	1310	34^0 27’33.7860E	0^0 27’20.4008N	Short rains

MA016	Busia	Malaba	1284	34^0 27’29.7123E	0^0 27’10.4775N	Short rains
MA017	Busia	Malaba	1284	34^0 27’24.3446E	0^0 27’18.4864N	Short rains
MA018	Busia	Malaba	1284	34^0 27’20.7854E	0^0 27’14.4700N	Short rains
MA019	Busia	Malaba	1280	34^0 27’21.7846E	0^0 27’17.9865N	Short rains
MA020	Busia	Malaba	1280	34^0 27’20.1246E	0^0 27’19.4554N	Short rains
MA021	Busia	Malaba	1280	34^0 27’27.7444E	0^0 27’18.4708N	Short rains
MA022	Busia	Malaba	1185	34^0 27’29.7555E	0^0 27’14.4734N	Short rains
MA023	Busia	Malaba	1193	34^0 27’29.7336E	0^0 27’19.6005N	Short rains
MA024	Busia	Malaba	1193	34^0 27’20.7000E	0^0 27’19.5432N	Short rains
MA025	Busia	Malaba	1193	34^0 27’24.1846E	0^0 27’17.8765N	Short rains
MA026	Busia	Malaba	1200	34^0 27’50.7006E	0^0 27’21.7004N	Short rains
MA027	Busia	Malaba	1210	34^0 27’80.7833E	0^0 27’33.4005N	Short rains
MA028	Busia	Malaba	1230	34^0 27’28.7877E	0^0 27’24.4755N	Short rains
MA029	Busia	Malaba	1256	34^0 27’28.7811E	0^0 27’42.7734N	Short rains
MA030	Busia	Malaba	1245	34^0 27’30.2346E	0^0 27’76.4004N	Short rains
MA031	Busia	Malaba	1246	34^0 27’26.4446E	0^0 27’86.4777N	Short rains
MA032	Busia	Malaba	1242	34^0 27’10.7336E	0^0 27’19.9008N	Short rains
MA033	Busia	Malaba	1243	34^0 27’25.3215E	0^0 27’10.8865N	Short rains
MA034	Busia	Malaba	1239	34^0 27’29.7846E	0^0 27’14.7640N	Short rains
MA035	Busia	Malaba	1242	34^0 27’20.3440E	0^0 27’19.4734N	Short rains
MA036	Busia	Malaba	1246	34^0 27’21.7846E	0^0 27’31.4755N	Short rains
MA037	Busia	Malaba	1246	34^0 27’22.7000E	0^0 27’32.4888N	Short rains
MH001	Kakamega	Muhonje	1654	34^0 36’23.9063E	0^0 35’46.4775N	Short rains
MH002	Kakamega	Muhonje	1637	34^0 36’24.8121E	0^0 35’76.4734N	Short rains

MH003	Kakamega	Muhonje	1654	34^0 36’26.9641E	0^0 36’84.4734N	Short rains
MH004	Kakamega	Muhonje	1620	34^0 36’28.7800E	0^0 36’72.5534N	Short rains
MH005	Kakamega	Muhonje	1614	34^0 36’20.2146E	0^0 35’19.4775N	Short rains
MH006	Kakamega	Muhonje	1615	34^0 36’70.4446E	0^0 35’19.4730N	Short rains
MH007	Kakamega	Muhonje	1621	34^0 36’28.7846E	0^0 35’10.4004N	Short rains
MH008	Kakamega	Muhonje	1607	34^0 36’29.7556E	0^0 35’23.6885N	Short rains
MH009	Kakamega	Muhonje	1593	34^0 36’20.7821E	0^0 35’43.4709N	Short rains
MH010	Kakamega	Muhonje	1585	34^0 36’20.5546E	0^0 35’75.4074N	Short rains
MH011	Kakamega	Muhonje	1585	34^0 36’20.7875E	0^0 36’19.4709N	Short rains
MH012	Kakamega	Muhonje	1605	34^0 36’26.5430E	0^0 36’16.4994N	Short rains
MH013	Kakamega	Muhonje	1604	34^0 36’20.7846E	0^0 36’17.4005N	Short rains
MH014	Kakamega	Muhonje	1609	34^0 36’28.8028E	0^0 36’18.9934N	Short rains
MH015	Kakamega	Muhonje	1612	34^0 36’21.7846E	0^0 35’19.4884N	Short rains
MH016	Kakamega	Muhonje	1594	34^0 36’20.7006E	0^0 35’18.4765N	Short rains
MH017	Kakamega	Muhonje	1599	34^0 36’25.7226E	0^0 35’13.4734N	Short rains
MH018	Kakamega	Muhonje	1600	34^0 36’27.7996E	0^0 35’17.0034N	Short rains
MH019	Kakamega	Muhonje	1587	34^0 36’29.7890E	0^0 35’13.4765N	Short rains
MT001	Kakamega	Matungu	1300	34^0 36’22.7850E	0^0 24’11.4734N	Short rains
MT002	Kakamega	Matungu	1298	$34^0$34’24.3440E	0^0 24’76.8888N	Short rains
MT003	Kakamega	Matungu	1286	34^0 34’26.7846E	0^0 25’98.9985N	Short rains
MT004	Kakamega	Matungu	1294	34^0 34’20.7846E	0^0 24’92.4005N	Short rains
MT005	Kakamega	Matungu	1296	34^0 34’28.7846E	0^0 24’56.4734N	Short rains
MT006	Kakamega	Matungu	1300	34^0 34’21.7846E	0^0 24’87.4777N	Short rains
MT007	Kakamega	Matungu	1304	34^0 34’22.7864E	0^0 25’16.8534N	Short rains

MT008	Kakamega	Matungu	1317	34^0 34’23.7726E	0^0 25’19.4775N	Short rains
MT009	Kakamega	Matungu	1313	34^0 34’24.7873E	0^0 24’10.4004N	Short rains
MT010	Kakamega	Matungu	1317	34^0 34’25.7869E	0^0 24’32.8865N	Short rains
MT011	Kakamega	Matungu	1308	34^0 34’26.7874E	0^0 25’56.9994N	Short rains
MT012	Kakamega	Matungu	1307	34^0 34’27.6646E	0^0 25’18.4785N	Short rains
MT013	Kakamega	Matungu	1307	34^0 34’28.7878E	0^0 25’19.4730N	Short rains
MT014	Kakamega	Matungu	1302	34^0 34’29.7804E	0^0 24’10.4704N	Short rains
MT015	Kakamega	Matungu	1311	34^0 34’20.2023E	0^0 24’17.4440N	Short rains
MT016	Kakamega	Matungu	1308	34^0 34’21.2019E	0^0 24’19.4734N	Short rains
MT017	Kakamega	Matungu	1319	34^0 34’23.2009E	0^0 24’43.8885N	Short rains
MT018	Kakamega	Matungu	1296	34^0 34’25.2014E	0^0 24’54.9009N	Short rains
MT019	Kakamega	Matungu	1300	34^0 34’27.2013E	0^0 24’76.4734N	Short rains
MT020	Kakamega	Matungu	1304	34^0 34’29.2005E	0^0 25’67.6665N	Short rains
MT021	Kakamega	Matungu	1317	34^0 34’22.2000E	0^0 24’98.7775N	Short rains
MT022	Kakamega	Matungu	1313	34^0 34’24.2005E	0^0 24’47.4734N	Short rains
MT023	Kakamega	Matungu	1317	34^0 34’26.7823E	0^0 25’19.4704N	Short rains
MT024	Kakamega	Matungu	1308	34^0 34’28.7236E	0^0 24’34.4730N	Short rains
MT025	Kakamega	Matungu	1307	34^0 34’21.3440E	0^0 24’65.4834N	Short rains
MT026	Kakamega	Matungu	1307	34^0 34’24.1446E	0^0 25’98.4725N	Short rains
MT027	Kakamega	Matungu	1311	34^0 34’28.0146E	0^0 24’19.8934N	Short rains
MT028	Kakamega	Matungu	1304	34^0 34’29.0646E	0^0 24’34.4994N	Short rains
MT029	Kakamega	Matungu	1300	34^0 34’27.7878E	0^0 24’56.4775N	Short rains
MM001	Kakamega	Mumias	1311	34^0 27’25.7800E	0^0 25’19.4999N	Short rains
MM002	Kakamega	Mumias	1241	34^0 27’23.0124E	0^0 25’56.9995N	Short rains

MM003	Kakamega	Mumias	1246	34^0 27’21.7846E	0^0 25’71.4734N	Short rains
MM004	Kakamega	Mumias	1261	34^0 27’22.7846E	0^0 25’19.4994N	Short rains
MM005	Kakamega	Mumias	1261	34^0 27’23.7846E	0^0 25’19.4740N	Short rains
MM006	Kakamega	Mumias	1241	34^0 27’24.7850E	0^0 25’13.4404N	Short rains
MM007	Kakamega	Mumias	1238	34^0 27’25.7860E	0^0 25’35.8734N	Short rains
MM008	Kakamega	Mumias	1272	34^0 27’21.7870E	0^0 25’96.9005N	Short rains
MM009	Kakamega	Mumias	1270	34^0 27’22.7806E	0^0 25’43.4222N	Short rains
MM010	Kakamega	Mumias	1272	34^0 27’23.7890E	0^0 25’92.9004N	Short rains
MM011	Kakamega	Mumias	1247	34^0 27’24.7850E	0^0 25’64.9005N	Short rains
MM012	Kakamega	Mumias	1449	34^0 27’25.5646E	0^0 25’19.4780N	Short rains
MM013	Kakamega	Mumias	1280	34^0 27’26.7456E	0^0 25’16.4735N	Short rains
MM014	Kakamega	Mumias	1262	34^0 27’26.7846E	0^0 25’10.4770N	Short rains
MM015	Kakamega	Mumias	1269	34^0 27’20.7506E	0^0 25’18.0984N	Short rains
MM016	Kakamega	Mumias	1261	34^0 27’10.7864E	0^0 25’17.0934N	Short rains
MM017	Kakamega	Mumias	1311	34^0 27’21.7875E	0^0 25’16.4884N	Short rains
MM018	Kakamega	Mumias	1241	34^0 27’21.7846E	0^0 25’15.4770N	Short rains
MM019	Kakamega	Mumias	1246	34^0 27’21.3440E	0^0 25’14.8008N	Short rains
MM020	Kakamega	Mumias	1261	34^0 27’20.7871E	0^0 25’13.4999N	Short rains
MM021	Kakamega	Mumias	1265	34^0 27’20.7626E	0^0 25’12.5002N	Short rains
MM022	Kakamega	Mumias	1261	34^0 27’25.7821E	0^0 25’11.4755N	Short rains
MM023	Kakamega	Mumias	1241	34^0 27’24.7531E	0^0 25’28.4994N	Short rains
MM024	Kakamega	Mumias	1238	34^0 27’24.4446E	0^0 25’24.4734N	Short rains
MM025	Kakamega	Mumias	1272	34^0 27’27.7846E	0^0 25’27.4777N	Short rains
MM026	Kakamega	Mumias	1270	34^0 27’28.7834E	0^0 25’26.4734N	Short rains

MM027	Kakamega	Mumias	1272	34^0 27’29.7830E	0^0 25’22.4765N	Short rains
MM028	Kakamega	Mumias	1264	34^0 28’24.7820E	0^0 25’19.8884N	Short rains
MM029	Kakamega	Mumias	1241	34^0 28’26.7810E	0^0 25’18.4700N	Short rains
MM030	Kakamega	Mumias	1251	34^0 28’22.8446E	0^0 25’10.0998N	Short rains
MM031	Kakamega	Mumias	1243	34^0 28’24.7846E	0^0 25’17.4770N	Short rains
MM032	Kakamega	Mumias	1239	34^0 28’26.0046E	0^0 25’13.4884N	Short rains
MM034	Kakamega	Mumias	1243	34^0 28’27.1046E	0^0 25’18.4777N	Short rains
MM035	Kakamega	Mumias	1240	34^0 28’28.7838E	0^0 25’15.4733N	Short rains
MM036	Kakamega	Mumias	1251	34^0 28’29.7432E	0^0 25’10.5005N	Short rains
MM037	Kakamega	Mumias	1250	34^0 28’20.7846E	0^0 25’19.4734N	Short rains
MM038	Kakamega	Mumias	1249	34^0 28’20.7000E	0^0 25’12.4554N	Short rains
MM039	Kakamega	Mumias	1245	34^0 28’20.7098E	0^0 48’17.4740N	Short rains
CHEB 1	Bungoma	Chebich	1666	34^0 36’56.0021E	0^0 48’18.4750N	Long rains
CHEB 2	Bungoma	Chebich	1643	34^0 36’21.7006E	0^0 48’17.0034N	Long rains
CHEB 3	Bungoma	Chebich	1609	34^0 36’23.7285E	0^0 48’29.4004N	Long rains
CHEB 4	Bungoma	Chebich	1668	$34^0$36’21.3440E	0^0 48’21.4775N	Long rains
CHEB 5	Bungoma	Chebich	1669	34^0 36’27.2646E	0^0 48’23.9834N	Long rains
CHEB 6	Bungoma	Chebich	1675	34^0 36’28.7006E	0^0 48’22.4730N	Long rains
CHEB 7	Bungoma	Chebich	1666	34^0 36’24.7822E	0^0 48’24.4734N	Long rains
CHEB 8	Bungoma	Chebich	1671	34^0 36’21.6540E	0^0 48’25.4794N	Long rains
CHEB 9	Bungoma	Chebich	1659	34^0 36’20.0986E	0^0 48’26.4735N	Long rains
CHE 10	Bungoma	Chebich	1634	34^0 36’25.7859E	0^0 48’19.5734N	Long rains
CHE 11	Bungoma	Chebich	1669	34^0 36’21.7846E	0^0 48’10.9009N	Long rains
CHE 12	Bungoma	Chebich	1643	34^0 36’29.8866E	0^0 48’12.4750N	Long rains

CHE 13	Bungoma	Chebich	1639	34^0 36’26.0046E	0^0 48’15.7634N	Long rains
KIM01	Bungoma	Kimilili	1600	34^0 35’21.7850E	0^0 48’18.4654N	Long rains
KIM02	Bungoma	Kimilili	1604	34^0 35’22.7860E	0^0 48’15.4770N	Long rains
KIM03	Bungoma	Kimilili	1603	34^0 35’23.6004E	0^0 48’10.4760N	Long rains
KIM04	Bungoma	Kimilili	1607	34^0 35’29.7846E	0^0 48’59.4733N	Long rains
KIM05	Bungoma	Kimilili	1610	34^0 35’21.7846E	0^0 48’18.9034N	Long rains
KIM06	Bungoma	Kimilili	1595	34^0 35’28.7008E	0^0 48’89.9739N	Long rains
KIM07	Bungoma	Kimilili	1597	34^0 35’22.1846E	0^0 48’99.4704N	Long rains
KIM08	Bungoma	Kimilili	1608	34^0 35’27.4840E	0^0 48’10.4934N	Long rains
KIM09	Bungoma	Kimilili	1604	34^0 35’23.7846E	$0^0$48’19.4730N	Long rains
KIM10	Bungoma	Kimilili	1614	34^0 35’20.5840E	$0^0$48’19.4734N	Long rains
KIM11	Bungoma	Kimilili	1617	34^0 35’20.7846E	0^0 48’19.4834N	Long rains
KIM12	Bungoma	Kimilili	1599	34^0 35’20.5840E	0^0 48’19.4750N	Long rains
KIM13	Bungoma	Kimilili	1589	34^0 35’20.6846E	0^0 48’19.4734N	Long rains
KIM14	Bungoma	Kimilili	1577	34^0 35’20.3440E	0^0 48’19.4770N	Long rains
KIM15	Bungoma	Kimilili	1600	34^0 35’27.2246E	0^0 48’44.4785N	Long rains
KIM16	Bungoma	Kimilili	1613	34^0 35’20.7846E	0^0 48’56.4004N	Long rains
Kap 01	Bungoma	Kapkateny	1650	34^0 36’29.7800E	0^0 48’78.4735N	Long rains
Kap 02	Bungoma	Kapkateny	1644	34^0 36’20.7886E	0^0 48’98.9734N	Long rains
Kap 03	Bungoma	Kapkateny	1643	34^0 36’21.2676E	0^0 48’19.4739N	Long rains
Kap 04	Bungoma	Kapkateny	1633	34^0 36’26.7830E	0^0 48’10.4734N	Long rains
Kap 05	Bungoma	Kapkateny	1643	34^0 36’20.2046E	0^0 48’23.4994N	Long rains
Kap 06	Bungoma	Kapkateny	1645	34^0 36’29.7116E	0^0 48’89.4750N	Long rains
Kap 07	Bungoma	Kapkateny	1639	34^0 36’28.7106E	0^0 48’71.4785N	Long rains

Kap 08	Bungoma	Kapkateny	1637	34^0 36’27.7833E	0^0 48’19.4784N	Long rains
Kap 09	Bungoma	Kapkateny	1634	34^0 36’28.7556E	0^0 48’67.0034N	Long rains
Kap 10	Bungoma	Kapkateny	1641	34^0 36’22.7833E	0^0 48’44.4004N	Long rains
Kap 11	Bungoma	Kapkateny	1644	34^0 36’27.5546E	0^0 48’32.4766N	Long rains
Kap 12	Bungoma	Kapkateny	1652	34^0 36’22.7800E	0^0 48’11.4875N	Long rains
Kap 13	Bungoma	Kapkateny	1667	34^0 36’26.7855E	0^0 48’12.9934N	Long rains
Kap 14	Bungoma	Kapkateny	1635	34^0 36’24.9946E	0^0 48’13.6574N	Long rains
Kap 15	Bungoma	Kapkateny	1637	34^0 36’25.7006E	0^0 48’14.4770N	Long rains
Kap 16	Bungoma	Kapkateny	1652	34^0 36’20.7890E	0^0 48’14.4730N	Long rains
Kap 17	Bungoma	Kapkateny	1634	34^0 36’21.7506E	0^0 48’15.4754N	Long rains
Kap 18	Bungoma	Kapkateny	1634	34^0 36’29.7830E	0^0 48’16.4770N	Long rains
Kap 19	Bungoma	Kapkateny	1625	34^0 36’21.7006E	0^0 48’17.4765N	Long rains
KL01	Bungoma	Kimalewa	1630	34^0 37’28.7855E	0^0 47’18.8534N	Long rains
KL02	Bungoma	Kimalewa	1628	$34^0$37’22.3440E	0^0 47’20.4796N	Long rains
KL03	Bungoma	Kimalewa	1627	34^0 37’26.7880E	0^0 47’19.9834N	Long rains
KL04	Bungoma	Kimalewa	1628	34^0 37’23.7555E	0^0 47’11.4734N	Long rains
KL05	Bungoma	Kimalewa	1625	34^0 37’26.7846E	0^0 47’31.4774N	Long rains
KL06	Bungoma	Kimalewa	1625	34^0 37’24.4002E	0^0 47’35.4785N	Long rains
KL07	Bungoma	Kimalewa	1621	34^0 37’25.5098E	0^0 47’46.4790N	Long rains
KL08	Bungoma	Kimalewa	1627	34^0 37’29.1978E	0^0 47’76.1034N	Long rains
KL09	Bungoma	Kimalewa	1624	34^0 37’21.1968E	0^0 47’19.4734N	Long rains
KL10	Bungoma	Kimalewa	1615	34^0 37’29.6008E	0^0 47’19.5634N	Long rains
KL11	Bungoma	Kimalewa	1602	34^0 37’22.7846E	0^0 47’11.4654N	Long rains
KL12	Bungoma	Kimalewa	1600	34^0 37’28.7996E	0^0 47’12.8634N	Long rains

KL13	Bungoma	Kimalewa	1602	34^0 38’23.4046E	0^0 47’13.4765N	Long rains
KL14	Bungoma	Kimalewa	1608	34^0 38’27.7850E	0^0 47’14.9834N	Long rains
KL15	Bungoma	Kimalewa	1606	34^0 37’24.7820E	0^0 47’15.4004N	Long rains
Chw01	Bungoma	Chwele	1611	34^0 36’27.7006E	0^0 45’16.4730N	Long rains
Chw02	Bungoma	Chwele	1615	34^0 36’20.7845E	0^0 45’18.4734N	Long rains
Chw03	Bungoma	Chwele	1603	34^0 36’21.7846E	0^0 45’13.4730N	Long rains
Chw04	Bungoma	Chwele	1603	34^0 36’28.7000E	0^0 45’10.4740N	Long rains
Chw05	Bungoma	Chwele	1605	34^0 36’22.4001E	0^0 45’19.4798N	Long rains
Chw06	Bungoma	Chwele	1605	34^0 36’29.6008E	0^0 45’19.5534N	Long rains
Chw07	Bungoma	Chwele	1602	34^0 36’23.7846E	0^0 45’19.4785N	Long rains
Chw08	Bungoma	Chwele	1609	34^0 36’27.7846E	0^0 45’19.4734N	Long rains
Chw09	Bungoma	Chwele	1618	34^0 36’23.7846E	0^0 45’19.5664N	Long rains
Chw10	Bungoma	Chwele	1620	$34^0$36’24.3440E	0^0 45’10.4740N	Long rains
Chw11	Bungoma	Chwele	1624	34^0 36’25.7846E	0^0 45’17.4750N	Long rains
Chw12	Bungoma	Chwele	1623	34^0 36’20.7006E	0^0 45’10.4730N	Long rains
Chw13	Bungoma	Chwele	1622	34^0 36’21.3009E	0^0 45’19.5005N	Long rains
Chw14	Bungoma	Chwele	1621	34^0 36’29.6700E	0^0 45’98.9999N	Long rains
Chw15	Bungoma	Chwele	1623	34^0 36’28.7000E	0^0 45’77.4994N	Long rains
Chw16	Bungoma	Chwele	1615	34^0 36’22.7855E	0^0 45’67.8534N	Long rains
Chw17	Bungoma	Chwele	1616	34^0 36’27.7976E	0^0 45’54.4755N	Long rains
MUM01	Kakamega	Mumias	1311	34^0 27’23.7840E	0^0 25’21.4770N	Long rains
MUM02	Kakamega	Mumias	1241	34^0 27’25.7852E	0^0 25’10.0014N	Long rains
MUM03	Kakamega	Mumias	1246	34^0 27’20.7006E	0^0 25’20.4784N	Long rains
MUM04	Kakamega	Mumias	1261	34^0 27’29.7809E	0^0 25’31.4785N	Long rains

MUM05	Kakamega	Mumias	1265	34^0 27’21.7054E	0^0 25’33.4775N	Long rains
MUM06	Kakamega	Mumias	1261	34^0 27’25.7846E	0^0 25’46.7734N	Long rains
MUM07	Kakamega	Mumias	1241	34^0 27’27.7809E	0^0 25’67.4654N	Long rains
MUM08	Kakamega	Mumias	1238	34^0 27’24.6746E	0^0 25’98.4740N	Long rains
MUM09	Kakamega	Mumias	1272	34^0 27’21.7006E	0^0 25’65.4554N	Long rains
MUM10	Kakamega	Mumias	1270	34^0 27’29.7809E	0^0 25’77.9000N	Long rains
MUM11	Kakamega	Mumias	1270	34^0 27’28.7822E	0^0 25’19.4734N	Long rains
MUM12	Kakamega	Mumias	1272	34^0 27’21.7116E	0^0 25’11.4770N	Long rains
MUM13	Kakamega	Mumias	1247	34^0 27’23.7846E	0^0 25’12.5004N	Long rains
MUM14	Kakamega	Mumias	1249	34^0 27’27.7116E	0^0 25’13.4755N	Long rains
MUM15	Kakamega	Mumias	1280	34^0 27’29.7822E	0^0 25’14.9534N	Long rains
MUM16	Kakamega	Mumias	1276	34^0 27’22.3440E	0^0 25’15.4904N	Long rains
MUM17	Kakamega	Mumias	1278	34^0 27’21.7846E	0^0 25’18.4770N	Long rains
MUM18	Kakamega	Mumias	1285	34^0 27’27.7333E	0^0 25’14.4780N	Long rains
MUM19	Kakamega	Mumias	1278	34^0 27’20.1146E	0^0 25’17.4004N	Long rains
MUM20	Kakamega	Mumias	1278	34^0 27’20.7846E	0^0 25’18.4734N	Long rains
MUM21	Kakamega	Mumias	1277	34^0 27’20.7006E	0^0 25’19.4734N	Long rains
MUM22	Kakamega	Mumias	1278	34^0 27’29.7800E	0^0 25’32.1734N	Long rains
MUM23	Kakamega	Mumias	1267	34^0 27’21.7226E	0^0 25’45.4734N	Long rains
MUM24	Kakamega	Mumias	1265	34^0 27’28.7555E	0^0 25’67.4730N	Long rains
MUM25	Kakamega	Mumias	1267	34^0 28’22.7865E	0^0 25’90.4934N	Long rains
MUM26	Kakamega	Mumias	1270	34^0 28’27.7876E	0^0 25’87.4704N	Long rains
MUM27	Kakamega	Mumias	1269	34^0 28’23.7850E	0^0 25’46.4730N	Long rains
MUM28	Kakamega	Mumias	1267	34^0 28’26.7870E	0^0 25’42.5034N	Long rains

MUM29	Kakamega	Mumias	1271	34^0 28’24.7009E	0^0 25’74.4750N	Long rains
MUM30	Kakamega	Mumias	1269	34^0 28’25.7846E	0^0 25’20.4734N	Long rains
MUM31	Kakamega	Mumias	1270	34^0 28’20.7846E	0^0 25’19.4554N	Long rains
MUM32	Kakamega	Mumias	1271	34^0 28’21.1765E	0^0 25’87.4004N	Long rains
MUM33	Kakamega	Mumias	1271	34^0 28’22.7846E	0^0 25’54.8734N	Long rains
MUM34	Kakamega	Mumias	1269	34^0 28’23.3094E	0^0 25’68.4740N	Long rains
MUM35	Kakamega	Mumias	1265	34^0 28’24.7846E	0^0 25’77.8735N	Long rains
Mat01	Kakamega	Matungu	1300	34^0 34’25.6003E	0^0 24’89.4730N	Long rains
Mat 02	Kakamega	Matungu	1324	34^0 34’26.7846E	0^0 24’72.9995N	Long rains
Mat 03	Kakamega	Matungu	1312	34^0 34’27.7846E	0^0 24’35.4795N	Long rains
Mat 04	Kakamega	Matungu	1300	34^0 34’28.3440E	0^0 24’56.4994N	Long rains
Mat 05	Kakamega	Matungu	1296	34^0 34’29.7846E	0^0 24’39.5534N	Long rains
Mat 06	Kakamega	Matungu	1300	34^0 34’28.1006E	0^0 24’09.4775N	Long rains
Mat 07	Kakamega	Matungu	1304	34^0 34’26.2046E	0^0 24’16.4004N	Long rains
Mat 08	Kakamega	Matungu	1317	34^0 34’24.7846E	0^0 24’54.4734N	Long rains
Mat 09	Kakamega	Matungu	1308	34^0 34’22.7840E	0^0 24’97.1234N	Long rains
Mat 10	Kakamega	Matungu	1307	34^0 34’21.7850E	0^0 24’19.4990N	Long rains
Mat 11	Kakamega	Matungu	1307	34^0 34’20.6780E	0^0 24’10.4740N	Long rains
Mat 12	Kakamega	Matungu	1307	34^0 34’22.7846E	0^0 24’17.2234N	Long rains
Mat 13	Kakamega	Matungu	1302	34^0 34’21.0046E	0^0 24’85.4774N	Long rains
Mat 14	Kakamega	Matungu	1311	34^0 34’22.1045E	0^0 24’66.4735N	Long rains
Mat 15	Kakamega	Matungu	1308	34^0 34’23.2041E	0^0 24’53.5534N	Long rains
Mat 16	Kakamega	Matungu	1319	34^0 34’24.7005E	0^0 24’23.4738N	Long rains
Mat 17	Kakamega	Matungu	1315	34^0 34’26.1006E	0^0 24’67.4884N	Long rains

Mat 18	Kakamega	Matungu	1314	34^0 34’27.7000E	0^0 24’10.4730N	Long rains
Mat 19	Kakamega	Matungu	1309	34^0 34’28.0046E	0^0 24’11.4740N	Long rains
Mat 20	Kakamega	Matungu	1308	34^0 34’29.7226E	0^0 24’12.4734N	Long rains
Mat 21	Kakamega	Matungu	1306	34^0 34’27.7098E	0^0 24’13.4554N	Long rains
Mat 22	Kakamega	Matungu	1309	34^0 34’25.0006E	0^0 24’14.4795N	Long rains
Mat 23	Kakamega	Matungu	1310	34^0 34’29.7850E	0^0 24’15.8734N	Long rains
Mat 24	Kakamega	Matungu	1312	34^0 34’21.7506E	0^0 24’17.4770N	Long rains
Mat 25	Kakamega	Matungu	1311	34^0 34’20.5046E	0^0 24’18.4780N	Long rains
Mat 26	Kakamega	Matungu	1309	34^0 34’20.7830E	0^0 24’10.4790N	Long rains
Mat 27	Kakamega	Matungu	1306	34^0 34’20.3440E	0^0 24’21.4785N	Long rains
Mat 28	Kakamega	Matungu	1304	34^0 34’26.7850E	0^0 24’33.4775N	Long rains
Mat 29	Kakamega	Matungu	1300	34^0 34’29.4005E	0^0 24’36.4790N	Long rains
Mat 30	Kakamega	Matungu	1301	34^0 34’26.7846E	0^0 24’87.4990N	Long rains
Muh01	Kakamega	Muhonje	1604	34^0 36’21.7006E	0^0 35’98.4734N	Long rains
Muh02	Kakamega	Muhonje	1609	34^0 36’28.7846E	0^0 35’75.4734N	Long rains
Muh03	Kakamega	Muhonje	1612	34^0 36’22.7830E	0^0 35’19.4734N	Long rains
Muh04	Kakamega	Muhonje	1594	34^0 36’29.7820E	0^0 35’10.4735N	Long rains
Muh05	Kakamega	Muhonje	1599	34^0 36’21.7810E	0^0 35’18.4775N	Long rains
Muh06	Kakamega	Muhonje	1600	34^0 36’20.7206E	0^0 34’13.4730N	Long rains
Muh07	Kakamega	Muhonje	1587	34^0 36’28.3046E	0^0 35’17.4734N	Long rains
Muh08	Kakamega	Muhonje	1574	34^0 36’25.7809E	0^0 35’10.0034N	Long rains
Muh09	Kakamega	Muhonje	1588	34^0 36’27.7506E	0^0 35’20.4795N	Long rains
Muh10	Kakamega	Muhonje	1574	34^0 36’24.7345E	0^0 35’19.4708N	Long rains
Muh11	Kakamega	Muhonje	1588	34^0 36’29.7846E	0^0 35’19.4750N	Long rains

Muh12	Kakamega	Muhonje	1634	34^0 36’20.7846E	0^0 35’19.4765N	Long rains
Muh13	Kakamega	Muhonje	1632	34^0 36’27.7846E	0^0 35’19.4798N	Long rains
Muh14	Kakamega	Muhonje	1612	34^0 36’29.7846E	0^0 35’19.1234N	Long rains
Muh15	Kakamega	Muhonje	1614	34^0 36’21.7846E	0^0 35’19.4738N	Long rains
Muh16	Kakamega	Muhonje	1609	34^0 36’22.7846E	0^0 35’19.4734N	Long rains
Muh17	Kakamega	Muhonje	1600	34^0 36’23.7846E	0^0 35’19.0034N	Long rains
Muh18	Kakamega	Muhonje	1606	34^0 36’29.7870E	0^0 35’19.4739N	Long rains
Muh19	Kakamega	Muhonje	1604	34^0 36’27.7846E	0^0 26’34.4734N	Long rains
Chak01	Busia	Chakol	1373	34^0 25’25.3440E	0^0 26’35.4765N	Long rains
Chak02	Busia	Chakol	1389	34^0 25’20.7850E	0^0 26’31.4114N	Long rains
Chak03	Busia	Chakol	1354	34^0 25’29.7906E	0^0 26’54.4770N	Long rains
Chak04	Busia	Chakol	1334	34^0 25’22.7846E	0^0 26’95.4730N	Long rains
Chak05	Busia	Chakol	1309	34^0 25’28.7890E	0^0 26’88.4765N	Long rains
Chak06	Busia	Chakol	1310	34^0 25’21.7846E	0^0 26’37.4554N	Long rains
Chak07	Busia	Chakol	1298	34^0 25’29.7815E	0^0 26’17.4740N	Long rains
Chak08	Busia	Chakol	1294	34^0 25’26.7846E	0^0 26’13.4750N	Long rains
Chak09	Busia	Chakol	1294	34^0 25’26.7850E	0^0 26’14.4765N	Long rains
Chak10	Busia	Chakol	1298	34^0 25’21.7850E	0^0 26’17.4770N	Long rains
Chak11	Busia	Chakol	1300	34^0 25’23.7306E	0^0 26’10.4713N	Long rains
Chak12	Busia	Chakol	1311	34^0 25’25.7812E	0^0 26’32.4735N	Long rains
Chak13	Busia	Chakol	1309	34^0 25’27.0046E	0^0 26’47.4004N	Long rains
Chak14	Busia	Chakol	1310	34^0 25’29.7006E	0^0 26’87.4509N	Long rains
Chak15	Busia	Chakol	1306	34^0 25’28.7855E	0^0 26’10.4734N	Long rains
Chak16	Busia	Chakol	1303	34^0 25’26.8005E	0^0 26’19.4735N	Long rains

Chak17	Busia	Chakol	1300	34^0 25’24.1006E	0^0 26’12.4750N	Long rains
Chak18	Busia	Chakol	1299	34^0 25’22.7844E	0^0 26’17.4854N	Long rains
Chak19	Busia	Chakol	1298	34^0 25’21.7226E	0^0 26’17.4725N	Long rains
Chak20	Busia	Chakol	1299	34^0 25’20.7800E	0^0 26’10.4730N	Long rains
Chak21	Busia	Chakol	1300	34^0 25’29.0006E	0^0 26’20.4884N	Long rains
Chak22	Busia	Chakol	1299	34^0 25’28.1006E	0^0 26’19.4799N	Long rains
Chak23	Busia	Chakol	1296	34^0 25’21.5006E	0^0 26’16.4889N	Long rains
Chak24	Busia	Chakol	1299	34^0 25’22.3440E	0^0 26’19.4786N	Long rains
Chak25	Busia	Chakol	1296	34^0 25’25.8846E	0^0 26’14.4740N	Long rains
Chak26	Busia	Chakol	1293	34^0 25’21.7556E	0^0 26’17.4779N	Long rains
Chak27	Busia	Chakol	1292	34^0 25’28.7840E	0^0 26’18.4789N	Long rains
Mal01	Busia	Malaba	1144	34^0 27’22.7128E	0^0 27’12.4994N	Long rains
Mal02	Busia	Malaba	1234	34^0 27’29.7556E	0^0 27’15.4753N	Long rains
Mal03	Busia	Malaba	1144	34^0 27’21.7000E	0^0 27’18.4095N	Long rains
Mal05	Busia	Malaba	1146	34^0 27’28.7340E	0^0 27’10.4775N	Long rains
Mal06	Busia	Malaba	1156	34^0 27’23.7846E	0^0 27’17.4730N	Long rains
Mal07	Busia	Malaba	1165	34^0 27’27.7846E	0^0 27’12.4740N	Long rains
Mal08	Busia	Malaba	1173	34^0 27’29.7846E	0^0 27’17.4770N	Long rains
Mal09	Busia	Malaba	1210	34^0 27’29.7846E	0^0 27’13.4734N	Long rains
Mal10	Busia	Malaba	1198	34^0 27’21.7846E	0^0 27’16.4730N	Long rains
Mal11	Busia	Malaba	1198	34^0 27’22.7846E	0^0 27’18.4790N	Long rains
Mal12	Busia	Malaba	1196	34^0 27’23.7846E	0^0 27’12.4790N	Long rains
Mal13	Busia	Malaba	1187	34^0 27’24.0006E	0^0 27’15.4780N	Long rains
Mal14	Busia	Malaba	1184	34^0 27’25.7850E	0^0 27’19.4730N	Long rains

Mal15	Busia	Malaba	1185	34^0 27’26.0046E	0^0 27’19.4784N	Long rains
Mal16	Busia	Malaba	1191	34^0 27’20.2106E	0^0 27’19.4934N	Long rains
Mal17	Busia	Malaba	1193	34^0 27’20.7100E	0^0 27’19.4794N	Long rains
Mal18	Busia	Malaba	1200	34^0 27’20.7820E	0^0 27’19.4738N	Long rains
Mal19	Busia	Malaba	1204	34^0 27’21.7846E	0^0 27’19.4730N	Long rains
Mal20	Busia	Malaba	1203	34^0 27’22.7846E	0^0 27’15.4734N	Long rains
Mal21	Busia	Malaba	1205	34^0 27’23.3440E	0^0 27’19.4734N	Long rains
Mal22	Busia	Malaba	1206	34^0 27’24.7809E	0^0 27’56.4734N	Long rains
Mal23	Busia	Malaba	1204	34^0 27’25.7890E	0^0 27’19.4734N	Long rains
Mal24	Busia	Malaba	1200	34^0 27’26.7106E	0^0 27’76.4857N	Long rains
Mal25	Busia	Malaba	1197	34^0 27’26.7820E	0^0 27’19.4777N	Long rains
Mal26	Busia	Malaba	1198	34^0 27’29.7830E	0^0 27’98.4735N	Long rains
Mal27	Busia	Malaba	1199	34^0 27’21.3336E	0^0 27’64.4770N	Long rains
Mal28	Busia	Malaba	1201	34^0 27’20.7800E	0^0 27’19.0034N	Long rains
Mal29	Busia	Malaba	1204	34^0 27’29.0046E	0^0 27’42.1734N	Long rains
Mal30	Busia	Malaba	1205	34^0 27’21.7206E	0^0 27’54.4784N	Long rains
Mal31	Busia	Malaba	1206	34^0 27’23.7850E	0^0 27’16.4735N	Long rains
ALP01	Busia	Alupe	1204	34^0 26’22.7860E	0^0 29’19.4730N	Long rains
ALP02	Busia	Alupe	1145	34^0 26’28.7556E	0^0 29’20.4794N	Long rains
ALP03	Busia	Alupe	1231	34^0 26’27.7870E	0^0 29’17.4749N	Long rains
ALP04	Busia	Alupe	1200	34^0 26’24.7878E	0^0 29’43.4709N	Long rains
ALP05	Busia	Alupe	1198	34^0 26’27.0002E	0^0 29’18.4934N	Long rains
ALP06	Busia	Alupe	1192	34^0 26’20.1650E	0^0 29’29.4770N	Long rains
ALP07	Busia	Alupe	1185	34^0 26’27.7846E	0^0 29’44.4730N	Long rains

ALP08	Busia	Alupe	1177	34^0 26’29.7840E	0^0 29’34.4734N	Long rains
ALP09	Busia	Alupe	1170	34^0 26’21.7850E	0^0 29’42.4734N	Long rains
ALP10	Busia	Alupe	1171	34^0 26’26.5046E	0^0 29’74.4734N	Long rains
ALP11	Busia	Alupe	1200	34^0 26’22.7830E	0^0 29’98.4734N	Long rains
ALP12	Busia	Alupe	1201	34^0 26’29.7846E	0^0 29’19.4730N	Long rains
ALP13	Busia	Alupe	1200	34^0 26’21.3440E	0^0 29’21.4734N	Long rains
ALP14	Busia	Alupe	1201	34^0 26’22.7846E	0^0 29’14.4734N	Long rains
ALP15	Busia	Alupe	1196	34^0 26’24.2006E	0^0 29’19.4794N	Long rains
ALP16	Busia	Alupe	1186	34^0 26’29.7000E	0^0 29’34.4736N	Long rains
ALP17	Busia	Alupe	1184	34^0 26’21.2005E	0^0 29’11.4734N	Long rains
ALP18	Busia	Alupe	1179	34^0 26’20.3090E	0^0 29’54.4775N	Long rains
ALP19	Busia	Alupe	1169	34^0 26’27.4008E	0^0 29’19.4734N	Long rains
ALP20	Busia	Alupe	1167	34^0 26’26.6005E	0^0 29’19.4708N	Long rains
ALP21	Busia	Alupe	1161	34^0 26’29.6005E	0^0 29’17.4798N	Long rains
ALP22	Busia	Alupe	1156	34^0 26’22.0046E	0^0 29’11.4702N	Long rains
ALP24	Busia	Alupe	1151	34^0 26’25.7986E	0^0 29’23.4994N	Long rains
ALP25	Busia	Alupe	1157	34^0 26’26.7005E	0^0 29’89.4708N	Long rains
ALP26	Busia	Alupe	1167	34^0 26’21.2009E	0^0 29’54.4703N	Long rains
ALP27	Busia	Alupe	1176	34^0 26’27.7098E	0^0 29’66.4777N	Long rains
ALP28	Busia	Alupe	1197	34^0 26’20.7846E	0^0 29’67.4734N	Long rains
ALP29	Busia	Alupe	1190	34^0 26’29.7006E	0^0 29’19.4798N	Long rains
ALP30	Busia	Alupe	1189	34^0 26’20.1846E	0^0 29’87.4765N	Long rains
ALP31	Busia	Alupe	1190	34^0 26’22.7840E	0^0 29’13.4734N	Long rains
ALP32	Busia	Alupe	1196	34^0 26’27.7850E	0^0 29’16.4711N	Long rains

ALP33	Busia	Alupe	1198	34^0 26’23.7306E	0^0 29’19.1134N	Long rains
ALP34	Busia	Alupe	1210	34^0 26’28.7860E	0^0 29’18.4754N	Long rains
ALP35	Busia	Alupe	1145	34^0 26’22.2048E	0^0 29’14.4684N	Long rains
ALP36	Busia	Alupe	1147	34^0 26’27.5842E	0^0 29’15.4730N	Long rains
ALP37	Busia	Alupe	1149	34^0 26’21.9840E	0^0 29’19.4750N	Long rains
ALP38	Busia	Alupe	1148	34^0 26’29.2845E	0^0 29’10.4734N	Long rains

Table 17: Region flagged with a blue star indicates farms that displayed the typical biological symptoms of *Groundnut ringspot virus*

Appendix xv:Institutional Ethics and Review Committee

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

Tel: 056-31375
Fax: 056-30153
E-mail: ierc@mmust.ac.ke
Website: www.mmust.ac.ke

P. O. Box 190-50100
Kakamega,
KENYA

Institutional Ethics and Review Committee (IERC)

REF: MMU/COR: 403012 Vol 5 (01)

Date: 16th August, 2021

To: Lubao Wanyonyi Murere

Dear Sir/madam

RE: SEROLOGICAL AND MOLECULAR CHARACTERIZATION OF GROUNDNUT RINGSPOT VIRUS INFECTING GROUNDNUTS IN WESTERN KENYA.

This is to inform you that ***Masinde Muliro University of Science and Technology Institutional Ethics and Review Committee (MMUST-IERC)*** has reviewed and approved your above research proposal. Your application approval number is **MMUST/IERC/001/2021.**The approval period is ***16th August, 2021 - 16th August, 2022.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including informed consents, study instruments, MTA will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***MMUST-IERC.***
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***MMUST-IERC*** within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***MMUST-IERC*** within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***MMUST-IERC***.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours Sincerely,

Dr. Gordon Nguka (PhD)
Chairman, Institutional Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)

Appendix xvi: Directorate of Postgraduate Studies

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY (MMUST)

Tel: 0702597360/61
: 0733120020/22
E-mail: deansgs@mmust.ac.ke
Website: www.mmust.ac.ke

P.O Box 190
50100 Kakamega
KENYA

Directorate of Postgraduate Studies

Ref: MMU/COR: 509079

4th June, 2021

Lubao Wanyonyi Murere,
SCP/H/01-53218/2018
P.O. Box 190-50100
KAKAMEGA

Dear Mr. Lubao,

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your Ph.D proposal entitled: ***"Serelogical and Molecular Characterization of Groundnuts Ringspots Virus (GRSV) infecting Groundnuts in Western Kenya"*** and appointed the following as supervisors:

1. Dr. Mario KollenBerg - SONAS, MMUST
2. Prof. Hassan K. Were - SAVET, MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director of Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Natural Sciences & Technology Graduate Studies Committee and Chairman, Department of Biological Sciences. Kindly adhere to research ethics consideration in conducting research.

It is the policy and regulations of the University that you observe a deadline of three years from the date of registration to complete your Ph.D thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours Sincerely,

DEAN
SCHOOL OF GRADUATE STUDIES
MASINDE MULIRO UNIVERSITY
OF SCIENCE & TECHNOLOGY

Dr Consolata Ngala
DEPUTY DIRECTOR DIRECTORATE OF POSTGRADUATE STUDIES