RESPONSE OF POTATO GENOTYPES TO VIRUS INFECTION AND EFFECTIVENESS OF POSITIVE SELECTION IN MANAGEMENT OF SEED BORNE POTATO VIRUSES

OKEYO ONDURU GIDRAF

BSc. Agriculture (Hons), University of Nairobi

THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE REQUIREMENTS OF THE DEGREE OF MASTER OF SCIENCE IN CROP PROTECTION

DEPARTMENT OF PLANT SCIENCE AND CROP PROTECTION

FACULTY OF AGRICULTURE

UNIVERSITY OF NAIROBI

©2017

DECLARATION

This thesis is my original work and has not been presented for award of a degree in any other University.

Okeyo Onduru Gidraf Signature.. Date..........................

This thesis is submitted with our approval as University supervisors

Prof. Rama D. Narla Signature.. Date..........................

Department of Plant Science and Crop Protection

University of Nairobi

Dr. Douglas W. Miano Signature.. Date..........................

Department of Plant Science and Crop Protection

University of Nairobi

Dr. Elmar Shulte - Geldermann Signature.. Date..........................

International Potato Centre (CIP), Regional Office, Sub-Saharan Africa

DEDICATION

I dedicate this thesis to my grandfather Mr. Zakayo Okeyo Orwa, uncles Tom and Oscar Ouma for their commitment to ensuring I got the merited education. I also dedicate this work to my lovely wife Mrs. Irine Nyambok and my beloved sons Fidel and Okeyo Junior for their patience during the hard times while I was away. Lastly I commend my siblings, friends and relatives for their social, moral and material support and also their prayers during the study period.

ACKNOWLEDGEMENT

I would like to thank the Almighty God for walking me through this coarse path of academic achievement. My sincere gratitude goes to my supervisors Prof. Rama Devi Narla, Dr. Douglas Watuku Miano and Dr. Elmar Schulte-Gelderman. I am highly indebted to their academic guidance, moral support and caring love throughout my academic period. Their commitments made this work complete and successful enabling me to be a better student.

Special gratitude also goes to the International Potato Center (CIP) for fully financing this study project and the Department of Plant Science and Crop Protection, Faculty of Agriculture in the University of Nairobi for awarding me a Masters scholarship. I would also like to thank Mr. Elly Atieno Ouma and Mr. Herman Machavuli both from CIP for their support in procurement of study materials, as well as other resources during the experimental period in the field. In a similar manner, I also wish to thank Dr. Maina Muiru for his counseling and guidance during difficult times of my research.

Finally, I humbly appreciate the support and love from my family members Zakayo Okeyo, Nesila Anyango, Tom Ouma, Oscar Ouma, Irine Nyambok, Fidel Okeyo, Okeyo Junior, Hermstone Opiyo, Leisha Awuor, Clinton Ombaka and Faith Odero. I am also grateful for the support from my friends; Shadrack Nyawade, Oliver Otieno, Geraldine Lengai and many others. I pray to the Almighty God to grant you mercies and good health to achieve your goals.

TABLE OF CONTENTS

DECLARATION ii

DEDICATION iii

ACKNOWLEDGEMENT iv

LIST OF TABLES ix

ACRONYMS AND ABBREVIATIONS xi

GENERAL ABSTRACT xii

CHAPTER ONE: INTRODUCTION 1

1.1 Background information 1

1.2 Problem statement 5

1.3 Justification 5

1.4 Objectives 7

1.4.1 Broad objective 7

1.4.2 Specific objectives 7

1.4.3 Hypothesis 7

CHAPTER TWO: LITERATURE REVIEW 8

2.1 Origin of potato 8

2.2 Potato production in Kenya 8

2.3 Potato production constraints 9

2.4 Seed potato degeneration and its management 10

2.4.1 Viruses affecting potatoes ... 11
2.5 Management of seed potato degeneration ... 15
2.5.1 Improving seed potato quality through positive selection ... 15
2.5.2 Adjustment of planting dates and field sanitation ... 16
2.5.3 Mulching, cover and barrier crops ... 17
2.5.4 Host plant resistance ... 18
2.5.5 Chemical control ... 19
CHAPTER THREE ... 20
RESPONSE OF POTATO GENOTYPES TO DIFFERENT VIRUS INFECTIONS ... 20
3.1 Abstract ... 20
3.2 Introduction ... 21
3.3 Materials and Methods ... 23
3.3.1 Description of the study area ... 23
3.3.2 Potato genotypes ... 24
3.3.3 Set up of the field experiment ... 25
3.3.4 Detection of viruses in seed potato tubers from Field Generation Four (FG4) ... 26
3.4 Data collection ... 28
3.5 Data analysis ... 29
3.6 Results ... 30
3.6.1 Emergence of seed potato tubers ... 30

3.6.2 Weather conditions during crop growth 32
3.6.3 Disease incidence 33
3.6.4 Plant height 34
3.6.5 Number of tubers per hill 35
3.6.6 Total Yield 36
3.6.7 Correlation among disease incidence, plant height, number of tubers per hill and yield in FG3 and FG4 37
3.6.8 Virus infection status of seed tubers from Field Generation Four (FG4) 38
3.7 Discussion 39
CHAPTER FOUR 47
EFFICIENCY OF POSITIVE SELECTION IN MANAGEMENT OF SEED BORNE VIRUSES AND YIELD OF POTATO 47
4.1 Abstract 47
4.2 Introduction 48
4.3 Materials and Methods 49
4.3.3 Set of the ffield experiment 50
4.3.4 Detection of viruses in seed potato tubers 51
4.4 Data collection 54
4.5 Data analysis 55
4.6 Results 55

4.6.2 Effects of positive selection on disease incidence 55

4.6.3 Effects of positive selection on plant height 56

4.6.4 Effects of positive selection on number of tubers 57

4.6.5 Effects of positive selection on yield 58

4.6.6 Correlations among disease incidence, plant height, number of tubers and yield in RSS and PS plots 59

4.6.7 Virus infections in seed potato tubers of different genotypes 60

4.7 Discussion 62

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS 66

5.1 Conclusions 66

5.2 Recommendations 66

REFERENCES 67

APPENDIX 79

LIST OF TABLES

Table 3. 1: Status of different potato genotypes with regard to major potato viruses 24

Table 3. 2: Percent emergence of potato genotypes at different Days After Planting 31

Table 3. 3: Percent emergence of potato seed tubers at different Days After Planting 32

Table 3. 4: Percent virus disease incidences at different Weeks After Planting (WAP) 33

Table 3. 5: Percent virus disease incidences at different weeks after planting (WAP) in 34

Table 3. 6: Plant height (cm) of different potato genotypes in Field Generation Three (FG3) and Field Generation Four (FG4) and percent decrease in FG4 35

Table 3. 7: Number of potato tubers per hill of different genotypes in Field Generation Three (FG3) and Field Generation Four (FG4) and percent decrease in FG4 36

Table 3. 8: Yield (t/ha) of potato genotypes recorded in Field Generation Three (FG3) and Field Generation Four (FG4) and percent decrease in FG4 37

Table 3. 9: Correlation coefficients among disease incidence, plant height, number of tubers per hill and total yield of different potato genotypes in FG3 and FG4 38

Table 3. 10: Incidences of potato viruses detected in seed tubers from Field Generation Four (FG4) 39

Table 4. 1: Effect of PS and RSS on disease incidence of different potato genotypes and percent decrease in disease incidence from positive selection 56

Table 4. 2: Effects of PS and RSS on plant heights of different potato genotypes and percent height increase from PS... 57

Table 4. 3: Effect of RSS and PS on number of tubers per hill of different potato genotypes and percentage tuber gain from PS 58

Table 4. 4: Effects of RSS and PS on total yield of different potato genotypes and percent yield increase from PS 59

Table 4. 5: Correlation coefficients among disease incidence, plant height, number of tubers and yield in Random seed selection and positive selection plots 60

Table 4. 6: Percent incidences of potato viruses in seed tubers from Random Seed Selection and Positive Selection plots 61

ACRONYMS AND ABBREVIATIONS

CABI	Center for Agriculture and Biosciences International
CIP	International Potato Center
DAS-ELISA	Double Antibody Sandwich - Enzyme Linked Immunosorbent Assay
DI	Disease Incidence
KALRO	Kenya Agricultural and Livestock Research Organization
NPT	National Performance Trials
PLRV	*Potato Leaf Roll Virus*
PS	Positive Selection
PVA	*Potato Virus A*
PVM	*Potato Virus M*
PVS	*Potato Virus S*
PVS^{A}	*Potato Virus S* Andean strain
PVS^{O}	*Potato Virus S* ordinary strain
PVX	*Potato Virus X*
PVY	*Potato Virus Y*
PVY^{C}	*Potato Virus Y* stripple strain
PVY^{N}	*Potato Virus Y* tobacco venial necrosis strain
PVY^{NTC}	PVY^{NTC} strain group
PVY^{O}	*Potato Virus Y* common strain
RSS	Random Seed Selection
WAP	Weeks After Planting
WP	Wettable Powder

GENERAL ABSTRACT

Potato seed degeneration resulting from accumulation of seed borne viral pathogens in seed-tubers is a major challenge limiting optimal yields in potato growing areas around the world especially in the tropics. A study was carried out at the Kabete Field Station, University of Nairobi to determine response of potato genotypes to natural virus infection in the field and to assess effectiveness of positive selection on the health of seed potato tubers with regards to potato viruses. Sprouted seed potato tubers of twelve genotypes harvested from Field Generation Two (FG2) were planted and the crop was subjected to natural virus infection in the field for two seasons (Field Generation Three (FG3) and Field Generation Four (FG4)). These genotypes consist of five commercial varieties (Tigoni, Kenya Mpya, Shangi, Asante and Sherekea) and seven clones (398190.200, 300046.22, 393371.157, 393077.159, 392797.22, 398098.65 and 397073.7) sourced from International Potato Center (CIP). Fungal diseases on the crop were controlled using appropriate fungicides with no control of insects to facilitate high vector movement. Ten weeks after planting of tubers from FG2, plants with no virus symptoms were pegged in each plot and regular checking to de-peg those with newly developed disease symptoms was done weekly until crop maturity. Symptomatic and asymptomatic plants were harvested separately; medium size (30-60 mm) and apparently healthy looking tubers were selected from each plot. These tubers from FG3 were stored in an insect proof diffused light store for two months to sprout. The sprouted tubers from positively selected plants were used as seed stocks for Positive Selection (PS) and tubers harvested from visually diseased plants were used as seed stock for Random Seed Selection (RSS) for FG4. At the end of FG4, 100 medium size and apparently healthy looking tubers were collected randomly from each genotype from both RSS and PS plots. These tubers were sprouted and tested for presence of six major potato

viruses using DAS-ELISA. Data was collected on percent seed emergence, disease incidence, plant height, numbers of tubers and total yield for each genotype in both FG3 and FG4. The study revealed a varied percent emergence, virus incidence, plant heights, number of tubers per hill and yield (t/ha) among the twelve genotypes from FG3 to FG4. High percent emergence, low disease incidence, higher plant heights, number of tubers per hill and yields were recorded in FG3 compared to FG4. Four potato viruses; *Potato leaf roll virus* (PLRV), *Potato virus S* (PVS), *Potato virus M* (PVM) and *Potato Virus Y* (PVY) were detected infecting tested potato tubers from FG4 either as single infection or as multiple infections. *Potato Virus S* (PVS) was the most dominant virus (67%) followed by PVY (20%), PLRV (12%) and PVM (7%) while PVA and PVX were not detected in any of the tested tubers. Use of PS reduced increase in virus disease incidence by 3 to 10%, increased plant height by 1 to 14%, number of tubers by 9 to 41% and yield by 4 to 56% depending on genotypes. ELISA results revealed *Potato Virus S* (PVS) as the most predominant virus followed by *Potato Virus Y* (PVY) and *Potato Leaf Roll Virus* (PLRV) in both RSS and PS plots, while *Potato Virus M* (PVM) was only detected in samples from RSS plots. Four clones; 397073.7, 398190.200, 393371.157 and 392797.22 were found tolerant to natural virus infections compared to five commercial varieties and other three clones in FG4 based on the yields obtained. Based on the results positive selection and use of resistant and or tolerant varieties can be used to manage seed borne potato viruses to some extent by farmers who produce their own seeds.

Key words: Potato, Seed degeneration, Seed-borne potato viruses, Genotype, tolerance

CHAPTER ONE: INTRODUCTION

1.1 Background information

Potato (*Solanum tuberosum L.*) is ranked third after rice and wheat among the most consumed crops worldwide (Wang'ombe and Dijk, 2013). The crop plays a major role in poverty alleviation through income generation and creation of employment to people working in potato industry especially small scale farmers (Gildemacher *et al*., 2011). The crop is grown by over 800,000 farmers and creates both direct and indirect employment opportunities to over 2.5 million households working as market agents, transporters and processors in the value chain (Onditi *et al*., 2012). Potato is grown in the high altitude and high potential areas of Kenya (1,500-3,000 m above sea level), which includes the slopes of Mt. Kenya, such as Meru, Embu and Kiringa and parts of Laikipia and Aberdare ranges that covers parts of Nyeri, Muranga, Kiamba and Nyandarua. Potatoes are also grown in the highlands on Mau Escarpment (Mau, Narok and Molo), Tinderet, Nandi Escarpment and Cherangani Hills. The crop is also grown in new areas such as Kericho in Rift Valley, Kisii in Western Kenya and Taita taveta at the Coast region (Janssens *et al*., 2013).

The Kenyan potato industry has been expanding rapidly every year, but availability of certified seeds has remains to be the main constraint leading to low yields. New potato varieties resistant to pests and diseases, high yielding, better seed and ware storability and processing qualities is a challenge to breeders and farmers since introduction of the potato crop in Kenya (Onditi *et al*., 2012). The Kenya Agricultural and Livestock Research Organization (KALRO) and International Potato Centre (CIP) have been working in

collaboration with other institutions like CIP to release high quality potato varieties to farmers after evaluation in National Performance Trials (NPT).

According to the potato seed variety catalogue published by the National Potato Council of Kenya (NPCK), forty varieties of potato are grown in Kenya. These varieties include Tigoni, Asante, Dutch Robjin, Kenya Mavuno, Kenya Karibu, Kenya Sherekea, Purple Gold, Kenya Sifa, Kenya Mpya, Kenya Pink, Desiree, Kenya Baraka and Annet among others (NCPK, 2015). Among the above mentioned, the most popular varieties in Kenya are Asante, Tigoni, Kenya Sifa and Shangi (Onditi *et al*., 2013).

Potato production in Kenya has been on the increase with increase in land areas devoted to crop production while the quality and yielding capacity of this crop has remained below potential (Wang'ombe and Dijk, 2013). Kenya produces 7.7 tons/ ha of potatoes which is far much less than about 40 tons/ha produced in developed countries. However, production has fluctuated in the recent years, from 9.5 tons/ha to less than 3 tons/ha (Muindi *et al*., 2013). These low yields have been attributed to poor agronomic practices, insufficient farm inputs, infertile soils, high prices of certified seed tubers, diseases and insect pests (Janssens *et al*., 2013). Potato seed tubers contribute to about 40-50% of potato production input costs and quality seed is the most important input (Kyamanywa *et al*., 2011). Unfortunately, more than 95% of seed potato is sourced from informal supply channels of poor quality status with accumulation of seed borne pathogens (Schulte-Geldermann 2013; Thomas-Sharma *et al.,* 2015). Less than 2% of farmers have access to certified seed potato tubers

due to high costs and inaccessibility of these certified seed potato tubers (Kyamanywa *et al*., 2011; Janssens *et al*., 2013).

In Kenya, National Potato Research Center (Tigoni) had been mandated to multiply and distribute clean potato seed tubers. Apart from commercial production of certified seed potato tubers, the Center is also mandated to carry out research on other crops hence its efficiency has not been optimized due to expansion of these other projects. The productivity of the institution has been no more than 25-50 tons of basic potato seeds per year thus the demand by farmers is not met (Janssens *et al*., 2013).

Seed potato tuber quality reduces through seed degeneration across seasons due to infection by tuber-borne diseases especially potato viruses (Gildemacher *et al*., 2011). Seed potato degeneration is a combined effect of increase in the number of infected seed tubers caused by a single or multiple pathogens such as viruses, and an increasing concentration of the virus particles in the seed tubers (Gildemacher *et al*., 2011). Infection by viruses has devastating effect on potato production significantly reducing yields depending on levels of resistance of the genotypes, the virus, stage of virus infection, and timing of virus exposure of the seed stocks in the field (Ali *et al*., 2013).

The universal solution to this problem has been to enhance availability and farmers' access to seed-tubers produced elsewhere by certified growers. Certified seed is often healthy and the rigorous certification process minimizes risks of infection with seed borne pathogens. However, due to reasons such as limited certified seed supply and high costs, farmers still prefer using their own farm-saved seed tubers from the previous seasons and therefore do

not maximize their yields (Thomas-Sharma *et al*., 2016). Management strategies such as positive selection of healthy looking plants to act as source of healthy seeds introduced by CIP (Gildemacher *et al*., 2011) and use of aeroponics in seed production (Tshisola, 2014) have been adopted in Kenya to reduce seed potato degeneration rate. Cultural control methods like use of mineral oils and borders crops such as planting soybean, wheat, maize and sorghum around the edges of potato fields has contributed as virus sinks. This interrupts non-persistence potato virus transmission by vectors such as aphids and other insects to the main potato crop (Dessureault *et al.,* 2011; Muindi *et al*., 2013).

Positive selection is an ancient technology that was used in formal seed potato multiplication to select healthy looking mother plants from potato growing fields in seed multiplication system (De Bokx and Van de Want 1987). This method was first used in Central Africa in 1980s as the starting point for seed multiplication (Haverkort 1986). However, it is not commonly used by ware potato producers, nor is its use promoted. This is because the technology is regarded as an obsolete technology in formal seed production and seed producers prefer to multiply seeds from tested, disease free, tissue culture material or from disease free nuclear stock (Gildemacher *et al*., 2011).

In Kenya positive selection was introduced by International Potato Center (CIP) in 2004 with the aim of training ware potato farmers on its importance (Gildemacher *et al*., 2007). The know-how of positive selection, if adopted and put into practice can provide additional options to small-holder famers in managing seed potato tuber infections and spread of seed borne pathogens mainly viruses at farm level (Gildemacher *et al*., 2011; Schulte-

Geldermann *et al*., 2012). However, it is not commonly practiced by farmers due to lack of knowledge on its importance, difficulties in diagnosis of different viruses in the field using visual symptoms and small-scale nature of their farming enterprises.

1.2 Problem statement

Potato is the main food and income generating crop cultivated by small scale farmers in the Kenyan highlands (Muthoni *et al*., 2013). The crop is ranked second after maize in terms of production volume and is a source of employment to people in potato industry (Muthoni and Nyamongo 2009). Its production is faced with several challenges mainly biotic factors leading to low yields in most potato growing areas (Janssens *et al*., 2013).

Viruses and aphid pests are major biotic constraint reducing yields in potato growing areas. Use of virus infected seed tubers has resulted up to 68% yield loss in fields free from fungal and bacterial diseases (Muindi *et al*., 2013). This reduction in yield arises from seed degeneration caused by recycling of the same seed stock across seasons leading to build up of viral pathogens in the fields (Lakra, 2010; Ali *et al*., 2013). Most potato varieties grown by Kenyan farmers have shown low levels of resistance and or tolerance to potato viruses and insect vectors. Unlike other potato diseases caused by fungal and bacterial pathogens, potato viruses cannot be managed by use of chemical pesticides and hence a feasible management method has to be adopted to help reduce yield loss (Njukeng *et al*, 2013).

1.3 Justification

Due to limited availability of certified seed potato tubers, most farmers in Kenya are forced to use seed tubers obtained from informal sources. These seed tubers are recycled from one

season to another without renewal or cleanup for diseases (Schulte-Geldermann 2013) and have resulted to high yield losses with few, small, deformed and misshaped tubers. Most of the potato varieties grown by Kenyan farmers were released in 1990s after they proved to be resistant to most potato diseases such as late blight, bacterial wilt and viruses. However, the resistance traits have deteriorated over time due to continuous exposure to natural inoculum pressure in potato growing fields and this necessitates screening of new clones for resistance to different pathogens especially viruses. These viruses cannot be controlled by chemical means leading to high yield losses in potato production fields.

The largest percentage of potato producers in Kenya are small scale farmers who cannot afford expensive virus management strategies. Due to smaller land holdings and favorable tropical climatic conditions, high insect vector populations mainly aphids have been reported predisposing potato crop to virus infection in the field (Carlos de Avila *et al.,* 2009). Farmers also find it difficult to diagnose virus infected plants in the field due to latent infection and symptom overlap by some viruses (Gildermacher *et al.,* 2011). Unlike fungal and bacterial diseases, potato viruses lack a proper chemical control strategy and this situation therefore calls for adoption of environmentally friendly and inexpensive management strategies such as positive selection of healthy looking mother plants to provide seed stock for propagation in subsequent seasons. This will help to reduce virus spread from one field to another as well as between seasons.

1.4 Objectives

1.4.1 Broad objective

The general objective of this study was to increase potato yields in Kenya through adoption of environmentally friendly potato virus management strategies such as use of resistant varieties and positive selection of healthy looking mother plants as source of healthy seed tubers.

1.4.2 Specific objectives

i. To evaluate response of selected potato genotypes to natural virus infections in the field.
ii. To investigate the effectiveness of positive selection on the health of seed potato tubers in response to potato viruses.

1.4.3 Hypothesis

i. Potato genotypes show different levels of resistance and or susceptibility to virus infection.
ii. Positive selection of healthy looking mother plants as seed source is a good management strategy with regard to seed borne potato viruses.

CHAPTER TWO: LITERATURE REVIEW

2.1 Origin of potato

Potato cultivation is believed to have originated from domestication of indigenous potato cultivars in South America; the highlands of Andes and lowlands of South Central Chile (Sukhotu and Hosaka, 2006). Potato then diffused from South America and was first reported in Europe at around16th century before spreading to all parts of the world (Huamán *et al*., 1997; Bradshaw and Ramsay 2009). In Africa potato cultivation began in 1830 with its first husbandry and movement experienced in South Africa and later in East Africa in 1880 by British and German colonialists (Black, 2008). In Kenya the crop was introduced in the late 19th century by British East African Trading Company and at that time, the crop was only grown by the white settlers in the white highlands (Durr and Lorenzl 1980). Today potato is ranked second most consumed crop after maize and the most important staple food crop in Kenya (Muthoni and Nyamongo, 2009).

2.2 Potato production in Kenya

Kenya is fifth among the biggest potato producing countries in Sub-Saharan Africa (Muthoni and Nyamongo, 2009) producing 7.7 tons/ ha. However, this has fluctuated in the recent years, from 9.5 to less than 3 tons/ha (Muindi *et al*., 2013). Potato is grown by over 800,000 farmers (Onditi *et al*., 2012) among which 500, 000 are small-scale farmers having land holdings of less than one hectare (Janssens *et al*., 2013). Potato production is practiced in the high-altitude areas (1,500-3,000 m above sea level)which includes the slopes of Mt. Kenya; Meru, Embu, Kiringa and parts of Laikipia, Aberdare Ranges; Nyeri, Muranga,

Kiamba and Nyandarua. The crop is also grown in the highlands on Mau Escarpment (Mau, Narok and Molo) and Tinderet, Nandi Escarpment and Cherangani hills. Small patches of potato are also grown in Kericho, Kisii and Taita hills among others since the cultivation of the crop is expanding (Janssens *et al*., 2013).

2.3 Potato production constraints

Seed potato sector in Kenya is challenged by various constraints such as lack of certified seeds, soil infertility, pest and diseases, lack of readily available market for potato products and poor seed packaging (Muthoni and Nyamongo 2009; Muthoni *et al*., 2013). Seed potato contributes to about 40-50% of potato production input costs (Kyamanywa *et al*., 2011). Shortage of certified seed potato tubers has resulted to reduced yields in potato fields, poor quality tubers and spread of seed borne pest and diseases between farms through recycling of infected seed tubers (Schulte-Geldermann, 2013). Kenya produces less than 1-2% of its nationwide certified seed requirement. This shortage has forced farmers to plant seeds from informal supply sources such as farm-saved seeds, seeds purchased from local markets and or from neighbors (Kyamanywa *et al*., 2011; Janssens *et al*., 2013).

Among pest and diseases, viruses and aphid pests which acts as the main vectors to most potato viruses, are the key constraints to certified seed potato production in Kenya contributing to reduced potato yields (Machangi *et al*., 2003). Six potato viruses namely *Potato Leaf Roll Virus* (PLRV), *Potato Virus A* (PVA), *Potato Virus M* (PVM), *Potato Virus S* (PVS), *Potato Virus X* (PVX), and *Potato Virus Y* (PVY) and four aphid species namely *Macrosiphum euphorbiae*, *Aphis gossypii*, *Myzus persicae*, and *Aphis fabae* are

known to affect potato production in Kenyan (Were *et al*., 2013). Cumulative infection by viruses has strong devastating effect on potato production by depressing yield potentials of the infected crop. This is seed potato degeneration and it is genotype specific because each variety reacts with different degrees of loss in tuber yield depending on the virus type, stage of infection, and time of field exposure of the seed stocks to the virus (Ali *et al*., 2013).

2.4 Seed potato degeneration and its management

Potato production in the world has been experiencing a decline in yield due to tuber deterioration caused by seed borne pathogens. The gradual degradation of genetic potential of potato seed is referred to as potato seed degeneration (Rahman *et al*., 2010). Degeneration effects are characterized by; decreased vigor yields and low resistance to diseases after consecutive recycling of infected seed potato tubers (Sanger *et al*., 1994).

Seed potato degeneration is mainly attributed to biotic factors like insect pests, bacteria, fungus, nematode and viruses infecting foliage and tubers triggering various symptomatic expressions such as reduced sprout emergence, poor plant vigour, pre and post emergence damping off and low quality tubers (Njukeng *et al*., 2013). Physiological degeneration which refers to the reduction in yield caused by unsuitable age of potato tubers at the time of harvest may also cause seed potato degeneration. When seed potato tubers are planted at the appropriate physiological age, they result to high growth vigor and high yields as opposed to when potato seed tubers are planted at unsuitable physiological age (Rahman *et al*., 2010). Physiological factors such as seed tubers injury, early and poor sprouting may

predispose the tuber to attack and invasion by potato pathogens accelerating deterioration process during storage and eventually poor field performance (Muthoni *et al*., 2013).

Diseases caused by plant pathogens are grouped into four namely bacterial diseases like Bacterial wilt by *Ralstonia solanacearum,* bacterial soft rot (*Erwinia carotovora* pv *carotovora*) and common scab (*Streptomyces scabies*), fungal diseases like late blight (*Phytophthora infestans*), early blight (*Alterneria solani*) and fusarium dry rot (*Fusarium spp*), viral diseases like PVY, PLRV, PVM, PVA, PVS as well as PVX and nematodes like Root-knot nematode (*Meloidogyne spp)* and potato Cyst Nematode (*Globodera rostochiensis*) (Warsito and Elseke 2006). Abiotic factors such as abnormal temperatures, nutrient deficiency and water/drought stress may also cause seed potato degeneration to some extent. These can affect and weaken the potato crop in the field predisposing them to attack and invasion by plant pathogenic microbes (Blom-Zandstra and Verhagen, 2015).

2.4.1 Viruses affecting potatoes

Viruses are the most important pathogens responsible for potato seed degeneration. Infection by potato viruses in production fields has resulted into decreased plant vigor, reduced levels of resistance to pests and diseases in potato cultivars after successive cultivation from the same seed lot and low yields (Nascimento *et al*., 2003). Over 37 viruses can infect potatoes naturally in the field causing diseases (Valkonen, 2007).

2.4.1.1 *Potato Virus Y*

Potato Virus Y is a member of the genus *Potyvirus,* family *potyviridae* and it consists of flexuous, long filamentous particles which measures approximately 740nm x 11nm in

length (Kumar, 2010). Four strains of PVY have been identified to infect potato plants and cause viral diseases namely PVY^{O} (common strain), PVY^{N} (tobacco venial necrosis strain), PVY^{C} (stipple streak strain) and PVY^{NTN} (strain group) (John *et al*., 2013). Under favorable environmental conditions, PVY causes 10-100% yield loss (Warren *et al*., 2005). Symptoms vary widely depending on the virus strains and potato cultivars (Jeffries 1998).

Overall symptoms of infection include leaf surface becoming uneven and brittle, shrunken leaves with midribs turning yellow but no symptoms are expressed during mild infections (Warsito and Elseke, 2006). Infection by PVY in the field may lead to numerous numbers of small tubers hence reduced yield but this is variety dependent (Hanne and Hamm, 1999).Despite some of the strains being restricted to certain countries, PVY is globally distributed (Kerlan, 2006). Apart from potatoes, PVY also infects other members of *Solanacea* family such as tobacco, tomato, pepper and wild species within the same family (Mc Donald, 1996). This virus is transmitted by more than 50 species of aphids in a non-persistent manner and winged aphids are the most effective vectors. Other modes of transmission include mechanically through plant contacts and cultural practices during crop growth (Warren *et al*., 2005; Wale, 2008).

2.4.1.2 *Potato Leaf Roll Virus*

Potato Leaf Roll Virus belongs to genus *Polerovirus* and family *Luteoviridae* (Harrison 1984). Infected potato plants exhibit primary symptoms such as reddening of leaf apex and affected leaves may roll inwards and becomes erect during their normal growth period in the field. Secondary symptoms usually appear when infected seed potato tubers are used for

propagation and these plants exhibit shoot stunting, lower and upper leaflets roll inwards, these leaves are usually dry and break easily. If touched by hands, the leaves produce a distinctive crackling noise (Kumar, 2010). Affected tubers develops phloem necrosis (Raza and Et, 2010). This virus only infects members of the *Solanaceae* family and is globally distributed in areas where potatoes are grown (Chiunga, 2013). *Myzuspersicae spp.* is the most efficient aphid vector of PLRV and it transmits the virus in a non-persistence manner (Thomas, 1984). Virus distribution occurs mainly through infected potato seed tubers and other vegetative parts including seedlings and micro-propagated plants (CABI, 2007).

2.4.1.3 Potato Virus X

Potato Virus X (PVX) is a member of the family Al*phaflexiviridae* and genus *Potexvirus* (Chiunga, 2013). Losses caused by this virus has been approximated to range between 15-20% (Kumar, 2010). Symptom expression due to PVX infection depends on the interaction of cultivars, virus strain and environmental conditions. At higher temperatures, above 25^0C, infection is asymptomatic. Symptoms such as mild mosaic, mottling and leaf crinkling may be observed during normal environmental conditions (Fribourg, 2007). This virus mainly affects plants from the *Solanaceae* family such as bell pepper, chili, tomato, tobacco, potato, turnip, purple clover, grape vine, red root pig weed and kangaroo apple among others(Chiunga, 2013). *Potato Virus X* is globally distributed and is transmitted through contact of infected and healthy plants or by farm machineries, clothes and animals (Fribourg 2007). Once inside the tuber, the virus remains active and accumulates in the tubers and can spread through tuber cutting process (Kumar, 2010).

2.4.1.4 *Potato Virus S*

Potato Virus S (PVS) belongs to the family *Betaflexiviridae*, genus C*arlavirus* and consists of slightly flexuous and filamentous particles measuring 660nm x 12nm. It has two recognized strains, PVS^O (Ordinary) which has a worldwide distribution and PVS^A (Andean) mainly found in Andean of South America (Hinostroza-Orihuela, 1973). This virus causes moderate yield reduction of about 20% (Kerlan 2008). The virus is usually asymptomatic but in severe cases, it produces minor symptoms on the leaf such as roughness, vein deepening and leaf browning (Kumar, 2010). *Potato Virus S* infects members of *Solanaceae* family and *Chenopodiaceae* spp. This virus is distributed worldwide (Chiunga, 2013) and is spread mechanically, by contact and in a non-persistent manner by aphid insect vectors mainly *Myzuspersicae* and *Aphisnasturtti* (Jeffries, 1998).

2.4.1.5 *Potato Virus M*

Potato Virus M consists of slightly curved filamentous particles measuring 650nm x 12nm and it belongs to genus *Carlavirus* and family *Flexviridae*. The virus causes yield loss ranging between 15-40% in potato fields if left uncontrolled (Chiunga, 2013). Infection by PVM is usually asymptomatic on most potato plants though symptoms like mottling, mosaic, crinkling, rolling of leaves and shoot stunting may be observed in the infested fields. Symptomatic expressions mainly occur in plants infected at early stage of growth (Kumar, 2010). Potatoes are the major host and other plant species like *Amaranthaceae, Caryophyllaceae* (Edwardson and Christie, 1997). This virus is distributed worldwide and is spread from plant to plant by aphid vectors in anon-persistent manner (Kumar, 2010).

2.4.1.6 *Potato Virus A*

Potato Virus A is a member of the genus *Potyvirus,* family *Potyviridae.* This virus causes 40% yield loss if left uncontrolled in potato growing fields. *Potato Virus S* is distributed worldwide in all potato growing areas. It consists of flexuous and filamentous particles 730nm x11nm long (Kerlan, 2008). Infected plants display minor mosaic symptoms similar to those caused by PVX. However, PVA infected leaves are shiny (wale, 2008). The virus is distributed worldwide and affects plants like potato, pepper and tobacco (CABI, 2007). *Potato Virus A* is spread by aphid vectors in a non-persistent manner (Wale, 2008).

2.5 Management of seed potato degeneration

Potato viral diseases if left uncontrolled in a potato production field can cause up to 50% yield loss (Nasir *et al*., 2012). The percentage yield loss is so high because no chemical treatment method can be used to reduce the disease to acceptable levels once it establishes in the field. Proper Integrated Pest Management (IPM) strategies should be put in place in order to maintain high yield in the field. Some of the Integrated Pest Management (IPM) strategies include use of positive selection, adjustment of planting dates, field sanitation, mulching, and use of barrier and or border crops, resistant varieties and chemical control.

2.5.1 Improving seed potato quality through positive selection

Potato productivity is declining due to insufficient quantities of healthy and certified seed tubers. More than 95% of potato seeds are sourced from farmers own harvests, markets and from neighbors (Gildemacher *et al.,* 2011). Such tubers are often of poor health status due to latent infections with bacterial wilt, viruses and other tuber-borne pathogens. This has

resulted in low on-farm yields estimated to be below 10 tons/ha compared to 40-60 tons/ha achievable under normal production conditions (Gildemacher *et al*., 2011).

Practicing positive selection as a technique of choosing healthy looking mother plants before harvesting to act as seed source in the next season has successfully reduced potato seed degeneration arising from farmer-seed selection. This method was introduced in Kenya by International Potato Center (CIP) in 2004 with the main focus on introducing the method and training of ware potato farmers on its importance (Gildemacher *et al*., 2007). The know-how of positive selection, if adopted and put into practice in potato production areas can provide additional options for small-holder famers in managing seed potato tuber infestations and spread of viruses at farm level (Gildemacher *et al*., 2011; Schulte-Geldermann *et al*., 2012). Gildemacher *et al*., (2011) and Schulte-Geldermann *et al*., (2012) also reported that use of positive selection technique resulted in 28-54% yield increases in comparison to conventional farmers' practice of random seed selection. However, it is not commonly practiced by farmers due to lack of knowledge on its importance, difficulties in diagnosis of different viruses at field condition using visual symptoms and small-scale nature of their farming enterprises.

2.5.2 Adjustment of planting dates and field sanitation

Potato viruses can be managed to some level by adjusting planting dates (Ragsdale *et al*., 2001). After thorough monitoring of virus vectors and studying their infestation time, planting dates can be altered and timely programmed in order to escape the vectors. Planting is done early if vectors infest at later growth stages and vice versa. Older plants are

less likely to be affected than younger plants since older mature plants offer some level of resistance to potato viruses (Warren, 2005).

Viral pathogens usually overwinter on plant debris, weeds and volunteer potatoes. These if left unrestrained, act as sources of inoculum to aphid vectors which can affect newly planted potato fields (Thomas and Richards, 2004). Therefore, these inoculum sources should be eliminated as early as possible before planting potato seeds in the field (Warren, 2005). Use of virus-free or certified seed potato tubers reduces introduction, establishment and spread of inoculum in potato growing fields and between crop seasons. This can be achieved through screening of sprouted tubers before planting for presence viruses (Njukeng *et al*., 2007). Field sanitation is a management strategy that relies mostly on symptomatology aspect. Once symptoms have been identified in the field, the affected haulms are pulled out and destroyed to reduce the spread of inoculum in the field. The only constraint is that some viruses have latent infection and can survive in the field for a whole season without detection acting as source of inoculum in the field (Warren, 2005).

2.5.3 Mulching, cover and barrier crops

Materials such as reflective mulches and stick yellow sheets have proven to reduce viral diseases such as PVY by between 51-80% (Ragsdale *et al*., 2001). Crop covers have also shown some positive results but increased temperatures below the cover can induce reduced tuber development and size especially where day time temperatures are high (Ragsdale *et al*., 2001). Barrier crops have also shown signs of reduction in viral transmission between potato plants e.g. sorghum and soya bean which reduces spread of PVY between potato

plants (Radcliffe and Ragsdale, 2002). Border crops acts as physical barriers forcing infective aphids to lose their virulence charge as they probe on these crops and consequently clean their mouthparts thus reducing their potential to transmit and spread viruses to protected potato crops (Muindi *et al*., 2013). Effectiveness of barrier crops depends on factors such as epidemiology of the virus, the height of the barrier crop and the extent of competition between the barrier and the protected crop (Fereres, 2000).

2.5.4 Host plant resistance

Host plant resistance to potato viruses has been classified in to two namely; Extreme Resistance (ER) and Hypersensitive Response (HR). In ER, the virus does not proliferate at the infection site while in HR a necrosis lesion develops around the infected tissue limiting spread to healthy tissues (Solomon-Blackburn and Barker, 2001). Genes for Extreme Resistance (ER) and Hypersensitive Resistance (HR) against major potato viruses like PVY, PVA, PVV, PVX, PVS and PVM have been identified and incorporated into different potato lines to help reduce yield loss caused by these viruses. Few genes for resistance to vectors of these viruses have also been obtained and some have been deployed successfully to help reduce spread (Palukaitis, 2012). For example, Kenyan potato varieties like Sherekea have been bred for PLRV and PVY resistance while Kenya Mpya has extreme resistance for PVX (NCPK 2015). Even though this has worked, some limitations such as high costs incurred, durability of resistance source has been experienced (Warren, 2005).

2.5.5 Chemical control

Unlike other plant disease causing pathogens like fungi, nematodes and bacteria, plant viruses cannot be controlled by use of chemicals once it establishes on the host plant. However timely application of chemical control methods can yield some positive results if volunteer plants which acts as alternate hosts and insect vectors are targeted (Warren, 2005). This method has some limitations like development of resistance among insect species and weeds in addition to environmental degradation and pollution (Radcliffe and Ragsdale, 2002). In addition, the efficiency of chemical control is dependent on virus pressure in the surrounding environment and mode of transmission by insect vectors (Milosevic *et al.,* 2015). Chemical insecticides used to control aphid vectors include imidacloprid, thiacloprid, acetamiprid from neonicotinoid group, pymetrozine from pyridine group and flonicamid from pyridinecarboxamide (Evans and Fenton, 2010).

CHAPTER THREE

RESPONSE OF POTATO GENOTYPES TO DIFFERENT VIRUS INFECTIONS

3.1 Abstract

Potato farmers in Kenya and around the world are faced with several production challenges that results in reduced yields and low quality tubers. Potato seed degeneration resulting from continuous use of infected seed tubers in successive seasons is believed to be the main cause of this problem. Previous surveys and studies in Kenya indicated six major potato viruses; *Potato leaf roll virus* (PLRV), *Potato virus A* (PVA), *Potato virus M* (PVM), *Potato virus S* (PVS), *Potato virus X* (PVX), and *Potato Virus Y* (PVY) as main viruses causing potato seed degeneration either through single or multiple infections in production fields. A field study was conducted to determine the reaction of potato genotypes to natural virus infection for two generations. Sprouted seed potato tubers harvested in Field Generation Two (FG2) of Twelve potato genotypes; five commercial varieties (Tigoni, Kenya Mpya, Shangi, Asante and Sherekea) and seven clones (398190.200, 300046.22, 393371.157, 393077.159, 392797.22, 398098.65 and 397073.7) sourced from International Potato Center (CIP) were subjected to natural virus infection in the field for two seasons (Field Generation Three (FG3) and Field Generation Four (FG4)). Fungicides were used to control fungal diseases except insect vectors in order to facilitate high virus infections and spread. Observations were made on disease incidence, growth performance of the crop and yield parameters of each genotype. From the final harvest in FG4, apparently healthy looking tubers were randomly selected per genotype from each plot, stored in an insect proof diffused light store for two months to sprout and sprouts used to test for presence of

viruses using CIP DAS-ELISA kit. The study revealed low percent emergence, plant heights, number of tubers per hill and total yields which varied among the genotypes in FG4 than FG3 while high disease incidence was recorded in FG4 in comparison to FG3. Three potato viruses; PLRV, PVS and PVY were detected infecting tested potato tubers either as single or as multiple infections. *Potato virus S* (PVS) was most dominant (67%) followed by PVY (17%) and PLRV (2%) respectively while PVA, PVM and PVX were absent in the tested tubers. Four clones; 397073.7, 398190.200, 393371.157 and 392797.22 recorded the highest plant heights ranging from 64cm to 83cm, high number of tubers per hill ranging from 7 to 8 tubers and high yields ranging from 20 to 25 tons/ha in FG4. Use of resistant varieties is a good management strategy with regards to seed borne potato viruses.

Key words: genotypes, natural virus infection, potato seed degeneration, virus tolerance.

3.2 Introduction

Potato (*Solanum tuberosum L.)* is the second most grown food crop in Kenya after maize (Muthoni *et al.,* 2013). The crop plays an important role in maintaining the country's food security as well as poverty alleviation through creation of employment and income generation to both farmers and people working in potato industry (Onditi *et al.,* 2013). The sector is however facing numerous production challenges mainly low yields and poor quality tubers. Average potato yields in Kenya have been reported at 7.7 tons/ha which is much less than the average 40 tons/ha produced in developed countries. This figure has fluctuated in the recent years, from 9.5 to less than 3 tons/ha in 2010 (Muindi *et al*., 2013).

Low yields are due to low soil fertility, infected seed potato tubers, unfavorable climatic conditions during crop growth, pests and diseases (Muthoni and Nyamongo, 2009).

Among pests and diseases, potato viruses have been reported as the major constraint in potato production fields (Muthomi *et al.,* 2009; Schulte-Geldermann *et al.,* 2012). Infection of seed potato tubers with viruses has led to high yield losses by up to 68% in potato fields free from bacterial and fungal diseases (Muindi *et al*., 2013). Yield loss following virus infection is variety specific. Each potato variety reacts with different degree of loss in tuber yield depending on the type of virus, growth stage, type of infection, and period of field exposure to that specific pathogen (Rahman *et al.,* 2010; Ali *et al.,* 2013). Different management options have been proposed to reduce virus infections in potato crops in the fields. These options include use of certified seed potato tubers, positive selection introduced by the International Potato Center (CIP) and aeroponics technology in seed production (Gildemacher *et al*., 2011; Tshisola, 2014; Thomas *et al*., 2015). Additional methods of virus control in potato include use of virus and vector resistant potato varieties and cultural control methods such as mineral oils and borders crops (Fereres, 2000; Dessureault *et al*, 2011; Palukaitis, 2012; Muindi *et al*., 2013). However, adoption of these management strategies especially use of certified seeds and positive selection has been a challenge to most farmers in Kenya (Gildemacher *et al*., 2011; Thomas *et al*., 2015).

Six types of potato resistances to viruses namely resistance to infection (field resistance), resistance to virus accumulation, resistance to virus movement, mature plant resistance, tolerance, and resistance to virus vectors have been reported in recent studies(Palukaitis,

2012). These are further subdivided into two namely; Extreme Resistance (ER) where there is little or no virus accumulation at the infection site as well as reduced movement to non-infected tissues and Hypersensitive Resistance (HR) where a necrotic lesion develops around the infected tissue preventing spread to surrounding tissues (Solomon-Blackburn and Barker, 2001). Extreme Resistance (ER) and Hypersensitive Resistance (HR) genes of major potato viruses such as PVY, PVA, PLRV, PVX, PVS and PVM have been identified and incorporated into different potato lines to help reduce yield loss (Palukaitis, 2012).

Most potato varieties that are grown by small scale farmers in Kenya have demonstrated low levels of resistance and tolerance to potato viruses and other disease causing pathogens due to their genetic inability to withstand physiological disorders caused by these pathogens. Therefore, new varieties which are tolerant to major potato pests and diseases, higher yields, better storability and processing qualities are required in order to reduce seed degeneration problem (Onditi *et al.,* 2012). The aim of this study was to assess the reaction of different potato genotypes to potato virus infection under field conditions.

3.3 Materials and Methods

3.3.1 Description of the study area

The field study was conducted at the Field Station of the University of Nairobi, Upper Kabete campus in two potato growth seasons long rains (March to July 2015) and short rains (October, 2015 to February, 2017). The station is located at an altitude of 1940 m, latitude 1° 15 S and longitude 36° 41’ E, in Lower Highland Zone II (LH2) of the Agro-Ecological Zone (AEZs) of Kenya (Jaetzold *et al.,* 2007). The site has two rainy seasons’

namely long rains between March and May and short rains between October and December per annum. The area receives annual rainfall of 1000 mm and average temperatures of 19°C. Kabete has humic nitisols derived from the Nairobi trachytic lava (Gachene, 1989).

3.3.2 Potato genotypes

Twelve potato genotypes used in this study were sourced from International Potato Center (CIP). These consisted of 7 advanced clones; 398190.200, 300046.22, 393371.157, 393077.159, 392797.22, 398098.65 and 397073.7 developed by CIP and 5 commercial varieties that are widely grown in Kenya namely Asante, Tigoni, Kenya Mpya, Shangi and Sherekea which were used as standard checks. The twelve genotypes were selected based on resistance and susceptibility to potato viruses like PLRV, PVX, and PVY (Table 3.1).

Table 3. 1: **Status of different potato genotypes with regard to major potato viruses**

Genotypes	PLRV	PVX	PVY
Shangi	Not tested	Not tested	Not tested
Asante	Not tested	Not tested	Not tested
Tigoni	Susceptible	Not tested	Susceptible
Sherekea	Resistant	Not tested	Resistant
Kenya Mpya	Not tested	Extreme resistance	Not tested
397073.7	Moderately susceptible	Extreme resistance	Extreme resistance
300046.22	Not tested	Extreme resistance	Resistant
392797.22	Resistant	Resistant	Extreme resistance
398098.65	Not tested	Extreme resistance	Susceptible
393371.157	Susceptible	Resistant	Extreme resistance
393077.159	Highly resistant	Resistant	Susceptible
398190.200	Not tested	Susceptible	Susceptible

Source: International Potato Center (CIP) catalogue of CIP advanced clones (2010) and National Potato Council of Kenya (NCPK), Potato Variety Catalogue (2015)

Certified seed potato tubers of the twelve genotypes produced through aeroponics were multiplied in the field for two seasons (Field Generation 1 (FG1) and Field Generation 2

(FG2). In both FG1 and FG2, selective fungicides were used to control fungal diseases except insect pests. The main aim of FG1 and FG2 was to acclimatize these materials to the climatic conditions of the study area as well as to increase seed volume. Medium (30-60 mm diameter) size and visually healthy looking tubers were selected from FG2 harvest, sprouted under diffused light store for two months and used as seed source for this study.

3.3.3 Set up of the field experiment

Land preparation was done prior to rains in order to achieve fine tilth. Experimental plots were laid down in a Randomized Complete Block Design (RCBD) of four blocks each measuring 33M by 20M with 1M spacing between blocks. Each block was further subdivided into twelve plots each measuring 7.5m by 6m, with spacing of 1M between each plot. In each, plot ten ridges were made at a spacing of 75cm from each other. N.P.K fertilizer blend (16:8:22 + 3MgO + 2S) was applied on the ridges at the rate of 2.53 kg per plot (562.22 Kg/ha) and mixed thoroughly with soil. The above twelve potato genotypes were randomly planted in each of the twelve plots per block and each genotype was replicated four times. Twenty sprouted and visually healthy looking seed potato tubers for each genotype from FG2 were planted manually after the onset of rains on ridges in the respective plots with the sprouts facing upwards at a spacing of 30cm between the tubers. Two hundred tubers were planted per plot making a plant population of 9600 plants for the whole experiment. This translates to 44,400 plants per hectare.

After plant emergence, preventive and curative fungicidal sprays were applied at regular intervals to control fungal diseases and the spray regime was dependent on symptom

appearance in the field. Redomil Gold 68WG, Milraz WP 76, Dithane M-45 and Oshothene 80WP were applied alternately after every fortnight in order to prevent late blight infection and spread in the field. Cultural practices such as weeding and earthing-up were conducted regulalary until plant maturity. During plant growth data was collected on parameters like disease incidence and plant height during crop growth until maturity. At maturity in FG3, dehaulming was done by cutting the above ground biomass in each plot and tubers harvested two weeks later. Medium sized and apparently healthy looking tubers were selected from each genotype and sprouted in insect proof diffused light store for two months. These tubers were used as propagative materials for planting in FG4. These tubers were planted in the field to produce FG4 and the whole cycle repeated as was done in FG3.

3.3.4 Detection of viruses in seed potato tubers from Field Generation Four (FG4)

During the final harvest in FG4, 100 medium size and apparently healthy looking tubers were randomly selected from each genotype per plot and sprouted in an insect proof diffused light store for two months. The sprouted tubers were then used to test for presence of viruses. Due to uneven sprouting among the twelve genotypes, sub-samples of thirty healthy looking tubers per genotype were selected randomly from each stock of sprouted tubers. One sprout was cored out from each tuber using sterilized knives and planted in a tray of sterilized sand medium in a greenhouse. Thirty sprouts of each genotype were planted per tray. As a result of varied emergence rates among the genotypes, five seedlings at three leaf stage were selected randomly from each genotype from which three leaves were sampled per seedling from top, middle and bottom and tested for presence of the six major potato viruses namely PVY, PLRV, PVM, PVA, PVS and PVX using a DAS-ELISA

kit sourced from CIP, Lima Peru. Standard stands followed in the kit during detection followed procedures by Clark and Adams (1977) and revised by CIP (Priou, 2001).

Six buffers were prepared using reagents provided in the kit before detection. The coating buffer was prepared at pH 9.6 by mixing 2 ml of buffer provided in the kit with 8 ml of distilled water. Phosphate Buffer Saline (PBS) was prepared at pH 7.4 by dissolving each packet of the second buffer in the kit in 1000 ml of distilled water. Washing Buffer in tween-twenty was prepared by adding 0.5 ml of buffer 2B (Tween-20) provided in the kit to buffer 2A (PBS PH 7.4) and mixing well using Pasteur pipette. Extraction buffer was prepared by y dissolving one packet of buffer 3 provided in the kit with 10 ml of PBS-Tween for every 100 samples and to the volume adjusted to 200 ml using phosphate buffer saline. The conjugate buffer was prepared by dissolving one packet of buffer 4 provided in the kit with 5 ml of phosphate buffer saline tween and the volume adjusted to 20 ml. The substrate buffer was prepared mixing 2 ml of buffer 5 provided in the kit with 8 ml of distilled water. After buffer preparation, 35µl of antibody specific to each virus was mixed with 10ml of coating buffer to form coating solution which was loaded to plates by adding 100µl of this solution to each well in the plates, labeled, covered with masking tape and incubated at 37^{0}C for 3-4 hours. After incubation, the plates coated with the antibodies were washed three times at three minute intervals using wash buffer. Three leaf samples collected randomly from three leaf stage potato seedlings were weighed and placed in labeled self-lock transparent crushing plastic bags. The extraction buffer was added to each crashing bag by measuring four times the volume (ml) of the sample. The samples plus extraction buffer in each plastic bag were then ground gently by rolling a thick test-tube on

the surface of the plastic bags until they were completely homogenized leading to release of the leaf extract. One hundred microliters of the extract from each leaf samples was added into the wells using a sterile pipette tip each time. The positive and healthy controls were also prepared and 100µl of each added to the last three wells of the plates. The wells were then filled with extraction buffer, sealed and incubated at 4^0C overnight.

After incubation, 35µl of each conjugate antiserum (IgG-AP) was mixed with 10ml of conjugate buffer to form conjugate solution. Then, 90µl of the conjugate solution was added to each well of the plate and incubated at 37^0C for 3-4 hours. After incubation, the plates were washed three times using washing buffer. One substrate tablet provided in the kit was dissolved in 10ml of substrate buffer to form substrate solution and 80µl of this substrate solution was added to each well of the plates. The plates were incubated for 30-60 minutes at room temperature for reaction to occur leading to development of yellow color in samples positive with viruses. The plates were read using an ELISA reader at 405nm and positive samples determined using the formula: $x \geq \bar{x}h$x2,

Where x= Threshold value and $\bar{x}h$=average value of healthy controls.

3.4 Data collection

Data on percentage emergence was collected randomly from the whole plot on a weekly basis from the 30th day after planting for three weeks. Data on virus disease incidence was scored by examining plants showing different virus disease symptoms like leaf roll and or mosaic in each plot. This was done weekly from the eighth week after planting, where data

on disease incidence was scored for four weeks. Disease incidence was determined using the following formula:

Percent disease incidence = (Number of symptomatic plants /Total number of plants) x 100.

At flowering, twenty potato plants were selected randomly from each plot and data on plant height was collected using a string and a tape measure. At harvesting, 40 plants were randomly selected per plot from which data on number of tubers and yield in grams per hill was collected. Data on weather parameters namely; total amount of rain fall, mean temperature and relative humidity were collected daily from the Meteorological Department, in the Faculty of Agriculture Upper Kabete Campus throughout the crop growth period. Threshold values for each of the six viruses were recorded per sample from the ELISA reader and a comparison was made between these values and that of calculated average value of healthy controls. Samples which displayed threshold values equal to or greater than twice the average values of healthy control of each virus were recorded as positive while those with threshold values less than twice the average values of healthy controls were recorded as negative samples for each virus. Virus positive tuber samples were checked for multiples infections.

3.5 Data analysis

All the collected data was analyzed using Genstat 15th version. Fisher's protected Test was used to separate treatment means and Least Significant Differences (LSD) at 5% probability level. Correlation analyses were conducted to establish the relationship between disease incidence, plants height, number of tubers and yield (t/ha) both in FG3 and FG4.

Daily weather data collected for the three parameters; total amount of rain fall, mean temperatures and relative humidity were computed to get monthly averages.

3.6 Results

3.6.1 Emergence of seed potato tubers

Percent sprout emergence varied significantly with genotypes at 30, 37, 44 and 58 days after planting in both seasons. In FG3, Shangi displayed high percentage of emerged seedlings by 90% while Sherekea had the lowest emerged seedlings with 0% at 30 days after planting. At 58 days after planting, Asante had the highest number of emerged plants at 98% while Sherekea had the lowest number of emerged plants at 61% (Table 3.2). In FG4; Asante, Tigoni, and Shangi showed high percent emergence in the range of 78 to 81% while Kenya Mpya, 398098.65, and Sherekea had low numbers of emerged plants in the range of 0 to 7% within 30 days after planting. Fifty-eight days after planting; genotypes 392797.22, 398190.200, Asante and 393371.157 attained high numbers of emerged plants in the range of 92 to 93% while Kenya Mpya, Sherekea and 398098.65 attained the lowest numbers of emerged plants in the range of 50 to 61% respectively (Table3.3).

Table 3. 2: **Percent emergence of potato genotypes at different Days After Planting (DAP) in Field Generation Three (FG3)**

	Percent emergence			
Genotypes	30 DAP	37 DAP	44 DAP	58 DAP
Shangi	89.5 j	92.0 j	94.9 h	96.3 ab
Tigoni	85.2 i	89.1 i	90.5 g	91.7 d
Asante	83.8 h	96.4 k	97.5 i	97.7 a
392797.22	54.8 g	91.3 j	94.5 h	96 .0 ab
393077.159	53.2 f	75.7 g	88.3 f	94.7 bc
300046.22	43.7 e	79.4 h	86.2 e	91.0 d
397073.7	24.5 d	60.5 e	85.3 e	95.3 abc
398190.200	24.3 d	70.2 f	86.5 e	95.3 abc
393371.157	18.2 c	51.2 d	80.7 d	93.3 cd
398098.65	9.2 b	28.8 b	49.5 a	67.3 e
Kenya Mpya	9.0 b	25.0 a	59.7 c	66.3 e
Sherekea	0.0 a	48.3 c	57.4 b	60.7 f
Mean	41.3	67.3	80.9	87.1
CV (%)	1.5	1.0	1.0	1.5
LSD P≤0.05	1.0	1.0	1.2	2.3

Means within the same column having a common letter(s) do not differ significantly at P ≤0.05, LSD=Least Significant Difference, CV (%) =Coefficient of Variation

Table 3. 3: Percent emergence of potato seed tubers at different Days After Planting (DAP) in Field Generation Four (FG4)

	Percent emergence			
Genotypes	30 DAP	37 DAP	44 DAP	58 DAP
Asante	81.0 j	86.1 h	89.2 gh	91. 8 gh
Tigoni	79.4 i	86.1 h	88.0 g	90.3 g
Shangi	78.4 i	820.0 g	85.3 f	87.1 f
392797.22	53.2 h	85.5 h	90.1 h	92.9 h
393077.159	50.0 g	72.3 f	84.5 f	90.5 g
300046.22	40.1 f	73.0 f	78.3 e	81.6 e
397073.7	22.7 e	55.0 d	67.8 d	75.4 d
398190.200	20.7 d	70.0 e	84.8 f	92.0 gh
393371.157	16.9 c	49.8 c	78.6 e	91.7 gh
Kenya Mpya	7.3 b	24.4 a	58.5 c	60.6 c
398098.65	7.0 b	25.3 a	38.7 a	49.6 a
Sherekea	0.0 a	41.7 b	53.3 b	54.8 b
Mean	38.1	62.6	74.8	79.9
CV (%)	2.5	1.8	1.2	1.6
LSD (P≤0.05)	1.4	1.6	1.3	1.9

Means within the same column having a common letter(s) do not differ significantly at P ≤0.05, LSD=Least Significant Difference, CV (%) =Coefficient of Variation

3.6.2 Weather conditions during crop growth

High amounts of rainfall were recorded during FG4 (909.1mm) (Appendix 2) and low in FG3 (760.7mm) (Appendix 1). Rainfall distribution also varied between the periods of crop growth with high rainfall amounts during the first month of growth which declined in the last months of growth in both seasons. Mean temperatures ranged between 19°C to 22°C in FG3 (Appendix 1) and 21°C to 22°C in FG4 (Appendix 2). Relative humidity ranged between 56 to 76% in FG3 (Appendix 1) and 63 to 75% in FG4 (Appendix 2).

3.6.3 Disease incidence

Disease incidence varied significantly among the two cropping seasons ranging from 8% to 85% in FG3 (Table 3.4) and from 26% to 88% in FG4 (Table 3.5). Disease incidence differed significantly at ($P \leq 0.05$) among seasons. All genotypes expressed varied levels of susceptibility to viruses based on percentage disease incidence in the two seasons. Disease incidence increased in FG4 and this varied among genotypes with Shangi showing the highest increase by 37% and 393077.159 showing the least increase by 3 % (Figure 3.2).

Table 3. 4: Percent virus disease incidences at different Weeks After Planting (WAP) in Field Generation Three (FG3)

	Percent disease incidence			
Genotypes	8 weeks	9 weeks	10 weeks	11 weeks
Asante	46.5 f	69.2 h	73.5 i	77.7 h
393077.159	30.0 e	45.9 g	78.8 j	84.85 i
398098.65	10.9 d	24.1 f	50.9 h	73.8 g
Tigoni	9.2 d	18.2 e	47.0 g	53.5 f
Sherekea	7.0 c	11.4 d	18.4 f	23.2 e
393371.157	6.5 c	10.4 cd	13.1 d	15.6 c
Kenya Mpya	5.1 bc	9.6 c	15.3 e	20.5 d
Shangi	4.5 b	10.0 c	10.7 c	11.6 b
300046.22	4.0 ab	7.3 b	15.2 e	24.8 e
392797.22	2.6 a	4.2 a	6.9 a	8.2 a
397073.7	2.4 a	6.1 b	9.1 b	11.4 b
398190.200	2.2 a	4.2 a	7.7 a	8.3 a
Mean	10.9	18.4	28.9	34.5
CV (%)	11.6	4.8	3	3.2
LSD ($P \leq 0.05$)	1.8	1.3	1.2	1.9

Means within the same column having a common letter(s) do not differ significantly at $P \leq 0.05$, LSD=Least Significant Difference, CV (%) =Coefficient of Variation

Table 3. 5: Percent virus disease incidences at different weeks after planting (WAP) in Field Generation Four (FG4)

Genotypes	Percent disease incidence			
	8 weeks	9 weeks	10 weeks	11 weeks
Asante	53.3 j	71.7 h	77.2 j	81.8 j
393077.159	49.5 e	70.4 h	81.7 k	87.5 k
Tigoni	28.8 h	49.7 g	63.4 h	70.7 h
398098.65	23.7 g	44.7 f	65.9 i	79.7 i
300046.22	17.7 f	29.7 e	49.0 g	55.2 g
Kenya Mpya	13.9 e	21.1 c	33.7 d	44.9 e
393371.157	12.5 d	22.9 d	30.6 c	37.0 c
Shangi	10.4 c	24.4 d	39.8 f	48.5 f
Sherekea	9.7 bc	18.5 b	27.8 b	39.8 d
398190.200	9.5 bc	28.4 e	36.6 e	45.3 e
392797.22	8.5 b	19.2 b	27.9 b	35.7 b
397073.7	7.0 a	13.3 a	19.9 a	26.3 a
Mean	20.4	34.5	46.1	54.4
CV (%)	4.7	3.4	2.8	1.6
LSD ($P \leq 0.05$)	1.4	1.7	1.9	1.3

Means within the same column having a common letter(s) do not differ significantly at $P \leq 0.05$, LSD=Least Significant Difference, CV (%) =Coefficient of Variation

3.6.4 Plant height

Plant height varied significantly depending on genotypes ranging from 40cm to 107cm in FG3 and 37 to 83cm in FG4. In both seasons, plant height differed significantly at ($P \leq 0.05$). Decline in plant height was observed in FG4 and the average percent decrease varied among the genotypes with Shangi showing the highest decrease at 27 % while Asante and 397073.7 recorded the least decrease at 4%. The remaining genotypes displayed a decline in plant heights ranging from 5% to 12 % (Table 3.6).

Table 3. 6: Plant height (cm) of different potato genotypes in Field Generation Three (FG3) and Field Generation Four (FG4) and percent decrease in FG4

Genotypes	FG3	FG4	Percent decrease
Shangi	106.6 g	78.1 c	26.8
398190.200	91.3 f	83.4 a	8.7
393371.157	86.4 f	81.1 b	6.2
Tigoni	75.9 e	71.1 d	6.4
392797.22	72.8 de	64.1 e	11.9
397073.7	66.6 cd	64.3e	3.5
393077.159	61.3 bc	56.8 g	7.3
Asante	60.7 bc	58.6 f	3.6
Sherekea	55.7 b	53.1 h	4.7
398098.65	46.2 a	41.4 i	10.3
300046.22	44.5 a	40.9 i	8.0
Kenya Mpya	40.4 a	36.6 j	9.4
Mean	67.4	60.8	
CV (%)	5.4	1.9	
LSD (P≤ 0.05)	6.2	1.6	

Means within the same column having a common letter(s) do not differ significantly at P≤0.05, LSD=Least Significant Difference, CV (%) =Coefficient of Variation

3.6.5 Number of tubers per hill

High numbers of tubers per hill were recorded in FG3 ranging from 4 to 13 compared to FG4 in which numbers per hill ranged from 3 to 10 and differed significantly at (P ≤ 0.05) between the two seasons. High numbers of tubers were recorded in Shangi, Tigoni, 397073.7 and 392797.22 ranging from 10 to 13 tubers per hill and the low in 393077.159, 398098.65 and Kenya Mpya ranging from 4 to 6 tubers per hill in FG3. In FG4, high numbers of tubers were recorded in Shangi, 392797.22 and Asante ranging from by 7 to 10 tubers per hill and the lowest in 398098.65 and Kenya Mpya with 3 tubers per hill. Reduction in number of tubers was observed in all the twelve genotype in FG4. Percentage

decline in number of tubers varied among the genotypes. Highest levels of decline were recorded in 397073.7 by 47% and lowest in 393077.159 by 3% (Table 3.7).

Table 3. 7: Number of potato tubers per hill of different genotypes in Field Generation Three (FG3) and Field Generation Four (FG4) and percent decrease in FG4

Genotypes	FG3	FG4	Percent decrease
Shangi	12.6 a	9.8 a	22.6
Tigoni	10.8 ab	5.8 de	46.5
397073.7	10.5 ab	7.0 bc	33.8
392797.22	10.3 bc	7.5 b	26.6
398190.200	9.0 bc	6.6 bcd	27.1
Sherekea	8.3 bc	5.1 e	39.1
300046.22	8.3 bc	5.1 e	42.9
Asante	7.8 bcd	7.5 b	4.0
393371.157	7.4 bcd	7.0 bc	5.9
393077.159	6.2 cde	6.0 cd	2.7
398098.65	4.7 de	3.4 f	27.1
Kenya Mpya	3.8 e	3.2 f	14.9
Mean	8.3	6.2	
CV (%)	22	10.8	
LSD (P≤ 0.05)	3.1	1.0	

Means within the same column having a common letter(s) do not differ significantly at (P≤0.05), LSD=Least Significant Difference, CV (%) =Coefficient of Variation

3.6.6 Total Yield

High yields were recorded in FG3 ranging from 10-49 t/ha compared to FG4in which yied ranged 5-25 t/ha. Yield differed significantly at P ≤0.05 across the genotypes in both seasons. In FG3, 392797.22 had the highest yield at 49.2 t/ha and 398098.65 the least at 10.2 t/ha. In FG4; 392797.22 had the highest yield of 25.0 t/ha and Sherekea the least yield of 5 t/ha. All the genotypes displayed low yields in FG4. The yield drop varied between the

genotypes with each showing different percentage decrease. Sherekea had highest decrease in yield with 68% while 398098.65 displayed the lowest by 48% decrease (Table 3.8).

Table 3. 8: Yield (t/ha) of potato genotypes recorded in Field Generation Three (FG3) and Field Generation Four (FG4) and percent decrease in FG4

Genotypes	FG3	FG4	Percent decrease
392797.22	49.2 a	25.0 a	49.09
398190.200	46.7 ab	19.8 c	57.56
397073.7	39.1 bc	18.7 d	52.27
393371.157	37.2 bc	22.2 b	40.35
Shangi	34.5 cd	14.4 f	58.43
Asante	33.1 cd	17.5 e	47.31
Tigoni	30.3 cd	11.8 i	61.09
300046.22	29.0 cd	12.9 h	55.34
393077.159	25.6 d	13.2 g	48.28
Sherekea	15.9 e	5.0 l	68.22
Kenya Mpya	11.1 e	5.6 k	49.34
398098.65	10.2 e	6.3 j	38.04
Mean	30.2	14.4	
CV (%)	18.6	1.1	
LSD ($P \leq 0.05$)	5.5	0.2	

Means within the same column having a common letter(s) do not differ significantly at $P \leq 0.05$, LSD=Least Significant Difference, CV (%) =Coefficient of Variation

3.6.7 Correlation among disease incidence, plant height, number of tubers per hill and yield in FG3 and FG4

Disease incidence displayed week negative correlation with plant height, number of tubers and yield ($r = -0.38$, $r = -0.38$ and $r = -0.42$ at $P \leq 0.05$), plant heights correlated positively to number of tubers and yield ($r = 0.71$ and $r = 0.64$ at $P \leq 0.05$) and number of tubers correlated positively to yield ($r = 0.51$ at $P \leq 0.05$) in FG3 (Table 3.10). Disease incidence correlated negatively to plant height, number of tubers and yield ($r = -0.28$, $r = -0.20$ and $r = -0.33$ at $P \leq 0.05$), plant heights correlated positively to number of tubers and yield (r =

0.72 and r = 0.67 at P ≤ 0.05) and number of tubers per hill correlated positively to yield in grams (r = 0.66 at P ≤ 0.05) in FG4 (Table 3.9).

Table 3. 9: Correlation coefficients among disease incidence, plant height, number of tubers per hill and total yield of different potato genotypes in FG3 and FG4

	Field Generation 3 (FG3)			
	Disease incidence	Plant heights	Number of tubers	Yield
Disease incidence	-			
Plant heights	-0.38*	-		
Number of tubers	-0.38*	0.71**	-	
Yield	-0.42*	0.64**	0.51**	-
	Field Generation4 (FG4)			
Disease incidence	-			
Plant heights	-0.28*	-		
Number of tubers	-0.20*	0.72**	-	
Yield	-0.33*	0.67**	0.66**	-

Coefficients denoted by * indicate negative correlations while coefficients denoted by ** indicate positive correlation at p≤0.05.

3.6.8 Virus infection status of seed tubers from Field Generation Four (FG4)

Results of tested tubers revealed PVS as the most dominant virus (67%) followed by PVY (20%), PLRV (12%) and PVM (7%). PVA and PVX were not found in the tested tubers. All potato genotypes tested positive for PVS; four genotypes for PVY and PLRV while only two genotypes tested positive for PVM. Two genotypes showed double infections by PVS + PVY, one genotype by PVS + PLRV, one genotype by PVM + PVS; one genotype showed triple infection by PLRV + PVM + PVS and finally two genotypes by PLRV + PVS + PVY in tested tubers (Table 3.10).

Table 3. 10: Incidences of potato viruses detected in seed tubers from Field Generation Four (FG4)

	Percent ELISA-Positive samples					
Genotype	PLRV	PVA	PVM	PVS	PVX	PVY
Shangi	0	0	0	60	0	0
Asante	0	0	0	40	0	0
Tigoni	20	0	40	100	0	0
Sherekea	0	0	0	20	0	100
Kenya Mpya	0	0	0	60	0	60
397073.7	0	0	40	40	0	0
300046.22	20	0	0	80	0	60
392797.22	80	0	0	20	0	20
398098.65	0	0	0	80	0	0
393371.157	0	0	0	100	0	0
393077.159	20	0	0	100	0	0
398190.200	0	0	0	100	0	0
Percent incidence (%)	11.67	0	6.67	66.67	0	20.0

0-no detection, PLRV-*Potato Leaf Roll Virus*, PVA-*Potato Virus A*, PVM-*Potato Virus M*, PVS-*Potato Virus S*, PVX- *Potato Virus X*, PVY-*Potato Virus Y*

3.7 Discussion

Percent plant emergence was low in FG4 compared to FG3. This may be attributed to infections of the seed potato tubers by seed borne viruses in the field during seed multiplication process in FG1 and FG2 in addition to fresh infections during FG3 and FG4 growth periods (Hutton *et al.,* 2015). Singh *et al*., (2012) also reported decline in percent plant emergence between seasons. The observed decline varied among the twelve potato genotypes among which 397073.7 showed the highest percent decline and Tigoni the least. This phenomenon may have occurred as a result of variation in tolerance of potato genotypes to virus infections in production fields (Ali *et al*., 2013). Recent studies have also documented a varied decline in plant emergence among different potato varieties in

experiment due to seed degeneration. In comparison to commercial varieties, clones like 393077.159, 398190.200, 392797.22 and 393371.157 had the lowest levels of percent decrease in plant emergence signifying high tolerance of these genotypes to potato viruses.

Variation in duration to attain maximum emergence also varied among the twelve genotypes in both seasons. This may have resulted from several factors like difference in dormancy periods among the genotypes which is dependent on cultivar, tuber ripening, growth conditions, storage conditions and size of tubers used in propagation (Lommen 1994; Germchi *et al*., 2011; Farshid *et al*., 2014). All the commercial varieties used in the study namely Asante, Tigoni, Kenya Mpya and Shangi are reported to have short dormancy under diffused light storage except Sherekea which has long dormancy ranging between four to five months (NPCK, 2015) while CIP clones have different dormancy periods under diffused light storage; 397073.7 at112 days, 398098.65 not reported, 300046.22 at 74 days, 393077.159 at 90 to ≥ 120 days, 398190.200 not reported, 392797.22 at 109 days and 393371.157 at 90 to ≥ 120 days (CIP, 2010).

High virus disease incidence which varied among the twelve genotypes was recorded in FG4. High virus incidences in FG4 might have occurred due to absence of insect vector (aphid) control strategies in the experimental field (Kabira *et al*., 2006) and abiotic factors like water stress (Batool *et al.,* 2011). Field Generation Four (FG4) was a short rain season with low rainfall amounts which could have led to drought stress and high insect vector populations predisposing the crop to high virus infections (Muthomi et al., 2009). In both seasons, disease incidence increased weekly and differed significantly at $P \leq 0.05$. All the

twelve genotypes expressed varied levels of susceptibility to potato viruses based on percentage disease incidence in the two growth seasons. This variation may have resulted from difference in levels of resistance of potato genotypes to infection by prevalent viruses in the field (Solomon-Blackburn and Barker, 2001). Ali *et al.* (2013) and Islam et al., (2014) also revealed varied virus disease incidences in different potato varieties. Similar results were reported by (Muthomi *et al*., 2009; Rahman *et al*., 2010; Ali *et al*., 2013). The increase in disease incidence was genotype dependent and was high in Shangi and 398190.200 and least in 393077.159 in FG4. Among the commercial varieties, Sherekea is reported to be resistant to PLRV and PVY while Kenya Mpya has extreme resistance to PVX (NPCK, 2015). Among the seven clones used in the study, 392797.22 and 393077.159 are reported to be resistant to PLRV, all the seven clones except 398190.200 are resistant PVX while 397073.7, 30046.22, 392797.22 and 393077.159 are resistant to PVY (CIP, 2010). Genotypes 393077.159, Asante and 398098.65 which showed high disease incidences in FG3, had low percent increase in disease incidence in FG4. This may be because these genotypes had almost reached their optimal virus infection levels in FG3 compared to other genotypes used in the study.

Plant heights in all the twelve potato genotypes were significantly high in FG3 compared to FG4. Low plant height in FG4 may have been as a result of high disease incidences recorded in FG4 and due to seed potato tuber infections in previous seasons (Rahman *et al*., 2010). Similarly, Salazar (1996) and Kumar, (2010) also documented dwarfism and stunted growth as the major symptoms of potato virus infections in production fields and the results of this study also showed similar growth habits. Rahman *et al*., (2010) and Islam *et al*.,

(2014) also reported decrease in plant heights due to *Potato Leaf Roll Virus* (PLRV) and *Potato Virus Y* infections in their experiments.

Decline in plant heights varied between the twelve genotypes with Shangi showing the highest percent decline and 397073.7 the least percent decline in FG4. This variation in decline of plant height observed in different genotypes was also reported in studies by (Hossain, 1999) and Islam *et al*. (2014) who revealed that there was varied reductions of plant heights in different varieties due to PVY infections. The variability in decline of plants heights can be attributed to difference in tolerance of these genotypes to infection by predominant potato viruses in the field (Islam *et al*., 2014; Hasan *et al*., 2015).

Low number of tubers per hill was recorded in FG4 compared to FG3 in all the genotypes. However, these phenomena varied among the twelve genotypes suggesting that different genotypes possess different resistance and or tolerance levels to potato viruses when exposed to natural virus infection in the field (Islam *et al*., 2014; Hasan *et al*., 2015). This variation can be attributed to difference in mechanisms supporting virus particle proliferation within the plant tissues of each genotype (Salazar, 1996). Also the decline in tuber numbers might have occurred due to increase in disease incidence observed in FG4 (Ali *et al*., 2013). John *et al*., (2013) and Islam *et al*., (2014) also reported that different potato varieties displayed varied decline in number of tubers per hill due to infection by different potato viruses. In comparison to commercial varieties used in the study, genotypes 398190.200, 397073.7, 393371.157 and 392797.22 had high average number of tubers per hill in FG4 signifying high tolerance levels to natural virus infections in the field.

Low yields were observed in FG4 compared to FG3. These low yields in FG4 can be attributed to increase in disease incidence recorded in FG4 (Rahman *et al*., 2010; Ali *et al*., 2013; Islam *et al*., 2014). Salazar (1996) reported that yield loss in potato fields increases with increasing symptom appearance on the foliage which was also observed in this study. Low yields might also have occurred as a result of low soil moisture availability during and after tuber initiation in FG4 (short rains). Potatoes are highly sensitive to water stress between plant emergence and flowering and any alteration in optimal moisture availability in the soil during this growth period can lead to low number of tubers resulting to low yield (O'brien *et al,* 1998; NPCK, 2013). In addition, water stress during vegetative growth may lead to reduced leaf area and plant heights resulting to low photosynthetic products stored in the tubers hence low yields (Alva, 2008). FG4 was a short rain season which received low rainfall amounts compared to FG3 (long rain season) and this could have resulted to drought stress predisposing the crop to high vector infestation and virus infection leading low yields. These findings were also reported by (Hane *et al*., 1999; Rahman *et al*., 2010; Ali *et al*., 2013; John *et al.,* 2013; Islam *et al*., 2014) in their experiments.

Yield varied among the twelve genotypes in both FG3 and FG4. In FG3, 392797.22 displayed the highest yield and 398098.65 displayed the lowest yield. In FG4, 392797.22 displayed the highest yield while Sherekea had the lowest yield. This variation in yield might have occurred as a result of difference in levels of susceptibility (Ali *et al*., 2013; Islam *et al.,* 2014) and resistance to potato viruses in the field. However, yield decline which varied among the twelve genotypes was observed in FG4. This variation in yield loss among the twelve genotypes can be attributed to differences in genotypes inherent reactions

to virus infections at field conditions (Salazar, 1996). Genotypes 392797.22 393371.157, 398190.200 and 397073.7 displayed high yields in FG4 signifying high levels of tolerance to natural potato virus infections in the field when compared to other genotypes.

Disease incidence displayed weak negative correlation to plant height, number of tubers and yield in grams per hill in both FG3 and FG4. The weak correlation can be an indication that reduction in growth and yield parameters might also have occurred as a result of other biotic and a biotic factors such as water stress during crop growth and insect pests infestation (Pereira and Nova 2008; Batool *et al.,* 2011). Rahman *et al*., (2010) and Islam *et al.*, (2014) also reported that increase in potato virus disease incidences resulted in reduction of plant height, number of tubers and yield.

Plant height, number of tubers per hill, and yield demonstrated strong positive correlations in both seasons which were also reported by Tuncturk *et al*., (2005) and Yousif *et al*., (2015). In their study, plant height, leaf number per plant, leaf area, dry weight, tubers number, tubers weight, and potato tuber yields displayed positive and significant correlations. Increased potato plant heights result to increase in foliage as well as photosynthetic products that lead to increased tuber numbers, size and yield. It can be assumed that any of these parameters can be used to carry out an investigative study on both abiotic and biotic factors affecting growth and yield performance of potatoes.

Four potato viruses namely PLRV, PVM, PVS and PVY were found to infect tested seed potato tubers collected from FG4 either as single infection or as multiple infections. Potato Virus S was the most predominant followed by PVY, PLRV and PVM. Were *et al*., (2013)

also reported PVS as the most detected potato viral disease followed by PVY, PVX, and PLRV in samples collected from potato growing districts in Kenya. Similarly, Yardimci *et al.* (2015) also reported PVS and PVY as the most prevalent potato viruses in potato growing areas in Turkey when ELISA tests were conducted on tubers. In addition, Yardimci *et al.,* (2015) reported PVY+PVS (9.17%) as one of the most common multiple infections in potato (tubers) as revealed in this study. ELISA results revealed that all the twelve genotypes were infected by PVS. This may be attributed to ability of the virus and probably other viruses to pass through tissue culture process which is usually adopted in production of certified seeds (Were *et al.,* 2013) or because PVS is mainly transmitted mechanically, it can easily be spread by farmers during cultural activities (Gul *et al.,* 2013).

Low incidences of PLVR and PVY as well as zero detection of PVM, PVA and PVX in RSS tuber samples may be attributed to restricted spread of these viruses, due to low numbers or absence of insect vectors and or absence of alternate hosts in the experimental site (Djilani-Khouadja *et al.,* 2010). Similarly, Njukeng *et al.*, (2013) reported that some viruses especially PLRV, are more prevalent on leaves than tubers and this could also explain why some of these viruses were not detected in the tubers. The situation may also have occurred as a result of differences in levels of susceptibility of these genotypes to different potato viruses in the experimental field (Wróbel, 2015).

Potato genotypes reacted differently to natural virus infections in the field. Some new clones like 392797.22 393371.157, 398190.200 and 397073.7 had high levels of tolerance to virus infection compared to commercial cultivars. Even though these genotypes had

significantly high yields, there was a decline in the total yield in FG4 suggesting seed degeneration due to accumulation of viruses in tubers. Varieties that are resistant to potato viruses should officially be released to farmers so as to curb seed degeneration arising from recycling of seed tubers across seasons. Farmers should also be sensitized on the importance of using potato varieties that are resistant to viruses so as to maximize yield.

CHAPTER FOUR

EFFICIENCY OF POSITIVE SELECTION IN MANAGEMENT OF SEED BORNE VIRUSES AND YIELD OF POTATO

4.1 Abstract

Most potato farmers in Kenya recycle seed tubers with latent infections by disease pathogens especially viruses resulting in low yields. A field study was conducted with the aim of assessing efficiency of positive selection in management of seed borne potato viruses. Sprouted seed potato tubers from Field Generation Two (FG2) sourced from international potato center (CIP) were subjected to natural virus infection in the field for two seasons (Field Generation Three (FG3) and Field Generation Four (FG4)). Ten weeks after planting, apparently healthy looking plants were selected and pegged in each plot. The plots were inspected weekly and pegs removed from plants with newly developed disease symptoms until maturity. At maturity, pegged plants were harvested separately, medium (30-60mm diameter) size and apparently healthy looking tubers selected, sprouted and used as propagative materials in Positive Selection (PS) plots while non-pegged plants provided seed tubers for Random Seed Selection (RSS) plots. Observations were made on disease incidence, growth performance and yield parameters of each genotype. Positive selection reduced disease incidence by 3 to 10%, increased plant height by 1 to 14%, number of tubers by 9 to 41% and yield by 4 to 56% depending on genotypes. ELISA tests revealed *Potato virus S* (PVS) as the most predominant virus followed by *Potato virus Y* (PVY) and *Potato Leaf Roll Virus* (PLRV) in both Random Seed Selection (RSS) and Positive Selection (PS) plots either as single or multiple infections. *Potato virus M* (PVM) was only

detected in tested tubers from RSS plots. Use of positive selection presented good management strategy with regards to seed borne potato viruses. Farmers should therefore be sensitized on the importance of PS and incorporate it in their management practices to help reduce seed potato degeneration resulting from tuber borne viruses.

Key words: Random Seed Selection (RSS), positive selection (PS), latent infection.

4.2 Introduction

Potato production is affected by many pests and diseases among which viruses are the most devastating and difficult to control (Salazar, 1996). More than 40 viruses are known to naturally infect potatoes in production fields among which PLRV, PVY, PVA, PVX, PVM and PVS are the major ones affecting yield either as single and or through multiple infections (Yardimici *et al.,* 2015). These viruses can be transmitted mechanically through physical contact with an infected plant, machinery and animals or by nematodes, fungi and insect vectors depending on the specific virus as well as through infected seed potato tubers selected from previous growth seasons (Yardimici *et al.,* 2015; Hutton *et al.,* 2015). Most potato viral diseases can often be diagnosed by visual symptoms such as mosaic patterns on leaves, stunted growth, and tuber malformations among others. However, some viruses such as PVS and PVX might have latent infections due to growth conditions in the field and stage of plant growth at the time of infection (Yardimici *et al.,* 2015).

Virus diseases unlike fungi and most bacteria cannot be managed by use of chemical pesticides and therefore a feasible way of managing them can only be achieved through good quality seed production and selection (Njukeng *et al*, 2013). Infection by viruses can

cause up to 68% yield loss in the field because their diagnosis is difficult and or poorly understood by most potato farmers (Kabira *et al.,* 2006). Several potato virus management strategies including use of certified seed potato tubers (Thomas-Sharma *et al*., 2016) positive selection (Gildemacher *et al*., 2011) and aeroponics in seed production (Tshisola, 2014) have been proposed to reduce virus infections in potato crops. Cultural control methods such as mineral oils and borders crops have also been effective in virus control in the field (Fereres, 2000; Muindi *et al*., 2013; Dessureault *et al*, 2011). However, adoption of these management strategies like use of certified seeds and positive selection in potato production fields is a challenge to most farmers due to high prices of certified seeds and farmer's lack of knowledge on application of positive selection (Gildemacher *et al*., 2011; Thomas-Sharma *et al*., 2016).

Positive selection technique was introduced jointly by CIP and KALRO (Gildemacher *et al*., 2011). It's being advocated for adoption by farmers who are unable to access certified seed potato tubers for propagation. However, it is difficult to identify virus free seed-tubers selected using this method especially for tubers with latent infections. These viruses can be passed across generations through seed tubers leading to potato seed degeneration. The study therefore aimed at assessing efficiency of positive selection on the health of seed potato tubers with regard to potato viruses.

4.3 Materials and Methods

Sections 4.3.1 and 4.3.2 are described in Chapter 3 section 3.3.1 and 3.3.2. Page 23-24.

4.3.3 Set of the ffield experiment

Land preparation in FG3 was done at the onset of rains in order to achieve fine a tilth. Experimental plots were laid down in a Randomized Complete Block Design (RCBD) with four blocks each measuring 33M by 20M with 1M spacing between blocks. Each block was further subdivided into twelve plots each measuring 7.5M by 6M with spacing of 1M between each plot. Ten ridges were made in each plot at a spacing of 75cm apart. At planting, N.P.K fertilizer blend (16:8:22 + 3MgO + 2S) was applied on the ridges at the rate of 2.53 kg per plot (562.22 Kg/ha) and mixed thoroughly with soil. The above twelve potato genotypes were allotted to each of the twelve plots per block and each genotype was replicated four times. Twenty sprouted and visually healthy looking seed potato tubers for each genotype from FG2 were planted manually on ridges in the respective plots with the sprouts facing upwards at a spacing of 30cm between the tubers. Two hundred tubers were planted per plot making a plant population of 9600 plants for the whole experiment. Planting was done at the onset of rain and the tubers were allowed to emerge. After emergence, preventive and curative fungicidal sprays were applied at regular intervals to control fungal diseases and the spray regime was dependent on prevailing weather conditions and symptom appearance in the field. Redomil Gold 68WG, Milraz WP 76, Dithane M-45 and Oshothene 80WP were applied alternately after every fortnight in order to prevent late blight infection and spread in the field. Intercultural practices such as weed control and earthing-up were conducted as recommended. Ten weeks after planting, healthy looking plants were selected and pegged in each plot. The plots were inspected every week and pegs removed from plants with newly developed disease symptoms. This activity was

done until plants began to show senescence symptoms towards physiological maturity. At maturity, pegged plants were harvested separately, medium (30-60 mm in diameter) size and visually healthy looking tubers selected, sprouted in an insect proof diffused light store for two months and used as propagative materials for Positive Selection (PS) while non-pegged plants provided seed tubers used as Random Seed Selection (RSS) in FG4.

In FG4, the experimental plots was ploughed to fine tilth and experimental plots laid down in a Randomized Complete Block Design (RCBD) of four blocks each measuring 33M by 20M with 1M spacing between blocks. Each block was further divided into twelve plots each measuring 7.5M by 6M with spacing of 1M between each plot. Each plot in the experimental field was subdivided into two equal portions. One hundred seed tubers obtained through PS were planted in one half of the plot and the other half planted with one hundred seed tubers sourced from RSS of FG3 harvest. All agronomic practices were conducted according to good potato production requirements except insect vector control to enhance high rates of virus transmission in the field. Data was collected on different parameters such as disease incidence and plant height during crop growth until maturity. At maturity data was collected on number of tubers and yield in grams per hill. The yield was computed to tons per hectare.

4.3.4 Detection of viruses in seed potato tubers

During the final harvest in FG4, 100 medium size and apparently healthy looking tubers were randomly selected separately from each genotype from both RSS and PS plots, stored in an insect proof diffused light store for two months to sprout and sprouted tubers used to

test for presence of viruses. Due to uneven sprouting among the twelve genotypes, subsamples of thirty tubers per genotype were selected from each stock of the sprouted tubers in both RSS and PS samples.

One sprout was cored out from each tuber using sterilized knives and planted in a tray of sterilized sand medium in the greenhouse. Thirty sprouts of each genotype were planted per tray and two different trays were used for each genotype; one for samples from RSS and the other for samples from PS plots. As a result of varied emergence rates among the genotypes, five seedlings at three leaf stage were selected randomly from each genotype from which three leaves were sampled per seedling from top, middle and bottom and tested for presence of the six major potato viruses namely PVY, PLRV, PVM, PVA, PVS and PVX using a DAS-ELISA kit sourced from International Potato Center, Lima Peru. Standard stands followed in the kit during detection followed procedures by Clark and Adams (1977) and revised by CIP (Priou, 2001).

Six buffers were prepared using reagents provided in the kit before detection. The coating buffer was prepared at pH 9.6 by mixing 2 ml of buffer provided in the kit with 8 ml of distilled water. Phosphate Buffer Saline (PBS) was prepared at pH 7.4 by dissolving each packet of the second buffer in the kit in 1000 ml of distilled water. Washing Buffer in tween-twenty was prepared by adding 0.5 ml of buffer 2B (Tween-20) provided in the kit to buffer 2A (PBS PH 7.4) and mixing well using Pasteur pipette. Extraction buffer was prepared by y dissolving one packet of buffer 3 provided in the kit with 10 ml of PBS-Tween for every 100 samples and to the volume adjusted to 200 ml using phosphate buffer

saline. The conjugate buffer was prepared by dissolving one packet of buffer 4 provided in the kit with 5 ml of phosphate buffer saline tween and the volume adjusted to 20 ml. The substrate buffer was prepared mixing 2 ml of buffer 5 provided in the kit with 8 ml of distilled water. After buffer preparation, 35µl of antibody specific to each virus was mixed with 10ml of coating buffer to form coating solution which was loaded to plates by adding 100µl of this solution to each well in the plates, labeled, covered with masking tape and incubated at 37^0C for 3-4 hours. After incubation, the plates coated with the antibodies were washed three times at three minute intervals using wash buffer. Three leaf samples collected randomly from three leaf stage potato seedlings were weighed and placed in labeled self-lock transparent crushing plastic bags. The extraction buffer was added to each crashing bag by measuring four times the volume (ml) of the sample. The samples plus extraction buffer in each plastic bag were then ground gently by rolling a thick test-tube on the surface of the plastic bags until they were completely homogenized leading to release of the leaf extract. One hundred microliters of the extract from each leaf samples was added into the wells using a sterile pipette tip each time. The positive and healthy controls were also prepared and 100µl of each added to the last three wells of the plates. The wells were then filled with extraction buffer, sealed and incubated at 4^0C overnight.

After incubation, 35µl of each conjugate antiserum (IgG-AP) was mixed with 10ml of conjugate buffer to form conjugate solution. Then, 90µl of the conjugate solution was added to each well of the plate and incubated at 37^0C for 3-4 hours. After incubation, the plates were washed three times using washing buffer. One substrate tablet provided in the kit was dissolved in 10ml of substrate buffer to form substrate solution and 80µl of this

substrate solution was added to each well of the plates. The plates were incubated for 30-60 minutes at room temperature for reaction to occur leading to development of yellow color in samples positive with viruses. The plates were read using an ELISA reader at 405nm and positive samples determined using the formula: $x \geq \bar{x}h$x2,

Where x= Threshold value and $\bar{x}h$=average value of healthy controls.

4.4 Data collection

Data on visual potato virus incidences was scored by examining plants showing different virus disease symptoms like leaf rolling, stunted growth, yellowing and leaf mosaic symptoms in both RSS and PS plots. This was done weekly from the eighth week after planting where data on disease incidence was scored for four weeks and the incidence calculated using the formulae shown below:

Percent disease incidence = (Number of symptomatic plants /Total number of plants) x 100.

At flowering (11 weeks after planting), twenty potato plants were selected randomly within each PS and RSS plots and data on plant height were collected using a string and a tape measure. At harvest, 40 plants were selected randomly from each PS and RSS plots and data on number of tubers and yield in grams per hill was collected. Data on weather parameters namely total rain fall, mean temperature and relative humidity were collected daily from the Meteorology Department in the Faculty of Agriculture, Upper Kabete Campus throughout the crop growth period.

Threshold values for each of the six viruses were recorded for each sample from the ELISA reader and a comparison was made between these values and that of calculated average

value of healthy controls as outlined in the kit. Samples which displayed threshold values equal to or greater than twice the average value of healthy control of each virus were recorded as positive while those with threshold values less than twice the average value of healthy controls were recorded as negative samples for each virus. Samples which tested positive for different potato viruses were checked for multiples infections.

4.5 Data analysis

All the data collected from the field were analyzed using Genstat 15th Edition. Fishers' protected Test was used to separate treatment means at 5% Least Significant Difference (LSD) probability level. Standard deviation from the means was calculated per parameter in each genotype using Microsoft excel 2010. T-Test; paired two means was also used to analyze means of each parameter per variety from both RSS and PS plots using Microsoft excel 2010 at 5% probability level. Daily weather data collected for the three parameters namely total amount of rainfall, mean temperature and relative humidity were computed to monthly averages for the two potato growth seasons.

4.6 Results

Section 4.6.1 is described in chapter 3 section 3.6.2. Page 31.

4.6.2 Effects of positive selection on disease incidence

Genotypes had significant differences on virus disease incidence both in PS and RSS. Low disease incidences were recorded in PS plots ranging from 24% to 85% compared to RSS plots where disease incidence ranged from 26% to 88% (Table 4.1). Disease incidence differed significantly at ($P \leq 0.05$) between RSS and PS plots. In both the plots, genotype

393077.159 had the highest disease incidence while 397073.7 had the least. All the twelve genotypes displayed a varied response to PS with regards to disease incidence. Genotype 397073.7 showed the highest response by 10% and 393077.159 the least by 3%.

Table 4. 1: Effect of PS and RSS on disease incidence of different potato genotypes and percent decrease in disease incidence from positive selection

	Disease incidence				Reduced (DI)	
Genotypes	PS	Std. Dev.	RSS	Std. Dev.	(%)	p (1 tailed)
393077.159	85.1 a	0.34	87.5 a	1.01	2.8	0.009
Asante	79.4 b	1.26	81.8 b	0.65	3.0	0.03
398098.65	76.6 c	0.98	79.7 c	0.94	4.0	0.014
Tigoni	68.0 d	1.37	70.7 d	0.9	4.0	0.011
300046.22	52.2 e	1.82	55.2 e	0.77	5.7	0.014
Shangi	46.3 f	0.89	48.5 f	0.38	4.8	0.006
398190.200	43.9 g	1.45	45.3 g	1.42	3.2	0.048
Kenya Mpya	42.8 g	0.58	44.9 g	0.76	4.9	0.011
Sherekea	38.0 h	0.97	39.8 h	1.16	4.7	0.032
393371.157	35.0 i	0.96	37.0 i	0.82	5.7	0.014
392797.22	33.2 j	0.72	35.7 j	1.16	7.5	0.002
397073.7	24.0 k	0.96	26.3 k	0.39	9.6	0.02
P-value	<.001		<.001			

Means within the same column having a common letter(s) do not differ significantly at P ≤0.05. RSS- Random seed selection, PS- positive selection, Std. dev.- standard deviation and DI- disease incidence

4.6.3 Effects of positive selection on plant height

Increase in plant height was observed in plots planted with positively selected seed tubers. Plant height in PS plots ranged from 38 to 87cm while in RSS plots, plant height ranged from 36 to 83cm (Table 4.2). Plant heights varied significantly at (P≤0.05) among RSS and PS plots. Increase in plant heights through use of PS varied between the twelve genotypes with 398098.65 showing the highest response by 14% and 393371.157 the least by 1%. T-

test analysis revealed no significant difference in plant heights from RSS and PS plots among genotypes; 300046.22, Asante, 392797.22, and Kenya Mpya (Table 4.2).

Table 4. 2: Effects of PS and RSS on plant heights of different potato genotypes and percent height increase from PS

	Plant height				Increased height	
Genotypes	PS	Std. Dev.	RSS	Std. Dev.	(%)	p (1-tailed)
398190.200	87.1 a	1.5	83.4 a	1.4	4.4	0.007
Shangi	85.8 a	0.9	78.1 c	0.4	9.9	0
393371.157	82.2 b	0.8	81.1 b	0.9	1.4	0.031
Tigoni	73.6 c	0.7	71.1 d	1.4	3.5	0.025
392797.22	65.5 d	0.9	64.1 e	0.6	2.2	0.056
397073.7	65.4 d	0.2	64.3 e	0.6	1.7	0.021
393077.159	61.4 e	1.2	56.8 g	1.1	8.1	0.018
Asante	60.0 e	1.1	58.6 f	0.8	2.4	0.13
Sherekea	54.3 f	1.2	53.1 h	0.8	2.3	0.017
398098.65	47.1 g	0.9	41.4 i	1.5	13.8	0.001
300046.22	42.1 h	1.2	40.9 i	0.5	2.9	0.15
Kenya Mpya	38.4 i	0.9	36.6 j	1.6	4.9	0.102
P-value	<.001		<.001			

Means within the same column having a common letter(s) do not differ significantly at P ≤0.05. RSS- Random seed selection, PS- positive selection, Std. dev. - standard deviation

4.6.4 Effects of positive selection on number of tubers

Low number of tubers per hill of different potato genotypes were recorded in RSS plots ranging from 3 to 10 tubers compared to PS plots in which number of tubers ranged from 4 to 13 (Table 4.3). Number of tubers per hill varied significantly at (P≤0.05) in most genotypes in both RSS and PS plots. Percent increase in number of tubers achieved through use of PS varied among the twelve genotypes with 398098.65 showing the highest response by 41% increase and Asante the least by 9%. T-test analysis revealed no significant difference in number of tubers per hill from RSS and PS plots among genotypes; Asante, 392797.22, 393077.159, 393371.157 and 398190.200 (Table 4.3).

Table 4. 3: Effect of RSS and PS on number of tubers per hill of different potato genotypes and percentage tuber gain from PS

	Number of tubers				Tuber gain	
Genotypes	PS	Std. Dev.	RSS	Std. Dev.	(%)	p (1-tailed)
Shangi	12.2 a	0.5	9.8 a	1.1	24.5	0.003
392797.22	9.0 b	1.2	7.5 b	0.7	20	0.101
397073.7	8.4 b	1.0	6.9 bc	0.3	21.7	0.025
Asante	8.2 bc	0.7	7.5 b	1.3	9.3	0.262
393371.157	8.1 bc	1.1	7.0 bc	0.3	15.7	0.068
398190.200	8.0 bc	0.7	6.6 bcd	0.6	21.2	0.055
393077.159	7.0 cd	0.8	6.0 cd	0.5	16.7	0.065
300046.22	6.7 d	0.5	5.1 e	0.4	31.4	0.014
Sherekea	6.6 d	0.9	5.1 e	0.7	29.4	0.045
Tigoni	6.5 d	0.2	5.8 de	0.6	12.1	0.029
398098.65	4.8 e	0.2	3.4 f	0.4	41.2	0.001
Kenya Mpya	4.0 e	0.7	3.2 f	0.4	25	0.012
P-value	<.001		<.001			

Means within the same column having a common letter(s) do not differ significantly at P ≤0.05. RSS-Random seed selection, PS- positive selection, Std. dev. - standard deviation

4.6.5 Effects of positive selection on yield

Low potato yield was observed in RSS plots ranging from 5 to 25 t/ha compared to PS plots in which yields ranged from 7 to 29 t/ha. Yield varied significantly at (P≤0.05) among most genotypes in RSS and PS plots. However, T-test analysis revealed no significant difference in yields from RSS and PS plots among genotypes Tigoni and 393371.157 (Table 4.4). Percent yield increase by use of PS varied between the twelve genotypes with 300046.22 showing the highest response by 56% and 393371.157 the least by 4%.

Table 4. 4: Effects of RSS and PS on total yield of different potato genotypes and percent yield increase from PS

	Yield (t/ha)				Yield increase	
Genotypes	PS	Std. dev.	RSS	Std. Dev.	(%)	p (1-tailed)
392797.22	29.2 m	1.4	25.0 l	0.1	16.8	0.005
393371.157	23.1 k	1.3	22.2 jk	0.2	4.1	0.145
Shangi	22.2 jk	1.3	14.4 f	0.2	54.2	0.001
397073.7	21.8 j	0.4	18.7 h	0.1	16.6	0.000
398190.200	20.8 i	0.5	19.8 i	0.1	5.1	0.018
Asante	20.2 i	0.2	17.5 g	0.1	15.4	0.000
300046.22	20.1 i	1.5	12.9 e	0.3	55.8	0.001
393077.159	14.8 f	1.0	13.2 e	0.2	12.1	0.021
Tigoni	12.9 e	1.5	11.8 d	0.1	9.3	0.11
Kenya Mpya	7.2 c	0.5	5.6 ab	0.1	28.6	0.002
Sherekea	6.9 c	1.2	5.0 a	0.0	38	0.027
398098.65	6.9 c	0.1	6.3 bc	0.1	9.5	0.007
P-value	<.001		<.001			

Means within the same column having a common letter(s) do not differ significantly at P ≤0.05. RSS- Random seed selection, PS- positive selection, Std. dev. - standard deviation

4.6.6 Correlations among disease incidence, plant height, number of tubers and yield in RSS and PS plots.

Disease incidence correlated negatively to plant heights (r = -0.28, r = -0.21 at P ≤ 0.05), number of tubers per hill (r = -0.20 and r = -0.27 at P ≤ 0.05) and yield (t/ha) (r = -0.33, r = -0.36 at P ≤ 0.05). Plant heights displayed strong positively correlation to number of tubers per hill (r = 0.72, r = 0.69 at P ≤ 0.05) and yield (t/ha) (r = 0.67, r = 0.55 at P ≤ 0.05). While number of tubers per hill correlated positively to yield (r = 0.66, r = 0.71 at P ≤ 0.05) in both RSS and PS plots (Table 4.5).

Table 4. 5: Correlation coefficients among disease incidence, plant height, number of tubers and yield in Random seed selection and positive selection plots

	Virus incidence		Height		No. of tubers		Yield	
	RSS	PS	RSS	PS	RSS	PS	RSS	PS
Virus incidence	-	-						
Height	-0.28*	-0.21*	-	-				
No. of tubers	-0.20*	-0.27*	0.72**	0.69**	-	-		
Yield	-0.33*	-0.36*	0.67**	0.55**	0.66**	0.71**	-	-

Coefficients denoted by * indicate negative correlations while coefficients denoted by ** indicate positive correlation at p ≤0.05, RSS- Random seed selection, PS- positive selection

4.6.7 Virus infections in seed potato tubers of different genotypes

Among the tested tubers from PS plots, PVS was the most predominant (50%) followed by PVY (17%) and PLRV (2%) while PVA, PVM and PVX were not detected. Ten genotypes tested positive for PVS; 5 for PVY and 1 for PLRV. Five genotypes showed mixed infections by PVS and PVY and one genotype by PVS and PLRV. In tested tubers from RSS plots, PVS was the dominant virus (67%) followed by PVY (20%), PLRV (12%) and PVM (7%). All potato genotypes tested positive for PVS, 4 for PVY and PLRV while only 2 genotypes tested positive for PVM. Two genotypes showed mixed infections by PVS + PVY, one genotype by PVS + PLRV, one genotype by PVM + PVS; one genotype showed triple infection by PLRV + PVM + PVS and two genotypes by PLRV + PVS + PVY in tested tubers (Table 4.6).

Table 4. 6: Percent incidences of potato viruses in seed tubers from Random Seed Selection and Positive Selection plots

	Percent virus incidences											
	PLRV		PVA		PVM		PVS		PVX		PVY	
Genotype	RSS	PS	RSS	PS	RSS	PS	RSS	PS	RSS	PS	RSS	PS
Shangi	0	0	0	0	0	0	60	0	0	0	0	20
Asante	0	0	0	0	0	0	40	20	0	0	0	0
Tigoni	20	0	0	0	40	0	100	80	0	0	0	20
Sherekea	0	0	0	0	0	0	20	20	0	0	100	80
Kenya Mpya	0	0	0	0	0	0	60	20	0	0	60	20
397073.7	0	0	0	0	40	0	40	60	0	0	0	0
300046.22	20	20	0	0	0	0	80	100	0	0	60	0
392797.22	80	0	0	0	0	0	20	40	0	0	20	0
398098.65	0	0	0	0	0	0	80	0	0	0	0	0
393371.157	0	0	0	0	0	0	100	100	0	0	0	0
393077.159	20	0	0	0	0	0	100	60	0	0	0	60
398190.200	0	0	0	0	0	0	100	100	0	0	0	0
Percent incidence (%)	12	2	0	0	7	0	67	50	0	0	20	17

0-no infection, RSS- Random Seed Selection, PS- Positive Selection, PLRV- *Potato leaf roll virus*, PVA- *Potato virus A*, PVY- *Potato virus Y*, PVM- *Potato virus M*, PVS- *Potato virus S*, PVX- *Potato virus X*

4.7 Discussion

The study revealed low disease incidences which varied with genotypes in PS plots compared to RSS plots. Low virus incidences in PS plots might have resulted from low viral loads in asymptomatic plants selected as seed source in PS plots. Based on visual virus symptoms observed in the field it can be assumed that positive selection displayed encouraging results in managing spread of potato viruses in the field via seeds tubers but this is difficult to prove since some potato viruses always have latent infections (Muthomi *et al*., 2009; Njukeng *et al*, 2013). Gildemacher *et al*., (2011) also reported low disease incidence in PS plots. The study also revealed high average plant heights in PS plots in all the twelve genotypes. This may have occurred as a result of low disease incidences observed in PS plots compared to RSS plots.

High numbers of tubers per hill and yield (t/ha) were recorded in PS plots compared to RSS plots. This can be attributed to low virus incidences recorded in PS plots. Percent increase in yield through use of positive selection also varied among genotypes signifying that efficiency of positive selection is genotype/variety dependent. Genotypes; 300046.22 and Shangi had the highest percent yield increase while 393371.157 had the least. Schulte-Geldermann *et al*., (2012) reported that positive selection out yielded farmer selection with an overall average of 30% while Gildemacher *et al*., (2011) reported a yield increase in the range of 28% to 54% which was similar to our findings. Similarly, Schulte-Geldermann *et al*., (2012) also reported varied response of different potato varieties to positive selection in their study.

Four potato viruses; PVS, PVY, PLRV and PVM were detected infecting potato tubers collected from RSS plots either as single infection or as multiple infections. Potato Virus S was the most predominant and was detected to infect all the twelve genotypes followed by PVY, PLRV and PVM respectively. High incidences of PVS may have occurred due to its ability to pass through tissue culture process which is usually adopted in production of certified seeds (Were *et al.,* 2013) or because PVS is mainly transmitted mechanically, it can easily be spread by farmers during farm operations (Gul *et al.,* 2013). Low incidences of PLVR, PVM and PVY as well as zero detection of PVA and PVX in RSS tuber samples may be attributed to restricted spread of these viruses due to low numbers or absence of insect vectors and or absence of alternate hosts in the experimental site (Djilani-Khouadja *et al.,* 2010). *Potato Leaf Roll Virus* and PVY are easily expressed on leaves while PVS and PVM are usually asymptomatic in most field conditions (Kumar, 2010; Njukeng *et al.*, 2013) and this could also explain the low incidences of these viruses in the tested tubers compared to visual incidences scored in the field or these viruses might have been present but in low concentrations which could not be detected by the kit (El-Araby *et al.,* 2009). Were *et al*., (2013) also reported PVS as the most detected potato virus followed by PVY, PVX, and PLRV in samples collected from potato growing districts in Kenya. Yardimci *et al.* (2015) reported PVS and PVY as the most prevalent potato viruses in potato growing areas in Turkey when ELISA tests were conducted on tubers. In addition, Yardimci *et al.,* (2015) reported PVY+PVS (9.17%) as one of the most common multiple infections in potato (tubers) as revealed in this study.

Three potato viruses; PVS, PVY and PLRV were detected infecting potato tubers from PS plots also either as a single infection or as multiple infections. However these tubers incidences were low compared to those detected in RSS plots. This might be because of low viral loads in the seed tubers collected from apparently healthy looking mother plants and used for propagation (Gildemacher *et al*., 2011). *Potato Virus S* (PVS) was predominant followed PVY and PLRV and this might be an indication that it is difficult to select seed potato tubers free from these viruses via PS method (Njukeng *et al*, 2013). Njukeng *et al.*, (2013) also reported that seed potato tubers free from PVA and PVX can be selected with a high degree of certainty using positive selection method which was confirmed with our findings. Positive selection was efficient in selecting PVS free seed potato tubers from Shangi and 398098.65, PVY free seed potato tubers from300046.22 and 392797.22, PLRV free seed potato tubers from Tigoni and 393077.159 and PVM free seed potato tubers from Tigoni and 397073.7 based on ELISA test results. This might be an indication that efficiency of positive selection technique is genotypes/variety and virus dependent. Detection of PLRV and PVY in some of the resistant genotypes like Sherekea, 392797.22, 393077.159 and 300046.22 in tested tubers from both RSS and PS plots may be an indication that these varieties lose their resistance after multiples seasons of exposure to natural virus pressure in the field.

Virus incidences in the field displayed weak negative correlation to plant height, number of tubers and yield in both RSS and PS plots. In general, PS plots had weaker values compared to RSS plots. This was similar to the findings Schulte-Geldermann *et al*., (2012) who reported that positive selection reduced virus spread among plant populations with

regards to virus incidences in his experiment. Rahman *et al*., (2010) and Islam *et al.*, (2014)) also reported increase in potato virus incidences and reduction in plant height, number of tuber numbers and yield in their study. Reduction in these growth and yield parameters may have resulted from reduced disease resistance mechanisms in potato plants accruing from increased infections by potato viruses in the experimental fields. Salazar (1996) and Kumar (2010) documented dwarfism, stunted growth and yield loss as the major symptoms expressed by virus infected potato plants as revealed in this study.

However, the weak negative correlation can be an indication that reduction in growth performance and yield parameters might also have occurred as a result of other biotic and abiotic factors such as insect pests, fungal diseases, bacterial diseases and water stress (Pereira and Nova 2008). Plant height, number of tubers per hill, and yield demonstrated strong positive correlations in both RSS and PS plots. Similarly, Tuncturk *et al*., (2005) and Yousif *et al*., (2015) reported that plant height, leaf number per plant, leaf area, dry weight, tubers number, tubers weight, and potato tuber yields displayed positive and significant correlations in their study. Increase in potato plant height increases foliage cover and photosynthetic products leading to increased tuber number and yield.

Positive selection proved to be an efficient management strategy with regard to seed borne potato viruses. However, its efficiency was genotype dependent. Serological assay using DAS-ELISA also revealed a reduction in incidences of PLRV, PVS, PVM and PVY in PS plots compared to the conventional farmers' practice of random seed selection.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

Potato genotypes reacted differently to natural virus infections in the field. Some new clones displayed high levels of tolerance to virus infection compared to commercial cultivars. Even though there was an increase in virus incidence in season two, these clones still maintained high plant heights, number of tubers per hill and yield (t/ha) in comparison to the commercial varieties used as checks.

Positive selection was effective in management of potato viruses in the field. In addition, there was a reduction in incidence of PLRV, PVS, PVY and PVM after serological assay using DAS-ELISA in positively selected plots compared to the conventional farmers' practice of random seed selection in all the genotypes. Yield also increased with the use of positive selection but percentage increase was genotype dependent.

From this study, the following can be recommended to improve potato production.

5.2 Recommendations

i. Farmers should be sensitized to use resistant varieties and positive selection in management of seed borne potato viruses.
ii. Positive selection should be carried out in multiple in order to produce healthy seeds.
iii. Molecular methods should be used alongside DAS-ELISA to detect viruses present under lower concentrations in the seed tissues.

REFERENCES

Ali, S., Kadian, M. S., Ortiz, O., Singh, B. P., Chandla, V. K. and Akhtar, M. (2013). Degeneration of Potato Seed in Meghalaya and Nagaland states in North-eastern hills of India. Potato Journal, 40 (2): 122-127.

Alva, Ashok K., (2008). "Water management and water uptake efficiency by potatoes: A review." Archives of Agronomy and Soil Science 54(1):53-68.

Batool, A., M. A., Khan, J., Farooq, S. M., Mughal and Y. Iftikhar. (2011). ELISA-based Screening of Potato germplasm against *Potato leaf roll virus*. Journal of Agricultural Research. 49:57-63.

Black, V., (2008). Hot Potato: GM Potatoes in South Africa, a Critical Analysis. African Center for Biosafety.

Blom-Zandstra, M., and Verhagen, A., (2015). Potato production systems in different agro ecological regions and their relation with climate change. Report No. 614, p:32.

Bradshaw, J. E., and Ramsay, G., (2009). Potato origin and production. Advances in potato chemistry and technology. p: 1-26.

CABI (2007). Crop Protection Compodium (online). AvailableFrom URL: WWW.cabi.org/compendia/cpc/commonwealth. Agricultural Bereau international (CABI),willingford, Uk. date accessed 4th April 2015.

Carlos de Avila, A ., P. Eduardo de Melo, L. R. Leite, and A. K. Inoue-Nagata, 2009. Virus occurrence in potatoes in seven Brazilian States. Horticultura Brasileira 27(4):490-497.

Chiunga, E., (2013). Viruses Occurring In Potatoes (*Solanum tuberosum*) In Mbeya Region, Tanzania. Masters Thesis, University of Helsinki.

Clark, M. F., and Adams, A. N., (1977). Characteristics of the Microplate method of Enzymelinked Immunosorbent Assay for the detection of plant viruses. Journal General Virology, 34: 475-483.

De Bokx J., Van de Want J., (1987) Viruses of potatoes and seed-potato production. http://phytopath.ca/wpcontent/uploads/2014/10/cpdsarchive/vol67/CPDS_Vol_67_No _2_ (53) 1987.pdf. Date accessed 24/03/2015.

Dessureault, M., Prasad, R., Meberg, H and Teasdale, C. (2011). Controlling aphid-vectored viruses for organic seed potato production: Literature and knowledge review. http://www.certifiedorganic.bc.ca. Date accessed: 13/05/2015.

Djilani-Khouadja, F., Glais, L., Tribodet, M., Kerlan, C., and Fakhfakh, H. (2010). Incidence of potato viruses and characterisation of Potato Virus Y variability in late season planted potato crops in Northern Tunisia. European Journal of Plant Pathology, *126*(4): 479-488.

Durr, G., and Lorenzl, G., (1980). Potato production and utilization in Kenya. http://pdf.usaid.gov . Date accessed: 16/04/205.

Edwardson J., R., Christie R., G., (1997). Viruses Infecting Papper and other Solanaceous Crops. University of Florida USA. Volume 1: 106-123.

El-Araby, W. S., Ibrahim, I., A., Hemeida, A., A., Mahmoud, A., Soliman, A., M., El-Attar, A., K., and Mazyad, H., M., (2009). Biological, Serological and Molecular diagnosis of three major potato viruses in Egypt. International Journal of Virology, *5*(2):77-88.

Evans A., and Fenton B. (2010). A Literature Review of Insecticide and Mineral Oil Use in Preventing the Spread of Non-Persistent Viruses in Potato Crops. https://potatoes.ahdb.org.uk . Date accessed 12/06/2017.

Farshid Hassani, Abbas Zareian and Enayat Rezvani. (2014). Effects of chemical treatments on dormancy breaking and sprouting characteristics of two potato cultivars in different tuber sizes. European Journal of Experimental Biology, 4(4):98-102.

Fereres, A. (2000). Barrier crops as a cultural control measure of non-persistently transmitted aphid-borne viruses. Virus Research Journal, 71(1):221-231.

Fribourg C., (2007). Virus Viroides Y Mollicutes Delas Pant Cultivades En el peru. Lima, peru p:219 .

Gachene C.K.K. 1989. Soils of the Erosion Research Farm, Kabete Campus, University of Nairobi. Soil Science Society of American Journal, 61: 565-569.

Germchi, S., Behroozi, F., G., and Badri, S., (2011). Effect of thiourea on dormancy breaking and yield of potato (Solanum tuberosum L.) minitubers Marfona cv. in greenhouse. International Conference on Environmental and Agriculture Engineering, 15: 19-24.

Gildemacher, P., R., Schulte-Geldermann, E., Borus, D., Demo, P., Kinyae, P., Mundia, P., and Struik, P., C., (2011). Seed potato quality improvement through positive selection by smallholder farmers in Kenya. Potato Research Journal, 54(3):253-266.

Gildemacher, P.,Demo, P., Nyongesa, M., and Mundia, P., (2007). Selecting the best Plant to Improve Seed Potato. LEISA, 23:10-11.

Gul, Z., Khan, A., A., Khan, A., U., and Khan, Z., U., (2013). Incidence of potato viruses in different districts of Khyber Pakhtunkhawa, Pakistan. International Journal of Phytopathology, *2*(1): 32-36.

Hane, D., C., and Hamm, P., B., (1999). Effects of seedborne potato virus Y infection in two potato cultivars expressing mild disease symptoms. Plant Disease Journal, 83(1):43-45.

Harison, B., D., (1984). Potato Leaf Roll virus. CMI/ AAB. Description of Plant Viruses , No. 291. Commonwealth Agricultural Bureaux, Farnhamn Royal Slough, UK.

Hasan, M., S., and Rashid, M., M., (2015). Viral Infections in Potato Fields in Relation to Aphid Population. World Applied Sciences Journal, 33 (1): 63-68.

Haverkort A (1986) Forecasting national production improvement with the aid of a simulation model after the introduction of a seed potato production system in central Africa. Potato Res 29 (1):119–130.

Hinostroza-Orihuela, A., M., (1973). Some properties of potato virus S isolated from Peruvian potato varieties. Potato Research Journal, 16 (3):244-250.

Hossain, M., H., (1999). Studies of different aspects of seed tuber infection by PLRV and PVY contributing degeneration of potato. Thesis of MS in Plant Pathology, BSMRAU, 128pp.

Huamán, Z., Golmirzaie, A., and Amoros, W., (1997). The potato. Biodiversity in trust: conservation and use of plant genetic resources in CGIAR Centres, p:21-28.

Hutton, F., Spink, J., H., Griffin, D., Kildea, S., Bonner, D., Doherty, G., and Hunter, A., (2015). Distribution and incidence of viruses in Irish seed potato crops. Irish Journal of Agricultural and Food Research, *54*(2):98-106.

International potato center (CIP). (2010). Catalogue of CIP advanced clone. https://research.cip.cgiar.org. Date accessed: 02/04/ 2017.

Islam, A., K., M., S., Akanda, A., M., Wazed, M., A., Chewdhery, M., R., A., and Rahman, M., J., (2014). Performance of fifth generation seed potato against Potato virus Y (PVY). Journal of Agricultural Science, 4(2):74-81.

J., G., Mc Donald and R., P., Singh. (1996). Host Range, Symptomology and Serology of Isolates of Potato Virus Y (PVY) that Share Properties with both the PVYN and PVY^{o} Strain Groups. Central Plant Laboratory, Agriculture and Agri- food Canada, 3851 Fallowfilled Rd., Nepean Canada K2H 8P9.

Janssens, S., R., M., Wiersema, S., G., and Goos, H., T., (2013). The value chain for seed and ware potatoes in Kenya: Opportunities for development. http://library.wur.nl. Date accessed: 04/05/2015.

Jeffries C., (1998). FAO/IPGRI Technical Guidelines for the sale Movement of Germplasm. No. 19. Potatoes. Rome, Italy: FAO/International Plant Genetic Resources Institute.

John, O., Kiarie, N., Solomon, S., Charles, L., Muthoni, J., Otieno, S., P., (2013). Potato virus Y (PVY) and potato virus X (PVX) resistance breeding in Kenya: applicability of conventional approaches. Agriculture and Biology Journal of North America, 4, 398–405.

Kabira, J. N., Wakahiu, M., Wagoire, W., Gildemacher, P., and Lemaga, B. (2006). Guidelines for production of healthy seed potatoes in East and Central Africa. Kenya Agricultural Research Institute, Nairobi, pp:1-26.

Kerlan,C., (2006). Potato Virus Y. http://www.dpvweb.net. Date accessed :11/06/2015.

Kerlan, C. (2008). Potato Viruses in Mahy, B.W and Regenmortel, M.H.V (Eds). 201, Desk Enciclopidia of Plant and Fungal Virology San Diego, California, pp:45-47.

Kumar, R., (2010). Viral diseases and their management in potato production. https://www.researchgate.net. Date accessed:15/5/2015.

Kyamanywa, S., Kashaija, I., N., Getu, E., Amata, R., Senkesha, N., and Kullaya, A., (2011). Enhancing food security through improved seed systems of appropriate varieties of cassava, potato and sweetpotato resilient to climate change in Eastern Africa. https://cgspace.cgiar.org. date accessed: 17/04/2015.

Labarta, R., A., (2013). Possibilities and Opportunities for Enhancing Availability of High Quality Seed Potato in Ethiopia: Lessons from the Successful 3G Project in Kenya. Seed Potato Tuber Production and Dissemination. https://cgspace.cgiar.org. date accessed: 13/04/2015.

Lakra, B., S., (2010). Degeneration of potato cultivars due to apical leaf curl virus disease under Hisar ecological conditions. Potato Journal, 37(3-4): 164-66.

Lommen, W., J., (1994). Effect of weight of potato minitubers on sprout growth, emergence and plant characteristics at emergence. Potato Research Journal, 37(3), 315-322.

M., Warren, K., K., and A., S., (2005). Potato Virus Y (PVY) and Potato Leafroll Virus (PLRV): University of Pretoria, pp:32.

Machangi, J., Olubayo, F., Nderitu, J., Njeru, R., El-Bedewy, R., and Obudho, E., (2003). Effect of aphids and virus diseases on yield of seed potatoes in Kenya. African Crop Science Conference Proceedings, 6:220-223.

Mathew R., E., F., (2012). Plant Virology Third Edition. New York. Harcourt Brace Jovanovich, Publishers.

Milosevic D., Stamenkovic S., and Peric P. (2015). Potential use of insecticides and mineral oils for the control of transmission of major aphid-transmitted potato viruses. Pestic Phytomed Belgrade Journal, 27(2): 97-106.

Muindi, Muthomi, J., W., Nderitu, J., H., Olubayo, F., M., Kabira, J., N., Cheminin'wa, G., N., Kiretai, S., M., E., M., (2013). Management of Potato Viruses In Seed Potato Production Using Border Crops. http://erepository.uonbi.ac.ke. Date accessed: 23/04/2015.

Muthomi, J., W., Nyaga, J., N., Olubayo, F., M., Nderitu, J., H., Kabira, J., N., Kiretai, S., M., ... and Wakahiu, M., (2009). Incidence of aphid-transmitted viruses in farmer-based seed production in Kenya. Asian Journal of Plant Sciences, 8(2):166-171.

Muthoni, J., and Nyamongo, D., O., (2009). A review of constraints to ware Irish potatoes production in Kenya. Journal of Horticulture Forestry, 1(7):98-102.

Muthoni, J., J., Shimelis, H., and Melis, R., (2013). Potato production in Kenya: farming systems and production constraints. Journal of Agricultural Science, 5(5), p182.

Nascimento, L., C., Pio-Ribeiro, G., Willadino, L., and Andrade, G., P., (2003). Stock indexing and Potato virus Y elimination from potato plants cultivated *in vitro*. Scientia Agricola, 60 (3):525–530.

Nasir, M., Sajjid, S., Zaidi, H., Batool, A., Hussain, M., Iqbal, B., Javed, M., M., (2012). ELISA-based detection of major potato viruses in tissue culture produced potato germplasm, 2(1), 75–80. International Journal of Agriculture Science, *2*(1): 075-080.

National Potato Council of Kenya (NCPK). (2013). A Guide to Potato Production and Postharvest Management in Kenya. https://www.npck.org. Date accessed: 21/03/2017.

National Potato Council of Kenya (NCPK). (2015). Nation Potato Variety Catalogue. https://www.npck.org. Date accessed: 04/05/2017.

Njukeng, A. P., Chewachong, M., G., Chofong, G., Demo, P., Sakwe, P., and Njualem, K., D., (2007). Determination of Virus-free Potato Planting Materials by Positive Selection and Screening of Tubers from Seed Stores in the Western Highlands of Cameroon. In African Crop Science Conference Proceedings, 8: 809-815.

Njukeng, A., Chewachong, M. G., Chofong, G., Demo, P., Sakwe, P., and Njualem, K., D., (2013). Determination of scanned virus-free potato planting materials by positive selection and screening of tubers from seed stores in the western highlands of Cameroon. International Journal of Biological and Chemical Sciences, 7(2):707-716.

O'brien, P., J., Allen, E., J., and Firman, D., M., (1998). A review of some studies into tuber initiation in potato (*Solanum tuberosum*) crops. The Journal of Agricultural Science, *130*(03):251-270.

Onditi John, Njoroge Kiarie and Shibairo Solomon. (2013). Improving potato tuber yields using genotypes with multiple virus resistance in Kenya. Agriculture and Biology Journal of North America, 4:406-412.

Onditi, J., O., Nderitu, S.,W., K., Landeo, J., A., Abong, G., O., Sikinyi, E., O., and Kabira, J., N., (2012). Release of three improved varieties for the expanded potato market in Kenya. Agriculture and Biology. Journal of North America, 3, 192-197.

Palukaitis, P., (2012). Resistance to Viruses of Potato and their vectors. The Plant Pathology Journal, *28* (3):248-258.

Pereira, A., and Nova, N., V., (2008). Potato Maximum Yield as Affected by Crop Parameters and Climatic Factors in Brazil. Journal of Horticultural Sciences, *43* (5): 1611-1614.

Priou, S., (2001 b). Laboratory Manual for Double Antibody Sandwitch Enzyme Linked Immunosorbent Assay (DAS-ELISA) kit for detection of potato viruses. international Potato Center(CIP), Lima , Peru.

Radcliffe, E.,B., and D., W., Ragsdale, (2002). Aphid -Transmitted Potato Viruses: The Importance of Understanding Vector Biology. American Journal of Potato Research 79: 353-386.

Ragsdale D., W, Radcliffe E., B, DiF onzo C., D., (2001). Epidemiology and Field Control of PVY and PLRV. In : Loebenstein G, Berger PH, Brunt AA, Lawson RH, eds. Virus-like Diseases of Potatoes and Production of Seed-Potatoes, Dordrecht, The Netherlands; Kluwer Academinc Publishers. pp. 237-270.

Rahman M., S., Akanda, A., M., Mian, I., H., Bhuian M., K., A., and Karim M., R., (2010). Growth and yield performance of different generations of seed potato affected by PVYand PLRV. Bangladesh Journal of Agricultural Research, 35(1): 37–50.

Rahman, M. S., and Akanda, A., M., (2010). Effect of PLRV infected seed tuber on disease incidence, plant growth and yield parameters of potato. Bangladesh Journal of Agricultural Research, 35(3):359-366.

Raza, A., L., I., and Et, A., (2010). Molecular Detection of Potato Leaf Roll Polerovirus through Reverse Transcription Polymerase Chain. Pakistan Journal of Botany, 42(5):3299–3306.

Salazar, L., F., (1996). Potato viruses and their control. International Potato Center. https://books.google.co.ke. Date accessed: 18/05/2016.

Sanger, R., B., S ., H., O., Agrawal and B., B., Nagich. (1994). Studies of Primary Infection of PVX and PVY among tubers of potatoes. Indian Journal of virology. 10(1): 22- 27.

Schulte-Geldermann, E., (2013). Tackling Low Potato Yields in Eastern Africa: an Overview of Constraints and Potential Strategies. Seed Potato Tuber Production and Dissemination. https://cgspace.cgiar.org. Date accessed: 26/05/2015.

Schulte-Geldermann, E., Gildemacher, P., R., and Struik, P., C., (2012). Improving seed health and seed performance by positive selection in three Kenyan potato varieties. American Journal of Potato Research, 89(6):429-437.

Singh, N., A., R., E., N., D., R., A., Chaudhary, S., M., and Patel, N., H., (2012). Degeneration of potato cultivars in North Gujarat agroclimatic zone. Indian Phytopathology, *67*(3):311-313.

Solomon-Blackburn, R., M., and Barker, H., (2001). Breeding virus resistant potatoes (*Solanum tuberosum*): a review of traditional and molecular approaches. Journal of Heredity, *86* (1), 17-35.

Sukhotu, T., and Hosaka, K., (2006). Origin and evolution of Andigena potatoes revealed by chloroplast and nuclear DNA markers. Journal of Genomics, 49(6):636-647.

Thomas J., E., (1984). Characterisation of Australian Isolate Tomato Yellow Top Virus. Annals of Applied Biology, 104: 79-86.

Thomas-Sharma, S., Abdurahman, A., Ali, S., Andrade-Piedra, J. L., Bao, S., Charkowski, A., O., and Torrance, L., (2016). Seed degeneration in potato: the need for an integrated seed health strategy to mitigate the problem in developing countries. Plant Pathology Journal, *65*(1): 3-16.

Thomas, P., E., and Richards. (2004). New Weed Hosts of Potato viruses and their Impact on Potato Virus Epidemiology. Phytopathology 94:154.

Thresh, J., M., (2003). Control of plant virus diseases in sub-Saharan Africa: the possibility and feasibility of integrated approach. African Crop Science Journal, *11* (3):199-223.

Tshisola, S., N., (2014). Improved potato (*Solanum tuberosum*) seed production through aeroponics system (Doctoral dissertation, Stellenbosch: Stellenbosch University). http://scholar.sun.ac.za/handle/10019.1/86240. Deate accessed: 13/05/2015.

Tuncturk, M., and Ciftci, V., (2005). Selection criteria for potato (*Solanum tuberosum* L.) breeding. Asian Journal of Plant Sciences, 4(1):27-30.

Volkonan J., (2007). Viruses: Economical Losses and Biotechnical Potential. In: Potato Biology and Biotechnology. Advances and Perspectives. Vreugdenhill D. (Ed) pp. 619-641.

Wale S, Platt H.,W, (Bud) and Cettlin N., (2008). Disease, Pests and Disorders of Potatoes. A color Handbook. Manson Publishers, London , Uk. pp. 176.

Wang'ombe, J., G., and van Dijk, M., P., (2013). Low potato yields in Kenya: do conventional input innovations account for the yields disparity?. Journal of Agriculture and Food Security, 2: 14.

Warsito Tantowinjoyo. (CIP), Elseke Van de Fliert.(FAO). (2006). All about Potatoes A Handbook to the Ecology and Intergrated Management of Potato.

Were, H., K., Kabira, J., N., Kinyua, Z., M., Olubayo, F., M., Karinga, J., K., Aura, J., and Torrance, L., (2013). Occurrence and Distribution of Potato Pests and Diseases in Kenya. Potato Research, 56(4):325-342.

Wróbel, S., (2015). The Rate of Virus Spread in New Potato Cultivars in the North of Poland. Potato Research, *58*(4): 329-342.

Yardimci, N., Çulal Kılıç, H., and Demir, Y, (2015). Detection of PVY, PVX, PVS, PVA, and PLRV on Different Potato Varieties in Turkey Using DAS-ELISA. Journal of Agricultural Science and Technology, *17*(3):757-764.

Yousif, S., A., Zamil, Q., M., Al Muhi, H., Y., and Al Shammari, J., A., (2015). Effect of Three Sand Types on Potato Vegetative Growth and Yield. World Academy of Science, Engineering and Technology, International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering, 9(3):300-303.

APPENDIX

Appendix 1: Monthly weather conditions during crop growth in Field generation Three (FG3) (March to July 2015).

	Field Generation Three (FG3)				
Weather conditions	March	April	May	June	July
Rainfall amount (mm)	30.1	323.9	298.3	84.2	24.2
Mean temperatures (^{0}C)	21.97	21.32	20.5	19.68	18.9
Relative humidity (%)	55.6	69.1	73.65	76.05	74.95

Appendix 2: Monthly weather conditions during crop growth in Field Generation Four (FG4) (October, 2015 to February, 2016).

	Field Generation Four (FG4)				
Weather conditions	October	November	December	January	February
Total rainfall (mm)	117.4	478.3	117.7	95.4	100.3
Mean temperatures (^{0}C)	21.69	20.63	20.9	21.68	21.82
Relative humidity (%)	63.25	74.75	71.25	71.4	63.97